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Pharmacology and Toxicology of Amphetamine and Related Designer Drugs

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Pharmacology and Toxicology of Amphetamine and Related Designer Drugs

Editors:

Khursheed Asghar, Ph.D.
Division of Preclinical Research
National Institute on Drug Abuse

Errol De Souza, Ph.D.
Addiction Research Center
National Institute on Drug Abuse

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Preface

The abuse of amphetamines is of national concern from a public health perspective. Review of this subject is timely and important, because the problem of amphetamine-like drugs has recently been amplified by the introduction of designer drugs in the illicit market. There has been an increasing number of attempts by chemists in clandestine laboratories to synthesize structurally altered congeners that might intensify the mood-altering property of this class of compounds. While attention over the last few decades has been centered on research related to amphetamine, methamphetamine, and clinically prescribed amphetamine derivatives including fenfluramine, recent attention has focused on a variety of amphetamine-related designer drugs. These designer drugs include ring-substituted derivatives of amphetamine and methamphetamine such as 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA "ecstasy"), respectively. MDMA has been the focus of a great deal of recent attention, since it represents one of a number of "designer drugs" that is being increasingly abused among certain segments of the population, especially among college students. This popularity is ascribed to the drugs' mixed central nervous system (CNS) stimulant and hallucinogenic effects. Furthermore, MDMA has been the subject of recent scientific and legal debate, as several psychiatrists have reported that MDMA may "enhance emotions" and "feelings of empathy" and thus serve as an adjunct in psychotherapy. While the psychotherapeutic usefulness of this drug remains to be determined, a great deal of research has been carried out on the abuse liability, behavioral effects, and neurotoxic effects of the amphetamine-related designer drugs.

A technical review meeting entitled "Pharmacology and Toxicology of Amphetamine and Related Designer Drugs" was held at the National Institutes of Health on August 2-4, 1988. The purpose of the technical review was to bring together scientists who have been carrying out research in the area to (1) summarize the research findings, (2) understand the neuronal mechanisms through which the amphetamines produce their effects, and (3) develop a consensus regarding future directions that may lead to better characterization of the effects of these drugs on various physiological parameters. An understanding of the mechanisms is critical to the development of therapeutic approaches for the treatment of intoxication, addiction, and adverse effects. The proceedings of this meeting are presented in the following chapters.

Khursheed Asghar, Ph.D.
Division of Preclinical Research
National Institute on Drug Abuse
Rockville, MD

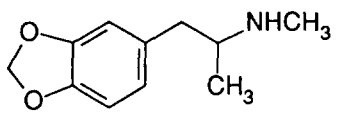
Errol B. De Souza, Ph.D.
Neurobiology Laboratory
Neurosciences Branch
Addiction Research Center
National Institute on Drug Abuse
Baltimore, MD

Structure-Activity Relationships of MDMA-Like Substances

David E. Nichols and Robert Oberlender

INTRODUCTION

There is virtually no one who is involved in drug abuse research, or who studies the properties of recreationally used drugs, that is not by now familiar with 3,4-methylenedioxymethamphetamine (MDMA) (figure 1). Over the past 4 years this substance, usually referred to in the popular press as "Ecstasy," has received widespread media attention, This chapter will relate recent fmdings with respect to the potential dangers attendant on the use of MDMA and explore its pharmacological properties.



MDMA (1)

FIGURE 1. *MDMA*

As the title implies, MDMA has pharmacological properties that set it apart from other classes of drugs. This is one of the most intriguing aspects of MDMA, largely overlooked as researchers examined the potential risks to health of MDMA use. Basic questions of how drugs work and why some are pleasurable and some are not are fundamental to our understanding of why humans use drugs. Although much of the popularity of MDMA can no doubt be attributed to curiosity following media attention, the drug itself must have some rewarding qualities.

MDMA typifies a central problem with the substituted amphetamine-type substances: The fact that we know so little about any of these kinds of drugs. What does MDMA actually do? What are the psychopharmacological properties that make it attractive for recreational use? Is it "just another hallucinogenic amphetamine," as some have asserted? In the following

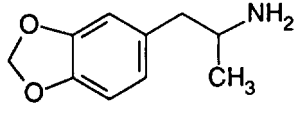
discussion, an attempt will be made to address some of these issues, and to put the questions into a broader perspective.

MDMA was patented in 1914 by a German pharmaceutical firm and evaluated as an appetite suppressant (Shulgin 1986). In that sense, MDMA is not a "designer drug." Its rediscovery in the late 1970s probably had little to do with the fact that it was, technically speaking, a legal drug. There were a variety of legal psychoactive drugs, many of which could probably have been synthesized and marketed with greater economic profit than MDMA, a substance with unremarkable quantitative potency, being only two to three times more active on a weight basis than mescaline (Shulgin and Nichols 1978). Nonetheless, no other substituted amphetamines with the popularity of MDMA have appeared. The explanation seems to be that MDMA has psychopharmacological properties that are deemed especially rewarding to the user.

MDMA is believed to have unique psychoactive properties that clearly distinguish it from hallucinogenic or psychostimulant phenethylamines. Not only have MDMA users consistently reported this distinctiveness, but subsequent studies of MDMA and similar compounds, in many laboratories. have shown that they do not fit within the structure-activity relationships that presently are understood to define the hallucinogenic amphetamines.

STRUCTURAL FEATURES OF MDMA

One of the structural features of MDMA that is somewhat unusual is the fact that it is 3,4-disubstituted. Both 3,4-methylenedioxyamphetamine (MDA) (figure 2) and MDMA possess the 3,4-methylenedioxy function, and there apparently are no other active compounds known that fall within the



MDA (2)

FIGURE 2. *MDA*

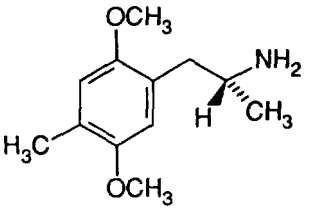
substituted amphetamine class and have substituents only in the 3 and 4 positions. The largest group of substituted amphetamines with significant hallucinogenic potency possess either 3,4,5- or 2,4,5- trisubstitution patterns. The parent compound MDA, although classified as a hallucinogenic amphetamine and available on the illicit market for about 20 years, had gained a reputation as the "love drug" (Weil 1976). It had been recognized for many years by both recreational drug users and clinicians (Turek et al. 1974) that

MDA had unique psychoactive properties that were different from hallucinogens such as LSD or mescaline. While MDA in high doses appears to be hallucinogenic or psychotomimetic, it seems not to have been used for this effect, but rather for its effects on mood: production of a sense of decreased anxiety and enhanced self-awareness. Even early reports described the desire of MDA users to be with and talk to other people (Jackson and Reed 1970). MDA is also the only substituted amphetamine that received serious clinical study as an adjunct to psychotherapy (Yensen et al. 1976).

A second structural feature of MDMA that distinguishes it from hallucinogenic amphetamines is the fact that it is a secondary amine. That is, the basic nitrogen is substituted with an N-methyl, while hallucinogenic amphetamines are most potent as primary amines. In either 3,4,5- or 2,4,5-substituted phenethylamine derivatives, N-methylation decreases hallucinogenic potency by up to an order of magnitude (Shulgin 1978). When MDA is ingested, the hallucinogenic effects are long lasting, typically 10 to 12 hours, similar to the duration of LSD or mescaline. By contrast, MDMA has a much shorter action, with perhaps a 3- to 5-hour duration of effects. There is no evidence that typical doses of MDMA lead to hallucinogenic effects in a significant proportion of users, although in high doses hallucinogenic effects have been reported (Siegel 1986). Thus, the simple addition of the N-methyl group limits the temporal course of the action to less than half that of MDA and attenuates or abolishes the hallucinogenic effects that occur with MDA itself.

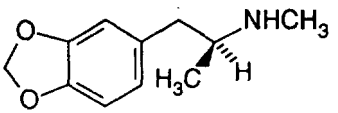
A third important difference between MDMA and the hallucinogenic amphetamines is the reversal of stereochemistry that occurs in MDMA. In every substituted hallucinogenic amphetamine that has been studied, the isomer with the *R* absolute configuration in the side chain is more potent in animal models, in a variety of in vim assays, and in man (figure 3). The two isomers differ in potency by a factor of 3 to 10, depending on the assay system (Nichols and Glennon 1984). By contrast, the *S* isomer of MDMA is more potent (figure 4). This was first reported in experiments with rabbits and in clinical studies (Anderson et al. 1978), and it has recently been confirmed in other animal models (Oberlender and Nichols 1988; Schechter 1987).

It is difficult to trivialize the significance of this argument, since the stereospecificity of biological receptors is accepted as a basic tenet of pharmacology. There is no rationale or experimental precedent for believing that the 3,4-methylenedioxy substitution should do anything that would cause the receptor(s) involved to accommodate a side chain stereochemistry reversed from that for phenylisopropylamines with other aromatic subtituents.



R-(-)-DOM

FIGURE 3. *The more active R-(-)-enantiomer of the hallucinogenic amphetamine DOM*



S-(+)-MDMA

FIGURE 4. *The more active S-(+)-enantiomer of MDMA*

Several studies have now clearly shown that the *R* enantiomer of MDA has the hallucinogenic effects of the racemate, while the *S* enantiomer possesses more potent MDMA-like properties than the *R* in animals models (Anderson et al. 1978; Shulgin 1978; Glennon and Young 1984a; Nichols et al. 1982; Nichols et al. 1986; Oberlender and Nichols 1988). Further, although (+)-MDA appears similar to amphetamine in the drug discrimination assay in rats (Glennon and Young 1984a), it is not generally realized that the effects of (+)-MDA in humans qualitatively resemble those of MDMA, rather than those of amphetamine (Shulgin, personal communication, 1985). This is a unique situation. Both enantiomers of MDA are active, having nearly equal quantitative potencies, but differing in qualitative effect. N-methylation of the racemic material dramatically and selectively attenuates the hallucinogenic effects of the *R* enantiomer, while essentially leaving intact the properties of the *S* enantiomer.

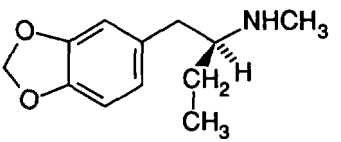
In earlier proposals (Anderson et al. 1978), based on this stereoselectivity for the *S* enantiomer of MDMA, it was suggested that, rather than having a direct effect at serotonin receptors, perhaps MDMA was a neurotransmitter-releasing agent, acting in a fashion similar to amphetamine, for which the *S* enantiomer is also more active than the *R* enantiomer. A subsequent study

in our laboratory indicated that the *S* isomers of MDA and MDMA were indeed potent releasers of [^{3}H]serotonin from prelabeled rat brain synaptosomes (Nichols et al. 1982). Recently, it was repotted that MDA and MDMA were potent releasers of serotonin from superfused hippocampal slices prelabeled with [^{3}H]serotonin (Johnson et al. 1986). In all studies to date, whether of release of monoamines from synaptosomes or brain slices, or of the inhibiting of monoamine reuptake into synaptosomes (Steele et al. 1987), the *S* enantiomer of MDMA is either equipotent to the *R* isomer or more potent.

THE ENTACTOGENS

As a consequence of these and other studies that have indicated that MDMA has a pharmacology different from the hallucinogenic amphetamines, and in view of the reports by certain psychiatrists (Greer and Tolbert 1986; Wolfson 1986) that MDMA could facilitate the process of psychotherapy, it was hypothesized that MDMA and related compounds represent a new pharmacological class, with as yet unexplored potential as psychiatric drugs (Nichols 1986; Nichols et al. 1986). This class of drugs has been called entactogens. Recently, efforts have been directed toward understanding the mechanism of action of MDMA and related compounds and testing the hypothesis that entactogens are a novel pharmacological class, distinct both from hallucinogenic agents and from central stimulants such as amphetamine or cocaine.

Important support for this hypothesis came from the discovery that the alpha-ethyl homolog of MDMA, MBDB (figure 5) possessed MDMA-like

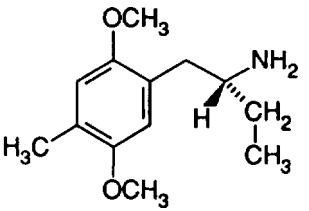


S-(+)-MBDB (3)

FIGURE 5. *The S-(+)-enantiomer of the alpha-ethyl homologue of MDMA, MBDB*

properties in man and in the drug-discrimination paradigm in rats (Nichols et al. 1986; Oberlender and Nichols 1988). It was known that homologation of the alpha-methyl of the hallucinogenic amphetamines completely abolished hallucinogenic activity (Standridge et al. 1976). For example, the alpha-ethyl homolog of *R*-DOM, BL-3912A (figure 6) was evaluated by a major pharmaceutical firm and found to lack hallucinogenic activity at doses more than a hundredfold higher than those effective for DOM (Winter 1980). This additional feature of the entactogens, that the alpha-ethyl

homologs retained activity, was a final and most powerful argument that MDMA, and certainly MBDB, could not lit within the well-established structure-activity relationships of the hallucinogenic amphetamines.



R-(-)-BL3912A (4)

FIGURE 6. *The nonhallucinogenic alpha-ethyl homologue of DOM, BL-3912A*

STUDIES OF STRUCTURE-ACTIVITY RELATIONSHIPS

EEG Studies

Recently, Dr. W. Dimpfel has used quantitative radioelectroencephalography in the rat to characterize the electroencephalograph (BEG) "fingerprint" of hallucinogenic amphetamines, MDMA, and MBDB. In this technique, four bipolar stainless steel electrodes are chronically implanted in each of four brain regions in rats: the frontal cortex, the hippocampus, the striatum, and the reticular formation (Dimpfel et al. 1986). The rats are freely moving; transmission of field potentials is accomplished using a telemetric device. The EEG is analyzed by Fourier analysis; power density spectra are computed for periods of 4 seconds, segmented into six frequency bands, and averaged on each channel over timeblocks of 15 minutes.

Using this method, a variety of hallucinogenic and nonhallucinogenic compounds were examined. As previously reported (Spüler and Nichols 1988), hallucinogens produce a marked increase of power in the a, frequency (7.0 to 9.50 Hz) in the striatum. The ability to increase power in this region of the EEG has been observed for other classes of serotoninergic drugs, including the 5-HT_{1A} agonists ipsapirone, gepirone, and buspirone, and with serotonin-uptake inhibitors (Dimpfel et al. 1988). With 5-HT_{1A} agonists, however, an increase in α_1 power is recorded only from the frontal cortex and hippocampus.

Doses of DOM, DOB, or DOI of 0.2, 0.1, and 0.1 mg/kg, respectively, produced a pronounced and long-lasting increase in a, power recorded from the striatum. By contrast, doses of (+)-MDMA and (+)-MBDB up to 1.6 mg/kg did not elicit this characteristic feature in the EEG. Thus, in this

sensitive quantitative EEG procedure, neither MDMA nor MBDB elicited an EEG fingerprint (four electrodes by six frequency bands per electrode) that resembled that produced by the hallucinogenic amphetamines DOM, DOB, DOI, or LSD. These data are consistent with the results obtained in other models and further support the hypothesis that MDMA and MBDB are not hallucinogenic phenethylamines.

Thus, for this class of psychoactive agent, preliminary structure-activity relationships are being formulated. Currently, four structural features contrast the structure-activity relationships of entactogens with those of hallucinogenic amphetamines.

(1) Ring substitution at only the 3,4- positions does not give active hallucinogens, except for MDA. However, this substitution is active for entactogenic agents.

(2) N-methylation greatly attenuates hallucinogenic activity, but has no significant effect on potency of entactogens. N-ethylation also seems to allow compounds to retain entactogenic activity.

(3) The more active stereochemistry of the entactogens is *S,* while that of the hallucinogenic amphetamines is *R.*

(4) Extension of the alpha-methyl to an alpha-ethyl abolishes hallucinogenic activity, but has only a minor effect on entactogens.

Drug Discrimination Studies

At the present time these contrasts seem sufficient to distinguish between the two drug classes. The stereochemical argument and the effects of alpha-ethylation are extremely powerful. A significant problem with the hypothesis remained: showing that entactogens differed from another structurally related class, the central nervous system (CNS) stimulants. Several studies have characterized MDMA as an amphetamine-like or cocaine-like agent, based on its stimulus properties or its self-administration in primates (Beardsley et al. 1986; Lamb and Griffiths 1987; Evans and Johanson 1986; Kamien et al. 1986). It is well known that both amphetamine and cocaine have powerful effects on dopamine pathways in the brain, and it seems likely that drugs that release dopamine, or stimulate dopamine receptors, have reinforcing properties that lead to self-administration and dependence liability (Wise and Bozarth 1987).

It could not be anticipated that the extension of the alpha-methyl of MDMA to an alpha-ethyl would also attenuate the effects of the compound on dopaminergic pathways in the brain. In contrast to MDMA, MBDB has no significant effect either on inhibition of uptake of dopamine into striatal synaptosomes (Steele et al. 1987) or on release of dopamine from caudate

slices (Johnson et al. 1986). In subsequent drug discrimination experiments in rats, the dopaminergic properties of MDMA were evident, while MBDB seemed to have a pharmacologically "cleaner" discriminative cue.

To characterize further the behavioral pharmacology of MDMA and MBDB, extensive drug discrimination studies were carried out using rats trained to discriminate saline from LSD, saline from (+)-amphetamine, saline from (±)-MDMA, and saline from (+)-MBDB. Table 1 summarizes the results of those experiments. As is the case with hallucinogens, the drug discrimination paradigm should not be considered, in strict terms, an animal model for entactogen activity. Yet, data from these experiments can provide a good initial behavioral evaluation of the qualitative and quantitative effects of a variety of compounds of interest.

It is clear from these results that, in MDMA- or MBDB-trained rats, complete generalization of the training cue to the typical hallucinogenic drugs LSD, DOM, and mescaline does not occur. Furthermore, transfer of the training stimulus does not occur to MDMA or MBDB in animals trained to discriminate LSD from saline (Nichols et al. 1986). Although MDMA has been shown to substitute for mescaline (Callahan and Appel 1987). (+)-MBDB-trained rats did not recognize the mescaline cue as similar to the training drug. These results are consistent with the conclusion that MDMA and MBDB are not hallucinogenic, as discussed earlier.

These data clearly illustrate the enantioselectivity of the (+)-isomers of MDA, MDMA, and MBDB in producing an MDMA-like stimulus and underscore the fact that *in vitro* studies of the biochemical pharmacology of these substances should reveal similar selectivity, once the primary pharmacological process underlying the interoceptive cue is identified. The data also indicate that (+)-MDA is the most potent of all the drugs tested in MDMA- or in (+)-MBDB-trained animals. The fact that (+)-MDA does not substitute in amphetamine-trained animals in our studies supports the argument that the pharmacology of this enantiomer of MDA is MDMA-like and is not like amphetamine.

Although amphetamine substitutes for MDMA in our studies, this occurs only at doses that disrupt a significant number of animals. Furthermore, the large ED_{50} for amphetamine substitution in MDMA-trained rats is certainly not consistent with the known potency of amphetamine in measures of its stimulant activity. That is, in man, or in animal assays of its activity as a CNS stimulant, amphetamine is perhaps 10 times more potent than MDA or MDMA. Thus, its large ED_{50} relative to that of the enantiomers of MDA or MDMA seems to suggest strongly that the primary discriminative cue of MDMA cannot simply be "amphetamine-like." Although some investigators have reported stimulus transfer with MDMA in animals trained to discriminate amphetamine from saline, in our paradigm no substitution occurred.

TABLE 1. *Results of drug discrimination transfer tests in LSD, (+)-amphetamine, (±)-MDMA, or (+)-MBDB-trained rats (ED_{50} expressed in micromoles per kilogram of body weight)*

Substitution Drug	Training Drug LSD	AMP	MDMA	(+)-MBDB
LSD	0.025	NS	PS[1]	PS[2]
DOM	0.61	NS	NS	NS
(+)-AMP	NS	1.68	4.22	NS
(+)-MDA	NS	NS	1.63	1.43
(-)-MDA	2.94	NS	2.27	3.09
(+)-MDMA	NS	NS	1.92	1.67
(-)-MDMA	NS	NS	5.03	3.09
(+)-MBDB	NS	NS	3.67	3.28
(-)-MBDB	NS	NS	6.71	6.51
Cocaine	NT	20.0	13.9	PS[3]
Mescaline	33	NS[b]	NT	NS
Fenfluramine	PS[4]	NS	NT	2.01

KEY: NS=no substitution occurred; PS=partial substitution; NT=not tested.

NOTE: Training doses: LSD tartrale 0.186 µmol/kg; (+)-amphetamine sulfate 5.43 µmol/kg; racemic MDMA.HCl 7.63 µmol/kg; and (+)-MBDB.HCI 7.19 µmol/kg. [1]78% at 0.372 µmol/kg; [2]57% at 0.186 µmol/kg; [3]63% at 29.42 µmol/kg; and [4]71% at 4.68 µmol/kg.

SOURCES: Stolerman and D'Mello 1981; Schechler and Rosecrans 1973.

Differences in experimental design or in numbers of animals and doses tested may account for this discrepancy. In our experiments, symmetrical transfer did not occur between MDMA and amphetamine.

These results show that the MDMA cue is complex and may have some similarity to amphetamine. However, suggestions that the pharmacology of (+)-MDMA is essentially the same as that of amphetamine are clearly not warranted by the data, This partial amphetamine-like action is believed to

be reflective of the effect that MDMA has on dopaminergic pathways (Johnson et al. 1986; Steele et al. 1987). Other workers have reached similar conclusions (Gold and Koob 1988).

Similarly, self-administration of MDMA in monkeys trained to self-administer amphetamine (Kamien et al. 1986) or in monkeys or baboons trained to self-administer cocaine (Beardsley et al. 1986; Lamb and Griffiths 1987) probably reflects a dopaminergic component to the pharmacology of MDMA. This would be consistent with current theories of dopamine involvement in the mechanism of action of drugs with dependence liability (Wise and Bozarth 1987).

In vitro studies have also shown that the alpha-ethyl congener MBDB lacks significant effects on dopamine systems in the brain. The drug discrimination data support this idea, and amphetamine does not substitute in (+)-MBDB-trained rats. Furthermore, while cocaine fully substitutes in MDMA-trained rats. it produces partial substitution in (+)-MBDB-trained rats. This is further evidence of the decreased effect of MBDB on catecholaminergic systems. If the data have been interpreted correctly, this might suggest that MBDB would not be self-administered in animal models of dependence behavior, and, hence, might have low abuse potential. It has been found, however, that (+)-MBDB produces serotonin neurotoxicity in rats, although MBDB is somewhat less toxic than MDMA (Johnson and Nichols, unpublished).

To summarize the data in table 1, neither MDMA nor MBDB has hallucinogen-like discriminative stimulus properties. Symmetrical transfer of the MDMA and MBDB stimulus indicates that their primary discriminative stimulus effects are very similar. For both MDMA and MBDB, there is enantioselectivity for the *S* isomer, with about a twofold eudismic ratio. Finally, the substitution of (+)-amphetamine and cocaine in MDMA-trained rats may indicate that MDMA has some psychostimulant-like properties, while MBDB seems to lack this activity.

Effect of the Side Chain Alpha-Ethyl

It seemed likely that an alpha-ethyl moiety would attenuate the ability of other phenethylamines to interact with dopaminergic systems. To test this hypothesis, the alpha-ethyl homolog of methamphetamine was synthesized. This compound (figure 7) was also tested in the drug discrimination paradigm in (+)-amphetamine trained rats, and compared with (+)-methamphetamine. While (+)-methamphetamine was found to have an ED_{50} of 1.90 micromoles per kilogram (µmol/kg), the racemic alpha-ethyl homolog only produced full substitution at high doses, and had an ED_{50} of 19.62 µmol/kg, making it approximately one-tenth the potency of (+)-methamphetamine. This confirmed our speculation, and illustrated that the alpha-ethyl group

was effective in reducing the effect of phenethylamines on catecholamine pathways.

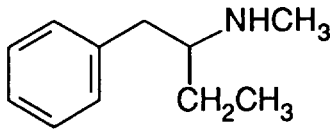


FIGURE 7. *The alpha-ethyl homologue of methamphetamine*

Thus, for structure-activity studies of MDMA-like substances, emphasis has been placed on the use of (+)-MBDB as the training drug, since it seems to possess a primary psychopharmacology similar to that of MDMA, but lacks the psychostimulant component of MDMA. That is, MBDB is pharmacologically less complex.

Table 2 is a summary of drug discrimination testing data for drugs that completely substitute in rats trained to discriminate saline from (+)-MBDB-HCl (1.75 mg/kg; 7.19 μmol/kg). These data are arranged in order of decreasing relative potency.

It is clear that the (+)-isomers of MDA and MDMA are the most potent in producing an MBDB-like cue. Furthermore, the stimulus produced by (+)-MDA is probably unlike that produced by amphetamine, based on the data presented in the earlier table. Thus, if the psychopharmacology of (+)-MDA is like that of MDMA, then N-methylation has little effect on the entactogenic properties of the molecule, but serves primarily to attenuate the hallucinogenic activity of (-)-MDA. Nevertheless, (-)-MDA also substitutes, and the psychopharmacology of racemic MDA might be viewed as comprised of the hallucinogenic and entactogenic properties of the (-)-isomer and the entactogenic and psychostimulant properties of the (+)-isomer. This illustrates why detailed studies of the mechanism of action of psychoactive compounds should be done on the pure optical isomers.

But what is the effect of MBDB or MDMA? We have been attempting to define this through the use of drug discrimination assays, with rats trained to a variety of drugs. Through the use of appropriate agonists and antagonists, we may be able to define the pharmacology of MBDB. Although there are some exceptions (e.g., fenfluramine), most of the substituted phenethylamines described in the literature can be categorized as hallucinogens or as stimulants. The psychopharmacology of MDMA perhaps represents a third category, and it is possible that other phenethylamine and amphetamine derivatives may possess similar pharmacology,

TABLE 2. *Compounds that completely substitute for (+)-MBDB in drug discrimination tests in rats*

Test Drug	ED_{50}(μmol/kg)	95% Confidence Interval
S-(+)-MDA	1.43	0.9 - 2.29
S-(+)-MDMA	1.67	0.98 - 2.86
Fenfluramine	2.01	1.30 - 3.09
(±)-MDA	2.09	1.36 - 3.21
(±)-MBDB	2.92	2.17 - 3.92
R-(-)-MDMA	3.09	1.80 - 5.32
R-(-)-MDA	3.09	1.88 - 5.07
S-(+)-MBDB	3.28	2.15 - 5.01
(±)-MDMA	3.35	2.35 - 4.77
R-(-)-MBDB	6.51	4.54 - 9.34

In view of the apparent pleasurable effects of MDMA, it becomes of considerable interest to understand the mechanism of action of substances with a similar effect. Major efforts have been directed toward the study of agents that have an effect on serotonin pathways, since that is the neurotransmitter system that seems most implicated in the mechanism of action of MDMA. This hypothesis is further reinforced by the observation that MDMA substitutes for fenfluramine (Schechter 1986). and fenfluramine substitutes for MBDB (Oberlender and Nichols, unpublished). The substitution data for (+)-amphetamine and cocaine in (+)-MBDB-trained rats are also similar to the data for substitution of these agents in fenfluramine-trained rats (White and Appel 1981).

However, the specific serotonin uptake inhibitor fluoxetine failed to produce an MBDB-like cue and failed to block the stimulus effects of MBDB when it was given prior to a training dose of MBDB. Table 3 summarizes results of fluoxetine testing in MBDB-trained rats. In other exploratory studies, pretreatment of MDMA-trained rats with either methysergide or ketanserin failed to block completely the MDMA-discriminative stimulus.

Based on the modest ability of the (+)-isomers of MDMA and MBDB to inhibit the reuptake of norepinephrine (NE) into hypothalamic synaptosomes (Steele et al. 1987). it seemed possible that noradrenergic pathways might be involved in the cue. In another series of drug discrimination experiments designed to test this hypothesis, the specific NE uptake inhibitor (-)-tomoxetine was tested for stimulus transfer in doses up to 10 mg/kg in MDMA-trained rats. At 5 mg/kg, 67 percent of the animals responded on, the drug lever. However, pretreatment with tomoxetine in six rats trained to discriminate MDMA from saline had no effect on the discrimination of a subsequent dose of MDMA.

TABLE 3. *Results of tests for fluoxetine substitution in (+)-MBDB.HCl-trained (1.75 mg/kg) rats*

Dose of Fluoxetine	N	Percentage Selecting Drug Lever
7.23 µmol/kg	8	38%
14.46 µmol/kg	8	50%
29.92 µmol/kg	7	43%

At the present time, a variety of other pharmacological agents are being tested for their ability either to antagonize or to potentiate the effect of MDMA in these animals. There is hope that appropriate pharmacological manipulations will eventually be found that will give useful information about the mechanism of action for entactogens.

ANALYSIS OF STRUCTURE-ACTIVITY RELATIONSHIPS

Medicinal chemists have a distinct advantage in pursuing mechanism-of-action studies because it is possible to synthesize a series of structurally related congeners and measure their biological activity. A correlation between activity and particular structural features not only helps to identify the pharmacophore, or active moiety imbedded within the molecule, but also may establish critical requirements or complementarity for the biological target or receptor for the particular drug class.

When a particular behavioral pharmacology is associated with a specific biochemical action within a series of congeners, it is likely that the biochemistry is a functional component of the observed behavioral activity. This is not necessarily the case if only one or a few molecules are available for study; they may well possess ancillary biochemical pharmacology that is

unrelated to the behavioral phenomenon being observed. However, the larger the series of structurally diverse molecules in which the two activities are associated, the stronger the basis for believing that a cause-effect relationship exists.

In designing studies of the structure-activity relationships of MDMA and related substances, there are at least three areas for structural modification. First, the nature of the amine substituents can be varied: other N-alkyls can be studied, or the nitrogen can be incorporated into a ring system. A second point for structural modification is the side chain. As already demonstrated, the alpha-methyl can be extended to an alpha-ethyl. Other modifications of the side chain would include incorporation into a variety of ring systems, or α,α-dialkylation. Finally, the nature and location of the ring substituents can be modified.

N-Alkylation

A number of investigators have examined the N-ethyl congener of MDMA, MDE (or MDEA), which has also gained popularity on the illicit market. Braun et al. (1980) have reported that, of the N-substituted MDA derivatives that were studied for analgesic action and human psychopharmacology, only the N-methyl, N-ethyl, and N-hydroxy compounds were active. The latter compound, the N-hydroxy, in all probability serves merely as a prodrug for MDA, being metabolically reduced to the primary amine, as has been observed for para-chloramphetamine (PCA) (Fuller et al. 1974). Since the range of modification of N-substitution seems so limited, it appears unlikely that studies of N-substituted MDA analogs will offer significant insight into mechanism of action. However, different N-alkyl groups may affect regional brain distribution and pharmacokinetic properties. For example, Boja and Schechter (1987) have found that the N-ethyl analog MDE has a much shorter biological half-life than does MDMA.

Ring Substituents

Little is presently known about requirements for particular aromatic ring substituents enabling a compound to possess MDMA-like activity. The 3,4-ethylidenedioxy and 3,4-isopropylidenedioxy compounds (figures 8 and 9) have been examined for ability to substitute in LSD- or MDMA-trained rats in the drug discrimination paradigm. Both compounds gave full substitution in rats trained to either drug. Those results and comparison data for MDA are given in table 4. Addition of steric bulk to the dioxole ring reduces CNS activity, whether defined as LSD-like or MDMA-like. Fenfluramine also produces a cue that is similar to both MDMA and MBDB, in that complete substitution occurs and does so at a relatively low dose of fenfluramine. This would seem to imply that the dioxole ring is

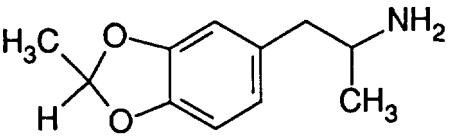


FIGURE 8. *The dioxole-ring methylated homologue of MDA, EDA*

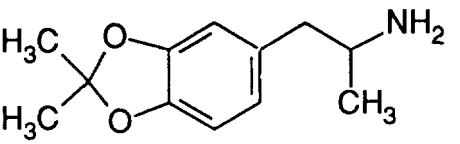


FIGURE 9. *The dioxole-ring dimethylated homologue of MDA, IDA*

not essential, and many workers have drawn comparisons between the neurotoxicity of fenfluramine and that of MDMA. However, the psychopharmacology of fenfluramine is quite different from that of MDMA.

TABLE 4. *ED_{50} values for substitution in LSD-trained or MDMA-trained rats, in the drug discrimination paradigm*

Compound	LSD ED_{50} (mg/kg)	MDMA ED_{50} (mg/kg)
MDA (figure 2)	0.97	0.88
EDA (figure 8)	3.07	1.86
IDA (figure 9)	7.12	5.21

NOTE: LSD tartrate=0.08 mg/kg, IP; (±)-MDMA.HCl=1.75 mg/kg, IP.

While MDMA produces CNS stimulation and euphoria, fenfluramine is more of a sedative and dysphoric. A detailed comparison of the pharmacology of fenfluramine and MDMA may be necessary to understand exactly how MDMA works.

Another study underway has begun to examine the effect of paramethoxyamphetamine (PMA) in MDMA-trained rats. After testing a few doses, it appears that full substitution may occur and that the *S* enantiomcr of PMA

is more potent. This result would also be consistent with a mechanism of action for MDMA where serotonin release is important, since PMA is a potent releasing agent of serotonin both in vitro (Tseng et al. 1978) and in vivo (Tseng et al. 1976; Nichols et al. 1982). PMA is also a potent releaser of NE in peripheral tissues (Cheng et al. 1974) but the blockade of its behavioral effects by chlorimipramine (Tseng et al. 1978) suggests that serotonin release may be important in the mechanism of action. PMA did make a brief appearance on the illicit market in the early 1970s but was responsible for several deaths (Cimbura 1974), and its use subsequently declined.

One might also speculate that PCA would have an effect similar to MDMA. Indeed, the early clinical data for PCA suggested that it possessed antidepressant activity (Verster and Van Praag 1970). This would suggest that the human psychopharmacology of PCA may well be closer to that of MDMA than fenfluramine, but it is unlikely that clinical studies can be carried out to study this.

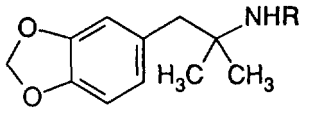
Side-Chain Modifications

A variety of side-chain modified analogs of MDMA and MBDB have begun to be examined. Very early studies were of the α,α-dimethyl analog, 3,4-methylenedioxyphentermine (figure 8a) and its N-methyl derivative (figure 10). This latter compound proved to lack MDMA-like activity (Shulgin, unpublished). Interestingly, this compound also lacked the ability to stimulate the release of [^{3}H]serotonin from prelabeled rat brain synaptosomes (Nichols et al. 1982).

Recently the tetralin and indan analogs of MDA have been examined (figures 11 to 14). It was previously shown that when hallucinogenic amphetamine derivatives were incorporated into similar structures, the hallucinogen-like activity in animal models was lost (Nichols et al. 1974). Thus. one might anticipate that a similar strategy with MDMA would lead to congeners that would lack MDA-like hallucinogenic effects. Furthermore, by examination of the two methylenedioxy positional isomers, one could infer the binding conformation of MDMA itself at the target site. As shown in table 5, one positional isomer is clearly preferred for MDMA-like activity. Furthermore, the indan derivative, figure 12, has a potency at least comparable to that of MDMA. This series has begun to define some of the conformational preferences of the receptor or target sites with which MDMA interacts, at least in producing its discriminative cue.

NON NEUROTOXIC ENTACTOGENS?

Although the problem of MDMA abuse has generated great interest because of MDMA's potential neurotoxicity, it is possible that nonneurotoxic entactogens can be developed. As in most areas of technology, this is a



R = H
R = CH_3

FIGURE 10. *The α, α-dimethyl homologues of MDA(a) and MDMA(b)*

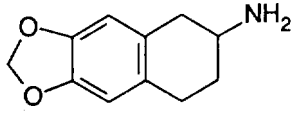


FIGURE 11. *Nonneurotoxic tetralin analogue of MDMA*

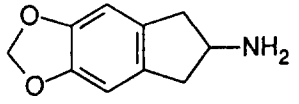


FIGURE 12. *Nonneurotoxic indan analogue of MDMA*

two-edged sword. A major concern might be that a nonneurotoxic entactogen could become popular as a recreational drug. A major deterrent to widespread use of MDMA should be the consideration by potential MDMA users that there is the possibility of neurotoxicity with unknown consequences, perhaps delayed for years before the consequences become manifest. On the other hand, researchers must give serious attention to the fact that any possible clinical utility for MDMA-like substances cannot be explored until the issue of neurotoxicity is resolved. Hence, a nonneurotoxic MDMA congener would allow clinical testing of the assertion that these compounds are useful adjuncts to psychotherapy.

Undoubtedly, nonneurotoxic entactogens can and will be discovered. Sufficient evidence already exists to support this hypothesis. We know, for example, from the work of Schechter (1986) that the discriminative stimulus properties of MDMA are largely dissipated within 4 hours of drug administration. On the other hand, Schmidt (1987) has shown that MDMA

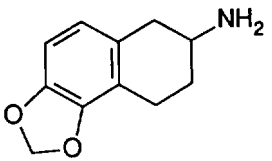


FIGURE 13. *Tetralin analogue of MDMA that lacks MDMA-like effects*

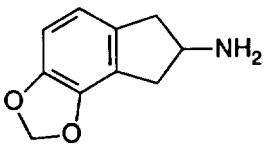


FIGURE 14. *Indan analogue of MDMA that lacks MDMA-like effects*

TABLE 5. *Drug discrimination results: Substitution tests in MDMA-trained rats*

Compound	Result in MDMA-Trained Rats
Figure 11	CS ED_{50}=1.29 mg/kg
Figure 12	CS ED_{50}=0.59 mg/kg
Figure 13	PS (75% drug responding @ 1.75 mg/kg)
Figure 14	PS (67% drug responding @ 0.5 mg/kg)

KEY: (±)-MDMA.HCl, 1.75 mg/kg IP, CS=complete substitution; PS=partial substitution.

has a biphasic depleting effect on cortical serotonin, with the later phase (more than 6 hours) associated with the long-term toxicity, a toxicity blocked by fluoxetine. Schmidt and Taylor (1987) administered the serotonin uptake inhibitor fluoxetine to rats 3 hours after treatment with MDMA and were able to prevent neurotoxicity. These workers suggested that the unique neurochemical effects of MDMA are independent of the long-term neurotox-icity. In our own studies, cited above, we have shown that fluoxetine does not antagonize the MDMA cue. Battaglia et al. (1988) reported that acute MDMA treatment decreased brain serotonin and 5-HIAA

levels, but that multiple MDMA treatments were required to decrease the number of 5-HT uptake sites, the latter presumably a reflection of neuron terminal degenera-tion. These studies indicate that the acute pharmacology can be dissociated from the long-term neurotoxic effects of MDMA.

Further, it is also known from work with the neurotoxin PCA that some structural congeners have an acute depleting effect on brain 5-HT, but lack the long-term neurotoxicity that is characteristic of PCA (Fuller et al. 1977). Since the psychopharmacological effects of MDMA have a relatively rapid onset and, in rodents, are largely dissipated at a time when a serotonin uptake inhibitor can still block neurotoxicity, it seems quite clear that molecules can be developed that will probably possess human psychopharmacology similar to MDMA, but will lack serotonin neurotoxicity. When this is accomplished we can look forward to a clearer definition of the primary pharmacology of entactogens. One would hope that, at that time, clinical studies with such a compound would be possible, to determine finally whether entactogens represent a new technology for psychiatry.

DISCUSSION

QUESTION: What are the criteria that you used for these newer compounds in order to classify these newer drugs as either sympathomimetic or hallucinogenic?

ANSWER: We are basically forced to deal with a variety of models. First of all, we have LSD-trained rats, and we have used that as our general screen for hallucinogen-like activity. If you are familiar with the drug discrimination literature, you can get false-positives, and perhaps Professor Glennon will correct me if I am wrong, but I am not aware of false-negatives. There are no cases where, in the drug discrimination paradigm, an animal has said this drug is not hallucinogenic when, in fact, in humans it is known that it is. So my feeling with drug discrimination is that we are detecting false-positives.

We are using I-125-labeled DOI as a radioligand and that has been shown, particularly by Professor Glennon and his coworkers, to be a good model for hallucinogenic activity. I think 5-HT_2 agonists, in terms of biochemical pharmacology, are the clearest indication that a compound is hallucinogenic.

We are routinely screening compounds for ability to displace I-125 DOI from frontal cortex homogenates. As far as the CNS stimulant effects, differentiating from psychostimulants, the present model we are using is substitution in amphetamine-trained rats, in drug discrimination. We have used synaptosomes and looked at their effect on dopamine release and reuptake. But basically they are correlative models.

And it is certainly true that these compounds could well be hallucinogenic but fall outside what we understand the structure-activity relationships of these compounds to be. For example, it may well be that MBDB in humans at some dose is hallucinogenic and is acting by some mechanism that is totally different from what we understand to be the mechanism of mescaline, DOM, or LSD. But at the present time, based on what we understand about structure-activity relationships, it should not be. That remains to be seen.

COMMENT: It might be advisable to stick to more operational definitions in talking about these compounds. One runs a risk if compounds have not been tested in people, and to refer to a compound as hallucinogenic when it is operative. A drug discrimination test might lead you to certain assumptions about the drug that are not true.

RESPONSE: Generally, it is safest to say there is LSD-like activity in drug discrimination profiles. Similarly, with these so-called entactogens, the name we have given them, we do know that we find in the tetralins and indans, for example, that a particular amino-indan we tested has fairly high potency in substituting for MDMA or MBDB. But we do not know what its effects would be in humans. There is no way to test that. Basically we are trying to develop correlative models based on what we know from the clinical data. But, again, it is speculative in the absence of clinical studies.

COMMENT: I would not rule out the possibility that MDMA or MDA produces effects at serotonin-2 receptors. Some of the data that I believe Dr. Battaglia has accumulated shows that of the 20 brain recognition sites that we have looked at, using standard radioligand binding procedures, MDMA has the highest affinity at serotonin-2 receptors as labeled by tritiated DOB. But I must qualify that. If you compare MDMA to something like DOI, it is about a hundredfold weaker. But its affinity is still 100 nanomolar in terms of an IC_{50}, concentration, which is still relatively potent considering the concentrations that may be achieved in brain at some of the doses used in animals.

RESPONSE: I have tended to think that things do not have affinity unless we see low nanomolar affinity. I think the EEG studies are fairly revealing in that regard. The fact that we see this increase in alpha-l power in the striatum is a characteristic of 5-HT_2 agonists. And we are clearly seeing EEG effects at doses that are not increasing that power in the alpha-l frequency. I tend to think that 5-HT_2 agonist effects are not that important in the action of these compounds.

COMMENT/QUESTION: I was very intrigued by your substitution data from the drug discrimination paradigm. But my question is not unlike Lou Seiden's. For with substances that are characterized by tremendously qualitatively different effects, biphasic in nature, and in many functional

assessments, I feel it may be premature to zero in on one selected training dose and give that a label.

I would like to know whether or not you have explored minimal discriminational doses of MDMA or MBDB and whether you have contrasted them with higher doses and have done experiments that are reminiscent of the Appel and White type approach where different mechanisms kick in at different dose ranges of the drug. Do we cover the relevant qualitatively different effects with that technique and with that approach, where one is zeroing in on one amphetamine dose and one MDMA dose?

I also have another question. When you compare release data from a slice preparation where it is in one application with discrimination data, are you comparing a creature that has received hundreds of injections every other day, on the average? I do not know what your protocol looks like, but I presume every other day is a drug and every other day is a control condition. Here you have an acute preparation and the relationship, of course, is quite tenuous.

ANSWER: Yes. We have looked at the lower doses of MDMA; the 1.75 mg/kg is the dose that gave us the best discriminability. We tried initially to train with 1 mg/kg but could not. We continued to increase the training dose by increments until we found the dose where we got reliable discrimination. It was 1.75 mg/kg. At least in our paradigm, I do not see how we could go much lower.

We have not explored all of the dose-response relationships. And with respect to the nature of the cue, we have studies underway now with a variety of serotonin agonists and antagonists, for example, fluoxetine. And have looked at MDMA. We cannot block the cue with fluoxetine. We are also looking at 8-hydroxy-DPAT, buspirone. PCPA pretreatment is on the way. So there are a variety of manipulations that we have in process. The treatments are all randomized, so a lot of them are only half finished, and no one can say what is happening. But in terms of pinning it down, I think that needs to be done.

We are looking at biochemical models as really pointing us in a particular direction. They are not rigorous; I recognize that. If we focused all our attention on drug discrimination we could do some complete studies. My emphasis in medicinal chemistry is to explore structure-activity relationships and synthesize tools to explore how the drugs work So we basically, more than focusing on pharmacological rigor, have tried to find quick screens that would point us in a direction so we could synthesize a drug to test this hypothesis.

Ultimately, these compounds will require a good deal of pharmacological evaluation, and we are in the early stages of that. In accordance with

Dr. Gibb's hypothesis regarding dopamine involvement, we thought that perhaps MBDB would not be neurotoxic because of a lack of effect on dopamine. But, in fact, it is neurotoxic as well, measured by whole-brain serotonin 5-HIAA and tritiated paroxetine binding sites. It is perhaps two-thirds the toxicity, on a molecular weight basis, of MDMA, but it is toxic.

A number of the studies that we have done are not completely rigorous, but their purpose is to see whether neurotoxicity is related to the nature of the cue. Your questions are well taken, but it has really been a choice between economy and rigor so that we could find the chemical structure to synthesize.

COMMENT/QUESTION: You have answered the first question, which was on the issue of whether or not MBDB produced long-term effects on the amine system. The second question has to do with the nature of the cue.

We have talked with people who participated in our study over the last year. As you know, many of them have experimented with a wide variety of psychoactive drugs, including MBDB. When asked about MBDB their response seems to be lukewarm in terms of how it compares to subjective effects, and whether these effects are comparable to those of MDMA. Is that accurate?

ANSWER: When we decided to make MBDB we felt the alpha-ethyl would attenuate hallucinogenic activity. Dr. Shulgin made that compound because he was looking at things that had a stimulant effect. He had made it but had not evaluated it at effective doses. After a discussion, he evaluated it in the group of people that worked with him.

Basically, the consensus was that the psychopharmacology was similar but that the compound lacked the ability to produce the kind of euphoria produced by MDMA. And he reported that there were at least one or two individuals who felt they never wanted to take the compound again.

My own bias is toward the therapeutic potential. I do not care whether anything we develop produces euphoria or dependence potential. I think from the point of view of a drug abuse problem or a dependence liability that the alpha-ethyl probably does not have the reinforcing qualities and is not as pleasurable as MDMA.

COMMENT: My question to these people would be directed toward this quality they regard as unique for MDMA--this rush. They admit that that is not the main reason for taking it. They do seem to be able to make that distinction. They do not dispute the fact that they enjoy the rush from an MDMA dose. Whatever this other quality is, they recognize it. And it is that quality that was less apparent in MBDB than in MDMA.

RESPONSE: I think this is an area where you would have to do detailed double-blind crossover studies and some fairly extensive testing to map out what the nature of that effect is.

In the drug discrimination assay we get symmetrical transfer. They seem to be the same. And the consensus, at least from Dr. Shulgin's group, is that it generally has the same kind of effect. Obviously it has not become a problem on the street. And I think if it was a very desirable compound we might well have heard something about it.

QUESTION: Have you done any studies of the metabolism of these compounds? As you probably know, there have been reports that MDMA is very quickly metabolized into MDA. Have you looked at MBDB to see if the ethyl group gets cleaved so that you essentially have an MDMA compound after you are through?

ANSWER: There is no chemical precedent for that kind of transformation. I really cannot think of an enzyme system that would cleave that down to the alpha-methyl. I think the effect is due to the alpha-ethyl.

In terms of other sites of metabolism, we are looking at the metabolism in the dioxole ring and in dealkylation. We have seen some interesting things, but I could not comment on this right now. With respect to the alpha-ethyl, I think that the parent compound is probably the one that is active.

QUESTION: I have two questions about your MBDB discrimination studies. It sounds as though you are doing experiments to investigate whether neuronal stores of serotonin are required for MBDB to be recognized. You mentioned that fluoxetine did not prevent the recognition. Does it prevent the release of serotonin *in vitro?* In other words. is that a carrier-dependent release by MBDB as it is, for example, in the case of *p*-toluylamphetamine?

My second question is this: You mentioned fenfluramine. I presume you used the racemic mixture. which would mean that in the brain you would have both *R* and *S* fenfluramine and *R* and *S* norfenfluramine present. And since these differ widely in their effects on dopamine versus serotonin neuronal systems, have you studied individual enantiomers of either fenfluramine or norfenfluramine?

ANSWER: Actually we used synthesized (+)-fenfluramine. The fluoxetine story is not clear. It does not block the discriminative cue, but other workers have shown that it blocks the neurotoxicity. We have not looked at it in enough detail or at any of the *in vitro* models to see whether it blocks or releases serotonin.

COMMENT: It seems as though that might be a good tool to determine whether the discrimination really does relate to serotonin release because, clearly, it has been shown to block the serotonin release *in vivo*. If it does not block the drug discrimination it seems that it is not consistent with the idea that that is a consequence of serotonin release.

RESPONSE: When you are in this business, you get letters from many strange people. I received an unsolicited letter from a fellow in Geneva, Switzerland, about a year ago, who told me that he had taken fluvoxamine, which I believe is available clinically in Geneva, and had subsequently taken MDMA. He said that the fluvoxamine had no effect on the action of the MDMA. I think this is an interesting question which, at least in one anecdotal account, suggests that there is a difference.

The biochemical followup would be interesting if it does prevent the release. And maybe the serotonin is a red herring. But that is the only thing we have seen consistently at this point

COMMENT [DR. SCHUSTER]: I am extremely pleased to see the sophistication of the animal studies and the medicinal chemistry studies. I lament the current lack of sophistication with regard to the available data in humans. It is feasible, as my colleagues and others have shown, to train drug discrimination in humans-to do as precise quantitative work there as is done in animals. In fact, probably more precise.

As far as subjective effects are concerned, and people's responses regarding why they take drugs, I have to say that I have a fair degree of skepticism that people are reporting in any way what is relevant. It may be, it may not be. But I can assure you that the contingencies that shape verbal behavior may be very different from the contingencies that shape the drug-taking behavior. And as a consequence there may not be any necessary correlation.

It is unfortunate, and this is a real deficit in this field, that we cannot do the very human studies that I know you would all like to do and, therefore, we rely upon whatever evidence we have to reach conclusions. But we have to be wary of the fact that the human data are weak in comparison.

QUESTION: Have you made any attempt to antagonize the MBDB stimulus with serotonin antagonists?

ANSWER: We have tried ketanserin, but it did not antagonize the stimulus. I do not believe we have tested fluoxetine in the MBDB-trained animals. It has only been tested in the MDMA-trained animals. We have not found an antagonist to the cue yet.

QUESTION: Did you measure tryptophan hydroxylase or just the 5-HT/5-HIAA depletion?

ANSWER: We used it in the 20 mg/kg twice a day for a 4-day regimen with MDMA, and then corrected for molecular weight and used an equimolar dose of MBDB, sacrificed the animals 2 weeks later, and then measured. We used basically HPLC and used serotonin and 5-HIAA from one hemisphere and then measured tritiated pyroxetine from the other hemisphere. And we got something like 60 percent depletion of serotonin, and the pyroxetine binding site B_{max}, decreased by about 60 percent. With MBDB it was decreased by about 40 percent. It was a clear and significant decrease, but not quite to the extent that we had. But we have not looked at tryptophan hydroxylase.

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AUTHORS

David E. Nichols, Ph.D.
Professor of Medicinal Chemistry

Robert Oberlender, Ph.D.
Research Assistant

Department of Medicinal Chemistry
and Pharmacognosy
School of Pharmacy and Pharmacal Sciences
Purdue University
West Lafayette, IN 47907

Self-Injection in Baboons of Amphetamines and Related Designer Drugs

C.A. Sannerud, J.V. Brady, and R.R. Griffiths

INTRODUCTION

Recent controversy about the recreational abuse and potential therapeutic use of "designer drugs" has focused attention on MDA (methylenedioxyamphetamine HCl) and structurally related phenylisopropylamine compounds, including MDMA *(d,l*-3,4-methylenedioxymethamphetamine HCl, "ecstasy"). These compounds are structural analogs of the psychomotor stimulant amphetamine and the hallucinogen mescaline, and produce stimulant and/or hallucinogenic effects (Shulgin 1978).

In humans, MDA and MDMA have been reported to produce positive mood changes, enhanced emotional awareness, and improved interpersonal communication (Greer and Tolbert 1986; Downing 1986; Shulgin 1986; Peroutka et al. 1988). Because of these psychotropic effects, MDA and MDMA have been used in psychotherapeutic situations (Naranjo et al. 1967; Yensen et al. 1976; Grinspoon and Bakalar 1986). In addition, presumably because of the same positive subjective effects, recreational use of MDMA on college campuses has increased in recent years (Peroutka et al. 1988).

Recreational abuse of "designer drugs" poses a major problem. Evidence concerning the safety of these drugs has shown that MDA and MDMA are toxic to serotonergic neurons in rodent (Ricaurte et al. 1985; Stone et al. 1987; O'Heam et al. 1988) and primate brains (Ricaurte et al. 1988). MDMA has also been associated with toxicity in humans. To date, there have been five cases reported in which MDMA has contributed to death in recreational users (Dowling et al. 1986).

Based in part on the neurotoxicity and recreational abuse, the Drug Enforcement Administration (DEA) has placed MDA, MDMA, and other "designer drug" analogs of stimulant/hallucinogens on Schedule I, used for drugs with high abuse potential and no recognized therapeutic usefulness.

Although MDA and MDMA were recently brought under legal regulation by scheduling under the Controlled Substances Act, other derivatives of these compounds can be synthesized easily, and these new "designer drugs" have begun to be used recreationally. Evaluation of these substituted phenylethylamine compounds for abuse liability should require an assessment of the reinforcing effects of the drugs and a comparison to structurally similar compounds, to determine relative potency and structure-activity relationships (SARs). This chapter will summarize previously published drug self-administration research with a variety of substituted amphetamine compounds, comparing the self-administration of stimulant/hallucinogenic analogs of MDA to standard anorectic phenylethylamine compounds in baboons.

METHOD FOR ASSESSING REINFORCING EFFECTS OF DRUGS

The use of nonhuman primates to assess abuse liability of test compounds is indicated, since there is a good correlation between the drugs that are abused by man and those that maintain self-injection behavior in animals (Schuster and Thompson 1969; Griffiths et al. 1980). Of the many different types of procedures developed to determine whether a drug will maintain self-injection, the substitution is the most common and reliable. The procedure involves establishing self-injection using a dose of a standard drug that is known to maintain reliable self-injection behavior. After this behavioral baseline is stable, a dose of test drug is substituted for the standard compound to determine whether the test drug will maintain self-injection.

PROCEDURE

The methods and procedures used to evaluate self-injection of these compounds were similar to those previously described by Griffiths and colleagues (Griffiths et al. 1976; Griffiths et al. 1979). Eighteen male baboons *(Papio cynocephalus)* weighing between 15 and 30 kg were used as subjects. Each animal was adapted to either a standard restraint chair (Findley et al. 1971) or a harness tether restrainmg system (Lukas et al. 1982). The chaired animals were housed individually in sound-attenuated chambers. The tethered animals were housed in standard stainless steel primate cages surrounded by a sound-attenuating, double-walled plywood external enclosure.

An aluminum "intelligence panel" used in self-injection studies has been previously described (Griffiths et al. 1975). Briefly, the panel containing levers and associated stimulus lights (approximately 1 cm in diameter) was mounted on the inside of the chamber (chaired animals) or on the rear wall of the cage (tethered animals). A Lindsley lever (lower left of panel), a leaf lever (lower right of panel), and a food hopper with stimulus light (lower left of panel) were mounted on the panel. A 5x5 cm translucent Plexiglas panel that could be transilluminated was mounted on the aluminum

panel in the upper left comer. A speaker for delivery of white noise and tones was mounted behind the panel. A feeder for delivering food pellets into the food pellet tray was mounted on the top of the wooden enclosure.

Baboons were surgically prepared with chronically indwelling silastic catheters implanted in either femoral or jugular veins under pentobarbital or halothane anesthesia using methods described in detail by Lukas et al. (1982). All baboons had served in studies of intravenous self-injection with a variety of drugs. They had continuous access to water via a drinking tube and to food pellets (as described below) and received two pieces of fresh fruit and a multivitamin daily.

The infusion system was similar to that described by Findley et al. (1972). The catheter was attached to a valve system that allowed slow continuous administration (55 to 60 mL in 24 hours) heparinized saline (5 units/mL) via a peristaltic pump to maintain catheter patency. Drug was injected into the valve system by means of a second pump and then flushed into the animal with 5 mL of saline from a third pump. This system necessitated a delay of approximately 20 seconds between the onset of drug delivery and actual injection into the vein. Drugs were delivered within a 2-minute period.

Food was available 24 hours per day under a fixed ratio 30 (FR 30) response schedule on the leaf lever; i.e. every thirtieth response delivered a 1 g banana-flavored food pellet and produced a brief flash of the hopper light.

Animals were trained to self-inject cocaine (0.4 or 0.32 mg/kg/injection) under an FR 160 response schedule on the Lindsley lever. Drug injections were available every 3 hours and were signaled by a 5-second tone, followed by the illumination of the jewel light over the Lindsley lever. When the jewel light was illuminated, each response on the Lindsley lever produced a brief feedback tone. Upon completion of the FR requirement, the jewel light was extinguished and the 5 mL drug injection was begun, followed by a 5 mL flush injection. Following the completion of the injections, the 5x5 cm translucent panel was illuminated for a 1-hour period, and the 3-hour timeout period was begun. There was no time limit for the completion of the response requirement.

When criterion cocaine self-injection performance (six or more injections per day for 3 consecutive days) was obtained a dose of drug or vehicle was substituted for cocaine for 12 to 15 days. Occasional equipment malfunction necessitated extending the period of substitution beyond 15 days. Cocaine self-injection performance was reestablished, and when criterion performance was obtained (typically in 3 to 5 days), another dose of drug was substituted This procedure of replacing cocaine with drug was continued through the study of a range of drug doses and their vehicles.

The order of exposure to different doses was either a mixed or an ascending sequence. The drug vehicle was generally examined immediately before or after the series of doses.

Drugs and Doses Tested. Drug solutions were prepared by dissolving the drug in physiological saline (0.9 percent sodium chloride) and were filter sterilized (Millipore). Drug doses (mg/kg/infusion) were calculated on the basis of the salt. The following drug doses were tested: *d*-amphetamine sulfate (0.01, 0.05, 0.1, 0.5); *l*-3,4-methylenedioxyamphetamine sulfate (MDA) (0.1, 0.5, 1.0, 2.0, 5.0); 4-methoxyamphetamine hydrochloride (PMA) (0.001, 0.01, 0.1, 0.1 1.0); 2,5-dimethyoxy-4-methylamphetamine hydrochloride (DOM) (0.001, 0.01, 0.1, 1.0); 2,5-dimethyoxy-4-ethylamphetamine hydrochloride (DOET) (0.001, 0.01, 0.1, 0.32. 1.0); *d,l*-3,4-methylenedioxymethamphetamine HCl (MDMA) (0.1, 0.32, 1.0, 3.2); phentermine hydrochloride (0.1, 0.5, 1.0); diethylpropion hydrochloride (0.1, 0.5, 1.0, 2.0); phenmetrazine hydrochloride (0.1, 0.5, 1.0); phendimetrazine tartrate (0.1, 0.5, 1.0, 2.0); benzphetamine hydrochloride (0.1, 0.5, 1.0, 3.0); *l*-ephedrine hydrochloride (0.3, 1.0, 3.0, 10.0); clotermine hydrochloride (0.1, 1.0, 3.0, 5.0); chlorphentermine hydrochloride (0.1, 0.5, 2.5, 5.0); and fenfluramine hydrochloride (0.02, 0.1, 0.5, 2.5).

Chemical Structures. Figure 1 shows the chemical structures for 14 phenylethylamine compounds. Nine of these compounds are used clinically as anorectics *(d*-amphetamine, phentermine, diethylpropion, phenmetrazine, phendimetrazine, clotermine, chlorphentermine, benzphetamine, and fenfluramine). Four of these compounds are not approved for clinical use and are reported to have hallucinogenic properties (MDA, PMA, DOM, and DOET). The final compound *(l*-ephedrine) is used clinically for bronchial muscle relaxation, cardiovascular, and mydriatic effects. Figure 2 shows the chemical structure for MDMA, the methyl analog of MDA. MDMA is not approved for clinical use and has been reported to produce both LSD-like and cocaine-like effects.

RESULTS

Figure 3 presents the mean levels of self-infusion for the 14 phenylethylamines shown in figure 1. Of all the drugs tested, *d*-amphetamine was the most potent, maintaining levels of drug self-injection above saline levels at doses of 0.05 and 1.0 mg/kg/mfusion. Phentermine, diethylpropion, phenmetrazine, phendimetrazine, benzphetamine, and MDA maintained levels of self-injection above saline at doses of 0.5 and 1.0 mg/kg/infusion. The compounds *l*-ephedrine, clotermine, and chlorphentermine were the least potent substances that maintained performance; self-injection rates were

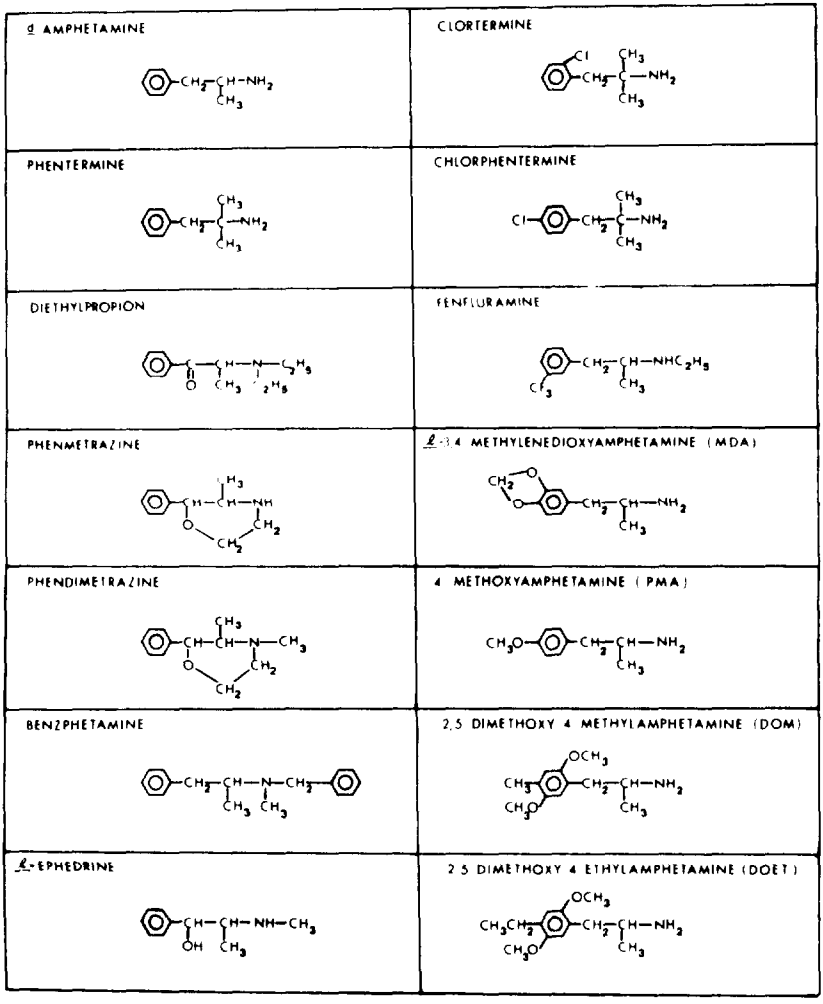

FIGURE 1. *Chemical structure of 14 of the 15 phenylethylamines tested to determine whether they maintain drug self-administration*

SOURCE: Griffiths et al. 1979, copyright 1979, Academic Press.

above saline control levels at doses of 3.0 and 10 mg/kg/infusion for *l*-ephedrine, 3.0 and 5.0 mg/kg/infusion for clortermine, and 2.5 and 5.0 mg/kg/infusion for chlorphentermine. In contrast to the other phenylethylamines tested, fenfluramine, PMA, DOM, and DOET did not maintain self-injection at levels greater than saline at any dose tested.

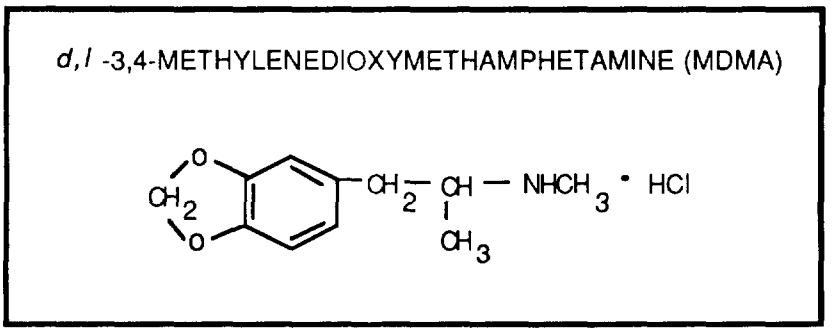

FIGURE 2. *Chemical structure of MDMA*

Figure 4 shows that MDMA maintained responding above vehicle level in the three baboons tested, the highest levels of self-injection were maintained by 0.32 or 1.0 mg/kg/injection; lower levels were maintained by 0.1 and 3.2 mg/kg/injection. During self-injection of cocaine, vehicle (saline), and low doses of MDMA, there were no unusual changes in gross behavior of the baboons. While self-injecting higher doses of MDMA, however, all three baboons engaged in notably unusual behaviors. Two animals appeared to track nonexistent visual objects (suggesting hallucinations), were uncharacteristically aggressive toward laboratory personnel, and engaged in repetitive scratching/self-grooming behavior.

Similar MDMA self-injection findings have been reported in rhesus monkeys (Beardsley et al. 1986). In three of the four animals trained to self-administer cocaine, substitution of at least one dose of MDMA resulted in rates of self-injection that exceeded vehicle rates; two animals self-administered MDMA at rates higher than cocaine rates.

CORRESPONDENCE OF BEHAVIORAL EFFECTS IN HUMANS AND ANIMALS

In a summary of the human abuse literature on anorectic phenylethylamines, Griffiths et al. (1979) found there was a good correlation between the results of self-administration studies in animals and information about the subjective effects and abuse in man. Specifically, amphetamine, diethylpropion, and phenmetrazine have been associated with numerous clinical case reports involving abuse, and these three compounds as well as benzphetamine and *l*-ephedrine have shown similar subjective effects in drug abuser populations (Griffiths et al. 1979). In addition, fenfluramine was associated with low incidence of abuse in humans and did not maintain self-injection responding in animals. Chlorphentermine was similarly associated with low incidence of abuse in man, but did not maintain self-injection uniformly in animals (Griffiths et al. 1979).

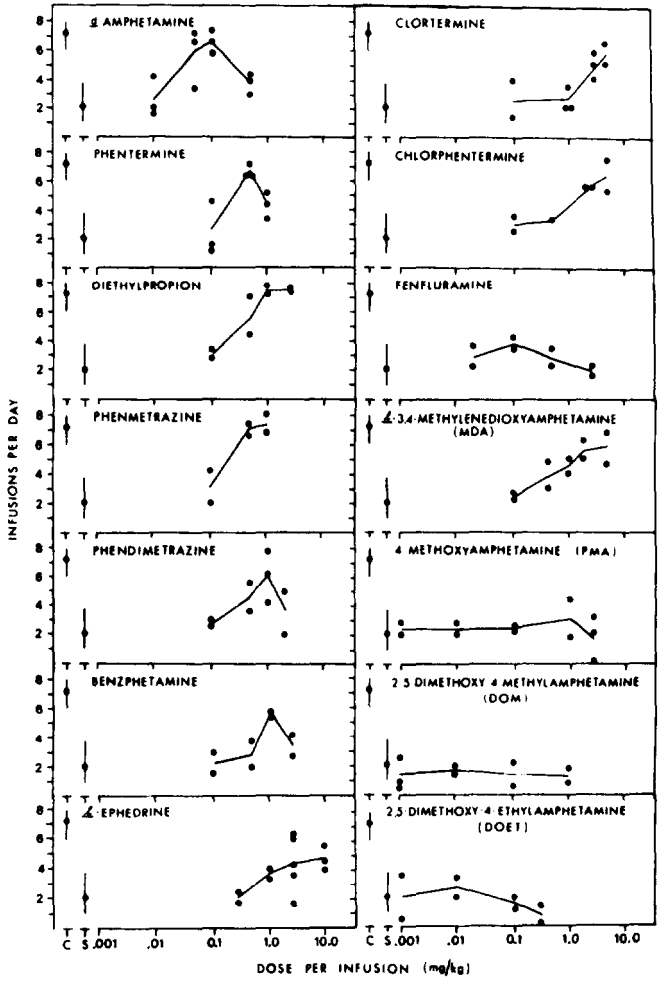

FIGURE 3. *Mean number of injections per day with 14 phenylethylamines for the last 5 days of drug or saline substitution under a 160-response T.0. 3-hour schedule of intravenous injection*

NOTE: The vertical axis represents the number of injections per day. The horizontal axis represents doses of drug (log scale). The points above "C" represent the mean of all 3-day periods of cocaine HCI (0.4 mg/kg/infection) availability that immediately preceded every drug dose or saline substitution. The points above "S" represent the mean of the last 5 days obtained during saline substitution (2 saline substitutions in each of 15 animals). Vertical bars indicate ranges of individual animal's means. Drug data points represent the mean of the last 5 days during substitution of a drug dose for individual animals.

SOURCE: Griffiths et al. 1979, copyright 1979, Academic Press.

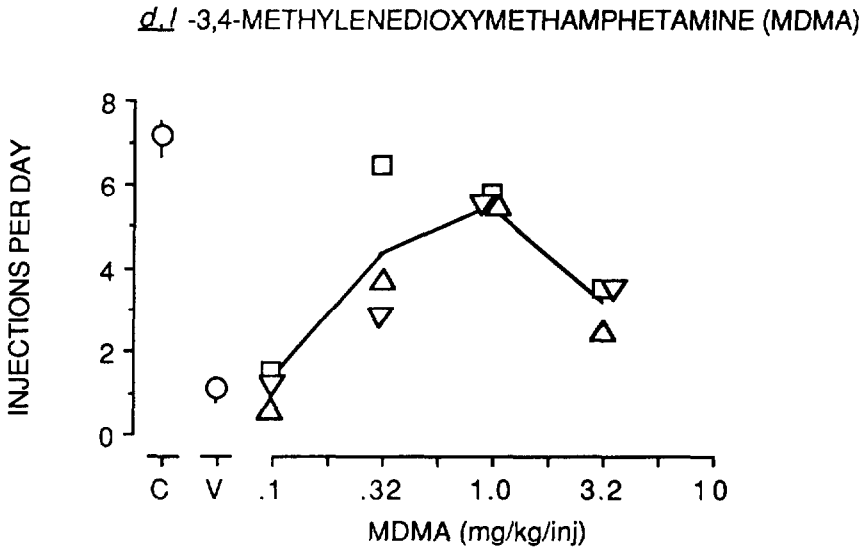

FIGURE 4. *Mean number of injections per day for the last 5 days of MDMA and MDMA vehicle (saline) substitution under a 160-response T.O. 3-hour schedule of intravenous injection*

NOTE: The points above "C" represent the grand mean of the 3 days of concaine HCl(0.32 mg/kg/injection) availability that preceded each MDMA dose or saline substitution. The points above "V" represent the mean of the last 5 days obtained during vehicle substitution. Vertical bars indicate ranges of individual animals' means.

SIMILARITIES AMONG AND DIFFERENCES BETWEEN PHENYLETHYLAMINE COMPOUNDS

A comparison of MDMA to *d*-amphetamine, MDA, and DOM can provide an understanding of the pharmacology of MDMA and its abuse liability. While there are differences between MDMA and amphetamine in the subjective effects in humans (Shulgin and Nichols 1978). the similarities in the self-injection and preclinical pharmacology profile between MDMA and *d*-amphetamine suggest that MDMA has abuse liability. Both MDMA and *d*-amphetamine maintain self-injection behavior above vehicle control levels, and high doses of both drugs are associated with a cyclic pattern of self-injection over days (Lamb and Griffiths 1987; Griffiths et al. 1976). At doses larger than those needed to maintain self-injections, both MDMA and d-amphetamine suppressed food intake and food-maintained behavior (Lamb and Griffiths 1987; Griffiths et al. 1976) and produced similar changes in gross behavior, such as tracking nonexistent visual objects and repetitive self-grooming (Lamb and Griffiths 1987; Lamb and Griffiths, unpublished observations). Both MDMA and amphetamine also sham discriminative

stimulus properties with *d,l*-MDA or amphetamine, but not with DOM, in rat drug-discrimination paradigms (Glennon and Young 1984a; Glennon and Young 1984b; Glennon et al. 1983).

MDMA has both similarities to and differences from *l*-MDA. MDMA and *l*-MDA are self-injected in baboons and share stimulus properties with MDA in the rat drug-discrimination paradigm (Glennon and Young 1984a). In addition, MDMA, but not *l*-MDA, shares discriminative stimulus properties with amphetamine in the rat drug-discrimination paradigm (Glennon and Young 1984a). Consistent with the reports of lesser hallucinogenic effects of MDMA as compared to MDA or LSD (Shulgin 1978), *l*-MDA, but not MDMA, shares discriminative stimulus properties with DOM in the rat drug-discrimination paradigm (Glennon et al. 1982; Glennon et al. 1983).

Although the substituted phenylethylamine compounds that have hallucinogenic properties in man (e.g., DOET, DOM, PMA, MDA, and MDMA) are commonly abused by humans, only MDA and MDMA maintained self-injection behavior in baboons. This suggests that this animal self-injection procedure may not be useful in predicting hallucinogenic drug effects. In addition, it suggests that the reinforcing properties of MDA and MDMA in baboons may be unrelated to the fact that these drugs produce hallucinogenic effects. Some phenyl-substituted phenylisopropylamines, such as MDA, PMA, and MDMA, have pharmacological properties distinct from those of amphetamine or DOM. Therefore, predictions about the abuse liability of these compounds based on their similarities to or differences from classic stimulants (such as cocaine or amphetamine) or hallucinogens (such as LSD or DOM) may provide inappropriate results.

STRUCTURE-ACTIVITY RELATIONSHIPS AMONG PHENYLETHYLAMINE COMPOUNDS

A comparison between the chemical structures of substituted phenylethylamine compounds and their potency in producing behavioral effects reveals an inverse relationship between the size of the substituent and central activity (Braun et al. 1980). Similarly, reports of SARs among phenylethylamine compounds have suggested that the size of the ring substitution in general may decrease potency of the phenylethylamines for maintenance of self-injection behavior. Research with a series of N-ethyl-aminates substituted at the meta position of the phenyl ring has demonstrated that the potency of these compounds, either to increase locomotor behavior in mice (Tessel et al. 1975) or to maintain self-injection behavior in rhesus monkeys (Tessel and Woods 1975; Tessel and Woods 1978). was inversely related to the size of the meta-substituted constituent. These findings indicate that the failure of fenfluramine (meta-trifluromethyl-N-ethyl-amphetamine) to maintain self-injection behavior is attributable to its meta-trifluromethyl group.

An examination of the SAR, comparing figures 1 and 3, also supports the suggestion that ring substitutions may decrease potency for maintaining self-injection behavior. The seven compounds shown in the right column of figures 1 and 3 have substitutions on the phenyl ring; these compounds were generally less potent in maintaining self-injection than were the compounds in the left columns of these figures, which do not have ring substitutions. In addition, phentermine differs structurally from both chlorphentermine and clotermine, which have the addition of a Cl at either the para or ortho positions of the phenyl ring; however, chlorphentermine and clotermine appear to be less potent than phentermine in maintaining self-injection behavior.

A similar SAR was found between side-chain substitutions and behavioral effects of phenylethylamines. A study using a series of *d*-N-alkylated amphetamines, synthesized in a series up to and including *d*-N-butylamphetamine, found that, for substitutes larger than ethyl, potency for maintaining drug self-administration in rhesus monkeys and for disrupting milk-drinking activity in rats of the *d*-N-alkylated amphetamines was inversely related to the N-alkyl length (Woolverton et al. 1980).

The pharmacological properties of phenylethylamines that control self-administration are complex. The effects of phenylethylamines on a variety of pharmacological measures do not appear to predict the reinforcing effects of these drugs, as measured by the cocaine substitution procedure in primates. Specifically, none of the following behavioral effects of these compounds accurately predict the results of self-administration experiments within the phenylethylamine class (Griffiths et al. 1976; Griffiths et al. 1979): the ability to suppress food intake (Griffiths et al. 1978); the ability to produce rate-dependent effects on schedule-controlled behavior (Harris et al. 1977; Harris et al. 1978); the ability to produce discriminative stimulus properties similar to amphetamine, DOM, or MDA (Glennon et al. 1982; Glennon et al. 1983; Glennon and Young 1984a; Glennon and Young 1984b Glennon et al. 1985; Glennon et al. 1988). Self-injection testing should remain an integral part of a continued analysis of abuse liability of these compounds.

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AUTHORS

Christine A. Sannerud, Ph.D.
Joseph P. Brady, Ph.D.
Roland R. Griffiths, Ph.D.

Department of Psychiatry and Behavioral Sciences
Department of Neuroscience
Johns Hopkins University School of Medicine
Traylor Building 624
720 Rutland Avenue
Baltimore, MD 21205

Stimulus Properties of Hallucinogenic Phenalkylamines and Related Designer Drugs: Formulation of Structure-Activity Relationships

Richard A. Glennon

INTRODUCTION

The purpose of the studies with phenalkylamine derivatives is severalfold: (1) to classify these agents by their primary effect; (2) to understand the structure-activity relationship (SAR) for each type of activity; and (3) to elucidate the mechanisms of action of these agents. Armed with information on SAR, one can, theoretically make predictions about the activity of agents yet to be synthesized; an understanding of the mechanisms of action can aid in the development of potential antagonists that could be useful for reversing the effects of these substances. Obviously, before one can investigate SAR and mechanisms of action, it is important to have some reliable method of classification. In the course of the studies, several different procedures have been used to examine the actions of these agents. Perhaps the most useful is the drug discrimination procedure. In this paradigm, animals are trained to recognize (or discriminate) the stimulus effects of a particular dose of a given agent; once trained, the animals can be administered doses of a test compound (i.e., a challenge drug) to determine if the challenge drug produces stimulus effects similar to those of the training drug. In such tests, referred to as tests of stimulus generalization, the animals essentially indicate whether or not a similarity exists between the actions of a new agent and those of a reference agent. Dose:response curves can be obtained and ED_{50} values determined. Thus, the procedure provides data that are both qualitative and quantitative. Needless to say, there are occasions when such studies produce results that are less than straightforward and are difficult to interpret. In other words, although drug discrimination studies provide very useful information on similarity of effect, potency, timecourse of action, mechanism of action, activity of metabolites, and other data, they cannot be used by themselves to characterize completely the pharmacological effects of a given agent. Reviews on the drug discrimination paradigm, particularly as it applies to the study of

phenalkylamines, appear in the following publications: Glennon et al. (1983), Glennon (1986), and Young and Glennon (1986).

In drug discrimination studies, groups of rats were trained to discriminate either the stimulant phenalkylamine (+)amphetamine (AMPH) or the hallucinogenic phenalkylamine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) from saline. Other structurally related training drugs that have been used include the iodo and bromo analogs of DOM, i.e., DOI and *R* (-)DOB, as well as methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA). Such investigations, coupled with the results of radioligand binding studies, have permitted the classification of a number of phenalkylamines (Glennon et al. 1983; Glennon 1986; Young and Glennon 1986) and have allowed proposal of a mechanism of action for the hallucinogenic phenalkylamines (Glennon et al. 1986a). The present review describes in detail some of the SARs that have been formulated on the basis of drug discrimination studies. This discussion of results is not meant to be comprehensive. Because species of animal, schedule of reinforcement, presession injection intervals, doses of training drugs, and other conditions have remained constant throughout the studies, it should be possible to make stricter comparisons than if data were compared across different laboratories. This SAR analysis is based, for the most part, on the results of discrimination studies already published.

Some of the agents used in the present study have not been previously reported in the literature. These agents were prepared in our laboratories, and details of their synthesis will be published elsewhere. However, three of these agents are potential metabolites of MDMA and are described here to the extent that such information might be helpful to other investigators studying the metabolism of MDMA. All three were isolated as their white, crystalline hydrochloride salts, and all were analyzed correctly for carbon, hydrogen, and nitrogen. Melting points and recrystallization solvents (in parentheses) are provided. N-methyl-1 -(4-hydroxy-3-methoxyphenyl)-2-aminopropane (N-Me 4-OH MMA): 210-212 °C (isopropanol/ ether); N-methyl-1-(3-hydroxy4-methoxyphenyl)-2-aminopropane (N-Me 3-OH PMA): 164-165 °C (isopropanol); N-methyl-1-(3,4-dihydroxyphenyl-2aminopropane (N,α-dimethyl dopamine or N-Me 3,4-diOH AMPH): 116-118 °C (isopropanol/ether).

Examination of the stimulus properties of a large number of phenalkylamines and related derivatives shows many can be characterized as producing either AMPH-like stimulus effects or DOM-like stimulus effects. The structures of some of these agents are shown in figure 1. Certain other agents could not be reliably classified as either AMPH-like or DOM-like because, at the highest dose tested, they either produced vehicle-appropriate (i.e., saline-appropriate) responding or resulted in disruption of behavior.

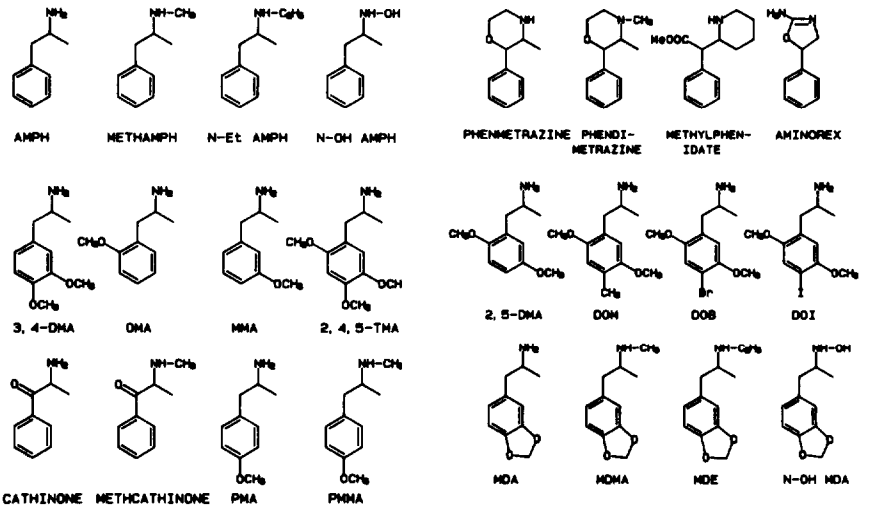


FIGURE 1. *Chemical structures of some of the agents employed in the present study*

Representative potency data (ED_{50} values) are presented in tabular form; data in these tables are given as μmol/kg so that direct potency comparisons can be made within a series. However, data presented in figures are given in mg/kg for the purpose of convenience.

Various phenallcylamines were shown to produce either DOM-like or AMPH-like stimulus effects; the structure-activity requirements for these activities are different from the standpoints of aromatic substitution patterns, terminal amine substituents. and optical activity. Thus, it has been possible to formulate two distinct SARs. It should be realized, however, that phenalkylamines need not produce only one of these two types of effects; certain phenallcylamines can produce pharmacological effects like neither DOM nor AMPH. Moreover, they can produce effects that are primarily peripheral, not central, in nature (Glennon 1987a). The fact that an agent produced DOM- or AMPH-like effects does not imply that it cannot produce an additional effect; conversely, if an agent does not produce either DOM- or AMPH-like stimulus effects, it is not necessarily inactive.

DOM-LIKE STRUCTURE-ACTIVITY RELATIONSHIPS

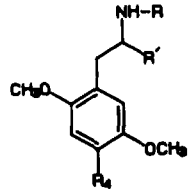
Aromatic Substituents

Substituents on the aromatic ring play a critical role in determining whether or not phenalkylamines possess DOM-like activity. Hallucinogenic activity is commonly associated with methoxy-substituted derivatives (Shulgin 1978); for this reason, much of this work has focused on these types of agents.

Methoxy-Substituted Derivatives. Phenalkylamines lacking aromatic substituents do not produce DOM-like stimulus effects. None of the three possible monomethoxy derivatives, 2-methoxy-(OMA), 3-methoxy-(MMA), or 4-methoxyphenylisopropylamine (PMA), produce DOM-like effects. Of the six dimethoxy analogs (DMAs) (i.e., 2,3-DMA, 2,4-DMA, 2,5-DMA, 2,6-DMA, 3,4-DMA, and 3,5-DMA), only the 2,4- and 2,5-dimethoxy derivatives 2,4-DMA and 2,5-DMA, respectively, are active. These two agents are essentially equipotent and are approximately one-tenth as potent as DOM. For purposes of comparison, the potencies of 2,5-DMA and DOM are 23.8 and 1.8 μmol/kg. Five trimethoxy analogs (TMAs) have been examined: 2,3,5-TMA is approximately one-third as potent as 2,5-DMA, and 2,3,4-TMA and 3,4,5-TMA are equipotent with 2,5-DMA. The other two, 2,4,5-TMA and 2.4.6-TMA, are about twice as potent as 2,5-DMA. None of the three possible tetramethoxy analogs has been investigated, and the pentamethoxy analog does not produce DOM-like stimulus effects. From these studies, it is apparent that the 2,4- and 2,5-dimethoxy substitution pattern plays an important role; certain 2.6-dimethoxy derivatives are also active, depending upon what substituents are present at the 4-position.

2,5-Dimethoxy Analogs. It should come as no surprise that methoxy groups at the 2- and 5-positions are important, when it is realized that DOM is a 2,5-dimechoxy-substituted derivative. Data for some representative 2,5-DMA analogs are provided in table 1. Removal of either one of the methoxy groups abolishes DOM-like stimulus effects. Introduction of a methyl group at the 4-position of 2,5-DMA, to produce DOM, enhances potency by more than an order of magnitude. Homologation of this alkyl group to ethyl (DOET) and *n*-propyl (DOPR) produces an increase in potency; further homologation to *n*-butyl (DOBU) decreases potency, and to amyl (DOAM) results in an agent that does not produce DOM-like stimulus effects. The relative potencies of these agents, as compared to 2,5-DMA, are: 2,5-DMA (1)<DOM (13)<DOET (27)<DOPR (43)>DOBU (7). Branching of this alkyl chain has varying effects. The isopropyl analog DOIP is eight times more potent than 2.5-DMA but is only about one-fifth as potent as its nonbranched counterpart DOPR. The tertiary butyl derivative DOTB does not produce DOM-like effects. In fact, it has been

TABLE 1. *Results of stimulus generalization studies using racemic DOM as a training drug*



Agent	Optical Isomer	R_4	R	R'	ED_{50} (μmol/kg)
2,5-DMA	(±)	H	H	CH_3	23.8
	(-)	H	H	CH_3	14.0
2,4,5-TMA	(±)	OCH_3	H	CH_3	13.7
DOM	(±)	CH_3	H	CH_3	1.8
	(-)	CH_3	H	CH_3	0.8
	(+)	CH_3	H	CH_3	6.9
N-Me DOM	(±)	CH_3	CH_3	CH_3	15.3
	(-)	CH_3	CH_3	CH_3	10.0
α-desMe DOM		CH_3	H	H	5.6
DOET	(±)	C_2H_5	H	CH_3	0.9
	(-)	C_2H_5	H	CH_3	0.3
	(+)	C_2H_5	H	CH_3	3.3
DOPR	(±)	nC_3H_7	H	CH_3	0.6
DOIP	(±)	iC_3H_7	H	CH_3	2.9
DOBU	(±)	nC_4H_9	H	CH_3	3.2
DOF	(±)	F	H	CH_3	5.8
DOC	(±)	Cl	H	CH_3	1.2
DOI	(±)	I	H	CH_3	1.2
	(-)	I	H	CH_3	0.6
	(+)	I	H	CH_3	2.6
DOB	(±)	Br	H	CH_3	0.6
	(-)	Br	H	CH_3	0.3
	(+)	Br	H	CH_3	2.6
N-Pr DOB	(±)	Br	nC_3H_7	CH_3	13.4
α-desMe DOB		Br	H	H	2.2
4-OH 2,5-DMA	(±)	OH	H	CH_3	NSG
4-COOH 2,5-DMA	(±)	COOH	H	CH_3	NSG
DOTB	(±)	tC_4H_9	H	CH_3	NSG
DOAM	(±)	nC_5H_{11}	H	CH_3	NSG
DOBZ	(±)	CH_2-C_6H_5	H	CH_3	NSG

KEY: NSg=no stimulus generalization at the highest dose tested.

NOTE: Training drug=DOMHCl (1.0 mg/kg, IP) administered 15 minutes prior to testing. In test of stimulus generalization, a presession injection interval of 15 minutes was employed. ED_{50} values are given where stimulus generalization occured.

found that DOTB acts as a partial agonist and can antagonize the stimulus effects of DOM (Glennon 1987b).

Certain polar substituents at the 4-position of 2,5-DMA render the compounds inactive; for example, the 4-COOH and 4-OH derivatives do not produce DOM-like effects. On the other hand, 4-halogenated compounds result in relatively potent derivatives. The 4-fluoro derivative DOF is 4 times more potent than 2,5-DMA. whereas the 4-chloro (DOC) and 4-iodo (DOI) analogs are about 20 times more potent than 2,5-DMA. The most potent halogenated derivative is the 4-bromo analog DOB, which is about 40 times as potent as 2,5-DMA.

The location as well as the nature of these substituents is important. For example, moving the methyl group of DOM, or the bromo group of DOB, from the 4-position to the 3-position (to produce isoDOM and isoDOB, respectively) results in agents that do not produce DOM-like stimulus effects. IsoDOB (or SL7161). for example, produces saline-appropriate responding at 100 times the ED, dose of DOB.

2,4-Dimetboxy Analogs. 2,4-DMA is approximately equipotent with 2,5-DMA. Introduction of a 5-methyl or 5-bromo group, to produce 5-methyl-2,4-DMA and 5-bromo-2,4-DMA, results in active agents, but they are not significantly more potent than 2,4-DMA itself. It seems that the methyl and bromo substituents are tolerated at the 5-position, but they do not produce the increase in activity seen in the 2,5-DMA series.

Terminal Amine Substituents

A primary (i.e., unsubstituted) amine appears to be optimal for DOM-like activity. Simple N-monomethylation of DOM results in a tenfold decrease in potency. Larger N-alkyl substituents produce an even greater decrease in potency; for example, N-n-propyl DOB is approximately one-thirtieth as potent as DOB itself (Glennon et al. 1986b). Using animals trained to discriminate *R* (-)DQB from saline, racemic DOB is 10 times more potent than N-monomethyl DOB, which, in turn, is 10 times more potent than N,N-dimethyl DOB (Glennon et al. 1987). The quaternary analog N,N,N-trimethyl DOB iodide (QDOB) is inactive.

Alpha-Methyl Group

The u-methyl group is important, but not usually necessary for activity. For example, the α-desmethyl analogs of DOM and DOB are both about one-third as potent as their parent agents. The ademethylation of 3,4,5-TMA, to produce mescaline, results in a similar (i.e., 2.5-fold) decrease in activity. Although these α-desmethyl analogs produce stimulus effects similar to those of DOM, there is some evidence that the spectrum of effects produced by these agents, in rats and in humans, is not

necessarily identical with that (spectrum) of their parent compounds (Shulgin and Carter 1975; Glennon et al., in press).

Optical Isomers

Due to the presence of the a-methyl groups, these agents exist as optical isomers. Both isomers usually produce DOM-like effects, and the *R* (-)isomers constitute the eutomeric series. In this regard then, the effects of these agents are stereoselective, but not stereospecific. In general, the *R* (-)isomers are twice as potent as their racemates and about 5 to 8 times more potent than their *S* (+)enantiomers. Some representative data are provided in table 1.

AMPHETAMINE-LIKE STRUCTURE-ACTIVITY RELATIONSHIPS

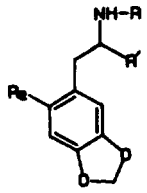
Aromatic Substituents

An unsubstituted aromatic ring appears to be optimal for AMPH-like stimulus effects. Using animals trained to discriminate 1.0 mg/kg of (+)amphetamine sulfate from saline (ED_{50}=1.8 µmol/kg), no aromatic-substituted derivative has yet been found to be more potent than AMPH itself. For example, each of the monomethoxy-substituted derivatives, i.e., OMA, MMA, and PMA, produce AMPH-appropriate responding but are 4 to 15 times less potent than AMPH itself (table 2). The (+)AMPH stimulus does not generalize to any of the above-mentioned DMAs or TMAs (or, for that matter, to any of the agents listed in table 1); however, several of these agents (notably 2,4-DMA, 2,5-DMA, 2,4,5-TMA, 2,4,6-TMA, and 3,4,5-TMA) result in partial generalization (40 to 50 percent AMPH-appropriate responding) suggesting that they may be capable of producing some AMPH-like activity in addition to their DOM-like effects (Glennon et al. 1985). The 4-OH derivative (parahydroxyamphetamine, or Paradrine), which is the O-desmethyl analog of PMA, produces saline-appropriate (2 percent drug-appropriate) responding at greater than 50 µmol/kg. The N-ethyl-3-trifluoromethyl derivative of AMPH, fenfluramine, produces saline-like effects at doses up to about 20 µmol/kg and disruption of behavior at doses greater than or equal to 24 µmol/kg. Complete reduction of the aromatic nucleus of AMPH does result in retention of activity, although potency is significantly decreased; that is, propylhexedrine produces AMPH-like stimulus effects (ED_{50}=15.5 µmol/kg).

Terminal Amine Substituents

In contrast to what was observed for DOM-like activity, N-monomethylation of AMPH-like agents does not decrease their AMPH-like character. Meth-AMPH (i.e., N-monomethylamphetamine) is slightly more potent than amphetamine; likewise, methcathinone (N-monomethylcathinone) is twice as potent as cathinone. N-methylation of DOM-like agents does not convert

TABLE 2. *Results of stimulus generalization studies using (+)amphetamine as a training drug*



Agent	Isomer	X	R_x	R	R'	ED_{50} (µmol/kg)
Amphetamine	(±)	H_2	H	H	CH_3	2.6
	(-)	H_2	H	H	CH_3	5.3
	(+)	H_2	H	H	CH_3	1.8
Methamphetamine	(±)	H_2	H	CH_3	CH_3	1.5
	(+)	H_2	H	CH_3	CH_3	1.2
Phenethylamine		H_2	H	H	H	NSG
Cathinone	(±)	0	H	H	CH_3	3.8
	(-)	0	H	H	CH_3	1.6
	(+)	0	H	H	CH_3	23.4
Methcathinone	(±)	0	H	CH_3	CH_3	1.8
N-OH AMPH	(±)	H_2	H	OH	CH_3	1.1
(+)N-Et AMPH	(+)	H_2	H	C_2H_5	CH_3	4.3
OMA	(±)	H_2	2-OCH_3	H	CH_3	38.7
MMA	(±)	H_2	3-OCH_3	H	CH_3	17.0
PMA	(±)	H_2	4-OCH_3	H	CH_3	9.5
PMMA	(±)	H_2	4-OCH_3	CH_3	CH_3	NSG
4-OH AMPH	(±)	H_2	4-OH	H	CH_3	NSG
Fenflummine	(±)	H_2	3-CF_3	C_2H_5	CH_3	NSG
3,4-DMA	(±)	H_2	3,4-di OCH_3	H	CH_3	NSG
N-Me 3,4-DMA	(±)	H_2	3,4-di OCH_3	CH_3	CH_3	NSG
2,4-DMA	(±)	H_2	2,4-di OCH_3	H	CH_3	NSG
N-Me 2,4-DMA	(±)	H_2	2,4-di OCH_3	CH_3	CH_3	NSG
2,5-DMA	(±)	H_2	2,5di OCH_3	H	CH_3	NSG
N-Me 2,5-DMA	(±)	H_2	2,5-di OCH_3	CH_3	CH_3	NSG

KEY: NSG=no stimulus generalization at the highest dose tested.

NOTE: Rats trained to dircriminate (+)amphetamine sulfate (1.0 mg/kg) from saline administered 15 minuted prior to testing.

them to AMPH-like agents; for example, see N-Me 2,4-DMA and N-Me 2,5-DMA (table 2). Homologation of the methyl to an ethyl group results in retention of AMPH-like activity, although potency is somewhat reduced;

for example, (+)N-Et AMPH is 2.5 times less potent than (+)AMPH itself. Although there have been no systematic investigations of N-alkylation, certain AMPH analogs bearing larger substituents are active. Mefenorex, the N-(3-chloro-n-propyl) analog of AMPH, produces saline-appropriate behavior at about 5.5 µmol/kg and disruption of behavior at 6 µmol/kg.The N-hydroxy analog of AMPH (i.e., N-OH AMPH), a metabolite of AMPH, also produces AMPH-like effects and is about twice as potent as AMPH (table 2).

Alpha-Methyl Group

Removal of the a-methyl group of AMPH results in phenethylamine (PEA). PEA does not produce AMPH-like effects. Likewise, removal of the a-methyl group of cathinone, resulting in α-desmethylcathinone, also results in an agent that does not produce AMPH-like stimulus effects. Huang and Ho (1974a) have demonstrated that pretreatment of the animals with a monoamine oxidase inhibitor prior to administration of PEA does lead to stimulus generalization, suggesting that the adesmethyl analogs may simply lack protection from metabolism.

Beta-Substituents

Very few ~-substituted analogs of AMPH have been investigated. Ephedrine, for example, produces weak AMPH-like activity (Huang and Ho 1974b). (+)Norpseudoephedrine (cathine) also produces AMPH-like stimulus effects. The oxidized analogs of norephedrine and ephedrine, cathinone and methcathinone, respectively, however, are potent AMPH-like agents (table 2).

Optical Isomers

Both optical isomers of AMPH are active (Schechter 1978). In general, for the few isomeric pairs that have been examined, the *S* isomers of AMPH-like agents are slightly more potent than the racemates and about 3 times more potent than the *R* isomers (Young and Glennon 1986). *S*(+)AMPH, for example, is 3 times more potent than *R*(-)AMPH (table 2); *S*(-)cathinone is 2.5 times more potent than racemic cathiione, but (unexpectedly) is nearly 15 times more potent than *R*(+)cathinone.

Miscellaneous Analogs

Certain agents with AMPH-related structures also produce AMPH-like stimulus effects. Agents in which the terminal amine has been incorporated into a cyclic structure, such as methylphenidate (Huang and Ho 1974b D'Mello 1981), phenmetrazine, and phendimetrazine, are active. These agents might be considered as N-alkyl β-substituted phefialkylamines. Aminorex is another agent that falls into this category and is essentially equipotent with AMPH.

METHYLENEDIOXY-SUBSTITUTED PHENALKYLAMINES

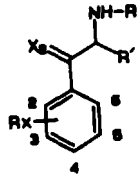
Methylenedioxy-substituted phenalkylamines are considered separately, because it has been shown that the parent 3,4-methylenedioxy analog of AMPH, MDA, is capable of producing both DOM-like and AMPH-like stimulus effects. Its ED, value in DOM-trained rats is 7.8 μmol/kg and in (+)AMPH-trained rats, 10.6 μmol/kg. The DOM-like properties reside primarily with the *R* (-)isomer (ED_{50}=3.8 μmol/kg), whereas the AMPH-like activity resides with the *S* (+)isomer (ED_{50}=4.2 μmol/kg) (figures 2 and 3). To this extent, 3,4-MDA is not a particularly potent agent; it is approximately one-sixth as potent as (+)AMPH and less than one-third as potent as DOM. A positional isomer of 3,4-MDA. 2,3-MDA, produces neither DOM- nor AMPH-like stimulus effects. The 2-methoxy analog of 3,4-MDA (i.e., 2-methoxy 4,5-MDA or MMDA-2) produces weak DOM-like effects (ED_{50}=13.7 μmol/kg), but does not produce AMPH-like stimulus effects.

3,4-MDA is unique. Not only does it produce both types of effects, but it seems to conflict with some of the above-mentioned SARs. For example, aromatic-substituted phenalkylamines such as the 3-methoxy and 4-methoxy derivatives MMA and PMA arc only weak AMPH-like agents, and the 3.4-dimethoxy analog 3,4-DMA (which is structurally very similar to 3,4-MDA) does not produce AMPH-like effects. The 3-OH, 4-OMe, and the 3-OMe 4-OH analogs of amphetamine are also inactive. Thus, it is surprising that 3,4-MDA possesses AMPH-like character. Likewise, neither MMA, PMA, nor 3,4-DMA produce DOM-like effects; yet 3,4-MDA does. 2-Methoxy 4,5-MDA (MMDA-2) and 2,4,5-TMA share a common substitution pattern; interestingly, these agents are essentially equipotent in producing DOM-like stimulus effects. Table 3 displays selected results.

CONTROLLED SUBSTANCE ANALOGS ("DESIGNER DRUGS")

One application of SARs is to make predictions concerning new agents. Assuming that the new agents are producing one of the above-mentioned effects, it should be possible to make approximate predictions of both activity and potency. Over the past decade, several new agents have appeared, and their activities and/or potencies have been consistent with these SARs. Some of these agents have been mentioned. Also encountered were some agents that do not fit the foregoing SAR; it is probably worthwhile considering these agents in depth. For example, PMMA, the N-monomethyl analog of PMA, should produce AMPH-like effects with a potency several times that of PMA itself. In fact, PMMA produces neither AMPH-like nor DOM-like effects. The animals' behavior, however, was disrupted at very low doses (<1 μmol/kg) suggesting that it may produce a central effect that is other than (or in addition to) AMPH-like or DOM-like.

TABLE 3. *Results of stimulus generalization studies with MDA analogs*



Agent	Isomer	R_2	R	R'	ED, Values (μmol/kg) AMPH-Like	DOM-Like
3,4-MDA (MDA)	(±)	H	H	CH_3	10.6	7.8
	(-)	H	H	CH_3	NSG	3.7
	(+)	H	H	CH_3	4.2	NSG
HPA		H	H	H	NSG	NSG
2-OMe 4,5-MDA	(±)	OCH_3	H	CH_3	NSG	13.7
N-Me 2-OMe 4,5MDA	(±)	OCH_3	CH_3	CH_3	NSG	-
MDMA	(±)	H	CH_3	CH_3	7.1	NSG*
	(-)	H	CH_3	CH_3	NSG	NSG*
	(+)	H	CH_3	CH_3	2.6	NSG*
MDE	(±)	H	C_2H_5	CH_3	NSG	NSG
	(-)	H	C_2H_5	CH_3	NSG	NSG
	(+)	H	C_2H_5	CH_3	NSG	NSG
MDP	(±)	H	C_3H_7	CH_3	NSG	NSG
N-OH MDA	(±)	H	OH	CH_3	NSG	NSG

*Partial generalization (i.e., 40 to 55 percent drug-appropriate responding, followed, at slightly higher doses, by disruption of behavior. NSG=no stimulus generalization at the highest dose tested.

NOTE: AMPH-like rats were trained to discriminate 1.0 mg/kg of (+)amphetamine sulfate from saline; DOM-like rats were trained to discriminate 1.0 mg/kg of DOM-HC1 from saline.

The N-monomethyl analog of 3,4-MDA is MDMA (XTC, "Ecstasy," "Adam"). It would be anticipated that N-monomethylation of MDA would reduce DOM-like character by at least an order of magnitude, simuitaneously enhancing the AMPH-like character. Thus, the AMPH stimulus should generalize to the racemate and to the *S* (+)isomer (with the latter being the more potent, and somewhat more potent than *S*(+)MDA), and the *R* (-)isomer might have, at best, some weak DOM-like character. Indeed, the (+)AMPH stimulus generalizes to racemic MDMA (ED_{50}=7.1 μmol/kg) (figure 4) and to *S*(+)MDMA (ED_{50}=2.6 μmol/kg), but not to *R*(-)MDMA.

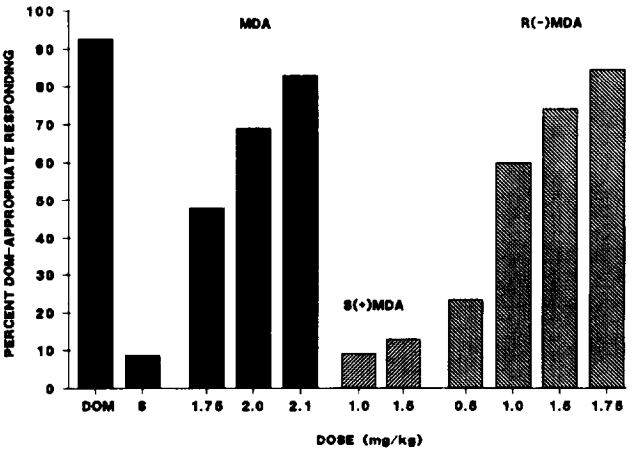


FIGURE 2. *The effects of racemic 3,4-MDA (MDA) and its optical isomers in rats trained to discriminate DOM from saline*

KEY: DOM=effect produced by the training dose, 1 mg/kg. of DOM; S=effect produced by 1 mL/kg of 0.9 percent saline.

NOTE: Doses of S(+)MDA greater than 1.5 mg/kg resulted in disruption of behavior. Results not shown for all doses evaluated. Where stimulus generalization did not occur, result of highest nondisruptive dose of test compounds is shown; slightly higher doses produced disruption of behavior.

More recently, others (Evans and Johanson 1986; Kamien et al. 1986) have published similar results with racemic MDMA. The DOM stimulus does not generalize to racemic MDMA or to either isomer. To this extent, the results appear to be consistent with established SARs.

MDE (MDEA, "Eve") is the N-ethyl analog of MDA. SARs would suggest that this agent should possess little, if any, DOM-like character and that it should be a rather weak AMPH-like agent, Interestingly, neither the racemate (figure 4) nor either optical isomer (figure 5) produces (+)AMPH-appropriate responding. As expected, DOM stimulus generalization does not occur with racemic MDE or with either of its optical isomers (figure 6). Another inconsistency is encountered with the N-hydroxy analog of MDA (i.e., N-OH MDA). Because N-hydroxylation of AMPH has relatively little effect on its stimulus properties, it was anticipated that N-OH MDA might behave in a manner similar to that of MDA. Figure 7 shows that N-OH MDA produces neither AMPH-like nor DOM-like stimulus effects. It should be noted, however, that the optical isomers of N-OH MDA have not yet been examined.

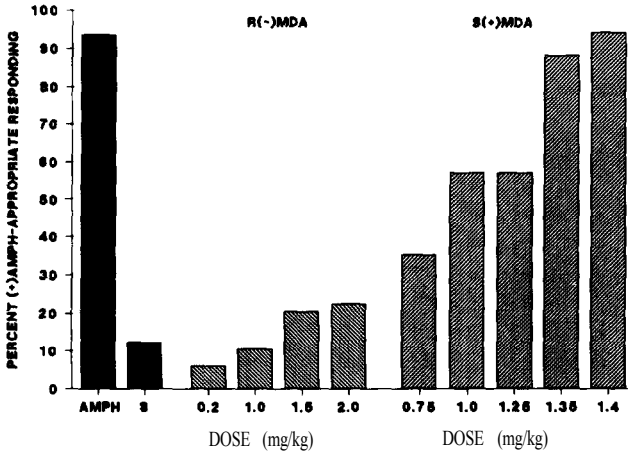


FIGURE 3. *Effect of* R*(-)MDA and* S*(+)MDA in rats trained to discriminate* S*(+)AMPH from saline*

KEY: AMPH=effect of the training dose. 1 mg/kg. of S(+)amphetamine sulfate; S=the effect of 1 mL/kg of 0.9 percent saline.

NOTE: Results not necessarily shown for all doses that were examined. Where stimulus generalization did not occur, result of highest nondisruptive dose is shown; evaluation of a slightly higher dose resulted in disruption of behavior.

At this point, the unexpected results cannot be readily explained with PMMA, MDE, or N-OH MDA. This is particularly confounding in view of the report that MDMA and MDE apparently produce similar psychopharmacological effects in humans (Braun et al. 1980). There are several possible explanations: (1) these agents may produce effects in rats that are different from those produced in humans; (2) these agents may produce in rats a central effect that somehow masks or obscures AMPH-like effects that might have otherwise been observed at higher doses had disruption of behavior not occurred at lower doses; and (3) some of these agents might be capable of producing a stimulus effect distinct from those produced by either AMPH or DOM (Glennon et al. 1988). The recent results of Oberlender and Nichols (1988) would tend to support the latter possibility.

To gain further insight into the stimulus properties of these agents, a group of rats was trained to discriminate MDA (1.5 mg/kg) from saline. Consistent with the generalization results described above, the MDA-trained animals recognized both racemic AMPH and DOM (table 4). MDA stimulus generalization also occurred with both isomers of MDA, with *S*(+)MDA (ED_{50}=2.4 μmol/kg) being about twice as potent as *R*(-)MDA

TABLE 4. *Results of stimulus generalization studies using rats trained to discriminate 1.5 mg/kg* of racemic *MDA from saline*

Agent	Optical Isomer	ED_{50} (μmol/kg)
MDA	(±)	3.0
	(-)	5.5
	(+)	2.4
AMPH	(±)	7.7
DOM	(±)	2.5
MDMA	(±)	4.2
3,4-DMA	(±)	23.2
2,3-MDA	(±)	13.4
Cocaine	(+)	17.3
LSD	(+)	0.07

(ED_{50}=5.5 μmol/kg). Because MDA produces both AMPH-like and DOM-like stimulus effects, it would be expected that MDA-trained animals would recognize both cocaine and LSD, this was found to be the case (Glennon and Young 1984a). Interestingly, the MDA stimulus also generalized to 3,4-DMA and 2,3MDA, agents to which neither the AMPH or DOM stimulus generalizes. These results suggest that MDA is indeed producing both AMPH-like and DOM-like effects and that it may also produce some other stimulus effect.

Next trained was a group of rats to discriminate racemic MDMA from saline. It was found that MDMA-trained animals (MDMA, ED_{50}=2.2 μmol/kg) recognize both *S*(+)MDMA (ED_{50}=1 μmol/kg) and *R*(-)MDMA (ED_{50}=4.3 μmol/kg). Thus, both isomers of MDMA appear to be active, with *S*(+)MDMA being 4 times more potent than *R*(-)MDMA (Glennon et al. 1986c). More recently, Schechter (1987) has reported an enantiomeric potency ratio of about 2, whereas Oberlender and Nichols (1988) obtained a ratio of 2.6. All three studies agree that the *S* (+)isomer is the more active isomer, and two of the three studies find that it is twice as potent as the racemate. Schechter (1987), on the other hand, has found that the racemate is twice as potent as the *S* (+)isomer.

It is probably important to note that although there may be differences between the effects produced by MDMA and MDE, there are also significant similarities. For example, preliminary data using MDMA-trained animals suggest that racemic MDMA and MDE produce similar stimulus

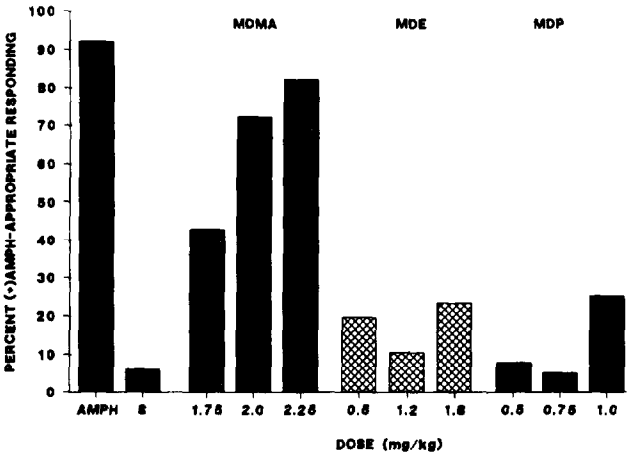


FIGURE 4. *Effect of racemic MDMA, MDE, and MDP in animals trained to discriminate (+)AMPH from saline*

KEY: AMPH=effect of the training dose., 1 mg/kg, of S(+)amphetamine sulfate; S=the effect of 1 mL/kg of 0.9 percent saline.

NOTE: Results not necessarily shown for all doses that were examined. Where stimulus generalization did not occur, result of highest nondisruptive dose is shown; evaluation of a slightly higher dose resulted in disruption of behavior.

effects, with MDE being slightly less potent than MDMA. At 8.2 µmol/kg. MDE produces stimulus effects comparable to those of 6.5 µmol/kg of MDMA. These results are consistent with those of Boja and Schechter (1987), who used animals trained to discriminate MDE from saline. On the other hand, whereas both MDMA and MDE are significantly less potent than (+)AMPH in increasing locomotor activity in mice, *S*(+)MDMA and *S*(+)MDE are about an order of magnitude more potent than their *R* (-)enantiomers, and S(+)MDMA is at least several times more potent than *S*(+)MDE (Patrick and Glennon, unpublished data).

Several recent reports allay fears that some progress is being made. For example. whereas MDA (Glennon and Young 1984b; Evans and Johanson 1986; Kamien et al. 1986). *S*(+)MDA (Glennon and Young 1984b), MDMA and/or *S*(+)MDMA (Glennon and Young 1984b; Evans and Johanson 1986; Kamien et al. 1986; Glennon et al. 1988) produce AMPH-like effects, *S*(+)MDA produces cocaine-like effects (Broadbent et al. 1987), and although MDMA-trained animals recognize *S*(+)AMPH (Oberlender and Nichols 1988), there are additional reports that AMPH-trained animals fail

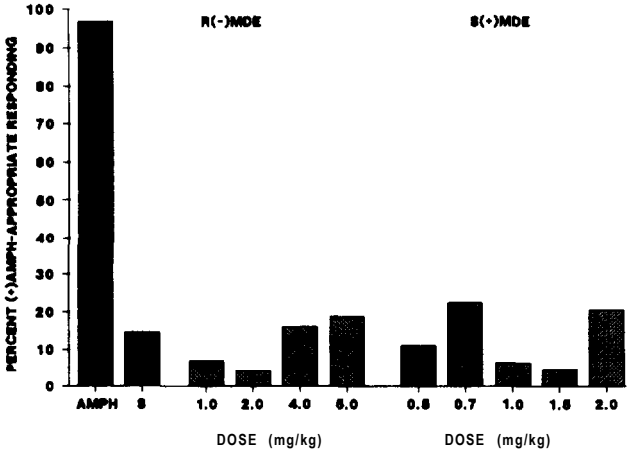


FIGURE 5. *Effect of* R*(-)MDE and* S*(+)MDE in animals trained to discriminate (+) AMPH from saline*

KEY: AMPH=effect of the training dose, 1 mg/kg, of S(+)amphetamine sulfate S=the effect of 1 mL/kg of 0.9 percent saline.

NOTE: Results not necessarily shown for all doses that were examined. Where stimulus generalization did not occur, result of highest nondisruptive dose is shown; evaluation of a slightly higher dose resulted in disruption of behavior.

to recognize *S*(+)MDA (Broadbent et al. 1987). MDMA, *S*(+)MDMA. and *R*(-)MDMA (Oberlender and Nichols 1988). Furthermore, Appel and coworkers have reported in one study that LSD-trained animals recognize both isomers of MDA (Broadbent et al. 1987) and, in another study, that LSD-trained animals recognize *R*(-)MDA but not *S*(+)MDA, *R*(-)MDMA, or *S*(+)MDMA (Callahan and Appel 1987). Consistent with results in DOM animals, Nichols and coworkers (1986) have found that LSD-trained animals recognize racemic MDA and *R*(-)MDA. In the latter study, half the animals tested also recognized *R*(-)MDMA. and 78 percent of a group of rats trained to discriminate MDMA from saline selected the drug-appropriate lever when administered LSD. However, *R*(-)MDA, *S*(+)MDA, *R*(-)MDMA, and *S*(+)MDMA all produced drug-appropriate responding in rats trained to discriminate mescaline from saline (Callahan and Appel 1987). These inconsistencies might be due to procedural differences, or they might be of greater significance. It is believed that *R*(-)MDA produces primarily, but not exclusively, DOM-like (or hallucinogenic) effects, and that *S*(+)MDA produces primarily, but not exclusively, AMPH-like effects. N-monomethylation of MDA enhances AMPH-like character and decreases DOM-like properties.

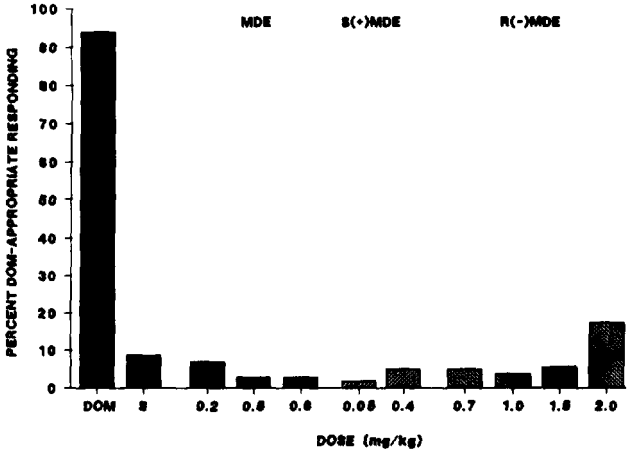


FIGURE 6. *Effect of MDE and its optical isomers in rats trained to discriminate DOM from saline*

KEY: DOM=effect produced by the training dose, 1 mg/kg of DOM; S=effect produced by 1 mL/kg of 0.9 percent saline.

NOTE: Doses of S(+)MDA (greater than 1.5 mg/kg resulted in disruption of behavior. Results not shown for all doses evaluated. Where stimulus generalization did not occur, result of highest nondisruptive dose of test compounds is shown; slightly higher doses produced disruption of behavior.

Evidence further suggests that MDMA (possibly MDA), and particularly MDE and MDP, can produce effects that are distinct from (or that are in addition to, but mask) AMPH-like and/or DOM-like effects.

Recent work shows that, in rodents, MDMA is metabolized, at least in part, to MDA, and that racemic MDMA is preferentially metabolized to *S*(+)MDA (Fitzgerald et al. 1987). The extent to which MDMA metabolites might contribute to the stimulus properties of MDMA is unknown at this time. Because *S*(+)MDA is capable of producing AMPH-like stimulus effects, involvement of this metabolite might explain some of the different results reported for MDMA (particularly if different animal species and various presession injection intervals were employed). In contrast, certain other potential metabolites of MDMA, such as 3-hydroxy-PMA, 4-hydroxy-MMA, 3,4-dihydroxy-AMPH (α-methyldopamine), N-methyl-3-hydroxy-PMA, N-methyl-4-hydroxy-MMA, N-methyl-3,4-dihydroxy-AMPH (N-methyl-α-methyldopamine) do not produce AMPH-like stimulus effects, but may be capable of producing other, distinct types of central activity or may somehow interfere with potential AMPH-like effects.

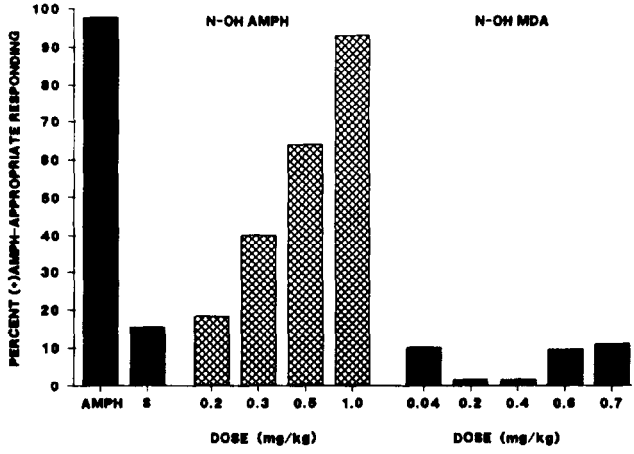


FIGURE 7. *Effect of N-OH AMPH and N-OH MDA in rats trained to discriminate (+)amphetamine from saline*

KEY: AMPH=effect of the training dose, 1 mg/kg. of S(+)amphetamine sulfate; S=the effect of 1 mL/kg of 0.9 percent saline.

NOTE: Results not necessarily shown for all doses that were examined. Where stimulus generalization did not occur, result of highest nondisruptive dose is shown; evaluation of a slightly higher dose resulted in disruption of behavior.

CONCLUSION

The drug discrimination paradigm is a powerful tool for studying centrally acting agents of the phenalkylamine type. It has been used to classify a large number of agents as being either AMPH-like or DOM-like, and it allows for the formulation of SARs. Though not discussed here, drug discrimination studies have proven invaluable in understanding the mechanisms of action of many of these agents (Glennon et al. 1986a; Young and Glennon 1986; Glennon 1988). The SAR can also be used to predict the activity (AMPH-like or DOM-like) and potency of novel agents. Two significant exceptions to established SARs have been encountered PMMA and the MDA analogs. Findings with PMMA were wholly unexpected. MDA analogs probably represent a special case; because no methylenedioxy analogs were included in the data set used to formulate the initial SARs, it may not be wholly valid to attempt extrapolation to these types of agents. Nevertheless, there are instances where the SARs correctly predict the activity and potency of certain analogs (e.g., MMDA-2, MDMA). However, other MDA analogs (i.e., N-OH MDA, MDE, MDP) seemingly lack all regard for the established SARs. Although differences in

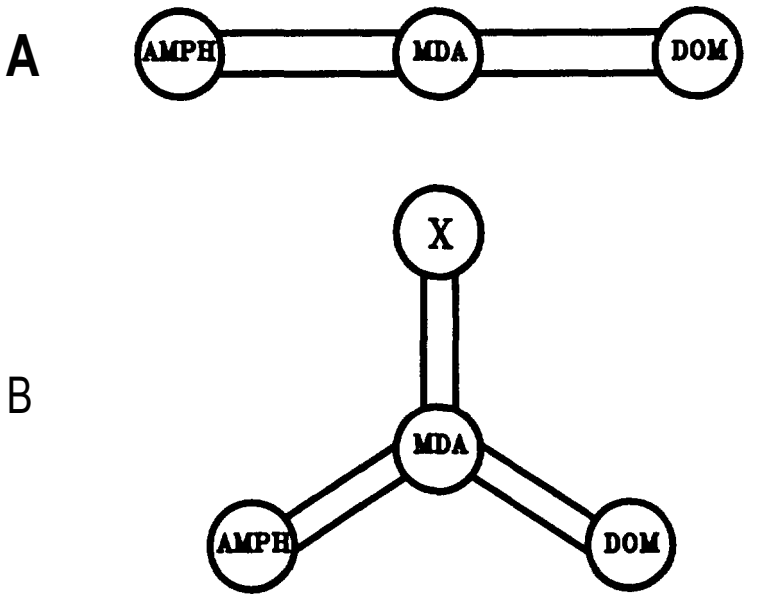


FIGURE 8. *Phenalkylamine analogs appear to produce central stimulus effects along AMPH-like to DOM-like continuum, depending upon the substituent groups present*

NOTE: (A.) MDA produces both types of stimulus effects. (B.) Trifurcated model is presented to account for a possible third, as yet undefined, type of central effect. Certain phenalkylamines may exert effects better described by the MDA/X component of this model than by either pure AMPH-like or DOM-like action.

metabolism and distribution may account for some of the observed results, it is entirely possible that some of these agents might also produce a unique effect that is neither AMPH-like nor DOM-like. Future drug discrimination studies, using the MDA analogs as training drugs, should be of immense benefit in understanding their stimulus effects.

Phenalkylamines are capable of producing a wide variety of pharmacological effects; prominent among the central stimulus effects produced by these agents are AMPH-like effects and DOM-like (or DOB-like) effects. Nearly a decade ago, the author proposed that such agents exist along an AMPH/DOM continuum and that the nature and location of pendent substituents determine where along this continuum an agent may lie (Glennon et al. 1980). It seems likely that MDA is positioned somewhere near the center of this continuum, because it produces both AMPH-like and DOM-like effects. Current evidence suggests the need for a revised model to explain the activity of MDE and MDP and the finding that 2,3-MDA and 3,4-DMA produce MDA-like, but not AMPH-like or DOM-like stimulus

effects. Certain of these agents may produce non-AMPH/non-DOM-like effects (with or without a certain amount of residual AMPH-like or DOM-like character); to account for this, a trifurcated continuum as shown in figure 8 is proposed. Agents such as MDE and MDP may exist somewhere along the MDA/X segment of this new model. The presence of the methylenedioxy group may not be a prerequisite for agents to exist along this arm of the model if, for example, agents such as PMMA can be shown to produce effects similar to those of MDE. In contrast, the amine substituents may play an important role. Obviously, additional studies will be necessary to support this working model. The model, as simplistic as it may be, accounts for the fact that certain of these agents possess some AMPH-like or DOM-like character but, at the same time, do not seem to follow the established SAP. The "X-like" activity (which could, in reality, consist of several different actions) may be a consequence of direct or indirect actions on dopamine and/or serotonin receptors (or populations of these receptors not normally involved in the actions of AMPH or DOM) or may represent actions at entirely different types of receptors.

DISCUSSION

COMMENT: I was surprised by the results with the N-hydroxy compounds, because a number of years ago N-hydroxy-p-toluylamphetamine was studied, and it was found that it was identical to ptoluylamphetamine in its properties because it was actually rapidly and essentially quantitatively converted to p-toluylamphetamine. You are finding that the N-hydroxy analog of MDMA is not MDMA-like in its properties. These data suggest that they certainly are not metabolized in a similar way, perhaps.

RESPONSE: I did not show that the N-hydroxy analog of MDA is not MDMA-like. I showed that it is not amphetamine-like.

COMMENT: That makes it not MDMA-like.

RESPONSE: You can extrapolate. I have problems with those extrapolations.

COMMENT/QUESTION: I was not using MDMA-like in the sense that you were using it--as a substitute. I am simply saying it did not have the pharmacologic effect that MDMA had; namely, substitution for amphetamine, which obviously must mean that the N-hydroxy compound is not converted to MDMA to the same extent at least as the p-toluylamphetamine analog was. I would like to know if you have any information about the metabolism of those N-hydroxy compounds.

RESPONSE/QUESTION: None whatsoever. We are looking at the metabolism of some of the other compounds, but we have not looked at the

N-hydroxy. Has the N-hydroxy MDA been identified as a metabolite of MDA from the earlier studies?

ANSWER: Not that I can remember.

RESPONSE: We have not looked at that at all. But it is surprising. I expected to see either hydroxylation or dehydroxylation, so I expected to see similar activities. But this is what we see. If you compare the activities on a milligram per kilogram basis, the N-hydroxy amphetamine appears to be equipotent. However, when you look at the molecular weights (it is a different salt), it is in fact twice as potent. I cannot explain that.

QUESTION: With respect to the N-hydroxy MDA, have you done a timecourse to see whether at longer times you might pick it up if it is a metabolic induction?

ANSWER: No, we have not. It is an idea.

QUESTION: I noticed that my group is the major one that disagrees with the amphetamine-like activity of MDMA. And when you look at the MDE and MDP you lose that. Is it possible that this MDMA-like activity is really an artifact? That is, that the rats are saying it seems to be amphetamine-like but, in fact, it really is not. And when you go ahead and put the ethyl or propyl, you do not see the amphetamine-like activity because that simply is not what it is?

ANSWER: Obviously no one knows what the rats are thinking. My opinion, based on what we have done so far, is that MDE and MDP may be doing something different. We may have a third wheel on this continuum, it may be a three-way continuum with MDA in the middle. Maybe there is a third kind of effect that MDA is capable of producing, but this is grossly overshadowed by its amphetamine-like or DOM-like activity.

If we start making analogs of MDA like the N-methyl, that moves it a little off center. It still retains some amphetamine-like activity. It may, at high doses, have DOM-like activity. We certainly do not see it. And then we have this third type of effect if we go even farther out to the ethyl homolog or to the types of compounds that you are making. You may have now gotten far enough from center that these compounds no longer have the amphetamine-like or the DOM-like character. But what we see with MDMA is that it is amphetamine-like.

QUESTION: Where does parachloroamphetamine fit in here?

ANSWER: We have never looked at parachloroamphetamine itself.

QUESTION: Substitution of lipophilic moieties on the phenyl ring of DOM makes the compounds more potent. Substitution of ionic-type moieties. like hydroxyl anions, makes them less potent. Is that a good generalization, that making the phenyl ring more lipophilic makes the compounds more potent in the DOM series?

ANSWER: No. There appears to be an optimal potency beyond which the compounds are no longer active as agonists but can, in fact, act as antagonists. So we have analogs of DOM that can antagonize the effects of DOM, because we have passed this optimal lipophilicity. The idea of lipophilicity at the four position is not new, and a number of investigators have looked at this over the years with regard to hallucinogenic activity.

Recently we have been looking at it with regard to binding at 5-HT_2 sites. And we see this correlation fits very well. As we get up to a certain point though, it stops. It appears that, in terms of discrimination and in humans, the propyl compound appears to be optimal. Once we get beyond that, lipophilicity continues to increase, 5-HT_2 receptor affinity continues to increase. The compounds start decreasing in potency and, in fact, they become inactive. So it may be that some of these are partial agonists, and ultimately we get out to antagonists of DOM. So it is not a strictly linear relationship.

QUESTION: Is there any evidence that a common effect of amphetamine and MDMA is mediated through a common biochemical mechanism; for example, antagonism studies in haloperidol?

ANSWER: No, we have not done anything in that regard in terms of drug discrimination.

QUESTION: You seemed to have looked through all of the various substituents in your amphetamine structure, with one exception. You did not touch the benzene ring itself. What would happen if you saturate the benzene ring and make a saccharide derivative of amphetamine?

ANSWER: It retains amphetamine-like activity.

RESPONSE: This is what, propylhexedrine? We have looked at propylhexedrine, and it does retain amphetamine-like activity, but it is less potent. In rats trained to discriminate 1.5 mg/kg of racemic MDMA from saline (ED_{50}=0.76 mg/kg), the ED_{50} values for stimulus generalization to MDE and N-OH MDA are 0.73 and 0.47 mg/kg, respectively (Glennon and Misenheimer, unpublished observations).

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AUTHOR

Richard A. Glennon, Ph.D.
Department of Medicinal Chemistry
School of Pharmacy
Medical College of Virginia
Virginia Commonwealth University
Richmond, VA 23298-0581

Amphetamines: Aggressive and Social Behavior

Klaus A. Miczek and Jennifer W. Tidey

INTRODUCTION

The potential of sudden, intense acts of violence is one of the most attention-getting facets of amphetamine action. Hippies of the 1960s warned: "Speed kills." At that time, reports from law enforcement personnel, psychiatrists, and drug abusers themselves could be viewed to indicate that "amphetamines, more than any other group of drugs, may be related specifically to aggressive behavior" (Ellinwood 1972). Neurotoxic effects of amphetamines and, more recently, their designer derivatives on neurons containing dopamine and serotonin--two neurotmnsmitters of paramount significance in neurobiological mechanisms of aggressive, defensive, social, and sexual behavior--have added a new dimension to the current wave of stimulant abuse (Seiden and Vosmer 1984; Ricaurte et al. 1985).

In fact, amphetamines may be associated with extreme changes in aggressive and social interactions: intense and sudden acts of aggression as well as total withdrawal from any social intercourse. These striking, seemingly opposite shifts in social and aggressive behavior under the influence of amphetamines and related substances are the product of numerous pharmacological, behavioral, and environmental, as well as genetic determinants. Another paradox about amphetamines and related psychomotor stimulants is their calming effect on excessively aggressive children and adolescents diagnosed with attention deficit disorder. The neurobiological mechanisms for the multiple effects of amphetamines on aggressive behavior have been most often related to those relevant to the motor-activating and motor-arousing effects of these drugs. Yet, mechanisms of amphetamine action specific to their effects on aggressive and social behavior have eluded a satisfactory delineation.

AMPHETAMINES AND HUMAN AGGRESSIVE AND SOCIAL BEHAVIOR

Case Reports and Surveys

Case reports and survey data provide a complex account of the link between amphetamines and aggressive behavior, leading to sharply differing

opinions on the severity and nature of the problem. As recently reviewed (Miczek 1987), a series of clinical observations and surveys of institutionalized drug abusers and delinquents point to greatly varying representation of amphetamines in these individuals during the commission of violent and criminal behavior. For example, several descriptions of murders and other intense violent behavior attribute these seemingly unpredictable and drastic changes in behavior to amphetamine abuse (Ellinwood 1971; Siomopoulos 1981). Frequently, clinical analyses suggest that chronic amphetamine intoxication, particularly by the intravenous route, produces a psychotic paranoid state, including frightening delusions that may result in aggressive acts (Kramer 1969; Angrist and Gershon 1969; Ellinwood 1971; Siomopoulos 1981).

Some surveys found sizable proportions of prison populations and juvenile delinquents to have committed their crimes of violence while intoxicated by amphetamines (Hemmi 1969; Simonds and Kashani 1979); conversely, others reported rare cases and very small percentages of juvenile delinquents and excessively hostile individuals as having abused amphetamine (Tinklenberg and Woodrow 1974; Tinklenberg et al. 1977; Gossop and Roy 1976). The reliability of several of these surveys is compromised by the lack of adequately matched samples in highly selected populations of institutionalized individuals. Reliability is also compromised by reliance on notoriously variable verbal reports for the details of the dose and frequency of amphetamine intake, as well as on the exact nature of the drug. It may very well be that the unusual and intense violent acts are more prominent among chronic high-dose abusers than they are among occasional amphetamine abusers. This possible distinction needs to be investigated systematically. So far, no reports have been published showing that substituted amphetamines are linked to a high incidence of excessively violent behavior or other offensive social behavior.

Attention Deficit Disorders

Reductions in aggressive behavior after treatment with amphetamine and other psychomotor stimulants are seen in children and adolescents who have been diagnosed with hyperkinesis or attention deficit disorder. There is considerable disagreement about these diagnostic categories and about whether the violent outbursts and uncontrolled episodes of aggressive behavior are limited to the early developmental period or continue into adulthood (Mendelson et al. 1971; Minde et al. 1972).

The early report by Bradley (1937) on beneficial treatment effects with amphetamine in aggressive, destructive, irritable, and hyperactive boys was repeatedly confirmed by double-blind, placebo-controlled studies, Significant reductions in aggressive behavior and improvements in social interactions were found after treatment with 10 to 40 mg/day of *d*- or *l*-amphetamine for boys and girls, 5 to 14 years of age, who had been diagnosed as

hyperkinetic, autistic, explosive, unsocialized, or emotionally disturbed (Conners 1969; Conners 1972; Winsberg et al. 1972; Winsberg et al. 1974; Arnold et al. 1973; Maletzky 1974).

Experimental Studies on Human Aggression

Earlier experimental studies on amphetamine and human behavior focused on performance measures as well as on eating and sleep disorders. None of these studies identified an increase in aggressive behavior as a problematic side effect (Leventhal and Brodie 1981; Laties and Weiss 1981). As a matter of fact, controlled studies on amphetamine and human social behavior, acute doses of *d*-amphetamine (5 to 30 mg) were found to increase socializing and speaking with no indications of aggressive acts (Griffiths et al. 1977). However, antifatigue and endurance-enhancing effects of amphetamines may contribute to the effects of these substances on aggressive behavior.

In an experiment that exposes a human subject to a competitive task leading to prize money, acute amphetamine doses (5 and 10 mg) increased aggressive responses such as delivering blasts of noise or subtracting money from the presumed competitor (Cherek et al. 1986). At the higher dose (20 mg), the rate of aggressive behavior declined, but the rate of money-winning responses increased, further indicating a dissociation between amphetamine effects on aggressive and nonaggressive responses. In contrast to amphetamine, acute administrations of caffeine only decreased aggressive responses, regardless of whether the subject was strongly or moderately provoked by loss of prize money (Cherek et al. 1983). This experimental approach to the study of human aggressive behavior under controlled laboratory conditions fulfills the demands for accurate, objective, and reliable behavioral measures. It is unclear, however, whether or not this experimental preparation is a valid model of clinically significant problem behavior. Future studies with hyperaggressive individuals or those prone to stimulant-induced aggressive behavior will be needed to validate the laboratory situation.

AMPHETAMINES AND AGGRESSION IN NONHUMAN SUBJECTS

Amphetamine Aggressiveness

More than four decades ago, Chance (1946a; Chance 1946b) observed episodes of rapid running, audible vocalizations, upright postures, biting, and, eventually, increased lethality after administration of near-toxic doses of amphetamine (greater than 10 mg/kg) to mice that were housed in groups. This so-called "amphetamine aggressiveness or rage," most often studied in laboratory rats and mice, but also in chicks, consists of fragmented agonistic acts and postures embedded in stereotyped motor routines (Randrup and Munkvad 1969; Hasselager et al. 1972). The phenomenon of amphetamine aggressiveness in otherwise placid laboratory rats or mice has limited

behavioral validity and appears to be primarily of pharmacological or toxicological interest; like motor stereotypies, the so-called amphetamine aggressiveness is reduced by experimental compromises of the nigrostriatal dopamine system such as synthesis inhibitors, receptor antagonists, and neurotoxic or electrolytic lesions in this region.

Traditional Research Methodologies

Amphetamine, cocaine, and other psychomotor stimulants have been examined with traditional research methodologies involving isolation-induced aggression in mice; pain-induced aggression in mice, rats, or squirrel monkeys; brain stimulation-induced aggression in cats; or mouse killing by rats. The results show an inconsistent mixture of increases, decreases, or no effects. Among the most important determinants of amphetamine effects on aggressive and defensive responses are the stimulus situation, species, prior experience with these types of behaviors (table 1) and, most critically, dosage and chronicity of drug exposure.

TABLE 1. *Doses of amphetamines for modulating behavior*

Aggression		Nonaggressive	
Increases	Decreases	Motor Activity	References
Isolation-Induced Aggression in Mice			
None	10.0 IP	10.0 IP	Melander 1960
None	ED_{50} > 3 IP	ED_{50} 3 IP	DaVanzo et al. 1966
None	5.0 IP	N/S	Valzelli 1967
2.0 IP	> 2.0 IP	> 2.0 IP	Charpentier 1969
None	4.0 IP	4.0 IP	Le Douarec and Broussy 1969
2.0 IP	6.0 IP	N/S	Welch and Welch 1969
None	10.0 IP	N/S	Scott et al. 1971
4.0 IP	8.0 IP	4.0, 8.0 IP	Hodge and Butcher 1975
None	8.0 IP	8.0 IP	Miczek and O'Donnell 1978
None	0.25-1 PO	> 1.0 PO	Krsiak 1979
None	5 IP	N/S	Essman and Valzelli 1984

TABLE 1. (Continued)

Aggression		Nonaggressive	
Increases	Decreases	Motor Activity	References
Pain-Induced Aggression in Mice			
8.4 PO	None	9.3 PO	Stille et al. 1963
0.1 IP	None	N/S	Kostowski 1966
0.5 PO	None	> 0.5 PO	Hoffmeister and Wuttke 1969
None	5.0 PO	2.5 PO	Tedeschi et al. 1969
Pain-Induced Aggression in Rats			
None	3.0 IP	N/S	Lal et al. 1968
0.25-1 IP	4.0 IP	N/S	Crowley 1972
1.0 IP	3.0 IP	N/S	Powell et al. 1973
3.48 IP	N/S	N/S	Mukherjee and Pradhan 1976
None	> 2.5 IP	N/S	Sheard 1979
Pain-Induced Aggression in Squirrel Monkeys			
None	0.3, 1 IM	0.03-1 IM	DeWeese 1977
0.125-1 SC	2.0 SC	> 2 SC	Hutchinson et al. 1977
0.125-1 SC	2.0 SC	> 2 SC	Emley and Hutchinson 1972; Emley and Hutchinson 1983
Extinction-Induced Aggression in Rats			
0.1 IM	0.5, 1.0 IM	0.1-1.0 IM	Miczek 1974
Brain Stimulation-Induced Aggression in Rats			
None	2.0 IP	2.0 IP	Panksepp 1971
Brain Stimulation-Induced Aggression in Cats			
5-7.5/cat IP	10/cat IP	N/S	Sheard 1967
None	>4 IP	N/S	Baxter 1968
None	0.3, 0.8 IP	N/S	MacDonnell and Fessock 1972
0.125-0.5 IP	1-1.5 IP	N/S	Marini et al. 1979
0.5-3 IP	N/S	N/S	Maeda et al. 1985

TABLE 1. (Continued)

Aggression Increases	Aggression Decreases	Nonaggressive Motor Activity	References
Drug-Induced Aggression in Mice			
l-dopa			
2.0 IP	N/S	N/S	Lal et al. 1970
Drug-Induced Aggression in Rats (Withdrawal from Opiates)			
2.0 IP	N/S	N/S	Florea and Thor 1968
ca. 3-11/day PO	N/S	N/S	Thor 1971
1-4 IP	N/S	N/S	Lal et al. 1971
2.0 IP	N/S	N/S	Carlini and Gonzalez 1972
2.0 IP	N/S	N/S	Puri and Lal 1973
2.0 IP	N/S	N/S	Gianutsos et al. 1975
Mouse Killing in Rats			
None	2-15 IP	4-5 IP	KarLi 1958
None	ED_{50} 1.5 IP	ED_{50} 6.6 IP	Horovitz et al. 1965; Horovitz et al. 1966
None	0.5-2 IP	> 2 IP	Kulkarni 1968
None	ED_{50} 0.8 IP	ED_{50} 4.2 IP	Sofia 1969
None	ED_{50} 1.8 IP	1-3 IP	Salama and Goldberg 1970; Salama and Goldberg 1973
None	5.0 IP	N/S	Valzelli and Bemasconi 1971
None	2, 4 IP	1, 1.5 IP	Vergnes and Chaurand 1972
None	ED_{50} 0.18 IP	> 0.18 IP	Malick 1975
None	1.5 IP	N/S	Gay et al. 1975
None	ED_{50} 0.6 IP	N/S	Malick 1976
None	0.75-3 IP	N/S	Gay and Cole 1976
None	2.0 SC	N/S	Posner et al. 1976
None	2 IP	N/S	Barr et al. 1976
None	ED_{50} 1.15 IP	N/S	Barr et al. 1977
None	0.5-2 IP	N/S	Barr et al. 1979
None	1-3 IP	2-3 IP	Russell et al. 1983

NOTE: All doses are expressed in mg/kg; N/S=Data not specified, PO=oral injection.

SOURCE: Miczek 1987.

Low acute amphetamine doses enhance pain-induced aggressive/defensive reactions in mice, rats, and squirrel monkeys (Kostowski 1966; Hoffmeister and Wuttke 1969; Crowley 1972; Powell et al. 1973; Emley and Hutchinson 1972; Emley and Hutchinson 1983). For example, squirrel monkeys subjected to electric shocks to their tails, bite a rubber hose more frequently after being administered amphetamine (0.06 to 1.0 mg/kg, SC) (Emley and Hutchinson 1972; Emley and Hutchinson 1983; Hutchinson et al. 1977). In rats, these pain-induced aggressive/defensive responses increase with doses of 0.1 to 1.0 mg/kg (Crowley 1972).

Intermediate to higher amphetamine doses routinely decreased or disrupted isolation- and extinction-induced aggressive behavior and pain-induced aggressive/defensive reactions in mice, rats, and squirrel monkeys while increasing nonaggressive motor activity (Melander 1960, DaVanzo et al. 1966, Miczek 1974; Hodge and Butcher 1975; Krsiak 1979). It may also be mentioned that amphetamines, as well as other psychomotor stimulants, reliably block mouse-killing behavior in selected laboratory rats (Horovitz et al. 1965; Kulkami 1968; Malick 1976; Russell et al. 1983). In this screening test for antidepressant drugs, the antimuricidal effect of amphetamines may be considered a false positive (Howard and Pollard 1983).

This complicated pattern of amphetamine effects in the traditional models of aggression, each relying usually on a single index, may be conveniently interpreted to reflect how amphetamine's effects on aggression depend on the particular measurement technique. Yet, such conclusions are not heuristic. More recently, an ethological approach to the study of drug action on aggression has focused on biologically valid test situations and detailed behavioral measurements, in an effort to gain insight into causative and functional determinants of aggressive, defensive, submissive, and flight behaviors (Miczek et al. 1984). In the following, an examination of the most important pharmacological and behavioral determinants of amphetamine effects on aggressive and defensive behavior in several animal species will emphasize the lawful, systematic nature of these drug behavior interactions and, at the same time, highlight their social and environmental constraints.

BEHAVIORAL DETERMINANTS OF AMPHETAMINE EFFECTS ON AGGRESSION

Differentiation Between Attack, Defense, Submission, and Flight

In animal species commonly used in laboratory research, social aggregation and dispersion are achieved by agonistic behavior patterns with various acts, postures, movements, and signals. Confrontations between a territorial resident and an intruder, between a dominant and lower-ranking group member, between rival males or females, between a lactating female and a

potential threat to her offspring can be reproduced and studied under controlled laboratory conditions. Amphetamine differentially alters attack and threat behaviors vs. defensive and flight reactions.

In situations of social conflict, amphetamine increases the frequency of escape and defensive responses to threats and attacks by a stimulus animal in mice, rats, cats, rhesus monkeys, and squirrel monkeys in a dosc-dependent manner (Hoffmeister and Wuttke 1969; Crowley et al. 1974; Miczek and O'Donnell 1978; Miczek 1979; Schlemmer and Davis 1981; Haber et al. 1981). Even in the absence of a distinctive behavioral stimulus from an opponent, amphetamine induces escape and defensive responses in mice. Krsiak considered these unprovoked defensive and escape responses as signs of "timidity" (Krsiak 1975; Krsiak 1979; Poschlova et al. 1977).

Amphetamines decrease attack and threat behavior by dominant animals toward lower-ranking group members, by territorial residents toward an intruder, by lactating females defending their litter, and play fighting by juveniles, mainly due to distortions in the perception of socially significant signals and the disruption of integrated sequences of threat and attack behavior (Miczek and Gold 1983; Miczek et al. 1989). Large and intense increments in aggressive behavior after amphetamine administration may occur suddenly in mice, rats, cats, and several primate species, under limited conditions. Several determinants for these infrequent but important amphetamine effects have begun to be identified, such as the base rate of aggressive behavior before any amphetamine administration, previous experiences with aggressive and defensive behavior, and the level of habituation to an aggression-provoking situation.

Baseline

Studies of amphetamine effects on behavior, mainly shaped and controlled by schedules of reinforcement, have led to the general principle of rate dependency; low rates of behavior tend to be increased by amphetamine-like drugs, intermediate rates are less altered, and high rates are decreased (Dews and Wenger 1977). This principle applies only rarely to the effects of amphetamines on aggressive behavior (Miczek and Krsiak 1979). In isolated mice, amphetamine increased the incidence of aggressive behavior only in those subjects that were selected for their near-zero levels during vehicle control tests. Amphetamine decreased aggressive behavior in animals with high rates during vehicle control tests (Krsiak 1975; Krsiak 1979). These results lend themselves to a rate-dependency interpretation. Comparisons between separate groups of subjects, one displaying a low rate of aggressive behavior, the other a high rate, however, are less persuasive evidence for rate dependency of amphetamine effects than is the demonstration of differential drug effects on low and high rates of behavior within the same subject.

A minute-by-minute analysis of rates of attack behavior during either a 5- or 28-minute confrontation between a resident and an intruder shows a high rate of aggression in the initial phase of the encounter and a gradual decline in the later phase (figure 1). This decrement from high to low rates

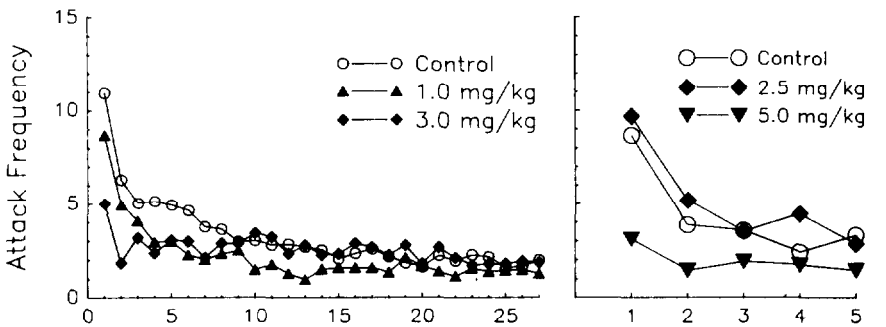

FIGURE 1. *Effect of* d*-amphetamine on the frequency of attack bites by a male resident mouse toward a male intruder during 28-minutes (left) or 5-minutes (right) confrontations*

NOTE: The resident mouse was adminstered an acute dose of amphetamine 30 minutes before confrontation. Frequency of attacks is minute-by-minute average.

of aggression could be due to fatigue, habituation, or changes in the stimulus qualities of the intruder animal. Contrary to the effects of drugs such as alcohol, there was no evidence that amphetamine increased either the high attack rates in the early phase of the encounter or the lower rates

of attack in the later phase (Miczek, unpublished observations). Also, higher amphetamine doses that decreased attack behavior at the start of an encounter did not lead to any rebound in the later phases, even during 28-minute encounters. Apparently, once an aggressive interaction has been initiated, and the opponent reacts with defensive and flight responses, amphetamine does not increase further the rate of aggressive behavior within the same encounter.

Habituation

A substantial increase in aggressive behavior is seen when amphetamine is administered to animals that are repeatedly confronting an intruder (Winslow and Miczek 1983). Specifically, during 2-hour sessions, resident male mice pursued, threatened, and attacked intruders 10 times, each 5-minute encounter being separated from the next by 5 minutes. 'The threat and attack behavior exponentially declined over the course of the 10 consecutive encounters; half of all aggressive behavior was displayed during the first 3 encounters, and the remaining 7 encounters were characterized by very low levels of aggressive behavior (Winslow and Miczek 1984). It is in this later phase of the habituation process that amphetamine more than doubled the rate of attack behavior (figure 2). These amphetamine effects on attack and threat behavior were dissociated from those on elements of motor activity such as walking, rearing, or grooming, in terms of timecourse and dose-effect curve. This pattern of effects suggests a direct action of amphetamine on the habituation process, an elementary form of learning, in addition to the well-known antifatigue effects of amphetamine.

Burst-Like Pattern of Aggressive Behavior

Amphetamine substantially alters the characteristic temporal pattern of agonistic behavior (Miczek 1983; Miczek et al. 1989). Normally, epochs or bursts of intense and frequent threat and attack behavior alternate with periods of relative behavioral quiescence, as, for example, in confrontations between a resident mouse and an intruder. The intervals that separate consecutive attacks are exponentially distributed, with 70 to 80 percent of all intervals being very short and constituting the steep portion of this distribution; the remaining long intervals represent the gaps that separate bursts of attacks. Amphetamine, at doses that did not alter the frequency or duration measures of aggressive behavior, increased the size of the aggressive bursts, and at higher doses abolished the characteristic burst pattern (figure 3).

Sequences of aggressive behavior that are composed of characteristic acts and postures following each other rapidly are disrupted. These disorganizing effects parallel the analysis of amphetamine effects on other intricately patterned behaviors such as feeding, maternal care, play behavior, or reproductive interactions. For example, amphetamine suppresses play

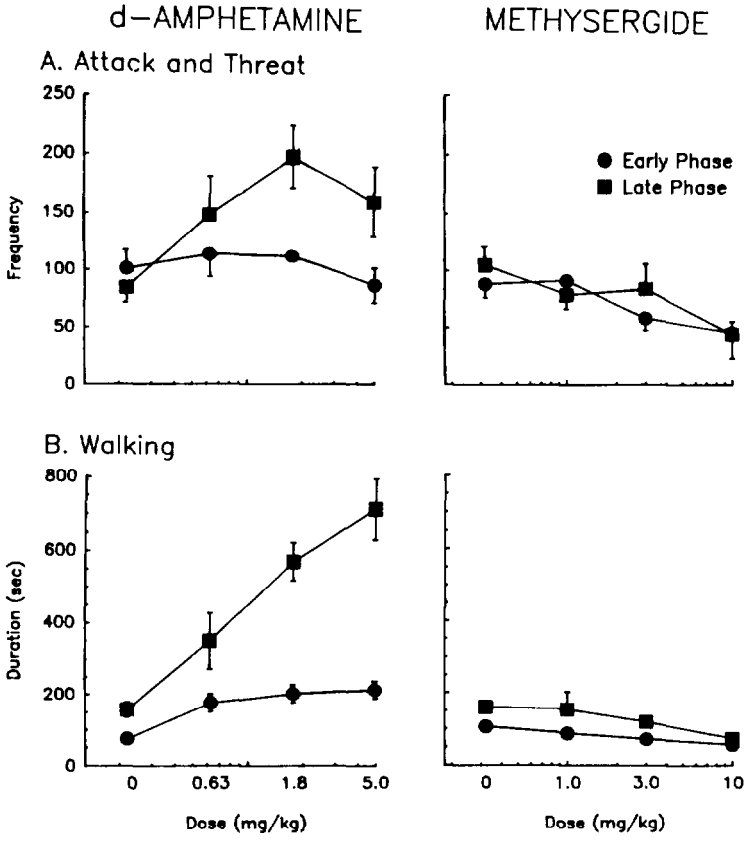

FIGURE 2. *Effects of* d*-amphetamine and methysergide on the cumulative frequency of attack bites and sideways threats (top) and walking duration (bottom) during the initial and later resident-intruder confrontations*

NOTE: Confrontations were in a sequence of 10 consecutive 5-minute trials. each trial seperated from the next by a 5-minute interval.

SOURCE: Winslow and Miczek 1983.

behavior in juvenile rats, an effect that is not antagonized by dopamine or norepinephrine receptor antagonists (Beatty et al. 1984). Similarly, maternal care is severely disturbed in female vervet monkeys under the influence of amphetamine (Schirring and Hecht 1979). These findings and those of others emphasize the disintegrative effects of amphetamine on patterns of

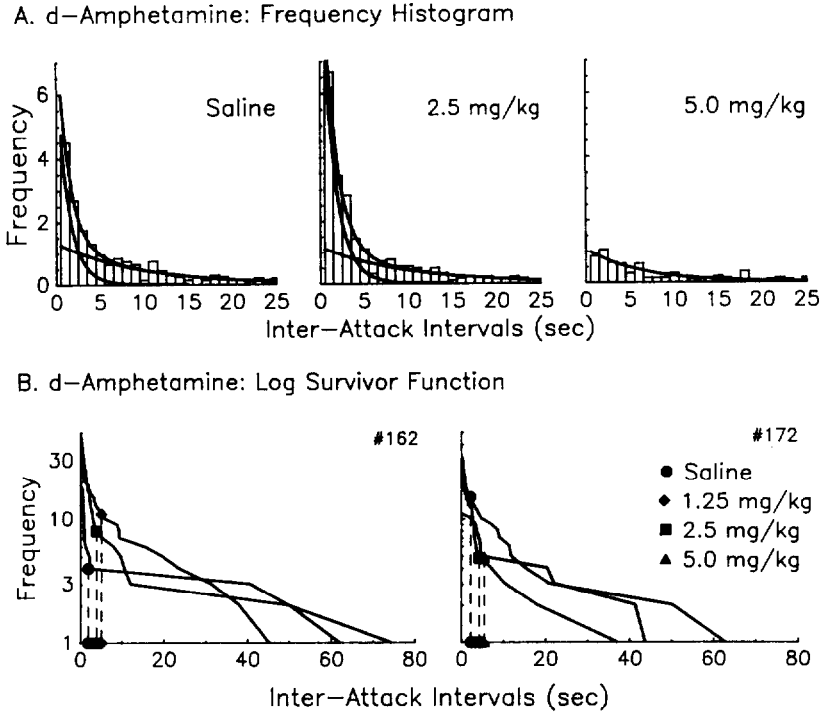

FIGURE 3. *Frequency historgrms of interval length between consecutive attack bites by a resident mouse toward an intruder after saline control, 2.5, or 5.0 mg/kg* d-*amphetamine (n=20). B. Number of interattack intervals surviving to increasing durations from single encounters under saline control conditions, 1.25, 2.5, and 5.0 mg/kg* d-*amphetamine.*

NOTE: Superimposed on the histograms are curves of a mixed exponential distribution and the component distributions. The length of attack bouts is estimated from the intersection of the component distributions. The intervals between attacks that represent the gaps between bouts are shaded.

SOURCE: Miczek et al. 1989.

social interaction (Kjellberg and Randrup 1971; Kjellberg and Randrup 1973; Garver et al. 1975; Miczek 1981b).

PHARMACOLOGICAL DETERMINANTS

Dose

Dose-dependent biphasic effects on aggressive behavior may be seen in several, but not all animal species and situations (Miczek and Krsiak 1979;

Miczek 1987). The paramount importance of dosage for amphetamine effects on aggressive and social behavior is illustrated by experiments in male rats confronting an opponent, either in a competitive situation or as an intruder into their homecage, showing aggression-enhancing effects at low acute doses (Miczek 1974; Miczek 1979). On occasion, increases in aggressive behavior after administration of low acute amphetamine doses have also been seen in fish, mice, and selected rhesus and stumptail macaque monkeys (Weischer 1966; Haber et al. 1981; Winslow and Miczek 1983; Smith and Byrd 1984; Kantak and Miczek 1988). A much more consistent observation, however, is the amphetamine-related increase in defensive, submissive, and flight reactions, which systematically increase with dose, up to a level at which motor stereotypies begin to interfere with the display of these behaviors (Hoffmeister and Wuttke 1969; Miczek 1974; Miczek and O'Donnell 1978).

Ongoing experiments with methylenedioxymethamphetamine (MDMA) show a systematic dose-dependent decrease in attack and threat behavior in mice confronting an intruder into their homecage (Miczek et al., unpublished observations). The decrement in aggressive behavior appears to be behaviorally specific; it is obtained at MDMA doses (0.3, 1, 3 mg/kg) that are lower than those necessary to decrease measures of conditioned performance under the control of schedules of positive reinforcement. Because of species-dependent neurotoxicity, MDMA's effects on aggressive behavior need to be explored in other species, including primates.

Chronicity

Tolerance or sensitization may result from repeated exposure to amphetamines, depending on the interval between consecutive amphetamine administrations (Segal et al. 1980; Robinson and Becker 1986). with continuous drug exposure resulting most often in tolerance, and intermittent administration in behavioral sensitization. Most of the evidence on the determinants of tolerance and sensitization to amphetamine derives from studies on the motor-activating effects of these drugs as measured in situations promoting locomotion, circling, or stereotyped movements.

Unfortunately, only a few experimental studies have focused on the effects of repeated amphetamine administration on aggressive and social behavior, although it is precisely this condition that is associated with the most troubling clinical experiences. Methamphetamine, given in daily increasing doses. decreased aggressive behavior in seven different mouse strains and genera, except for grasshopper mice (Richardson et al. 1972). Daily administration of *d*-amphetamine or cocaine for 2 to 4 weeks to resident mice confronting an intruder failed to shift the dose-effect function for these drugs' effects on any element of threat and attack behavior, while augmenting the stereotypy-inducing effects (O'Donnell and Miczek 1980). Slow-release amphetamine capsules, implanted subcutaneously in rats that

lived in large all-male colonies, produced hyperactivity and social withdrawal in the initial phase of drug exposure; after about a week a high incidence of startle, threat, and defensive responses was seen (Ellison 1978; Eison et al. 1978). Similar, chronically implanted amphetamine capsules in vervet monkeys again resulted in hallucinatory-like grooming, grasping, and head movements, and disrupted social interactions without evidence for tolerance development (Nielsen and Lyon 1982). These progressively more pronounced social withdrawal and motor stereotypies are also seen in groups of macaques or marmosets that are administered amphetamine daily (Garver et al. 1975; Ridley et al. 1979). So far, neither tolerance nor sensitization to amphetamine's effects on withdrawal from all social and aggressive interactions has been seen in the very few studies that either examined changes in the ongoing rate of these behaviors during the course of repeated amphetamine administration or that tested for shifts in dose-effect functions before, during, and after chronic amphetamine exposure.

The only evidence on chronic amphetamine administration and heightened aggressiveness derives from the studies, discussed earlier, on group-housed placid laboratory rats or mice. The behavioral validity of these phenomena under near-toxic dosage conditions, however, needs to be resolved.

Opiate Withdrawal

Amphetamine effects on aggression are markedly modulated by opiates and opioid peptides. Withdrawal from prolonged exposure to opiates may lead to increased defensive and aggressive responses in mice and rats and increased hostility in humans (Lal et al. 1971; Gossop and Roy 1976; Kantak and Miczek 1986). Amphetamine and cocaine, as well as dopaminergic agonists, increase further the already high levels of defensive responses in aggregated rats undergoing withdrawal from opiates, leading in extreme cases to the death of the subjects (Lal et al. 1971; Puri and Lal 1973).

Locomotor-activating effects of amphetamine have previously been linked to dopamine release (Iversen 1977), and it has been suggested that the aggression-enhancing effects may be mediated by a similar mechanism (Gianutsos and Lal 1976). Enhancement of aggression by treatment with a combination of *l*-dopa and *d*-amphetamine can be blocked with the dopamine receptor antagonist haloperidol (Lal et al. 1975); aggression induced by challenge with amphetamine during morphine withdrawal is blocked by either haloperidol or alpha-methyl-para-tyrosine (Lal 1975; Puri and Lal 1973).

The dramatic heightening of aggressive behavior in morphine-withdrawn animals may be due to dopamine receptor upregulation (Gianutsos et al. 1975; Lal et al. 1975). Morphine and methadone inhibit dopamine receptors in the central nervous system (CNS) suggesting possible disuse

supersensitivity and hyperactivity of the receptor during withdrawal (Puri and Lal 1973; Martin and Takemori 1986). Further enhancement of morphine-withdrawal aggression by amphetamine has been interpreted to reflect stimulation of supersensitive dopamine receptors (Puri and Lal 1973; Kantak and Miczek 1988).

Recently, it was found that single-housed mice that had been undergoing withdrawal for 48 hours (after removal of a subcutaneously implanted 75-mg morphine pellet) showed an elevation of attack and threat behavior that was doubled when these mice were challenged with amphetamine, cocaine, *l*-dopa, or apomorphine (figure 4) (Kantak and Miczek 1986;

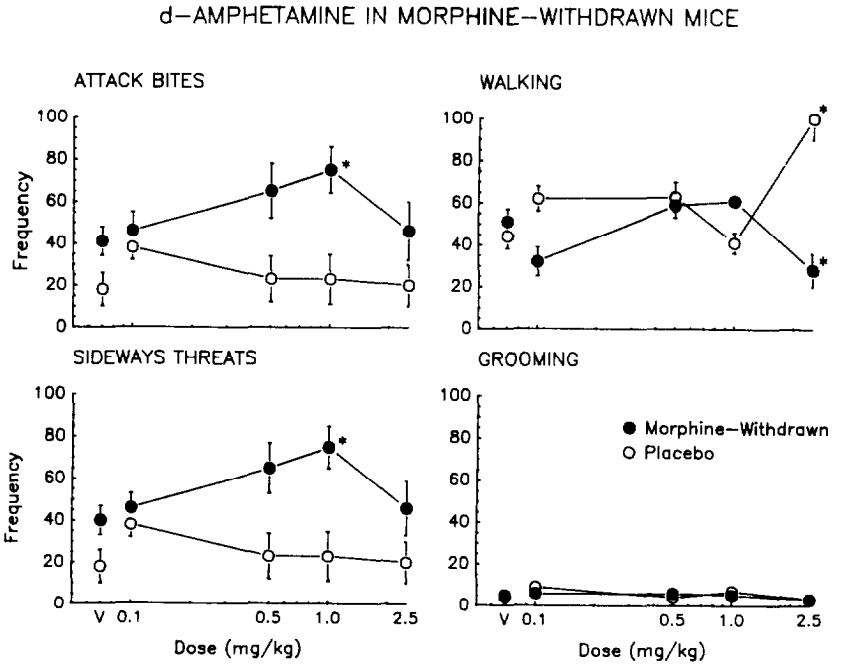

FIGURE 4. *The frequency of attack, threat, walking, and grooming (mean ±SEM per 5 minutes) following saline or 0.1, 0.5, 1.0, or 25 mg/kg* d-*amphetamine*

$p<0.05$ compared to vehicle control.

NOTE: These doses were administered to male resident mice implanted with either placebo pellets (open circles) or morphine pellets (solid circles) subsequently withdrawn 48 hours prior to testing.

SOURCE: Kantak and Miczek 1988.

Kantak and Miczek 1988). Similarly, Lal et al. (1971) and Thor et al. (1970) found that in aggregated rats, amphetamine enhances defensive

upright postures and audible squeals most strongly about 72 hours after termination of a chronic morphine injection schedule. Mice that have been in withdrawal for 5 hours, however, do not show this enhancement when challenged with amphetamine (Miczek and Tidey, unpublished observations). This difference in the reaction to amphetamine may reflect changes in sensitivity of dopamine receptors over time: shortly after withdrawal from opiates, a lessened sensitivity to amphetamine's heightening effects on aggression is seen; later a supersensitivity emerges.

To assess this possibility, selective dopamine receptor agonists were administered to mice 5 hours after subcutaneous morphine pellet removal (Miczek and Mohazab 1987). Challenge with either quinpirole, a selective D2 agonist, or SKF 38393, a selective D1 agonist, or a combination of both did not result in heightened aggression. In fact, the studies with combined administration of D1 and D2 agonists indicate that, in the presence of D1 receptor activation by a small dose of SKF 38393 (3.0 mg/kg), very large doses of D2 receptor agonists are necessary to modify aggressive behavior in these mice, suggesting a subsensitivity of D2 receptors. This particular timecourse relates solely to the aggression-enhancing effects; the authors and others (Bläsig et al. 1973; Lal 1975; Kantak and Miczek 1988) have noted that different autonomic and somatic opiate withdrawal signs emerge at earlier times after morphine pellet removal or termination of a chronic injection schedule.

The sub- and supersensitivity to amphetamine's aggression-modulating effects during withdrawal from morphine depend on the time since the last exposure to opiates; it will be intriguing to determine how the relevant opioid and dopamine receptor populations are altered at these behaviorally critical phases of opiate withdrawal. The display of aggressive, defensive, and submissive behavior is accompanied by marked changes in the functioning of brain opioid peptides in the absence of any drug exposure (Miczek et al. 1986); it will also be interesting to determine how amphetamine's effects in individuals with differential experiences with aggressive or submissive behavior may involve alterations in brain opioid peptides and their receptors.

ANTAGONISM OF AMPHETAMINE EFFECTS ON SOCIAL AND AGGRESSIVE BEHAVIOR

The most consistent and potent antagonism of amphetamine effects on increased motor activity and stereotyped movements is obtained with antagonists at dopamine receptors of the D2 subtype (Creese et al. 1982). This is not the case with amphetamine's disruptive effects on social and aggressive behavior, So far, no antagonists have been identified that reverse amphetamine's disruption of sexual, play, maternal, or aggressive behavior. In many ways, this situation parallels the clinical experiences, in being

unable to reverse the negative symptoms of both amphetamine-induced and endogenous psychoses with classic neuroleptics (Crow 1985).

Dopamine Receptor Antagonists

Haloperidol and chlorpromazine potently decrease aggressive and social behavior as well as many other behavioral functions in various animal species and humans. The marked potency and long-lasting nature of the antiaggressive effects of the neuroleptics with dopaminergic receptor-blocking properties may be the reason why these types of drugs are most frequently used in treating pathologically violent individuals (Itil 1981; Leventhal and Brodie 1981; Sheard 1984; Tupin 1985). The poor behavioral specificity of their antiaggressive effects, however, renders the phenothiazines, butyrophenones, or thioxanthines as less than ideal choices; this pattern of effects is already apparent in preclinical studies (Malick 1979; Miczek and Winslow 1987).

Recently, the effects of more selective dopamine receptor antagonists on aggressive behavior were explored. In resident mice confronting an intruder into their homecage; quinpirole (0.1 to 1.0 mg/kg) potently reduced pursuit, threat, and attack behavior; however, it also reduced concurrent motor activity. This pattern of effects paralleled haloperidol effects in the same species and situation. However, the D1 receptor agonist SKF 38393 more selectively, although less potently, decreased aggressive behavior by resident mice, in the absence of concurrent changes in motor functions. These studies highlight the problem of identifying a dopamine antagonist that could be useful in the blockade of amphetamine effects, but would not suppress behavior on its own.

Dopaminergic receptor antagonists do not antagonize the disruptive effects of amphetamine on aggression. In squirrel monkeys, *d*-amphetamine (1.0 mg/kg) disrupted agonistic and social behavior; haloperidol pretreatment did not prevent this disruption (figure 5, right) (Miczek and Yoshimura 1982). Similarly, *d*-amphetamine decreased attack and threat behavior in resident mice confronting an intruder haloperidol pretreatment failed to reverse this disruption, but further decreased aggressive behavior in amphetamine-treated mice (figure 5, left) (Miczek 1981a). By contrast, the large activation of motor activity, as evidenced by increased time spent in locomotion, was effectively antagonized by haloperidol in mice as well as in squirrel monkeys (figures 5). Similarly, play fighting in juvenile rats is profoundly disrupted by amphetamine, and this disruption is not reversed by haloperidol or chlorpromazine (Beatty et al. 1984). By contrast, in those situations where low, acute doses of amphetamine enhance aggressive behavior, dopaminergic receptor antagonists attenuate this enhancement. These observations suggest differential mechanisms for the aggression-heightening effects of amphetamine as distinct from the disruptive actions on social and aggressive behavior. The neurobiological mechanisms for

amphetamines' disruption of social and aggressive behavior remain to be elucidated.

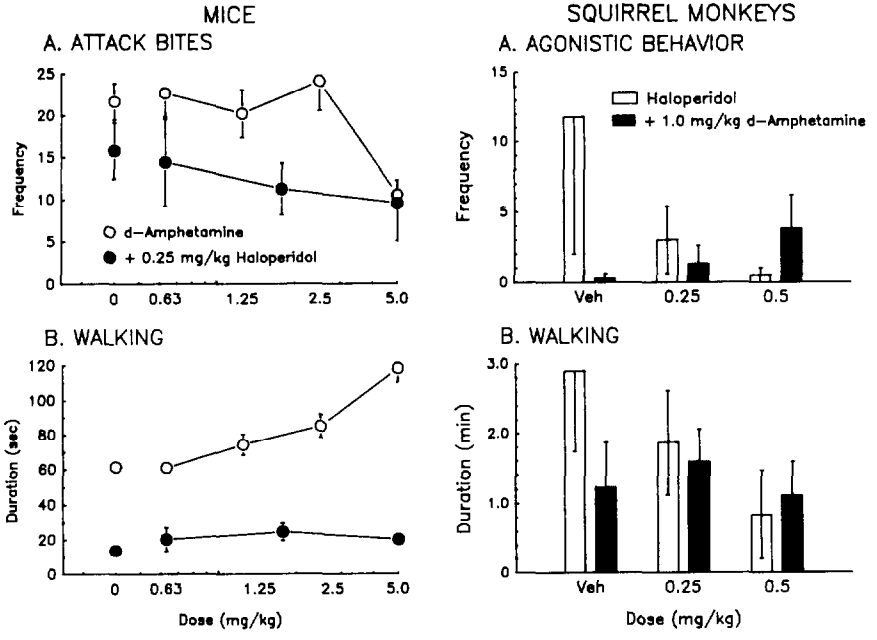

FIGURE 5. *Mice: Frequency of attack bites (A.) and the duration of walking across cage (B.) by resident male mice after administration of* d-*amphetamine alone (open circles), and after pretreatment with haloperidol (0.25 mglkg, solid circles). Squirrel monkeys: Frequency of aggressive behavior (A.) and walking (B.) by dominant squirrel monkeys in established social groups following administration of amphetamine alone (open bars), and combined with haloperidol (0.25, 0.5 mg/kg, IM, solid bars).*

KEY: Vertical lines at each data point represent ± 1 SEM

Noradrenergic Receptor Antagonists

Antagonism of several characteristic effects of amphetamine and cocaine by the alpha adrenergic receptor antagonist prazosin is a most recent example of noradrenergic mechanisms in the actions of psychomotor stimulants (Tessel and Barrett 1986). We investigated whether or not prazosin may attenuate the disruptive effects of amphetamine on social and aggressive behavior in mice and squirrel monkeys (Miczek, unpublished observations). Pretreatment with prazosin (0.4 mg/kg) attenuated the disruption of attack

bites and sideways threats in resident mice treated with higher doses of amphetamine, but no such attenuation was found of amphetamine-disrupted aggressive behavior by dominant squirrel monkeys after prazosin pretreatment (figure 6). By contrast, amphetamine's hyperactivity, measured

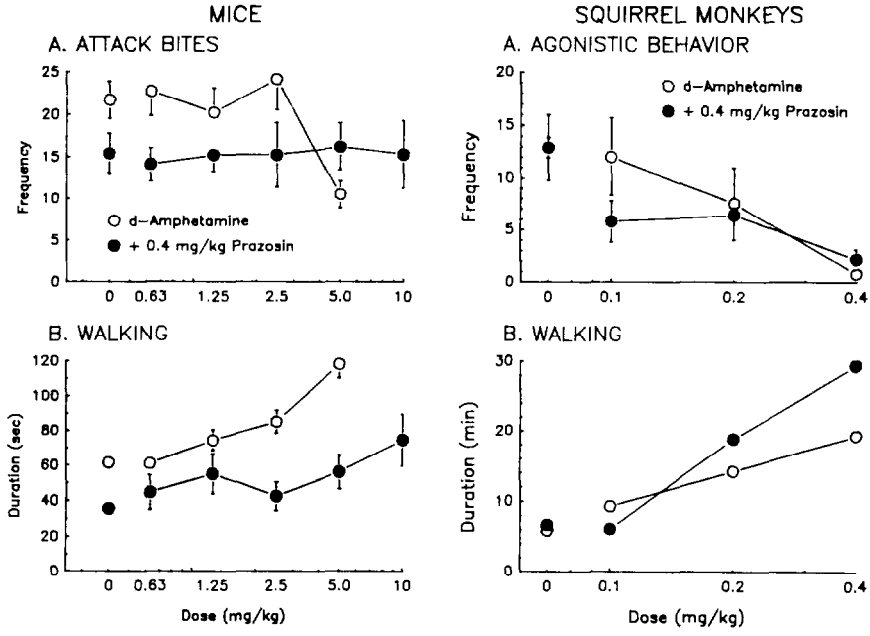

FIGURE 6. *Left: Frequency of attack bites (A.) and duration of walking across cage (B.) by resident male mice after administration of* d-*amphetamine alone (open circles), and after pretreatment with 0.4 mglkg prazosin (solid circles). Right: Frequency of aggressive behavior (A.) and walking (B.) by dominant squirrel monkeys in established social groups following administration of amphetamine alone (open circles), and after pretreatment with 0.4 mg/kg prazosin, IM (solid circles).*

KEY: Vertical lines at each data point represent ± 1 SEM.

as time spent in locomotion, was attenuated by prazosin pretreatment both in mice and squirrel monkeys. Previously, we have observed that pretreatment with phenoxybenzamine or propanolol did not attenuate the suppression of aggressive behavior in amphetamine-treated resident mice (Miczek 198la). In juvenile rats, the suppression of play fighting by amphetamine was also not reversed by phenoxybenzamine or propranolol (Beatty et al. 1984). Again, although the evidence is limited to a few

receptor antagonists and to laboratory rodents, so far there is no evidence pointing to the possible attenuation or reversal of amphetamine's disruptive effects on social and aggressive behavior by noradrenergic receptor antagonists. The negative evidence from efforts to antagonize amphetamine's effects on aggressive behavior with noradrenergic receptor antagonists suggests that these amphetamine effects do not involve noradrenergic mechanisms.

Opioid Antagonists

Opioid receptor antagonists have been found to modulate brain dopamine-mediated behavioral and cellular functions such as motor activity, drug self-administration, and brain stimulation reward (Koob and Bloom 1988).

Naloxone has been found to attenuate the increased motor activity in rats and guinea pigs after amphetamine administration (Holtzman 1974; Haber et al. 1978; Hitzemann et al. 1982; Andrews and Holtzman 1987). Similarly, opiate antagonists reduced the enhancement of rewarding electrical brain stimulation by amphetamine and cocaine (Bain and Kometsky 1987), and intracerebral injections of opiate antagonists into the nucleus accumbens selectively blocked heroin self-administration and motor activation in rats (Amalric and Koob 1984; Vaccarino et al. 1985). Although independent studies have found marked changes in social, aggressive, defensive, and submissive behavior after either opiate antagonists or psychomotor stimulants, the potential antagonism of amphetamine effects on these behaviors by opiate receptor antagonist has not been investigated until recently.

In experiments with mice and squirrel monkeys, we confirmed and extended the antagonism of amphetamine-induced motor hyperactivity by naltrexone; at the same time, however, amphetamine's disruption of aggressive and social behavior was not reversed by naltrexone (Winslow and Miczek, in press). Specifically, in mice, the resident's attack and threat behavior toward an intruder was even further reduced by amphetamine after naltrexone pretreatment (figure 7). Squirrel monkeys that are dominant within their social group exhibit significantly lower levels of aggressive display toward other group members and initiate fewer social interactions after amphetamine treatment; naltrexone did not block these effects. The interactive effects of amphetamine and naltrexone on locomotor behavior are consistent with the proposed modulation of dopamine-mediated functions by opioids; however, the interaction between amphetamine and naltrexone on social behavior appears to involve a different mechanism.

SUMMARY

Clinical case reports and survey data point to incidences of intense violence in certain individuals self-administering high doses of amphetamine via the

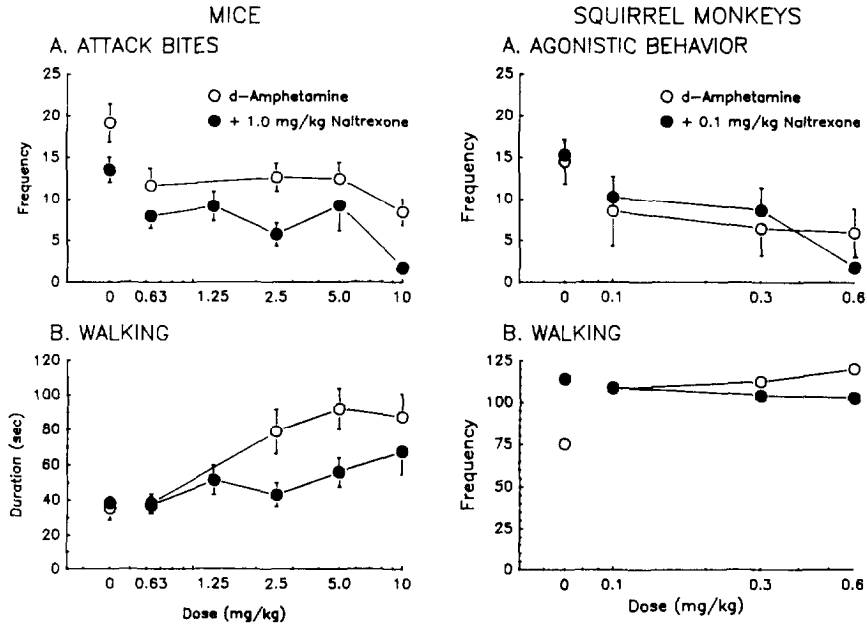

FIGURE 7. *Left: Frequency of attack bites (A.) and duration of walking across cage (B.) by resident male mice after administration of* d-*amphetamine alone (open circles), and after pretreatment with 1.0 mg/kg naltrexone (solid circles). Right: Frequency of aggressive behavior (A.) and walking (B.) by dominant squirrel monkeys in established social groups following administration of amphetamine alone (open circles), and after pretreatment with 1.0 mg/kg, IM, naltrexone (solid circles).*

KEY: Vertical lines at each data point represent ± 1 SEM.

SOURCE: Winslow and Miczek 1988.

intravenous route. It is unclear how common this amphetamine effect is, what circumstances promote its occurrence, and which characteristics predispose an individual to exhibit this effect,

Amphetamine may engender a dose-dependent biphasic effect on aggressive behavior in experimental situations, both with human and animal subjects, as, for example, in subjects that have habituated to an aggression-provoking stimulus. Most often, however, amphetamines disrupt social, sexual, matemal, and aggressive behavior patterns in a dose-dependent manner;

neither tolerance nor sensitization appears to develop to these disruptive effects.

Amphetamine consistently enhances defensive and flight reactions in various experimental situations and animal species. This effect appears to be mediated by brain dopaminergic systems. So far, no dopaminergic, noradrenergic, or opioid antagonists have been found that attenuate, reverse, or prevent the disruptive effects of amphetamines on social and aggressive behavior. The evidence from opioid-withdrawn subjects strongly suggests a profound modulatory influence by opioid peptides on the aggression-altering effects of amphetamines.

DISCUSSION

QUESTION: You know the serine compound is very potent. Have you tried lower doses on a rate-decreasing effect of the stimulant drug?

ANSWER: I tried 0.3 and 1.0. In mice, 0.3 does not have an effect in itself. In rats, 0.3 could be quite disruptive. So there is quite a bit of a species difference. The range of dose is very different in mice and rats.

QUESTION: What do you think causes the aggressive decreasing effects? Are the mice stereotyping or perseverating on some other object?

ANSWER: In the studies we did in mice, rats, and monkeys, we looked carefully at motor changes that might intrude into the behavior and prevent the animals from showing the behavior, not in this dose range. They are nonoverlapping dose ranges. You have to go to higher doses to see stereotypic and motor-activating effects.

In fact, Cherek made that point in one of the very first studies. You cannot see further increases in monetary reinforced behavior. But you see a decline in aggressive behavior. And that is true in other species and humans, too. So the most significant point is that the disruptive effects are due to the intrusion into the repertoires of other repetitive routines.

COMMENT: One of the first studies that was done with SCH compound 23390 showed that it had pronounced antiaggressive effects. This was a Canadian study of people who were in backward, isolated conditions. It had a fairly pronounced effect there.

I think one of the things that is confusing in the aggressive homicide literature is the fact that at low doses, i.e., 10, 20, 30 milligrams for a 70-kilogram person, there is a calming effect. This was one of the things that we used to see with hyperactive children. Many of those hyperactive children were indeed aggressive-hyperactive children, and the amphetamines

had a very pronounced effect on that. This probably represents a low-level activity.

In really aggressive people who have taken amphetamines a long time, you see what is called the reactive phase of aggressiveness.

Let me give you an example of this, which is particularly true in homicides. The individual is engaged in an activity and suddenly misinterprets something. He wakes up in the back of a car and smells poison gas and hits someone over the head with a pipewrench. Or he is robbing a store and someone smiles. There is a sudden impulse and he kills an individual.

If you look at the court records, you see that story repeatedly, i.e., this reactive component. And you can see the same thing in chronic animals. You do have to take them out to a 3- or 6-month period to see those effects. During long-term chronic use, the dopamine at that point is markedly depleted. We are talking about animals that have 20 or 30 percent of the original dopamine levels a month or so after they have been given the last dose of amphetamine.

So I think we are talking about two or three different phenomena, and I think it is very important that we make those distinctions.

RESPONSE: I left aside the hyperactivity issue because that is a literature study in itself. It is also limited to adolescents, children, and juveniles, although there are some reports in adults as well. But there the therapeutic range for amphetamine is 20, 30, or 40 milligrams, and for methylphenidate it is slightly higher, which is actually the preferred agent.

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AUTHORS

Klaus A. Miczek, Ph.D.
Jennifer W. Tidey, B.S.

Tufts University
Medford, MA 02155

Neurochemical Mechanisms Involved in Behavioral Effects of Amphetamines and Related Designer Drugs

Lisa H. Gold, Mark A. Geyer, and George F. Koob

INTRODUCTION

The psychoactive drug 3,4-methylenedioxymethamphetamine (MDMA) has become increasingly popular as an abused substance (Beck and Morgan 1986; Peroutka 1987). Biochemically, MDMA is thought to release serotonin and to a lesser extent dopamine (Johnson et al. 1986; Nichols et al. 1982; Schmidt et al. 1987), while structurally, MDMA resembles both mescaline and amphetamine (Nichols et al. 1986; Shulgin 1978). MDMA is the N-methylated form of 3.4-methylenedioxyamphetamine (MDA), another substituted phenylethylamine with psychotropic properties that may have contributed to its popular name, "the love drug." MDA is considered to be frankly hallucinogenic and has been found to be highly toxic to serotonergic neurons (Ricaurte et al. 1985). Recently, long-term depletions of serotonergic markers have also been observed following single and multiple injections of MDMA in experimental animals, indicating a neurotoxic potential similar to that associated with MDA (Mokler et al. 1987; Schmidt 1987; Stone et al. 1986).

Interestingly, some psychotherapists have been using MDMA to enhance the psychotherapeutic process and to promote easy emotional communication in their patients (Grinspoon and Bakalar 1986). MDMA is characterized as evoking an altered state of consciousness with emotional and sensual overtones (Shulgin and Nichols 1978). This state is described as a pleasant state of introspection, a highly controllable experience that invites intensification of feelings (Grinspoon and Bakalar 1986) and greatly facilitates interpersonal communication (Nichols et al. 1986). Encouraged by these properties, the advocates of MDMA-assisted therapy argue that MDMA is a useful therapeutic tool. Unfortunately, sympathomimetic side effects are occasionally mentioned (Barnes 1988; Grinspoon and Bakalar 1986; Shulgin and Nichols 1978), and concern over a potential to induce arrhythmias in individuals with underlying cardiac disease has been expressed (Dowling et al. 1987).

To better understand the behavioral effects of MDMA, this drug and various analogs have been tested in several behavioral procedures in animals. Significant abuse potential for MDMA was demonstrated by animal self-administration of MDMA (Beardsley et al. 1986, Lamb and Griffiths 1987) and a lowering of self-stimulation thresholds by MDMA (Hubner et al. 1988). MDMA has also been reported to generalize to amphetamine in drug discrimination studies, indicating that MDMA may have subjective effects similar to those of amphetamine (Evans and Johanson 1986; Kamien et al. 1986; Oberlender and Nichols 1988). A more complex mechanism of action has been suggested by one report of generalization to the serotonin agonist fenfluramine (Schechter 1986) and another report that described drug-like responding following MDMA in rats trained on mescaline (Callahan and Appel 1987). Indeed, several authors have concluded that MDMA may produce discriminative stimulus effects that are different from both stimulants and hallucinogens (Glennon et al. 1988; Oberlender and Nichols 1988).

LOCOMOTOR ACTIVITY AND PSYCHOSTIMULANT EFFECTS

Locomotor activity has historically been used as an index of psychostimulant effects. Simple assessment of amount of locomotor activity can provide the basis for anatomical as well as pharmacological analysis of the neural substrates that mediate the behavioral expression of stimulant action. More sophisticated behavioral measurement systems can record multiple measures of activity and describe spatial and temporal patterning of locomotion. In such systems, qualitative aspects of behavioral activation can be evaluated by examining the entire activity profile. A comparison of the effects of novel drugs with those produced by well-characterized substances may lead to a better understanding of their mechanisms of action and subjective properties.

Neural Substrates of Psychostimulant Locomotion

The neural substrates of locomotor activation produced by psychomotor stimulants have been linked for some time to dopamine function in the nucleus accumbens. An early finding reported that direct injection of dopamine into the nucleus accumbens produced enhanced locomotor activity in rats (Pijnenburg and Van Rossum 1973), and the unconditioned motor activation produced by amphetamine was shown to be blocked by dopamine receptor antagonists (Pijnenburg et al. 1975). Destruction of dopamine terminals within the nucleus accumbens with 6-hydroxydopamine (6-OHDA) was found to attenuate the locomotion produced by indirect sympathomimetics (Joyce and Koob 1981; Kelly et al. 1975; Kelly and Iversen 1976) but not to disrupt the locomotor-activating properties of caffeine, scopolamine (Joyce and Koob 1981) (figure 1). corticotropin-releasing factor (CRF) (Swerdlow and Koob 1985). or heroin (Vaccarino et al. 1986) (figure 2) in rats. Thus, the locomotor stimulation produced by psychostimulant drugs

has been hypothesized to result from release of dopamine from the mesolimbic dopamine terminals in the region of the nucleus accumbens, but other drugs with locomotor-activating properties may interact with other parts of the limbic-nucleus accumbens-ventral pallidal circuitry known to be important for psychostimulant activation (Swerdlow et al. 1984; Swerdlow et al. 1986).

Neural Substrates of Psychostimulant Reinforcement

The locomotor-activating properties of psychomotor stimulants have been hypothesized to be one aspect of their reinforcing properties (Mucha

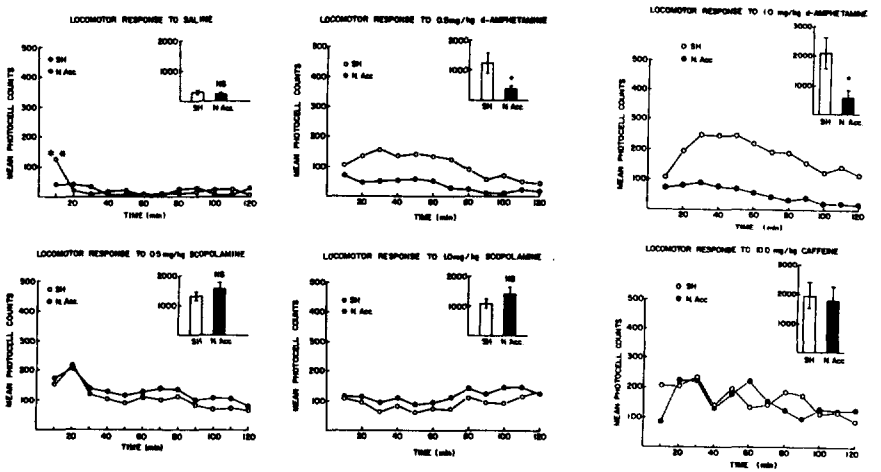

FIGURE 1. *Effects of amphetamine, scopolamine, caffeine, and saline on locomotor activity in rats with 6-OHDA lesions of the nucleus accumbens or sham-operated controls (n=8 rats/group)*

*Refers to a signficant group effect.

**Refers to a significant difference between the groups at 10 minutes postinjection, simple main effects.

KEY: Values in upper right corner of each panel represent mean ± SEM for the total activity over the 2-hour drug test.

SOURCE: Joyce and Koob, 1981, Copyright 1981, Springer-Verlag.

et al. 1982; Spyraki et al. 1982; Swerdlow and Koob 1984). Animals will learn to prefer an environment previously associated with drugs that produce hyperactivity, and pharmacological or surgical manipulations that block the locomotor-activating properties of psychomotor stimulants block this place preference. The nucleus accumbens, which has been demonstrated to be

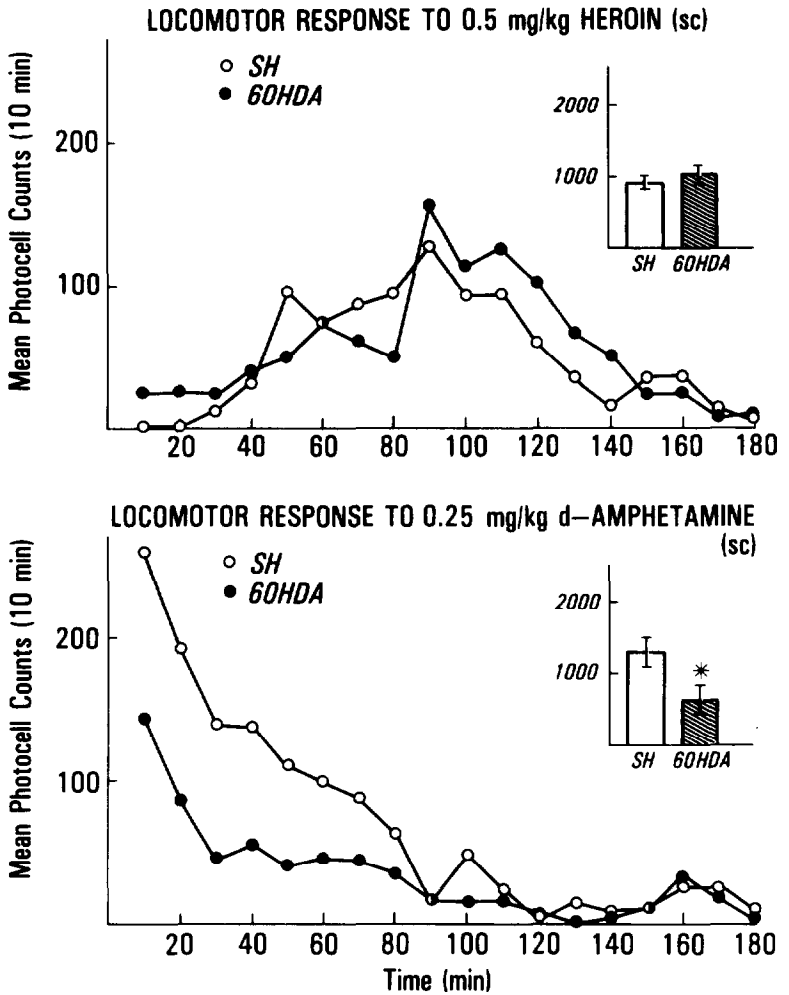

FIGURE 2. *Effects of 6-OHDA lesions of the nucleus accumbens on the locomotor response ajier SC injection of heroin (0.5 mg/kg) or amphetamine (0.25 mg/kg)*

*Significantly different from sham group. $p<0.05$.

NOTE: Rats were habituated to the photocell cages for 90 minutes. after which they were injected. Inserts show the mean ± SEM total counts for 180 minutes for eight rats in the sham and lesion group, respectively.

SOURCE: Vaccarino et al. 1986, Copyright 1986, Pergamon Press.

involved in a variety of the behavioral actions of stimulants and opiates, may act as a bridge between the limbic system and the extrapyramidal motor system, integrating limbic influences and motor activity (Mogenson and Nielson 1984; Swerdlow et al. 1986).

The reinforcing properties of psychomotor stimulants have also been linked to the activation of central dopamine neurons and their postsynaptic receptors. When the synthesis of catecholamines is inhibited by administering alpha-methyl-para-tyrosine, an attenuation of the subjective effects of euphoria associated with psychomotor stimulants occurs in man (Jonsson et al. 1971), and a blockade of the reinforcing effects of methamphetamine occurs in animals (Pickens et al. 1968). Furthermore, low doses of dopamine antagonists will increase response rates for intravenous injections of *d*-amphetamine (Risner and Jones 1976; Yokel and Wise 1975; Yokel and Wise 1976).

Noradrenergic antagonists such as phenoxybenzamine, phentolamine, and propranolol had no effect on stimulant (amphetamine) self-administration (DeWit and Wise 1977; Risner and Jones 1976; Yokel and Wise 1976). Wise and coworkers hypothesized that a partial blockade of dopamine receptors produced a partial blockade of the reinforcing effects of *d*-amphetamine. Thus, animals were thought to compensate for decreases in the magnitude of the reinforcer by increasing their self-administration behavior. Similar results have been observed with alpha-flupenthixol (Ettenberg et al. 1982) and many other dopamine receptor antagonists, including haloperidol, chlorpromazine, metoclopramide, thioridazine, and sulpiride (Roberts and Vickers 1984). Recently, the selective D-1 antagonist SCH 23390 was shown to increase cocaine self-administration at doses that did not impair motor function (Koob et al. 1987a), whereas spiperone, a D-2 selective compound, produced only small increases in responding at doses close to those that produced motor dysfunction. These results suggest that dopamine receptor blockade, particularly D-l receptor blockade, may be involved in the reinforcing effects of psychomotor stimulants in rats. It should be noted, however, that the SCH 23390 compound failed to produce this action consistently when administered intravenously to rhesus monkeys self-administering cocaine (Woolverton 1986).

The role of dopamine in the reinforcing properties of psychomotor stimulants was extended by the observations that 6-OHDA lesions of the nucleus accumbens produce extinction-like responding and a significant and long-lasting reduction in self-administration of cocaine and *d*-amphetamine over days (Lyness et al. 1979; Roberts et al. 1977). These effects were thought to be due largely to the depletion of dopamine, since rats pretreated with desmethylimipramine before the nucleus accumbens lesion (to protect norepinephrine neurons from destruction with the 6-OHDA) showed an identical extinction-like response (Roberts et al. 1980). Similar results were obtained following 6-OHDA lesions of the ventral tegmental area (Roberts and Koob

1982). Subsequent studies have shown that 6-OHDA lesions of the frontal cortex (Martin-Iverson et al. 1986) and corpus striatum (Koob et al. 1987b) do not significantly alter cocaine self-administration. Interestingly, lesions of specific subsets of the dopamine forebrain projections have been associated with facilitated acquisition of amphetamine self-administration (Deminiere et al. 1984; Deminiere et al. 1988), suggesting that some specific neuropathology within the dopamine system could sensitize individuals to the reinforcing actions of psychostimulants.

These results, showing facilitated acquisition of psychostimulant self-administration with lesions of subsets of the dopamine projections, emphasize the need for other measures of reinforcement besides a continuous reinforcement schedule. To this end, rats that had been trained to self-administer cocaine intravenously were subjected to a progressive-ratio procedure following 6-OHDA lesion to the nucleus accumbens or corpus striatum. The rats with a lesion of the nucleus accumbens showed a significant decrease in the highest ratio for which they would respond to obtain cocaine (figure 3) (Koob et al. 1987b). Complementary results have been obtained using a similar progressive-ratio procedure in which rats with 6-OHDA nucleus accumbens lesions increased significantly the highest ratios for which they would self-administer apomorphine (Roberts and Vickers 1988). This motivational probe thus avoids many of the problems associated with measuring local rates of responding. For example, the rats with 6-OHDA lesions showed a decrease in cocaine self-administration while on a continuous reinforcement schedule that superficially could be interpreted as either a decrease or increase in the reinforcing value of cocaine. The results in the progressive-ratio test suggest that this decrease in local rates of responding, previously observed with lesions to the region of the nucleus accumbens, does in fact represent a motivational deficit.

Both amphetamine and cocaine have also been reported to support intracranial self-administration in the mesolimbic/mesocortical dopaminergic system. Rats will self-administer cocaine into the medial prefrontal cortex (Goeders and Smith 1983). while amphetamine is self-administered into the orbitofrontal cortex of rhesus monkeys (Phillips and Rolls 1981) and the nucleus accumbens of rats (Hoebel et al. 1983; Monaco et al. 1981). These data indicate that the mesolimbic/mesocortical dopaminergic system is involved in the initiation of stimulant reinforcement processes, and this work suggests that the region of the nucleus accumbens, more specifically the mesolimbic dopamine system, may be an important substrate for reinforcing properties of several psychomotor stimulant drugs.

Behavioral Profile of MDMA

Both quantitative and qualitative aspects of the behavioral profile of motor activation produced by MDMA and methylenedioxyethylamphctamine

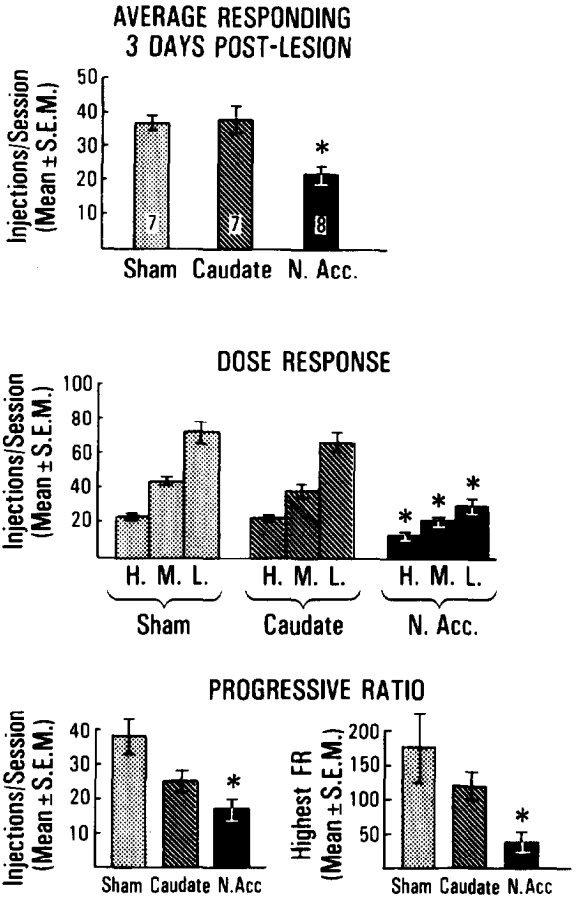

FIGURE 3. *Effects of 6-OHDA lesions to the nucleus accwnbens and corpus striatum on responding for rats self-administering cocaine*

*Significantly different from sham group. p<0.05 Newman-Keuls test.

KEY: H.=2 times the normal 0.75 mg/kg/injection dose; M=middle dose range, 0.75 mg/kg/injection; L=1/2 the 0.75 mg/kg/injection dose.

NOTE: Top panel shows continuous reinforcement data averaged over the first 3 days postlesion (means ± SEM). Sham, vehicle-injected (0.1 mg/mL ascorbic acid in saline) controls. Caudate, rats receiving 8 µg in 2 µL of 6-OHDA injected into the corpus striatum. N. Acc rats receiving 8 µg in 2 µL of 6-OHDA injected into the nucleus accumbens. Middle panel shows the dose-effect functions for each group. Bottom panel shows the mean rewards and mean highest ratio obtained by each group on the progressive ratio probe

SOURCE: Koob et al. 1987b. Copyright 1987, Raven Press.

(MDE) have been characterized and, the effects of these drugs compared with those of classic stimulants and hallucinogens (Gold et al. 1988). Exploratory activity was monitored in eight separate behavioral pattern monitor (BPM) chambers, each consisting of a 30.5 by 61 cm black Plexiglas holeboard with three floor holes, seven wall holes, and a steel touchplate 15 cm above the floor that detected rearings against the wall (figure 4) (Geyer et al. 1986). The frequency of photobeam breaks was used as a general measure of motor activity, and the number and duration of holepokes and rearings were cumulated.

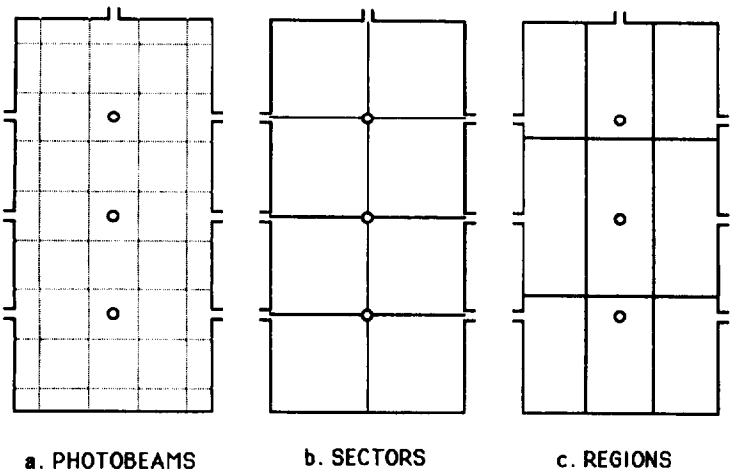

FIGURE 4. *Diagrammatic representation of the behavioral pattern monitor chamber. The positions of the seven wall and three floor holes are shown in each diagram*

KEY:
a. Infrared photobems are arranged in a Cartesian coordinate system on 7.6-cm centers and are sampled five times per second
b. Sectors are equal 15-cm squares and are used to define crossovers, a measure of horizontal locomotion.
c. Regions are unequal in size and are used primarily to define entries into the center region and for the CV9 analysis of spatial patterns of locomotion.

SOURCE: Geyer et al. 1987. Copyright 1981. Pergamon Press.

MDMA significantly altered the behavioral activity profile of rats. Figure 5A illustrates the timecourse of the effects of MDMA on crossovers resolved into 30-minute blocks across the 2-hour test session. Doses of 1.25, 2.5, 5.0, and 10.0 mg/kg produced significant increases in crossovers, which remained elevated at the end of the session at the two highest doses studied. Interestingly, during the first 10 minutes in the chamber (10 to 20 minutes postinjection), doses of 2.5 to 10 mg/kg did not significantly increase crossovers.

Concomitant with this total increase in horizontal locomotion, MDMA (1.25 to 10.0 mg/kg) caused alterations in measures of investigatory behavior. MDMA had a profound effect on the distribution of investigatory holepokes over time. Whereas the control animals exhibited a decrease in the number of investigatory holepokes as they habituated to the chambers during the session, the MDMA-treated rats demonstrated an initial decrease in the number of holepokes, followed by the tendency to increase investigatory holepoking over time (figure 5B). Similarly, MDMA (1.25 to 10.0 mg/kg) dramatically reduced the amount of rearing behavior measured. The amount and duration of this suppression of rearing was related to the dose of MDMA studied (figure 5C). Rearing in rats heated with MDMA was markedly reduced compared to control rats during the first 30 minutes.

More descriptive measures of the animals' behavior were provided by cumulating entries into and time spent in each of nine unequally sized regions, which included the center and the four comer regions (Geyer et al. 1986). Accompanying these changes in the amount of rearing and investigatory holepoking was an observable avoidance of the center of the experimental chamber. Thus, a significant decrease in average duration of center entries for the first 30 minutes was obtained following MDMA doses of 1.25 to 10.0 mg/kg.

MDE, the N-ethyl derivative of MDA, produced a behavioral profile similar to that described for MDMA. MDE increased the number of crossovers measured during a 1-hour experimental session (figure 5A). A transient decrease in the number of crossovers during the first 10 minutes in the chambers (0:549.1, 1.0:645.7, 3.0:313.1, 10.0:284.4) was noted for MDE at doses of 3.0 and 10.0 mg/kg. As with MDMA, the two highest doses of MDE tested (3.0 and 10.0 mg/kg) significantly decreased the total number of holepokes for the first 30 minutes. Rearing was also suppressed by these doses of MDE over a similar timecourse (figures 5B and 5C). At the 10 mg/kg dose of MDE, avoidance of the center was again observed as a significant decrease in the average duration of center entries.

For spatial pattern analyses, the data were reduced to sequences of X,Y positions as described elsewhere (Geyer et al. 1986). These sequences were used to produce video displays of the animal's position, rearings, and holepokes, which could be viewed from 1 to 20 times teal-time speed. The transition frequency between any of five areas (two ends, center, and two long wall areas) was calculated, as was the coefficient of variation (CV) for the relative transition frequencies (Geyer 1982). A related but slightly different procedure evaluated the sequence of position changes by calculating the number of occurrences of each of the 40 transitions among any of 9 specified regions. As an animal preferentially repeats certain transitions, the CV increases, while a more random pattern produces a lower CV. T'he CV thus reflects the extent to which the animal establishes a preferred pattern of locomotor activity over time.

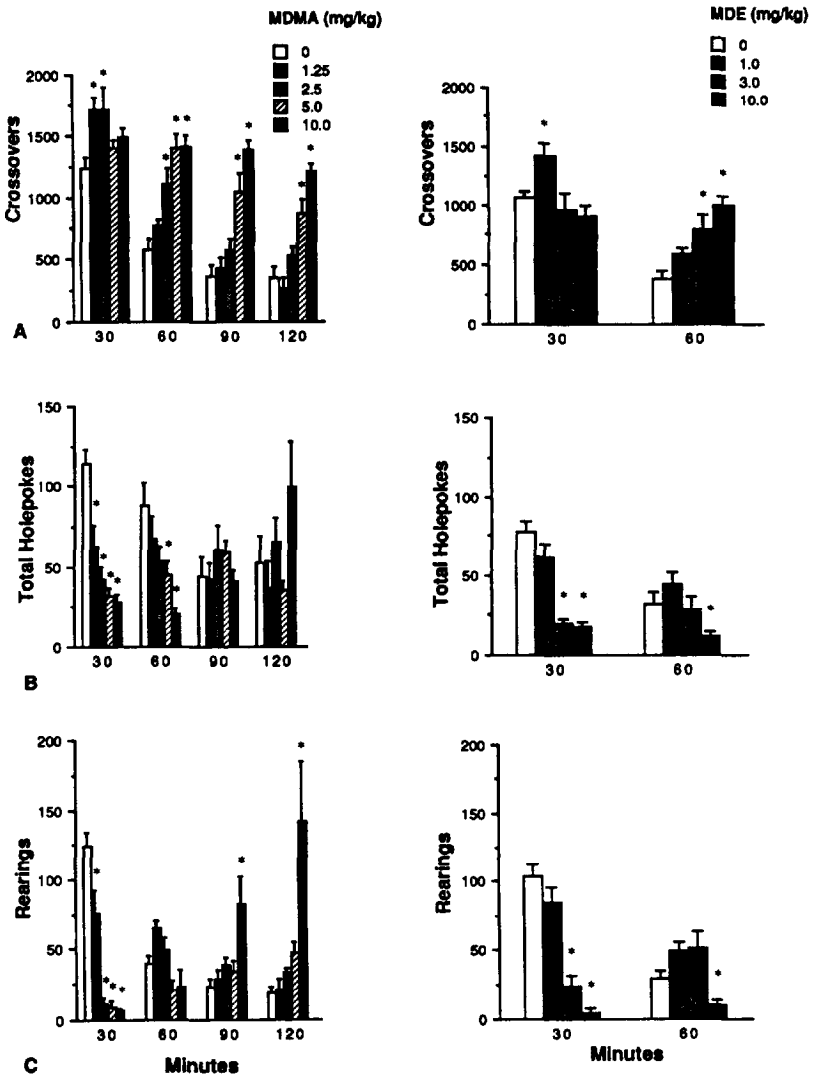

FIGURE 5. *Timecourse effects of MDMA (n=7 to 8 rats/group) and MDE (n=9 to 12 rats/group) on A (crossovers), B (total holepokes), and C (rearings) per 30 minutes in the BPM*

$p<0.05$.

NOTE: Animals were injected 10 minutes before being placed in the chambers. Effects of selected doses are shown as group means ±SEM.

Both MDMA and MDE caused some obvious qualitative changes in the locomotor patterns of rats. At moderate to high doses of MDMA, a definite avoidance of the center of the experimental chamber was frequently seen, and circling around the perimeter was the dominant behavior. This thigmotaxis is similar to that previously observed with apomorphine or scopolamine (Geyer et al. 1986). Although most rats had a predominant direction of rotation, occasionally the rats reversed direction for one or more revolutions. The impression of a disruption in locomotor patterns described above was corroborated by a significant change in the spatial CV measure. Both MDMA and MDE increased the spatial CV, which suggests a more perseverative nature of locomotor patterns (table 1). In contrast, doses of amphetamine (AMPH) that produced similar increases of horizontal locomotion tended to induce highly varied patterns of directional changes, which were reflected in a reduced spatial CV (Geyer et al. 1986).

Neural Substrates for the Psychostimulant Actions of MDMA

The neurochemical mechanisms for the stimulant properties of MDMA were examined in a photocell cage apparatus following pharmacological and neurochemical manipulations. Locomotor activity was measured in a bank of 16 wire cages 20 cm by 25 cm by 36 cm, each cage with two horizontal infrared beams across the long axis 2 cm above the floor. Total photocell beam interruptions and crossovers were recorded by a computer every 10 minutes. Before the drug series, each rat was habituated to the photocell cages overnight, and, prior to drug injection, the rats were habituated again to the photocell cages for at least 90 minutes.

A role for serotonin in the stimulant actions of MDMA was tested by examining the effects in rats of the serotonin antagonist methysergide on MDMA activation (Gold and Koob 1988). The locomotor-activating properties of MDMA. amphetamine, and methysergide are seen in figure 6. Drug doses for amphetamine and MDMA were selected to produce similar increases in activity, although MDMA appears to have a longer duration of action (Gold et al. 1988). Once the rats were habituated to the photocell apparatus, saline injection produced only transient arousal (lasting less than 20 minutes) followed by relative inactivity (figure 6C). MDMA at 10 mg/kg produced an increase in beam interruptions that lasted for at least 2 hours (figure 6A). Methysergide (2.5, 5, 10 mg/kg) significantly potentiated the locomotor hyperactivity produced by MDMA (10 mg/kg) when compared to MDMA injection alone (figure 6A). This enhancement of MDMA's locomotor effects was evident within the first 10 minutes and lasted for the full 2-hour session. In contrast, methysergide only slightly and nonsignificantly increased the locomotor hyperactivity produced by 0.5 mg/kg of amphetamine (figure 6B). Methysergide alone at these doses had no effect on locomotor activity (figure 6C).

TABLE 1. *Effects of MDMA, MDE, and AMPH on spatial CV*

Dose (mg/kg)	CV5	CV9
0	.485 ± .02	
MDMA 1.25	.709 ± .1	
MDMA 2.5	.730 ± .17	
MDMA 5.0	1.063 ± .21*	
MDMA 10.0	.995 ± .13*	
0		1.723 ± .02
MDE 1.0		1.737 ± .05
MDE 3.0		2.209 ± .18*
MDE 10.0		2.007 ± .11
0	.522 ± .04	
AMPH 0.25	.379 ± .06*	
AMPH 0.5	.376 ± .03*	
AMPH 1.0	.373 ± .04*	
AMPH 2.0	.506 ± .03	

*$p<0.05$, Dunnett's *t*-test

NOTE: Group means ± SEM are shown: an increase in spatial CV indicates a more repetitive pattern of movements in the BPM: a decrease indicates a more highly varied pattern.

The role of the mesolimbic dopamine system, which is known to be critical for amphetamine-stimulated locomotion, was investigated in MDMA-treated rats with 6-OHDA lesions of the nucleus accumbens (Gold et al., in press). Rats received bilateral injections of 6-OHDA (8 µg/2 µl, expressed as the free base) dissolved in saline containing ascorbic acid (0.1 mg/mL; lesion group) or injections of saline-ascorbic acid vehicle alone (sham group). Approximately 9 days following surgery, rats were injected with saline, and locomotor activity was measured for 120 minutes. In one study, rats (sham: n=8, lesion: n=8) were injected on the following day with 5 mg/kg MDMA, 3 days later with 0.5 mg/kg AMPH, and again with saline. Locomotor hyperactivity produced by MDMA was attenuated in the group with 6-OHDA lesions (figure 7A). When the rats were injected with 0.5 mg/kg AMPH, the sham-operated group showed a large increase in locomotor activity; this effect was significantly reduced in the rats with lesions.

Moreover, the hyperactivity seen in the sham rats was somewhat greater than that usually observed following this dose of AMPH, suggesting a cross-sensitization between MDMA and AMPH. The day after the AMPH injections, all the rats were reinjected with saline. At this time there was no significant difference between the sham- and lesion-operated rats.

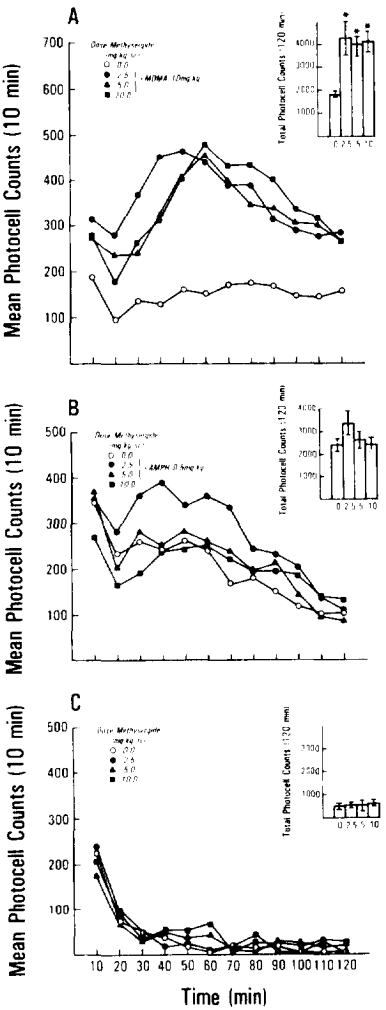

FIGURE 6. *Locomotor activity during 120-minute test session in the photocell cage apparatus*

*Significantly different from 0 methysergide dose, Newman-Keuls test following significant ANOVA main effect

KEY: Values in the upper right corner of each panel represent mean ±SEM for the total activity over the 2-hour drug test.

NOTE: Following a habituation period, rats were injected with methysergide (0-10 mg/kg, SC; C) and 2 minutes latex by (A) MDMA (10 mg/kg. SC). (B) amphetamine (0.5 mg/kg. SC).

SOURCE: Gold and Koob 1988, Copyright 1986, Pergamon Press.

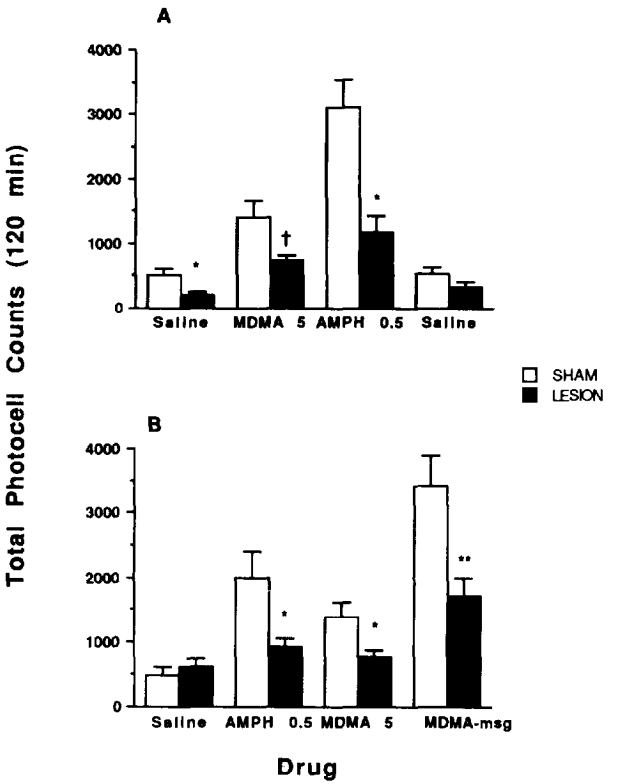

FIGURE 7. *Effects of 5 mg/kg MDMA, 0.5 mg/kg AMPH, or 5 mg/kg MDMA plus 25 mg/kg methysergide on locomotor activity in rats with 6-OHDA or sham lesions of the nucleus accumbens*

*p<0.05.

**Significant interaction.

†p.055.

KEY: Total photobeam interruptions, measured in photocell cage apparatus, for 120-minute test session, shown as group means ± SEM.

NOTE: A. The significant difference between sham-operated rats and those with 6-OHDA lesions following saline injection was attributed to a reduced response to the injection procedure in the lesion-operated group. Note that means for the two groups were almost identical (sham=576±84, lesion=524±55) for the 90-minute habituation period preceding saline injection. Sham group, n=8; lesion group, n=8. B. Sham group. n=8; lesion group, n=6.

SOURCE: Redrawn from Gold et al., in press.

In an additional study, following recovery and saline injection, rats (sham: n=8, lesion: n=8) were injected with 0.5 mg/kg AMPH on day 9 or 10 and 5 mg/kg MDMA 3 days later. On day 16 or 17 these rats received two injections: a serotonin antagonist, 2.5 mg/kg methysergide; and 5 mg/kg MDMA. Rats were injected with 0.5 mg/kg AMPH on the next day and, as in previous experiments, the locomotor hyperactivity produced by AMPH was attenuated in the group with 6-OHDA lesions (figure 7B). The mean ± SEMs per 120 minutes for the sham and lesion groups were 1,995.9±389.3 and 906±132, respectively. In the first experiment described above, the means for these groups were 3.111.9±421.8 and 1,176.5±248.1, respectively. When the rats were injected with 5 mg/kg MDMA 3 days later, the sham-operated group showed a large increase in locomotor activity; this effect was significantly reduced in the rats with lesions. The mean ± SEMs for the sham and lesion groups were 1,368±249.5 and 754.1±107.4, respectively. These values were not different from those described in the first experiment (sham: 1,401.3±257.8, lesion: 745.2±81.7). Therefore, a cross-sensitization from AMPH to MDMA was not evident. On the next day, locomotor activity was measured following injections of a serotonin antagonist and MDMA. Here, methysergide potentiated the effects of MDMA. Both the effects of surgery and the influence of methysergide were observed. However, a log transformation of the data eliminated the significant interaction between the two, which suggests that the interaction effect was due to scaling differences. Thus, the response of both the sham rats and the rats with lesions was increased by the serotonin antagonist.

Biochemical analyses of 6-OHDA-injected animals revealed a 93 percent depletion of dopamine. The tissue was assayed using electrochemical detection following separation by high-pressure liquid chromatography (Felice et al. 1978). recorded as ng/mg protein in the nucleus accumbens and compared to control rats with sham lesions (sham=65.5±4.4, lesion=4.9±1.5; t(39)=23.4). A lesion was defined as complete if 75 percent or more of the dopamine was determined to be depleted from the nucleus accumbens compared to mean sham group values.

SUMMARY

The motor activation produced by psychomotor stimulants has been long associated with the midbrain dopamine systems. While focused stereotyped behavior produced by high doses of indirect sympathomimetics is blocked by removal of dopamine terminals in the corpus striatum (Creese and Iversen 1975), the locomotor activation produced by low doses of indirect sympathomimetics is blocked by removal of dopamine terminals in the region of the nucleus accumbens (Kelly et al. 1975). This dopaminergic substrate for psychostimulant effects appears selective for the indirect sympathomimetics in that dopamine lesions to the region of the nucleus

accumbens do not block caffeine, scopolamine, heroin, or CSF-induced locomotor activation (Swerdlow and Koob 1985; Vaccarino et al. 1986).

The neurochemical sites for psychomotor stimulant reward are likely to be the presynaptic dopamine terminals located in the region of the nucleus accumbens, frontal cortex, and other forebrain structures that originate in the ventral tegmental area. Note, however, that intracranial self-administration of cocaine is elicited from the frontal cortex, but not from the nucleus accumbens (Goeders and Smith 1983). Thus, concomitant activation of structures other than the nucleus accumbens may be an important part of the circuitry involved in initiation of cocaine intravenous self-administration, as has been hypothesized for the opiates (Smith and Lane 1983; Smith et al. 1982).

In addition, these neuropharmacological studies provide evidence to show that, in the rat, the neural/neurochemical substrates for processing the reinforcing and stimulant properties of psychomotor stimulants may be similar, if not identical. Parallel manipulations using dopamine receptor antagonists and 6-OHDA lesions produce parallel results. How far this parallelism continues in further processing is under current investigation; however, such an overlap brings additional impetus to earlier hypotheses relating reinforcement and motor function (Glickman and Schiff 1967).

The motor activation produced by MDMA and MDE has similarities to classic psychostimulants, but also some important differences. In the BPM system, the stimulant-like properties of these drugs were reflected in significant increases in horizontal locomotor activity measured across a wide dose range. Interestingly, medium to high doses of MDMA or MDE produced a transient decrease in horizontal locomotion for the first 10 minutes, followed by a sustained increase. The increase in holepokes and rearings that typically accompanies the increase in ambulation seen with amphetamine itself or other indirect sympathomimetics (Geyer et al. 1986) was not observed with MDMA or MDE. Instead, initial decreases in holepokes and rearings and a tendency to avoid the center were evident, a behavioral profile that is characteristic of hallucinogenic indoleamine or phenylethylamine derivatives (Adams and Geyer 1985a; Adams and Geyer 1985b; Geyer et al. 1979).

MDMA and MDE also produced locomotor patterns that differed significantly from other stimulants. Previous studies in rats have demonstrated that amphetamine-induced hyperactivity involves complex patterns of widely distributed locomotion with frequent directional changes (Geyer et al. 1986; Geyer et al. 1987). In contrast, similar levels of behavioral activation produced by scopolamine or apomorphine are associated with relatively smooth locomotor paths, in which the same movement patterns are frequently repeated. Other stimulants, such as caffeine or nicotine, increase the amount of locomotor activity without significantly altering its pattern (Geyer

et al. 1986). With LSD and other hallucinogens, the behavioral profile is characterized by an increase in the diversity of locomotor patterns and a concomitant suppression of the exploration of novel and open areas (Adams and Geyer 1985a). When evaluated on this basis, MDMA and MDE are similar to hallucinogens in producing an avoidance of the center. This effect is particularly notable in light of the simultaneous increases in the total amount of locomotor activity. Unlike LSD, however, MDMA produced a scopolamine- or apomorphine-like increase in perseverative and thigmotactic patterns of locomotion, reflected by increases in the spatial CV measures. The MDMA profile was also similar to that of apomorphine insofar as both drugs reduced holepoking and rearing, behaviors that are increased by scopolamine. However, relative to apomorphine, the MDMA-induced rotational patterns were less strictly unidirectional, and the reductions in investigatory responses were less complete. Rather, most animals injected with MDMA changed directions and exhibited investigatory responses at least occasionally, effects similar to those observed following various doses of scopolamine (Geyer et al. 1986). Hence, the behavioral profile engendered by MDMA and MDE appears to be unique among the various drugs that have been so characterized to date.

Investigation of the neurochemical substrates for the psychostimulant effects of MDMA suggests a role for the mesolimbic dopamine system. Destruction of dopamine terminal fields in the nucleus accumbens significantly attenuated the locomotor activation produced by MDMA. A similar blockade of amphetamine-induced locomotor hyperactivity is known and was observed following amphetamine injection in these same rats. Such results support the hypothesis that at least one component of MDMA-induced hyperactivity is dopamine mediated and suggest that mesolimbic dopamine specifically is the critical substrate. In this way, MDMA resembles other classical psychostimulants like amphetamine and cocaine. Interestingly, evidence for functional cross-sensitization was suggested in the study in which an injection of amphetamine followed MDMA injection.

The stimulant properties of MDMA were enhanced by the presence of a serotonin antagonist, methysergide. Thus, following serotonin-receptor blockade, profound locomotor hyperactivity was observed. This result can be viewed as a disinhibition of the dopamine neurons from serotonin modulation. These data are consistent with the hypothesis that MDMA acts predominantly as a serotonin agonist with weak dopamine activity. In this study, methysergide did not potentiate the effect of amphetamine. However, Hollister et al. (1976) reported that methysergide potentiated locomotion produced by 2 mg/kg amphetamine intraperitoneally. In fact, an enhancement of an amphetamine response after prior exposure to MDMA has also been observed. It is possible that previous exposure to MDMA may have resulted in neurotoxic damage to some serotonin neurons. Depletions of serotonin and its metabolites have been repotted following single injections of MDMA (Mokler et al. 1987; Schmidt 1987; Stone

et al. 1986). A decrease in serotonergic tone would also result in a disinhibition of dopamine neurons and may explain the enhanced amphetamine response. Indeed, evidence for such a "functional lesion" has been reported in an operant procedure in which MDMA-induced serotonin depletion was found to potentiate its psychomotor stimulant effects (Li et al. 1986). In the case where amphetamine was given first, followed by MDMA, no change in responsiveness would have been expected.

The stimulation of locomotor activity by MDMA and the importance of mesolimbic dopamine in this response reflect similarities with the prototype phenylethylamine stimulant, amphetamine. It is important to note that these parameters are frequently associated with rewarding aspects of drugs and drug abuse. Additionally, the behavioral profiles of MDMA and MDE share certain characteristics with hallucinogen-like agents. This unique mixture of stimulus properties and neurochemical actions may contribute to a dangerous behavioral toxicity and neurotoxic potential for drugs like MDMA.

DISCUSSION

QUESTION: Can you get animals to self-administer cocaine into the nucleus accumbens?

ANSWER: No, I never tried that, but the literature there is complicated, as you know. Animals will, however, self-administer cocaine into the frontal cortex. Amphetamine is self-administered into the nucleus accumbens.

You have to know that we take out most of the mesocorticolimbic dopamine system with that lesion; we are not just taking out the nucleus accumbens dopamine projection. I am very careful to put the region of the nucleus accumbens on my slide.

I think the way Jim Smith and I have discussed this paradox is a follows: He thinks that the frontal cortex has something to do with initiation of cocaine self-administration, and I think probably the whole system may be involved in maintaining the behavior once the animals have learned it.

QUESTION: Have you tried MDMA into the nucleus accumbens?

ANSWER: No, we haven't tried self-administration of MDMA. I am not sure we would get rats to switch from cocaine to MDMA.

QUESTION: Have you had the opportunity to look at the impact of methysergide pretreatment on MDMA's effects on exploration and rearing?

ANSWER: No, we just put that on the books. We would really like to look in the behavioral pattern monitoring system. I predict that the lesion

is going to block the crossovers. But I don't know what methysergide would block. I believe it would be incredibly interesting if it would turn those rats into amphetamine-like rats, and they would explore and show less thigmotaxis and more nosepokes.

QUESTION: How can you dissociate the locomotor effects from the reinforcing effects? It has been agreed that lesions of the mesolimbic system affect locomotor activity and shown by Eberson with respect to the dopaminergic system. How do you know you don't have a rat that is motorically compromised and can't press the lever to get the cocaine? How can you dissociate that from the reinforcement efficacy?

ANSWER: I think the only thing I can really argue strongly is that we have made similar lesions in rats the lever pressed for heroin, so they are lever pressing in the exact paradigm as a reinforcer, and continue to take heroin, although the cocaine self-administration extinguishes. I have a slide of a rat who keeps plugging along on heroin self-administration and at the same time every other day is tested on cocaine. The paradigm was heroin on Monday, cocaine on Tuesday, heroin on Wednesday, cocaine on Thursday, His cocaine self-administration was completely extinguished, yet his heroin self-administration continued. This is one piece of evidence that looks like a real dissociation. The animals can lever press for another reinforcer, but they choose not to lever press for cocaine.

And the other part would be that they show locomotion with other drugs. It is just the indirect sympathomimetics where locomotion is blocked.

It could be said that the reason they are not pressing the lever for cocaine is that it doesn't do anything for them. And then you get into a circuit where it is the psychostimulant effects that produce the reinforcing effects.

COMMENT: Your data showed that, at least with that one model rat, there was a good extinction pattern and high levels of activity. I would consider that to be a better piece of evidence.

RESPONSE: Yes, it means that the animal is capable of moving around for apomorphine. But then it gets subtle. In people, too, there is initially a high level of activity to exhaust residual dopamine stores and then the activity goes down to a very low level. The apomorphine can reinstate some locomotion. I think the most convincing evidence is the heroin. It is not true motor activity.

QUESTION: Do you have any explanation for sensitization within the MDMA or the methysergide? There has been evidence of serotonergic inhibition.

ANSWER: I think there are two ways to look at it I would say one reason is MDMA doesn't look like amphetamine. Why doesn't MDMA look like amphetamine without any other drugs added on? The animal has the psychedelic repertoire, whatever that is, interfering. This is what Klaus Miczek and I were discussing. The animal perhaps has another behavioral repertoire interfering with the expression of motor activity, and that happens to be that the animal is hanging close to the side of the cage and for whatever reason, if it is LSD-like, he doesn't want to go into the center because it is frightening. He is hallucinating. I am speculating here. And if you then take away that competing behavior or competing brain Gestalt of psychedelic activity, then you are turning the drug into basically amphetamine. That is one way of looking at it.

Another way of viewing it would be as levers going off. Serotonin is inhibitory, dopamine is excitatory. That is naive, but there is evidence to suggest that in the neurochemistry of those compounds there has been a kind of yin and yang.

QUESTION: How do you explain MDMA sensitization for amphetamine?

ANSWER: There is evidence that even one exposure of MDMA at 10 mg/kg can cause some serotonin neurotoxicity. Dr. Seiden has shown in DRL procedures with repeated exposure that there is more of an amphetamine-like effect after some of the serotonin has been depleted.

QUESTION: Had you looked at that at all?

ANSWER: In terms of the biochemistry itself, no, not at all.

COMMENT: I am not sure the serotonin is inhibitory and dopamine excitatory is all that naive. There were clinical studies published where they showed that serotonin agonists could completely suppress the CNS stimulant effects of amphetamine clinically in humans. So you may be seeing the same thing. I am not sure that it has to be a psychedelic activity superimposed. It may simply be some kind of a synergistic attenuation.

COMMENT: Both Campbell and Harvey, in independent experiments, have shown if you take away the serotonin input you can exacerbate the psychomotor stimulant effects of amphetamines.

RESPONSE: Yes, and there is some recent study too that was done by Lyness showing that if the serotonin system is destroyed, toxicity and self-administration of amphetamines are increased. There is a lot of evidence that some of this interaction does occur at some level, but we don't know where yet.

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AUTHORS

Lisa H. Gold, B.S.
Predoctoral Fellow
Division of Preclinical Neuroscience and Endocrinology
Department of Basic and Clinical Research
Research Institute of Scripps Clinic
La Jolla, CA 92037

Mark A. Geyer, Ph.D.
Associate Professor of Psychiatry
Department of Psychiatry, T-004
University of California, San Diego
La Jolla, CA 92093

George F. Koob, Ph.D.
Associate Member
Division of Preclinical Neuroscience and Endocrinology
Department of Basic and Clinical Research
Research Institute of Scripps Clinic
La Jolla, CA 92037

Neuronal Actions of Amphetamine in the Rat Brain

Philip M. Groves, Lawrence J. Ryan, Marco Diana, Stephen J. Young, and Lisa J. Fisher

INTRODUCTION

Amphetamine and related designer drugs have widespread actions on neuronal activity in the brain. This is believed to be due in part to the enhanced release and blockade of reuptake of catecholamines (Kuczenski 1983). The sites of action of such stimulant drugs of abuse include presynaptic neurons, by virtue of the action of released catecholamines on autoreceptors (Tepper et al. 1985), and postsynaptic targets of catecholamine axons, including neurons in the cerebral cortex, basal ganglia, cerebellum, reticular formation, and other neuron systems of the brainstem (Groves and Rebec 1976).

The consequences of amphetamine administration include widespread neuronal pathology in the brains of experimental animals (Groves et al. 1987; Seiden and Kleven, this volume; Gibb et al., this volume) and significant changes in the pattern and intensity of neuronal activity throughout the brain. One particularly useful approach to understanding the sites and mechanisms of action underlying the behavioral effects of amphetamine has been to record the electrophysiological consequences of amphetamines administration in the rat brain (Groves and Rebec 1976; Groves and Tepper 1983).

EFFECTS OF AMPHETAMINE ON CATECHOLAMINERGIC NEURONS

One of the most widely known electrophysiological actions of amphetamine on the brain is to decrease the firing rate of dopaminergic and noradrenergic neurons recorded in vivo from the rat brain (Bunney et al. 1973; Graham and Aghajanian 1971). The underlying mechanisms include possible inhibition by afferent systems and by local inhibitory mechanisms involving local release of catecholamine (Groves et al. 1975). In the case of dopaminergic neurons, this release occurs from dendrites, whereas, in noradrenergic nuclei, release occurs from axonal collateral innervation as well as from dendrites (Groves et al. 1979). The effect of amphetamine on monoamine neurons is

dose dependent. Noradrenergic neurons of the locus coeruleus are most sensitive, with a mean dose for 50 percent inhibition of firing of 0.25 mg/kg, IV (Engberg and Svenson 1979; Ryan et al. 1985). Dopamine neurons are less sensitive, requiring a mean dose of approximately 1.6 mg/kg, IV (Bunney et al. 1973). Serotonin neurons are the least sensitive, requiring a mean dose for 50 percent inhibition of 3.0 mg/kg, IV (Rebec et al. 1982).

The inhibition of firing of catecholamine neurons resulting from amphetamine administration is likely due to activation of somatodendritic autoreceptors. This causes a hyperpolarization of the somatodendritic membrane of both locus coeruleus noradrenergic and substantia nigra dopamine neurons, probably as a consequence of an increase in potassium conductance (Lacey et al. 1987; Williams et al. 1985).

Because the cell body is hyperpolarized by autoreceptor stimulation, it seemed plausible that stimulation of autoreceptors located on the synaptic endings of such neurons might similarly lead to a decrease in excitability. Over the past several years, we have determined the excitability of the axonal synaptic endings of single monoaminergic neurons in vivo by electrical stimulation of the axon while recording the antidromic responding of the neuron at the level of the cell body. It is now apparent that, in addition to causing a decrease in the amount of transmitter released by each action potential (Langer 1977), stimulation of autoreceptors located at the terminal field results in a decrease in terminal excitability in all monoaminergic neuron systems that have been tested (Tepper et al. 1985).

The effects of amphetamine on catecholamine terminal excitability are very similar to the effects of direct-acting agonists. Nigrostriatal dopamine neurons, for example, show a decrease in terminal excitability following the systemic administration of doses of amphetamine ranging from 0.25 to 5.0 mg/kg, IV (Groves et al. 1981). Direct infusions of amphetamine into the terminal fields also decrease excitability. A similar decrease in excitability is seen after the administration of the direct-acting D_2 agonist apomorphine (Tepper et al. 1984) and the D_1 agonist SKF 38393 (unpublished data). The action of amphetamine may be blocked by dopamine antagonists, including haloperidol, fluphenazine, and sulpiride, as well as by pretreatment with the dopamine synthesis blocker α-methylparatyrosine (Tepper et al. 1984). These actions of amphetamine occur only in regions of the dopamine axon containing presynaptic autoreceptors; infusions of amphetamine into the medial forebrain bundle were without effect, whether excitability was tested by stimulation at the terminal field or along the axon in the medial forebrain bundle.

The activation of presynaptic autoreceptors, as revealed by changes in terminal excitability, suggests that amphetamine releases dopamine at every tested dose. This observation is consistent with recent direct demonstrations using

dialysis, which show that amphetamine induces dopamine release in the neostriatum in a dose-dependent manner (Hemandez et al. 1987; Impcrato and Di Chiara 1984). Amphetamine-induced release is opposed, to some extent, by the action on autoreceptors of released dopamine. Activation of somatodendritic autoreceptors decreases dopamine neuron firing, which, in combination with dopamine terminal autoreceptor activation, will decrease impulse-dependent release. Thus, the net effect of amphetamine on catecholamine release will be a compromise between release-inducing actions and release-diminishing actions.

Indeed, at some doses, amphetamine may actually reduce norepinephrine release from terminals of locus coeruleus neurons, Amphetamine is a less potent releaser of norepinephrine than it is of dopamine, and norepinephrine release is provoked only at high doses (Kuczenski 1983). Since locus coeruleus neurons are very sensitive to inhibition of neuronal firing, the net effect of amphetamine at low doses may be to reduce impulse-dependent release enough that amphetamine overwhelms the amphetamine-induced nonimpulse-dependent release. Huang and Maas (1981). for instance, observed a biphasic dose effect of amphetamine on hippocampal neuron firing, which they interpreted as indicating that amphetamine reduced norepinephrine release at low doses. A biphasic dose effect on amphetamine has been observed on the terminal excitability of locus coeruleus axons in frontal cortex, which we also interpreted in this manner (Ryan et al. 1985). Thus, amphetamine may have quite different dose-dependent effects on noradrenergic and dopaminergic neurons.

EFFECTS OF AMPHETAMINE ON NEOSTRIATAL NEURONS IN BEHAVING RATS

Amphetamine can alter neostriatal unit activity directly by enhancing the release of dopamine from terminals of the midbrain dopamine projections of the substantia nigra and, at higher doses, by increasing serotonin release in the neostriatum. It may also indirectly alter neostriatal activity by changing activity in systems that project into the neostriatum, including the neocortex. thalamus, and amygdala. The net effect of amphetamine on neostriatal activity will be determined by the relative magnitudes of these various influences.

In anesthetized rats, amphetamine causes dose-dependent changes in neostriatal unit activity. Spontaneously active neostriatal cells are uniformly inhibited at low (<2.0 mg/kg, IP) doses. At intermediate doses, an initial excitation precedes the inhibition, and, at high doses (>5.0 mg/kg, IP), the predominant effect is excitation (Groves and Rebec 1976). Regional differences in the direction, magnitude, and duration of the response of neurons in the neostriatum exist (Rebec and Curtis 1983).

In contrast. initial studies of neostriatal unit activity in unanesthetized, behaving animals suggested that neostriatal units were excited by even small doses of amphetamine (Hansen and McKenzie 1979; Rebec and Gardiner 1985; Trulson and Jacobs 1979; Warenycia and McKenzie 1984a; Warenycia and McKenzie 19848; Warenycia and McKenzie 1984c; Warenycia et al. 1984; West et al. 1985). and that the firing rate did not change with the transition between amphetamine-induced locomotion and stereotypies (Hansen and McKenzie 1979). These studies, though. did not report the behavioral correlates of the unit activity recorded in the predrug period. Since the firing of neostriatal neurons can vary widely with different behaviors, the observed changes in neuronal firing following amphetamine could reflect solely the change in behavior produced by amphetamine. Furthermore, in these studies, primarily spontaneously active neurons were recorded from, either as single- or multiple-unit responses. Since the majority of neostriatal neurons are very slowly firing, rapidly firing neurons were undoubtedly overrepresented in these studies.

In our studies, we examined how amphetamine altered the firing of identified neostriatal projection neurons during specific pre- and postdrug behaviors. Neurons were identified as projection neurons by antidromic activation from the substantia nigra, using criteria that we previously established (Ryan et al. 1986b). Of 41 antidromically identified neostriatal cells, only 1 fired faster than 1 Hz during any of the four behaviors that we analyzed, namely locomotion: face washing; quiet, nonmoving waking; and sleep. The median firing rate during locomotion was 0.02 Hz (Ryan et al., in press). An additional group of 24 nonantidromically activated neurons was also studied. Most of these neurons also fired infrequently; the median rate during locomotion was also 0.02 Hz. Indeed, with the exception of two cells that fired over 6 Hz, the nonantidromically activated cells resembled the antidromic cells in all respects. Many antidromic and nonantidromic neurons showed tenfold or greater changes in rate across the four different behaviors.

The effect of amphetamine on these neostriatal neurons was relatively uniform. Four doses of amphetamine were tested: 0.25, 1.0, 2.5, and 5.0 mg/kg, SC. At all four doses, amphetamine reduced the firing rate of both antidromic and nonantidromic neurons during the initial drug-induced period of locomotion as compared to the rate during predrug locomotion (figure 1).

At the higher doses, several stages of stereotyped behaviors were seen. The transition from amphetamine-induced locomotion to locomotion associated with stereotyped side-to-side head movements was accompanied by a further reduction in firing rate. In those animals in which focused stereotypy was observed following this period of locomotion plus head movements, neurons showed a still further reduction in firing rate (figure 2).

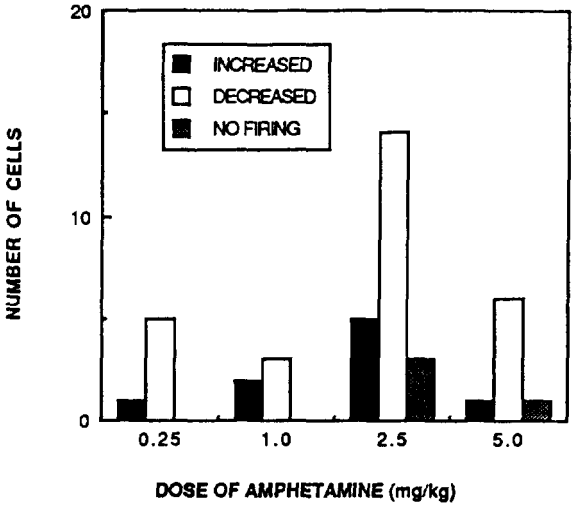

FIGURE 1. *Change in firing rate of both antidromically and nonantidromically activated neostriatal neurons following amphetamine administration in the freely moving, behaving rat*

NOTE: Firing rate during predrug locomotion compared to firing rate during initial drug-induced locomotion shows the majority of neurons inhibited by amphetamine. Some cells fired no action potentials either pre- or postdrug.

These effects were seen for both antidromic and nonantidromic neurons. However, of the three most rapidly firing neurons, two showed an acceleration in firing rather than a reduction, much as has been previously reported for spontaneously active neurons (Hansen and McKenzie 1979; Rebec and Gardiner 1985; Trulson and Jacobs 1979; Warenycia and McKenzie 1984a; Warenycia and McKenzie 1984b Warenycia and McKenzie 1984c; Warenycia et al. 1984; West et al. 1985). Thus, amphetamine may induce a divergence in firing rate, exciting rapidly firing neurons and inhibiting slowly firing neurons.

Both the degree and pattern of neostriatal activity are altered by amphetamine. Since identified striatonigral projection neurons are inhibited by amphetamine, the inhibitory control of the substantia nigra pars reticulata may be decreased. The rapid tonic firing of these neurons may be enhanced, ultimately resulting in increased inhibition of the targets of the pars reticulata, namely the ventromedial thalamus, the superior colliculus, and the pendunculopontine (PPN) nucleus. Thus, amphetamine may cause the inhibition of these structures, thereby locking in a particular behavioral pattern.

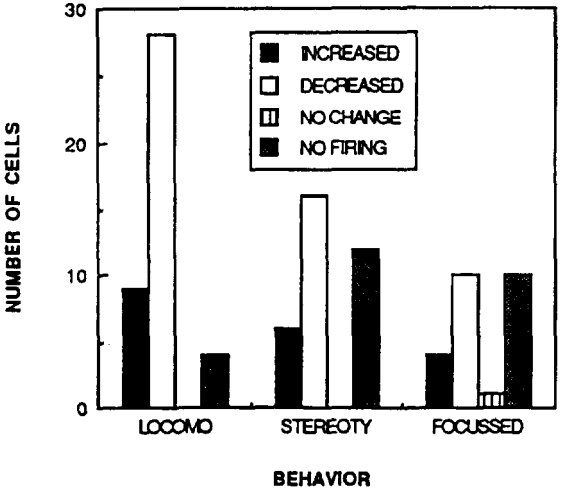

FIGURE 2. *Change in firing rate of activated neostriatal neurons, data combined across all doses*

NOTE: Amphetamine-induced inhibition of neostriatal unit firing during postdrug compared to predrug locomotion may be clearly observed. As locomotion gives way to locomotion with stereotyped side-to-side head movements. there is a further decline in firing rate. When locomotion with head movements was followed by focused stereotypies. there was a still further decrease in firing rate.

EFFECTS OF AMPHETAMINE ON TERMINAL EXCITABILITY OF STRIATONIGRAL PROJECTION NEURONS IN BEHAVING RATS

Amphetamine may exert its effects not only by altering the firing rate of neurons, but also by altering the coupling between action potentials and neurotransmitter release, by acting on presynaptic terminal receptors. Dopamine receptor activation has been shown to increase GABA release in the substantia nigra (Reubi et al. 1977; Starr 1987). It is possible that this modulation of GABA release occurs by dopamine acting on D_1 receptors known to reside on the presynaptic terminals of striatonigral projection neurons (Altar and Hauser 1987; Aiso et al. 1987). If so, amphetamine may activate these receptors by inducing the local release of dopamine within the substantia nigra (Groves et al. 1975), and this change may be detected by measuring the electrical excitability of the axon terminal (Groves et al. 1981). We have recently shown that, in urethane-anesthetized rats, local infusions of the specific D_1 agonist SKF 38393 (10 μM) into the substantia nigra decrease the electrical excitability of suiatonigral neuron terminals (unpublished data). In contrast, amphetamine, at doses ranging from 0.5 to 5.0 mg/kg, SC, did not alter terminal excitability in either unanesthetized, freely moving rats (Ryan et al., in press) or in

urethane-anesthetized rats (unpublished data). Thus, it seems unlikely that amphetamine alters GABA release in the substantia nigra by acting at presynaptic D_1 receptors on striatonigral terminals.

EFFECTS OF AMPHETAMINE ON SUBSTANTIA NIGRA PARS RETICULATA NEURONS IN BEHAVING RATS

The substantia nigra pars reticulata represents one of the major targets of the neostriatum. This projection has been demonstrated anatomically (Grofova 1979) and has been shown electrophysiologically to inhibit the firing of its target neurons (Deniau et al. 1976). Thus, since amphetamine inhibits the firing of neostriatal neurons, it is plausible that peripheral administration of amphetamine could alter the tonic activity of substantia nigra pars reticulata cells across behavioral states. It is, therefore, of some interest to study the effects of amphetamine on the activity of pars reticulata neurons.

In anesthetized animals, the iontophoretic application of dopamine increases the firing of pars reticulata neurons (Matthews and German 1986; Ruffieux and Schultz 1980; Waszczak and Walters 1983), and dopamine attenuates the inhibitory effects of GABA, which is the transmitter used by some of the striatonigral projection. Little is known, though, about how amphetamine changes activity in this structure in freely moving animals. One recent study by Olds (1988) suggested that the activity of nondopamine neurons of the substantia nigra increases after amphetamine administration. However, the behavioral mix of the 90-minute predrug period was uncontrolled; since, as we have observed, the firing rate of these neurons varies widely with behavior, it is unclear whether this action reflects the effects of amphetamine on these neurons or the behavioral activation induced by amphetamine. We have recently begun a series of experiments to elucidate the relationship between firing rate of pars reticulata neurons and specific behaviors and to demonstrate how amphetamine alters these correlations. Preliminary data suggest that the firing rate of pars reticulata neurons during several behaviors is increased by amphetamine, compared with the same preamphetamine behavior. The activity of a single pars reticulata neuron during pre- and postamphetamine locomotion and face washing is shown in figure 3.

In this cell, the tonic firing of the neuron during this behavior is increased by amphetamine. This result is consistent with our finding that neostriatal units projecting to the pars reticulata are inhibited by amphetarninc. The resulting disinhibition of these nigral units may, in turn, increase their tonic inhibitory control over their target structures, such as the deep layers of the superior colliculis (Chevalier et al. 1985). the thalamus (Deniau and Chevalier 1985) and the PPN. These output structures are known to affect the motor behaviors that amphetamine influences (Di Chiara et al. 1979).

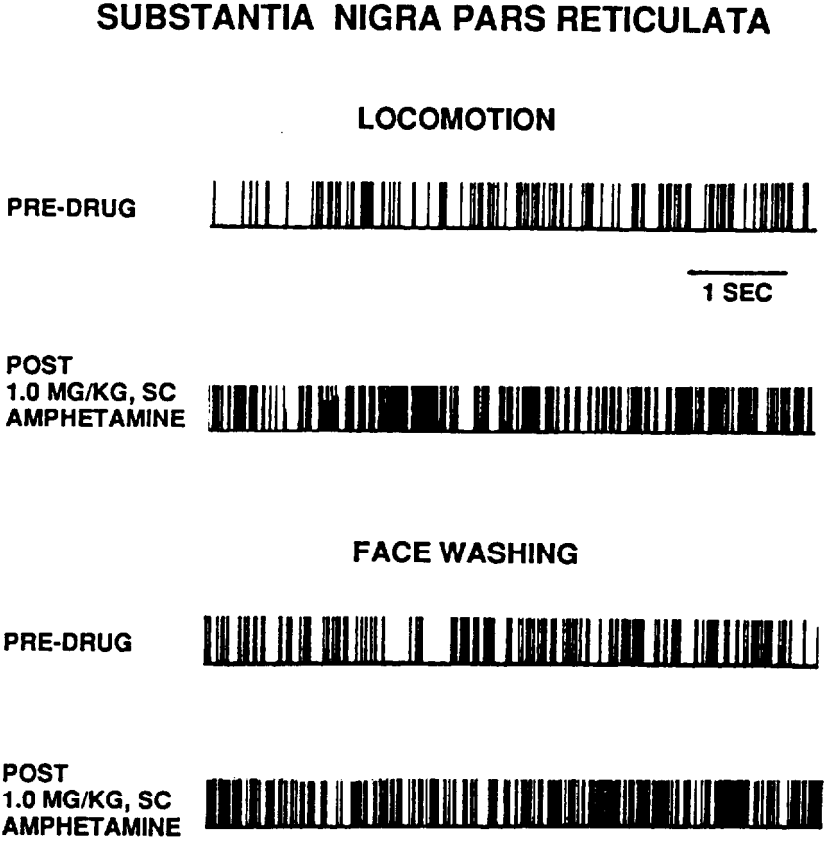

FIGURE 3. *Amphetamine increases the firing rate of a substantia nigra pars reticulata neuron in the chronically implanted, behaving rat*

NOTE: Action potentials are represented as a pulse output from a spike-height discriminator: Each vertical line represents one action potential. The firing rate of this neuron during similar pre- and postdrug behaviors is increased by injection of 1.0 mg/kg, SC, amphetamine. This increase occurs for both locomotion (from a mean of 28.3 to 39.0 spikes/sec) and face washing (from a mean of 43.7 to 46.5 spikes/sec).

EXTRASTRIATAL EFFECTS OF AMPHETAMINE ON THE CORTICALLY EVOKED STRIATAL RESPONSE IN ANESTHETIZED RATS

The rat neostriatum receives massive input from the cerebral cortex and thalamus (Chung 1979; Kemp and Powell 1971; Somogyi et al. 1981).

Neostriatal processing may not be solely influenced by effects of amphetamine on intrastriatal dopamine systems but may also be influenced by actions within these other major afferent systems. Thus, amphetamine, which produces, among other effects, increased firing of mesencephalic reticular neurons (Boakes et al. 1971), depression of response in locus coeruleus (Graham and Aghajanian 1971). and cortical desynchronization (Arushanian and Belozertsev 1978) may have marked effects on activity within the neostriatum. As an approach to understanding the contribution of these extrastriatal actions on striatal functioning, the effect of amphetamine on cortically evoked intracellular events and field potentials in the neostriatum is being studied.

Electrical stimulation of the neocortex evokes a regular sequence of intracellular and extracellular potentials in the neostriatum (Liles 1973; Hull et al. 1973). In intracellular striatal recordings, single-pulse stimulation of cortical afferents elicits an initial depolarizing postsynaptic potential (DPSP), which is followed by a long-lasting afterhyperpolarization and a rebound depolarization (figure 4).

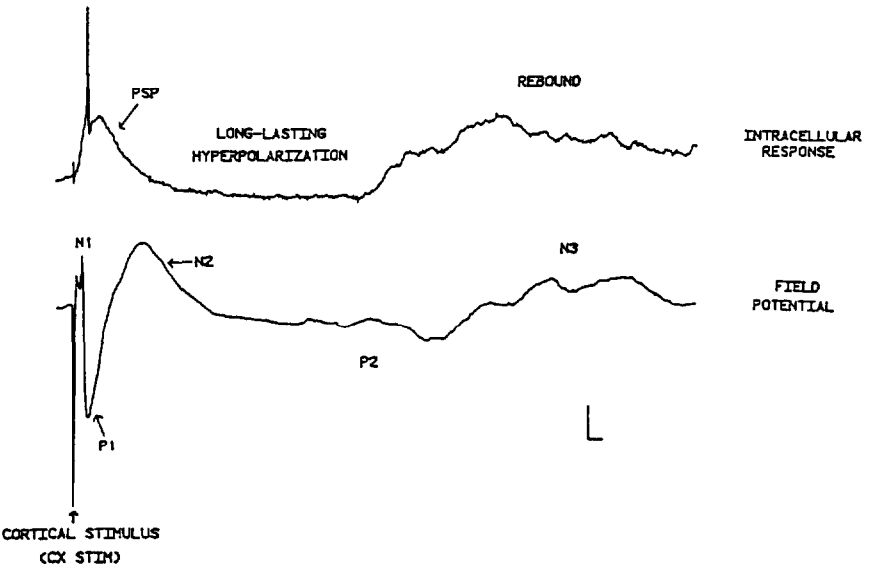

FIGURE 4. *Illustration of the correspondence between components of the cortically evoked neostriatal intracellular (top, positive up) and field potential (bottom, negative up) response*

NOTE: Time calibration: 5 milliseconds for both traces. Intracellular amplitude. calibration: 10 mV.

A correspondence between these intracellular events and components of the cortically evoked neostriatal field potential (Ryan et al. 1986a) have recently been demonstrated. As shown in figure 4, the initial extracellular positive wave Pl is associated with the intracellular DPSP. The negative wave N2, which might reflect intrastriatal collateral inhibition, occurs during the initial period of intracellular hyperpolarization. The later period of hyperpolarization, which has been attributed to a loss of tonic excitation from cortical and thalamic inputs (Wilson et al. 1983). is seen to overlap with the second extracellular positive wave P2. A late negative wave, N3, occurs in phase with the rebound depolarization. These correspondences between intracellular and extracellular events encouraged employment of the cortically evoked field potential as an index of striatal population response to the effects of amphetamine. Systemic amphetamine (0.5 to 5.0 mg/kg) has been found to reduce the amplitude of Pl and dramatically decreases the latency to N3. Interestingly, these effects appear to be due, at least in part, to the action of amphetamine at extrastriatal sites, since they could be mimicked by high-frequency, low-current stimulation of thalamic afferents in the mesencephalic reticular formation (Ryan et al. 1987a). Further, these changes in the cortically evoked neostriatal field potential following either systemic amphetamine or high-frequency reticular stimulation were abolished by kainic acid lesion of the medial thalamus. The pharmacological characterization of this response supports the extrastriatal origin of these effects of amphetamine. Dopaminergic antagonists, such as haloperidol and fluphenazine, do not block or reverse the effects of amphetamine on wave Pl or N3. In contrast, amphetamine's actions are potentiated by the α-2 noradrenergic autoreceptor antagonist yohimbine and are reversed by the beta antagonists propranolol and metoprolol (Ryan et al. 1987b). In addition, the latency to a positive wave recorded in the region of somatosensory cortex overlying the neostriatum and temporally coincident with the neostriatal wave N3 is reduced by amphetamine by the same amount as is wave N3. These temporally similar actions in neostriatum and neocortex also indicate an extrastriatal site of action for amphetamine. To characterize further the extrastriatal effects of amphetamine, cortically evoked neostriatal field potentials and intracellular responses have been examined after either local application of amphetamine or high-frequency stimulation of the reticular formation (RF) (Fisher et al. 1987). Brief, low-intensity (0.1 mA) 60 Hz stimulation of the PPN produces a depolarization of cell-resting-membrane potential and a resulting decrease of the DPSP. This depolarization is reflected in a parallel reduction of the Pl wave in the evoked field potential. In addition, a decrease in the latency of the rebound depolarization is observed following RF stimulation, corresponding to a shift in N3 in the extracellular response. Notably, a local infusion of amphetamine into the PPN (10^{-6}M, total volume 0.2 uL over 4 minutes) produces alterations in the intracellular and extracellular evoked responses, similar to those observed with RF stimulation (figure 5) and systemic amphetamine administration.

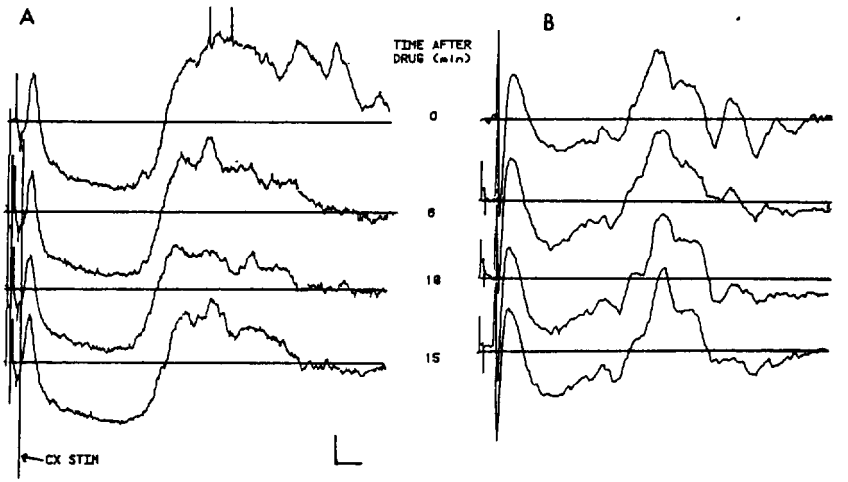

FIGURE 5. *Cortically evoked intracellular (A, positive up) and field potential (B, negative up) response in the neostriatum at increasing times following local infusion of amphetamine (10^{-6}M) into the pedunculopontine reticular nucleus*

NOTE: Following amphetamine, the intracellular postsynaptic potential (PSP) is reduced as is P1 in the field potential. A reduction in the latency to and a modification in components of the rebound potential can also be observed. Time calibration: 50 milliseconds. Intracellular amplitude: 5 mV.

Activation of the RF presumably alters neostriatal functioning via its effects on thalamocortical pathways. These alterations may affect striatal excitability and timing. Results suggest that important alterations in striatal functioning can result from extrastriatal actions of amphetamine.

CONCLUSION

The actions of amphetamine are widespread throughout the brain. Amphetamine's immediate effect is to alter the release of monoamines in a dose-dependent manner that is specific for each monoamine transmitter neuronal system. The net effect of amphetamine on monoamine release is complex, with some mechanisms tending to increase monoamine release (e.g., blockade of reuptake and nonimpulsedependent release), and several mechanisms tending to diminish release (e.g., activation of somatodendritic and terminal autoreceptors).

It is important to consider that the behavioral outcome of amphetamine-induced alterations in monoamine release is determined by changes induced in postsynaptic targets of monoamine neurons. The consequences of

amphetamine on the activity of these target cells reflects both direct actions of monoamines and changes in the pattern and intensity of interactions of afferents and intrinsic neurons. In the neostriatum, for instance, amphetamine affects neostriatal cells directly by altering the release of dopamine and serotonin, and indirectly by changing activity patterns in cortical, thalamic, and amygdalar afferents. One of the greatest challenges facing neuropharmacologists is to dissect these multitudinous influences to understand how amphetamine and related designer drugs produce their important behavioral consequences.

DISCUSSION

QUESTION: Have you looked at the globus pallidus yet?

ANSWER: We have looked at the globus pallidus in anesthetized animals, which appears to be uniformly increased by amphetamine administration. I might mention that Jean Walters has also looked at the globus pallidus in the behaving animals, and it is routinely increased by amphetamine administration.

QUESTION: Do you think that there is a similar feedback regulation system of the reticulata part for the nigra?

ANSWER: I think that is controversial right now. Yes, I think there is a projection system that runs from the pars reticulata to pars compacta, but the consequences of activation of that system and how you would get access to it are not well understood at this time.

QUESTION: Is there a parallel to the reticulata component of the striatum, the dorsal striatum in the ventral tegmental area?

ANSWER: I wish I knew that. It certainly seems so, and we are trying to record nucleus accumbens at this time. It seems that nucleus accumbens is similar to neostriatum, but ventral tegmental area has a much greater heterogeneity of nerve cells than does substantia nigra. It may be that the nondopaminergic neurons of the ventral tegmental area are just sprinkled through and that, of course, is the neurophysiologist's nightmare because you don't know where electrodes pick up, although we think there are neurophysiological criteria. Ultimately, that question will be amenable to analysis. I wish I could answer it now.

QUESTION: Are the effects of amphetamine in the anesthetized preparation confounded by the anesthetic agent?

ANSWER: I am not entirely sure, but people in my lab believe that the effects are related to two different populations of nerve cells, that those excited by amphetamine administration represent a different population, and

there are only five or seven or so different types of nerve cells in the neostriatum, with 95 percent of them in one morphological class.

But there are those in my lab who believe that the excitation is being seen by a bias toward large cells and that they represent a large cell population in the neostriatum. I don't necessarily believe that. I don't know why, in the anesthetized animal, you can flip a nerve cell that is inhibited by amphetamine by increasing the dose. It has been postulated that the excitation is related to the occurrence of both the stereotyped behaviors, and that this may be provoked at doses that produce neurotoxicity. We have also done a number of studies looking at the neurotoxicity of amphetamine administration in animals, most of which replicate Lou Seiden's work.

QUESTION: Do you find a population of autoreceptors at the axonal endings using your techniques?

ANSWER: I think it is very clear that there is a population of autoreceptors at the axonal end, but there is no population of autoreceptors in the axon that passes through the medial forebrain level. The antidromic stimulation is up there, and it looks as if amphetamine and related dopamine agonists cause a decrease in excitability of the terminal field in the same way that they cause a decrease in excitability in the cell body. And we believe that this fact, only recently shown by Allan North's group, is that they cause an increase in potassium conductance and as a consequence hyperpolarization of the cell body.

QUESTION: Does dopamine do it?

ANSWER: Yes.

QUESTION: Whose paper is that?

ANSWER: That is Lacey et al., Allan North's group. It was published in the *Journal of Physiology* last year. It was also an abstract in the Society 2 years ago. It is the consequence of that application of the agonists, recording intracellularly in the slice of the dopamine neuron. He gets the same thing by virtue of application of norepinephrine agonists to noradrenegic slice preparation. That is a conventional way to create a hyperpolarization of the cell, to increase the potassium conductance, and so forth. This is presumably the way that much of the polarization of the cells occurs.

QUESTION: Do you think it is necessary to have an intact cell to have this hyperpolarization?

ANSWER: I believe very strongly that you need an intact system. Now there are certain questions that you can answer in a slice. You deprive the

cells of all their normal afferents and in many cases you cut their axons. You cut the axon of the very cell you are recording, but you are unaware that has occurred. But there are many different traumatic events that take place when you extract the slice. The behavior of the cells is quite abnormal if you look at it carefully.

QUESTION: If you slice the medial forebrain bundle, is there a change in the resistance at the terminal area? Which way does it go?

ANSWER: I don't know. There is a change at the cell body; the resistance goes way up, and it will set itself into a repetitive firing mode, which is quite an abnormal looking mode of firing, but the dopamine neuron or the norepinephrine neuron or virtually any neuron in slice will go into this bizarre repetitive mode of firing.

Still, activation of autoreceptors causes an inhibition of the activity. So, there are certain qualitative similarities that lead us to believe that the slice is a useful preparation for entering certain types of neuropharmacologic questions. But as far as answering how it is that the brain works, I don't think the slice is going to solve it for us.

COMMENT: You were showing your reticulata cell showing an increased activation. Some people think that they may have this feedback mechanism. Several years ago Koob and others were postulating that this was indeed part of an outflow system. And if you put GABA or GABAergic drugs into that outflow system, that you could also produce behaviors.

RESPONSE: Yes.

QUESTION: Could it be either/or?

ANSWER: Well, I don't think it is either/or.

QUESTION: Do you think it is a feedback system?

ANSWER: I don't think it is a feedback system per se. I think the feedback system goes a long way. I think Steve Bunney and George Aghajanian believe that it is a feedback system and that it routes itself from the neostriatum through the pars reticulata and that the pars reticulata then causes the inhibition of firing in the dopamine system. Now, as far as feedback is concerned, I think it is much more likely. for example, that the globus pallidus causes an inhibition of firing of the dopamine neuron when amphetamine is injected because amphetamine causes this dramatic increase in firing of globus pallidus, and we know anatomically that the globus thalamus projections end up in substantia nigra pars compacta. So it is much more likely that the globus pallidus is

influencing the activity of the dopamine neuron by virtue of an afferent system.

And there are others who believe that dopamine is indirectly or directly influencing pars reticulata by virtue of being released from dendrites and exciting the pars reticulata neuron, so that is another theoretical approach that has not, in my opinion, been adequately tested.

QUESTION: Do you find autoreceptors in mesolimbic structures?

ANSWER: Yes, we do. As far as our evidence goes, we have tested many hundreds of mesolimbic neurons, and there is a theory that a certain group of mesolimbic dopamine, cortically projecting neurons lack auto-receptors. We have studied the cortically projecting tegmental area of dopamine neurons ad nauseam, and in our hands they look exactly the same as the substantia nigra pars compacta. They have autoreceptors and they are influenced by low doses of amphetamine, which we know is used in the ventral tegmental area, operated by virtue of autoreceptor activation. They are influenced by low doses; both the excitability at the terminal and the excitability of the cell body are inlluenced by autoreceptor activation through the tegmental area in the cortically projecting cells. So our evidence does not agree with that point of view.

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AUTHORS

Philip M. Groves, Ph.D.
Lawrence J. Ryan, Ph.D.
Marco Diana, M.D.
Stephen J. Young, Ph.D.
Lisa J. Fisher, Ph.D.

Department of Psychiatry
University of California, San Diego
La Jolla, CA 92093

Methamphetamine and Related Drugs: Toxicity and Resulting Behavioral Changes in Response to Pharmacological Probes

Lewis S. Seiden and Mark S. Kleven

INTRODUCTION

Some substituted phenethylamines are toxic to certain neurons in the brain. In view of this neurotoxicity, we will review some data relevant to this process. First, we will review data showing that methamphetamine (METH), a prototypic psychomotor stimulant, which has been widely used for nonmedical purposes at doses often a good deal higher than therapeutic doses, is neurotoxic to dopamine (DA) and serotonin (5-hydroxytryptamine (5-HT)) systems. Second, we will examine the evidence that other substituted phenethylamines are also neurotoxic to certain transmitter systems. Last, we will examine the behavioral and pharmacological consequences of neurotoxicity that result from exposure to some of these amphetamine-related drugs.

Phenethylamines can be ring- and/or side chain-substituted, and many of these derivatives show potent pharmacological effects (Weiner 1985). Of phenethylamines without ring substitutions, pharmacologically active compounds tend to be mainly psychomotor stimulants, possessing sympathomimetic, antifatigue, and reinforcing effects in humans using the drugs. The antifatigue and reinforcing properties are likely to be responsible for their abuse potential. In very high doses, such as those used by human amphetamine abusers, the amphetamine-type drugs can lead to a psychotic state, which has paranoid delusional symptoms that are very often indistinguishable from an acute psychotic episode seen in patients with schizophrenia (Jonsson and Gunne 1970). Outbreaks of METH epidemics have occurred in several countries including the USA, Sweden. and Japan (Kramer et al. 1967; Inghe 1969; Brill and Hirose 1969).

The prototype amphetamine enhances release and blocks reuptake of DA, norepinephrine (NE), and 5-HT and is also a monoamine oxidase inhibitor. As a result of these effects, drugs in this class are potent indirect agonists at monoaminergic receptors. In experimental animals, amphetamine

stimulates locomotor activity at low doses and causes stereotypic activity at higher doses; amphetamine also interferes with food and water consumption. These behavioral effects are related to amphetamine's actions on DA, NE, and 5-HT systems (Lewander 1977; Moore 1978).

Amphetamine-related drugs such as 3,4-methylenedioxyamphetamine (MDA) or 3,4-methylenedioxymethamphetamine (MDMA), with substitutions on the benzene ring, tend to show hallucinogenic activity at lower doses and psychomotor stimulation at higher doses (Climko et al. 1986). In contrast to the behavioral effects of ring-substituted amphetamines, side-chain analogs show psychomotor stimulation at low doses and hallucinogenic activity at higher doses. Their abuse potential raises the question of whether amphetamine-like drugs may have long-lasting and/or toxic effects on the central nervous system (CNS). The data show that some of these ring-substituted compounds have toxic effects on the DA and 5-HT systems, whereas others seem to be toxic mainly to the 5-HT system. Also of interest are some structure-activity relationships, the toxicity to DA and/or 5-HT fibers, and a possible mechanism by which these drugs are toxic. Data from behavioral tests using pharmacological probes show that these neurotransmitter systems are compromised.

NEUROTOXIC EFFECTS OF AMPHETAMINE-RELATED DRUGS

Although the symptoms produced in humans suggested that the amphetamine type of drugs may engender a neurotoxic response in the CNS, there were no signs of brain pathology until the early 1970s. Koda and Gibb (1973) reported that 18 hours after a dosing regimen in which METH was injected every 5 hours, the V_{max} for tyrosine hydroxylase was reduced. Seiden et al. (1975-76) reported that, in monkeys administered amphetamine for several months and sacrificed 3 to 6 months after the last injection, the levels of DA in the brain were reduced. This long-term reduction in brain DA suggested but did not prove that METH was toxic to DA cells.

Neurotoxicity of METH was shown to occur in rats by virtue of the facts that: (1) levels of DA and activity of the enzyme that is rate limiting for DA synthesis were decreased for a long period after cessation of drug treatment (Ricaurte et al. 1980; Ricaurte et al. 1982; Hotchkiss et al. 1979); (2) the number of reuptake sites for DA were reduced (Ricaurte et al. 1980; Ricaurte et al. 1982); and (3) there was shown to be neuronal degeneration in DA-rich areas of the brain (Ricaurte et al. 1982; Ricaurte et al, 1984). It is important to stress that these three criteria must be met before neurotoxicity can be established. Similar effects upon 5-HT levels, reuptake sites, and morphology must also be observed before it can be concluded that 5-HT neurotoxicity has occurred. In this regard, multiple doses of METH have been shown to produce long-lasting reductions in tryptophan hydroxylase activity (Hotchkiss et al. 1979) as well as 5-HT content and uptake sites (Ricaurte et al. 1980) in the rat brain.

The criteria for neurotoxicity have been met for the hallucinogenic amphetamines MDA and MDMA (table 1). Levels of 5-HT were depleted as long as 8 weeks following a repeated administration of either MDA or MDMA (Ricaurte et al. 1985; Schmidt et al. 1986; Schmidt 1987a; Stone et al. 1987a; Stone et al. 1987b). Additionally, both MDA and MDMA reduced numbers of 5-HT uptake sites (Ricaurte et al. 1985; Commins et al. 1987; Schmidt 1987a), depressed tryptophan hydroxylase activity (Stone et al. 1986; Schmidt and Taylor 1987) and produced signs of neuronal degeneration (Ricaurte et al. 1985; Commins et al. 1987). It is clear from these data that neurotoxicity is directed primarily toward the 5-HT system. Much higher doses are required to produce significant reductions in the levels of DA or its metabolites.

TABLE 1. *Effects of amphetamine-related compounds on monoaminergic neurons*

Drug	Levels DA	Levels NE	Levels 5-HT	Uptake Sites DA	Uptake Sites 5-HT	Fink-Heimer
Amphetamine	⇓	⇔	⇓	⇓	⇓	+
Cathinone	⇓	⇔	⇔	⇓	⇔	-
METH	⇓	⇔	⇓	⇓	⇓	+
MDA	⇔	⇔	⇓	⇔	⇓	+
MDMA	⇔	⇔	⇓	⇔	⇓	+
Fenfluramine	⇔	⇔	⇓	⇔	⇓	-
Dethylpropion	⇔	⇔	⇓			
Mazindol	⇔	⇓	⇔			
PPA	⇔	⇔	⇔			
Cocaine	⇔	⇔	⇔			
MPH	⇔	⇔	⇔	⇔		

KEY: METH=methamphetamine PPA=phenylpropanolamine; MPH=methylphenidate.

More recently, the N-ethyl analog of MDA (MDE) was examined for possible neurotoxic effects (Stone et al. 1987a; Schmidt 1987b). In comparison to MDA and MDMA, MDE was much less potent in causing depletions of 5-HT 2 weeks after a multiple dose regimen (10 mg/kg every 6 hours for five consecutive intervals). This regimen also failed to reduce tryptophan hydroxylase activity; additionally, 5-HT uptake sites were not reduced 1 week after a single 20 mg/kg injection, unlike the reduction found with similar doses of MDA and MDMA (Schmidt 1987b). However,

as observed with MDA and MDMA, no effects of MDE on DA neurons were reported in these studies.

Fenfluramine, like MDMA and MDA, is a ring-substituted amphetamine derivative that has been found to meet all the criteria for neurotoxicity. When administered in doses higher than 12 mg/kg/day, depletions of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) last up to 6 months after cessation of drug treatment (Harvey and McMaster 1975; Harvey et al. 1977; Clineschmidt et al. 1978; Steranka and Sanders-Bush 1979; Schuster et al. 1986; Kleven et al. 1988). Other long-lasting effects of fenfluramine include a decrease in 5-HT uptake sites (Schuster et al. 1986) and tryptophan hydroxylase activity (Steranka and Sanders-Bush 1979). Previous studies have also indicated that fenfluramine produces signs of neuronal degeneration (Harvey and McMaster 1975; Harvey and McMaster 1977; Harvey et al. 1977). Recent immunohistochemical studies also indicated that fenfluramine produced morphological damage to 5-HT terminal fields (Appel and De Souza 1988). Collectively, the neurochemical and histological data support the idea that fenfluramine is neurotoxic to 5-HT.

Cathinone and phenylpropanolamine are side-chain-substituted amphetamines that have thus far met only some of the criteria for neurotoxicity. Like *d*-amphetamine, cathinone releases and, at very high concentrations, blocks uptake of DA (Wagner et al. 1982). Similarly, cathinone mimics *d*-amphetamines long-lasting toxic effects on DA levels and uptake sites (Wagner et al. 1982). The possibility that neuronal damage occurs following neurotoxic doses of cathinone has not been examined. Neurotoxic effects of phenylpropanolamine are unlike those reported for other substituted phenethylamines. When very high doses (200 mg/kg by injection) are administered, slight decreases in frontal cortex DA have been observed (Woolverton et al. 1986). with no effects on other monoamine systems.

Several substituted phenethylamines, such as methylphenidate (MPH) and mazindol, are notably lacking in toxic effects on DA or 5-HT (Wagner et al. 1980). However, mazindol does produce a slight decrease in NE, following repeated administration. None of the other neurotoxic amphetamine derivatives have been found to have long-lasting effects on the NE system. Both amphetamine and MPH are potent monoamine-releasing agents; however, MPH appears to act primarily on a pool that does not depend upon recent synthesis (Scheel-Kruger et al. 1977). The newly synthesized transmitter pool seems to be required for the neumtoxicity produced by METH, since inhibition of DA synthesis blocks the neurotoxicity of METH (Commins and Seiden 1986; Wagner et al. 1983).

The similarities in acute neurochemical effects of cocaine and amphetamine-like compounds raise the possibility that repeated exposure to cocaine might

produce long-term neurotoxic changes similar to those produced by METH. Most previous studies have examined effects of repeated doses of cocaine within 24 hours after the last injection (Roy et al. 1978; Taylor and Ho 1976; Taylor and Ho 1977). These studies reveal that exposure to cocaine for up to 45 days causes only small decreases in DA and/or 5-HT levels. Longer lasting consequences of repeated exposure to cocaine have only recently been examined (Trulson and Ulissey 1987; Trulson et al. 1986; Trulson et al. 1987). For example, it has been reported that repeated cocaine administration to rats reduced striatal and mesolimbic tyrosine hydroxylase activity 60 days after the last injection (Trulson et al. 1986; Trulson et al. 1987), a finding that suggests that prolonged exposure to cocaine might produce long-lasting damage to DA-containing neurons. A reduction in tyrosine hydroxylase activity for several days or weeks is consistent with toxicity to catecholamine neurons. However, using neurochemical methods, we have not found such evidence of neurotoxicity (Kleven et al., in press). Repeated injections of either moderate (20 mg/kg/day) or high doses (100 mg/kg/day) of cocaine for 10 days failed to produce long-term reductions in the concentration of monoamines or metabolites in any of the brain regions examined, including the striatum. Similarly, increasing exposure to 21 days of continuous infusion of a high dose of cocaine (100 mg/kg/day) failed to significantly deplete DA and 5-HT in the striatum or other regions examined. Higher doses of cocaine were lethal within 4 days of exposure. Therefore, long-lasting depletions of monoamines do not seem to occur following cocaine administration.

The data reviewed above indicate that amphetamines and a number of their analogs are neurotoxic to DA and/or 5-HT. Although both ring- (MDA, MDMA, MDE) and side-chain-substituted amphetamines (METH, cathinone, mazindol) have been found to produce neurotoxicity, some differences are apparent. First, side-chain-substituted phenethylamines are primarily toxic to DA neurons, with effects on 5-HT appearing at higher doses. On the other hand, ring-substituted phenythylamines are selectively toxic to 5-HT neurons, with effects on DA evident only at much higher doses. Second, these two classes of substituted phenethylamines may also differ in terms of potency, either absolute or relative to other behavioral effects.

Table 2 summarizes results of neurotoxicity studies that have utilized the same regimen of drug injections (twice daily for 4 days) and survival times (2 weeks). In addition, the ability of these drugs to suppress milk intake in rats is also presented. It is clear that ring-substituted amphetamines are more potent in terms of absolute dose required to reduce amine content than is the parent compound amphetamine. With regard to relative potency, METH is toxic to DA and 5-HT neurons at doses that are more than tenfold higher than doses that produce anorexia, whereas fenfluramine, MDA, and MDMA are toxic to 5-HT neurons at doses that are only three

TABLE 2. *Relationship between behavioral and neurotoxic potency*

Drug	Dose††	Neurotoxicity* DA-striatum	5-HT-hippo	Anorectic ED_{50}†
METH	100	65%	57%	1.6
Cathinone	100-200	50%	⇔	3.9
PPA	100-200	64%	⇔	>100
Dethylpropion	25	⇔	67%	10.0
MDA	10	⇔	41%	2.5
MDMA	20	⇔	30%	2.8
Fenfluramine	6.25	⇔	43%	5.0

*Levels of transmitter % of control.

†Reduction of intake of sweetened condensed milk during 15-minute sessions.

††mg/kg injected twice daily for 4 days, rats were sacrificed 2 weeks after the last injection.

KEY: PPA=phenylpropanolamine; ⇔=no effect.

to four times higher than those needed to suppress milk-drinking activity in rats. These data suggest that ring substitution increases neurotoxic potency to a greater extent than increasing behavioral potency.

BEHAVIORAL EFFECTS OF NEUROTOXIC AMPHETAMINES

The most robust behavioral change that has been observed after a brief regimen of amphetamine-related drugs is altered sensitivity to subsequent administration of the same or a related drug. The underlying effect is not apparent until it is unmasked by pharmacological challenge. Thus, long-lasting behavioral effects of neurotoxic amphetamines may be more subtle than those produced by monoamine neurotoxins such as 6-hydroxydopamine (6-OHDA) or 5,6-dihydroxytryptamine (5,6-DHT). Perhaps this is because, with the toxic amphetamines, levels of neurotransmitter are usually depleted to about 50 percent of normal. Studies in which monoamine neurons are lesioned using 6-OHDA or 5,6-DHT show behavioral deficits when levels are depleted to 80 or 90 percent of normal. Even in these studies, the most common finding is a change in sensitivity to pharmacological probes (Heffner and Seiden 1979; Levine et al. 1980).

The original observation of long-term depletions of DA in the rhesus monkey was made during a study of the development of tolerance to the effects of daily injections of METH (Fischman and Schuster 1977). In this study, it was found that behavioral tolerance to METH on a differential-reinforcement-of-low-rate (DRL) task persisted long after the repeated METH regimen. In a similar study conducted later, monkeys treated with repeated METH showed reduced sensitivity to apomorphine and increased

sensitivity to haloperidol (Finnegan et al. 1982). Increased sensitivity to haloperidol and tolerance to METH's effects on locomotor activity of rats (Lucot et al. 1980) and a force-lever task in rhesus monkeys (Ando et al. 1985) have also been reported following a regimen of METH. Since, in each of these studies, repeated administration of METH produced substantial decreases of DA, tolerance to subsequent METH injections is most likely related to selective destruction of DA terminals.

In contrast to studies in which tolerance to METH was observed, sensitivity to the effects of MDMA on DRL performance in rats has been found to increase as a consequence of a neurotoxic regimen of MDMA (Li et al., in press). Acute administration of MDMA at 2, 4, and 6 mg/kg increased the response rate and decreased the reinforcement rate of rats performing under a DRL 72-second schedule, similar to that observed with other psychomotor stimulants. Repeated administration of MDMA for 4 days (6 mg/kg, SC, twice daily), to rats performing on the DRL schedule produced a shift to the left of the MDMA dose response curve and also increased the maximal response to MDMA at all dosages. Since levels of 5-HT but not NE or DA were significantly depleted following the MDMA regimen, the behavioral results suggest that 5-HT neurons normally exert an inhibitory action upon the psychomotor stimulant effects of MDMA. Since the psychomotor stimulant effects of amphetamines appear to be mediated primarily by the DA system, these results provide evidence that 5-HT and DA may represent opposing systems insofar as they play a role in DRL schedule-controlled behavior.

It has recently been found that sensitivity to the analgesic effects of morphine is altered in rats previously treated with a neurotoxic regimen of MDMA (Nencini et al. 1988). Rats were injected twice a day for 4 days with 20 mg/kg MDMA or saline, and, after 14 days, nociception was determined by measuring reaction time to the tail immersion in heated water (55 °C). After determining baseline reaction times, rats were randomly assigned to four groups receiving saline or morphine (2.5, 3.55, or 5 mg/kg, SC), and the nociceptive test was repeated at various times after drug or saline administration. Morphine administration produced an analgesic effect that was more potent and prolonged in MDMA- than in saline-pretreated rats. These data indicate that morphine was more potent as a consequence of a neurotoxic regimen of MDMA.

During evaluation of neurotoxicity produced by fenfluramine, an apparent transitory depletion of 5-HT was observed, with recovery of levels occurring at 16 weeks for most regions except the hippocampus. It was of interest to examine this finding in greater detail because of previous work that had suggested irreversible effects of the drug. Tolerance to the anorectic effects of fenfluramine was observable 2 but not 8 weeks following a standard 4-day regimen of fenfluramine (6.25 mg/kg. twice daily). Because levels of 5-HT are apparently returning toward control values by 8 weeks in striatum

and hypothalamus, it is possible that tolerance to fenfluramine's anorectic effect is due to 5-HT depletion. Thus, sensitivity to the anorectic effect may be related to existing levels of 5-HT. Rats previously allowed to drink sweetened condensed milk during daily 15-minute sessions were treated with fenfluramine (6.25 mg/kg twice daily for 4 days) or saline. After 2 to 8 weeks, rats were administered fenfluramine acutely, tested for milk intake, and sacrificed 2 hours later. Acute adminitration of fenfluramine produced a dose-related decrease in milk intake and 5-HT levels in various brain regions. The milk intake data indicated that tolerance to the anorectic effect of fenfluramine occurred as a result of prior exposure to fenfluramine. However, levels of 5-HT were also depleted 2 and, to a lesser extent, 8 weeks after the fenfluramine regimen. There was apparent tolerance to the acute 5-HT-depleting effect of fenfluramine as a result of the 4-day fenfluramine regimen; partial recovery of this neurochemical tolerance was observed at 8 weeks. The results suggest that tolerance to the anorectic effects of fenfluramine may be due to a selective depletion of 5-HT.

CONCLUSION

The results of behavioral studies reviewed are summarized in table 3. It is clear that a neurotoxic regimen of METH produced tolerance to the effects of subsequent injections of METH on either conditioned or unconditioned behaviors. The regimen of METH produced long-lasting depletions of DA in each of these studies. Similarly, repeated administration of fenfluramine also produced decreases in 5-HT and tolerance to the anorectic effects of fenfluramine. Repeated administration of MDMA to rats performing a DRL schedule resulted in sensitization to the effects of MDMA. This latter finding is interpreted as being due to the effects, MDMA on DA release, in

TABLE 3. *Evidence of neurotoxin-induced behavioral changes in response to pharmacological probes*

Drug	Regimen	Test	Effect	Reference
METH	0.5-16 mg/kg/day	DRL	⇓ MA ⇑ Haloperidol	Fischman et al. 1977
METH	1-32 mg/kg/day	DRL	⇓ MA ⇑ Haloperidol	Finnegan et al. 1982
METH	100 mg/kg/day * 4	Locomotor	⇓ MA ⇑ Haloperidol	Lucot et al. 1980
METH	4-40 mg/kg/day * 4	Force Lever	⇓ MA	Ando et al. 1985
MDMA	6.0 mg/kg/inj * 8	DRL-40 sec	⇑ MDMA	Li et al., in press
MDMA	20 mg/kg/inj * 8	Tail Flick	⇑ Morphine	Nencini 1988
FEN	6.25 mg/kg/inj * 8	Milk Intake	⇓ FEN	Kleven et al. 1988a

Key: METH=methamphetamine; FEN=fenfluramine; ⇓=tolerance; ⇑=sensitization.

the absence of effects on 5-HT release, as a consequence of the prolonged depletions. Each of these studies has utilized pharmacological techniques to unmask the behavioral deficits produced by the neurotoxic regimen of drug. It should be noted that persisting behavioral effects of the chronic regimen of drug, in the absence of such pharmacological challenges appear not to have been reported. While pharmacological probes reveal an underlying change in DA and/or 5-HT function, the nature of behavioral deficits in the absence of drug challenge remains to be determined.

DISCUSSION

QUESTION: Have you or anyone else had the opportunity to look at the changes in the neurochemical parameters in animals that self-administer some of the amphetamines?

ANSWER: No, not to my knowledge. What we have done is look at what the consequences are on self-administration from these chronic regimes.

In other words, we do not have them self-administering these toxic doses. We have done it with some rhesus monkeys that were self-administering methamphetamine. If you give them a regime that depletes the dopamine and serotonin, and then see what alterations there are in self-administration, it does go down, but we have not looked at that.

QUESTION: Do you get bigger effects on some of the behavioral parameters after the amphetamine treatments if you pretreat the animals with a low dose of alpha methyltyrosine during that period, during the post-amphetamine period? In the old days, when we had a partial lesion, we gave a low dose of AMFT, and it would reexpose the lesion. Have you tried that?

ANSWER: No. It is a good idea, though.

QUESTION: I find the technique of challenging the animals with various "typic" agents quite intriguing for assessment after prolonged exposure to MDMA or amphetamine. How long do those changes last? I saw in your slide something on the order of 2 weeks or a few days after the MDMA treatment. If you would come back a few months later, would that supersensitivity still exist?

ANSWER: We are not sure. We have not systematically looked at later times; we have done so accidentally, however. Sometimes we are not ready to do an experiment, and we have repeated the MDMA experiment, We have also, by chance, done tests 6 weeks later, and we have gotten essentially the same results.

QUESTION: Is that reasonably concordant then with the depleting effects of these treatments?

ANSWER: For MDMA, it is. It certainly would not be for fenfluramine. But we haven't looked functionally yet.

QUESTION: Because by 8 weeks you already see some sort of recovery?

COMMENT: I would like to show a slide because I believe the data are interesting. It has to do with the strategy of looking for a functional change after serotonin is lost following fenfluramine treatment.

There is a recent clinical report by Emil Coccaro and colleagues that I think might be relevant to the kind of thing you have done in rats. They have been looking at endocrine responses to fenfluramine in humans as a marker of central serotonergic function. And they have observed an increase in serum prolactin concentration, which is felt to be due to serotonin release. They reported that, in subjects who received a second dose of fenfluramine within 12 days after the first dose, that there was a blunted response to serum prolactin.

There are probably a multitude of explanations, but clearly one would be a possible persistent depletion of serotonin, the substrate whose release is required for the acute response to prolactin.

So I think the accumulation of the additional data as you have presented in rats and perhaps additional data like that in humans may help to clarify whether there are functional consequences of that loss of serotonin following fenfluramine.

RESPONSE: That is very interesting. I should mention that, for fenfluramine, the toxic dose as for MDMA is very close to the therapeutic dose.

I didn't go in much detail into what the effects of different doses were, but with fenfluramine we are getting toxicity in the range of 3, 6, and 10 mg/kg and to interfere with feeding behavior in the rat you arc dealing with an order of 2.0 mg/kg. So there isn't much of a window there. Similarly, for MDMA, the neurotoxic dose range is 10, 20 mg/kg and a human is taking approximately 2.0 mg/kg. Behaviorally effective doses are in the neighborhood of 4, 5, and 6 mg/kg.

In contrast to methamphetamine, where we are dealing with behaviorally effective doses that are in the range of 1 to 4 mg/kg, toxicity doses are in the range of 50 to 100.

So you see, according to our thinking, some of these drugs are more dangerous because the toxic doses are so very close to the behaviorally active therapeutic doses.

COMMENT: Just a very brief comment that originally followed up on the idea of pharmacologic challenge. It is a very powerful technique with which one can detect underlying or covert neurochemical deficits,

The beauty of it is not only can it uncover an otherwise unapparent deficit, but it is a technique that can be readily applied to humans.

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AUTHORS

Lewis S. Seiden, Ph.D., Professor
Department of Pharmacological and Physiological Sciences
and Department of Psychiatry

Mark S. Kleven, Ph.D., Research Associate (Assistant Professor)
Department of Pharmacological and Physiological Sciences

The University of Chicago
947 East 58th Street
Chicago, IL 60637

Role of Dopamine in the Neurotoxicity Induced by Amphetamines and Related Designer Drugs

James **W.** *Gibb, Donna M. Stone, Michel Johnson, and Glen R. Hanson*

INTRODUCTION

In 1971, extensive excitement about the increasing abuse of amphetamines piqued the authors' interest in the effects of amphetamine and its analogs on biogenic amine metabolism; specifically, whether the biosynthesis of biogenic amines may be altered. In the prior year, Mandell and Morgan (1970) reported that methamphetamine (METH) produced an increase in adrenal tyrosine hydroxylase (TH) activity. Fibiger and McGeer (1971) also observed that chronic treatment with METH caused an increase in TH activity in the adrenal gland and a decrease in enzyme activity in the neostriatum.

METHAMPHETAMINE STUDIES

METH Effects on the Dopaminergic System

In an attempt to simulate in rats the dosage regimen commonly employed by abusers of amphetamines, METH was administered (10 or 15 mg/kg every 6 hours; four to six doses), after which the animals were killed (Koda and Gibb 1971: Koda and Gibb 1973). TH activity and catecholamine concentrations were measured in various brain regions and in the adrenal. Neostriatal TH activity was depressed in a dose-dependent manner and reached its nadir at 36 hours. Dopamine (DA) and norepinephrine concentrations were initially elevated, but then decreased in parallel with TH activity. Adrenal TH activity was elevated. presumably because of stress associated with the toxic doses of METH.

It was later determined whether this was a generalized response to METH of all transmitter systems or whether it was characteristic of specific systems. In the dosage used, METH did not affect striatal choline

acetyltransferase or glutamic acid decarboxylase; however, tryptophan hydroxylase (TPH) activity was dramatically decreased (Hotchkiss et al. 1979). These observations suggested that METH selectively altered the dopaminergic and serotonergic systems, but did not change the striatal cholinergic or GABAergic systems.

METH Effects on the Serotonergic System

While there are significant similarities in the response of the dopaminergic and serotonergic systems to METH, there are also qualitative and quantitative differences. Decreases that occur in the serotonergic parameters are much larger than the changes in parameters of the dopaminergic system; moreover, the doses required to obtain the response in the serotonergic system are lower. Furthermore, the decrease in striatal TH activity is not observed until approximately 12 hours after METH administration, while there is a pronounced effect in the serotonergic system within 15 to 30 minutes. (figure 1). TPH activity and serotonin (5-HT) concentrations declined rapidly, while concentrations of 5-hydroxyindoleacetic acid (5-HIAA) were transiently elevated after a single dose of METH; tryptophan content was also elevated after a single dose of METH. Similar responses occurred with *p*-chloroamphetamine and amphetamine (Peat et al. 1985). Since dopaminergic fibers are confined to fewer regions of the brain, while the serotonergic system is present in many brain areas, the effect of METH on the brain serotonergic system is more widespread than is the dopaminergic response (Hotchkiss and Gibb 1980).

When only a single dose of METH (10 mg/kg) was administered, TPH activity returned to normal in all areas within 2 weeks after administering the drug (Bakhit and Gibb 1981). However, with repeated administration of METH (five doses, given every 6 hours), TPH activity in the neostriatum, cerebral cortex, nucleus accumbens, and hippocampus recovered to some extent but remained significantly depressed 110 days after the last dose of the drug had been administered; neostriatal TH activity was also depressed after 110 days.

These findings suggest that, although a single exposure to METH does not result in permanent alteration of the serotonergic system, repeated administrations of large doses of METH result in sustained damage. These observations, together with the METH-induced morphological alterations (Ricaurte et al. 1982) and the compromised uptake of DA (Wagner et al. 1980), suggest that METH, given in repeated, large doses, is neurotoxic to brain serotonergic and dopaminergic neurons.

Role of DA and Its Reactive Metabolites

After the response to METH had been relatively well characterized, the mechanism responsible for the effect was still unidentified. Since DA

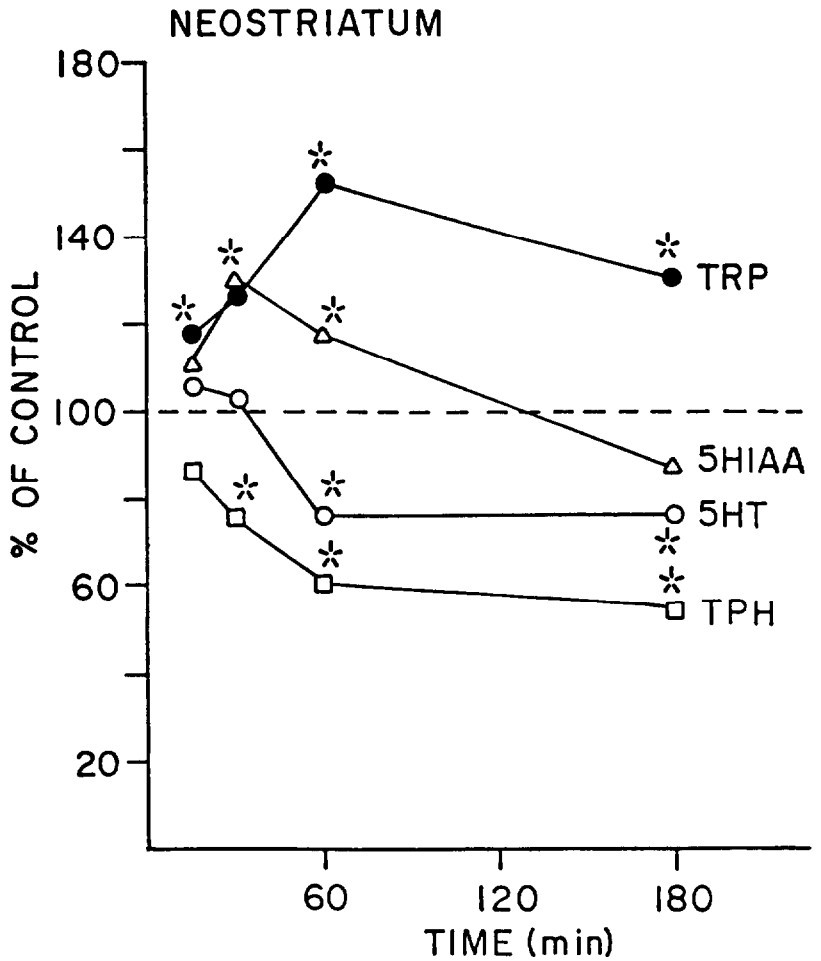

FIGURE 1. *Effect of acute METH on serotonergic parameters*

*Significantly different from control, $p<0.05$ by Student's *t*-test.

NOTE: A single dose of METH (10 mg/kg. SC) was administered and rats were killed 3 hours later. TPH activity and concentration of tryptophan (TRP), 5-HT. and 5-HIAA in the neostriatum were determined; saline control values (means ± SEM): 5-HT. 0.75±0.l; 5-HIAA, 0.72± 0.06, TRP, 5.68± 0.17 ng/mg; TPH activity, 24.5± 1.4 nmol/g tissue/hr (n=6 or more).

antagonists had previously been reported to attenuate other effects of amphetamines (Lasagna and McCann 1957; Randrup et al. 1963; Espelin and Done 1968), the influence of chlorpromazine or haloperidol on the METH-induced decreases in TH activity (Buening and Gibb 1974) was

investigated. Either drug prevented the response of TH to METH in the neostriatum; moreover, at the dosage used, haloperidol prevented and chlorpromazine attenuated the elevation of adrenal TH activity. More recently, it was found that the decrease in striatal TH activity is both a D_1- and D_2-mediated event (Sonsalla et al. 1986).

Whether the serotonergic responses to METH could be attenuated by DA antagonists was next examined (Hotchkiss and Gibb 1980). Surprisingly, haloperidol, administered concurrently, prevented the METH-induced decrease in neostriatal TPH activity (figure 2). Similar responses were observed in the cerebral cortex.

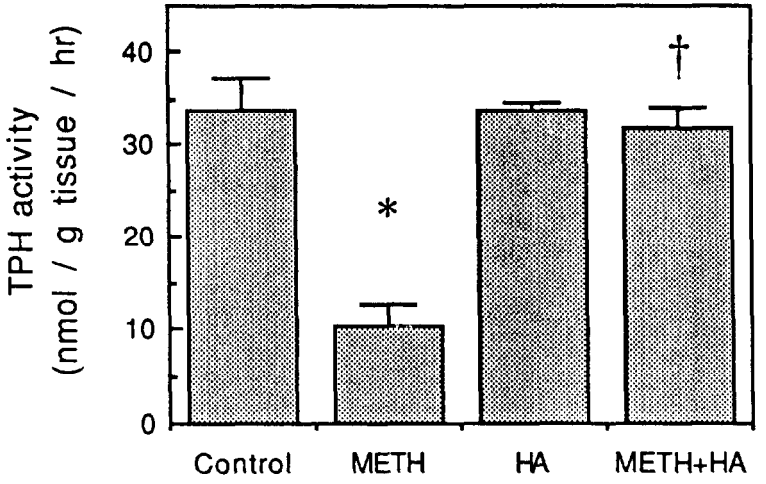

FIGURE 2. *Effect of HA on METH-induced decrease in neostriatal TPH activity*

*p<0.05 compared to control.

†p<0.05 vS. METH done by Student's *t*-test

NOTE: METH (15 mg/kg, SC) was administered four times at 6-hour intervals and rats were killed 5 days after the first administration. HA (3 mg/kg. IP) was administered on the same schedule (n=4 to 10).

Subsequent experiments revealed that DA is necessary for METH to cause neurotoxicity; when DA synthesis was inhibited with α-methyl-*p*-tyrosine (MT), the usual decrease in striatal TH activity observed after METH was prevented (figure 3A) (Kogan and Gibb 1979). Reinstatement of DA synthesis by administering, *l*-dopa. which circumvents the inhibited TH step, returned the METH-induced decreased in TH activity.

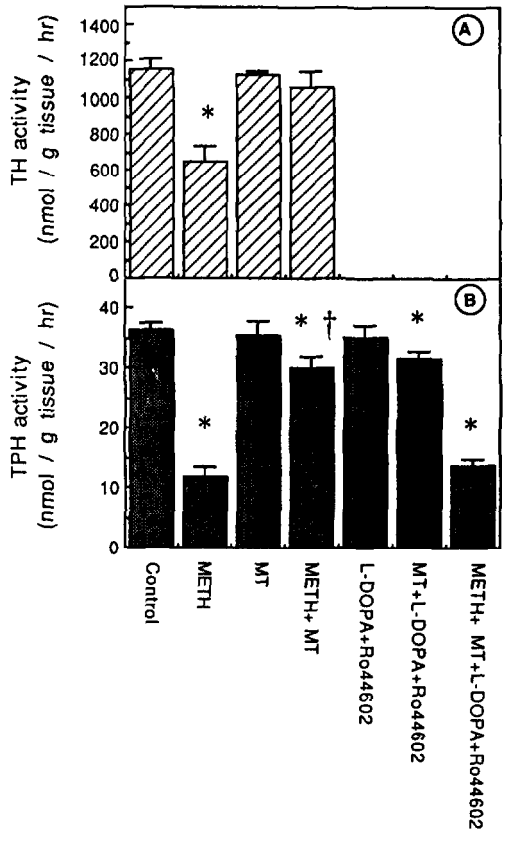

FIGURE 3. *A. Inhibition of DA synthesis on the METH-induced decrease in neostriatal TH activity. B. Inhibition of DA synthesis on the METH-induced &crease in neostriatal TPH activity*

*p<0.05 vs. Control

†p<0.001 vs. METH alone, by Student's *t*-test

NOTE: A. METH (15 mg/kg, SC) was administered four times at 6-hour intervals, and rats were killed 5 days after the first administration. MT (60 mg/kg, lP) was administered on the same schedule. B. METH (IS mg/kg. SC) was administered five times at 6-hour intervals, and rats were killed 18 hours after the first administration. MT (60 mg/kg. IP), *l*-dopa (50 mg/kg. IP), and RO 44602 (25 mg/kg, lP) were administered concurrently (n=4 to 10).

More fascinating was the response of the serotonergic system to MT and METH. When DA synthesis was interrupted by concurrent administration of MT, TPH activity remained normal after METH; however, when DA synthesis was reinstated by administering concurrently *l*-dopa, a peripheral

dopa decarboxylase inhibitor (RO 44602). MT, and METH, the METH-induced depression of TPH activity was again observed (figure 3B).

It was thought that additional evidence for involvement of DA in the serotonergic response to METH could be obtained by selectively destroying the dopaminergic input to the neostriatum with bilateral injections of the neurotoxin 6-hydroxydopamine (6-OHDA) into the substantia nigra. METH was then administered to determine whether the decrease of TPH activity caused by METH would be absent in the neostriatum but present in the other regions. 6-OHDA was injected bilaterally into the substantia nigra 11 days prior to METH administration. In the neostriatum, deprived of dopaminergic input, there was no decrease in TPH activity. In the frontal cortex and hippocampus, however, the METH-induced decrease in TPH activity still occurred (Johnson et al. 1987).

In summary, three different approaches were used to examine the role of DA in the METH response: first, blockade of DA receptors by haloperidol or more specific DA antagonists prevented the METH-induced alteration of the dopaminergic and serotonergic systems, suggesting that DA may be involved in these alterations; second, when DA synthesis was inhibited, the METH-induced changes were prevented in both monoaminergic systems; finally, when dopaminergic input to a specific brain region was interrupted, the METH-induced decrease in TPH activity in that brain region was selectively abolished.

OTHER DRUG STUDIES

Effect of MDMA on Serotonergic and Dopaminergic Systems

In 1985, Seiden and his coworkers reported that 3,4-methylenedioxyamphetamine (MDA) caused a decrease in brain 5-HT and 5-HIAA concentrations; 5-HT uptake was also compromised (Ricaurte et al. 1985). We compared the effects of the methylenedioxy derivatives of METH and amphetamine on the serotonergic and dopaminergic parameters previously demonstrated as altered by METH administration (Stone et al. 1986).

When 3,4-methylenedioxymethamphetamine (MDMA) or MDA was administered to rats in a single dose, TPH activity was markedly depressed. Multiple doses of MDMA or MDA resulted in a further decline in TPH activity (figure 4). In contrast to METH, however, neither MDA nor MDMA altered neostriatal TH activity. The decrease in TPH activity was accompanied by a dramatic decrease in 5-HT and 5-HIAA concentrations; these changes in TPH activity and in 5-hydroxyindole content also occurred in other serotonergic terminal areas such as the hippocampus and cerebral cortex. Both neostriatal DA and homovanillic acid (HVA) were initially elevated 3 hours after a single dose of MDMA, but had returned to normal

by 24 hours. Dihydroxyphenylacetic acid (DOPAC) concentrations were initially decreased (15 minutes) and had recovered by 24 hours.

Subsequent experiments were designed to characterize further the response of the serotonergic system to MDMA. When a single low dose (5 mg/kg) of MDMA was administered, there was an initial decrease in TPH activity and concentrations of 5-HT and 5-HIAA. These serotonergic parameters

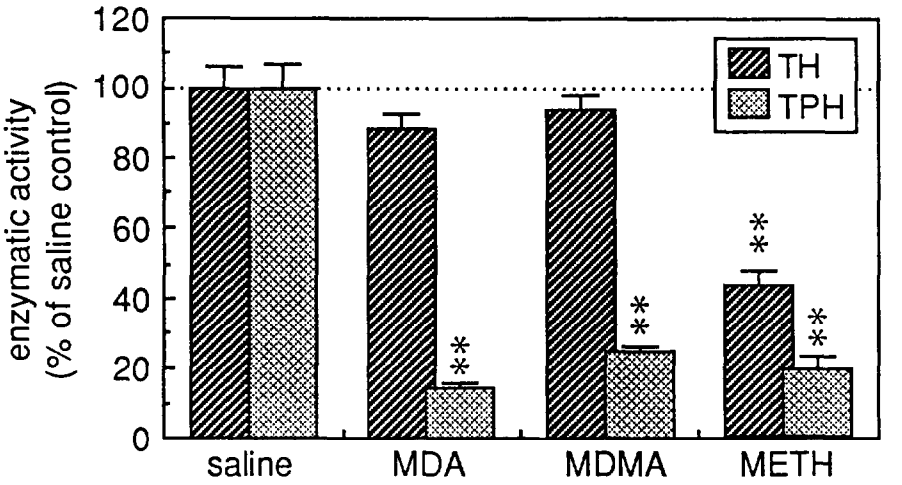

FIGURE 4. *Effect of multiple-dose drug treatment on neostriatal TH and TPH activity*

**p<0.01 vs. saline control, by Student's *t*-test.

NOTE: Rats were administered five SC doses of MDA (10 mg/kg). MDMA (10 mg/kg). or METH (15 mg/kg), one dose every 6 hours. and killed 18 hours after the last dose. Results are presented as the means ± SEM, expressed as a percent of saline control. Control values were: TH, 2645± 163 nmol tyrosine oxidized/g tissue/hr and TPH, 45.0± 3.5 nmol 14CO2 liberated/g tissue/hr

returned toward control and were essentially normal 2 weeks after the single dose. If, however, higher doses of MDMA (10 mg/kg, given every 6 hours) were administered and the serotonergic parameters were monitored for varying periods of time after discontinuing treatment, neostriatal TPH activity and concentrations of 5-HT and 5-HIAA remained significantly depressed for at least 110 days (figure 5) (Stone et al. 1987). The timecourse of recovery was similar in other serotonergic terminal regions examined.

Role of DA in MDMA-Induced Changes in the 5-HT System

The possible role of DA in the MDMA-induced alterations of the serotonergic system was then examined. Techniques previously used in studying the role of DA in the METH-induced neurochemical effects were employed. When DA synthesis was inhibited with MT, the effect of multiple doses of MDMA on TPH activity (figure 6) and concentrations of 5-HT and 5-HIAA was attenuated. The degree of protection with MT seemed to be a function of the size and number of doses of MDMA used as well as a function of the serotonergic parameter that was measured.

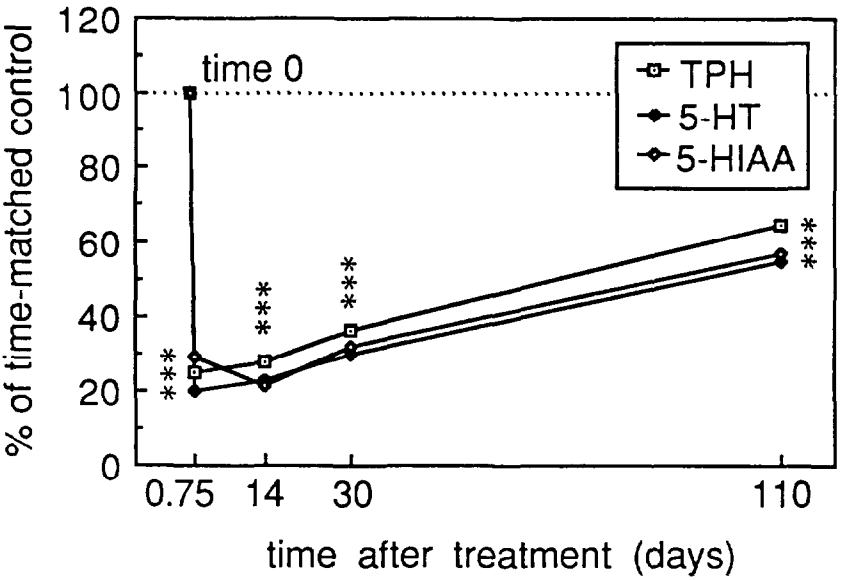

FIGURE *5. Long-term recovery of neostriatal serotonergic parameters after multiple doses of MDMA*

*p<0.01 vs. time-matched saline, by Student's *t*-test

NOTE: Rats were administered live doses of MDMA (10 mg/kg). one dose every 6 hours, and killed at specified times thereafter. Results are the means ± SEM (n=6 to 10). expressed as a percent of time-matched saline-treated control.

Moreover, the early transient response to a single dose of MDMA was less attenuated by MT than was the persisting response that occurred after multiple doses of MDMA.

DA was then depleted by using a different drug to determine whether the response to MDMA would be attenuated. When a single high dose of MDMA (20 mg/kg) was administered after reserpine, MT, or MT plus reserpine pretreatment, the usual decrease in neostriatal TPH activity

remaining 3 days after MDMA treatment was completely prevented (figure 7). Additionally, when animals were injected with 6-OHDA bilaterally into the substantia nigra and a single dose of MDMA (10 mg/kg) was administered 11 days later, the acute (3 hours) MDMA-induced decline in neostriatal, but not hippocampal or cortical, TPH activity was significantly attenuated (figure 8).

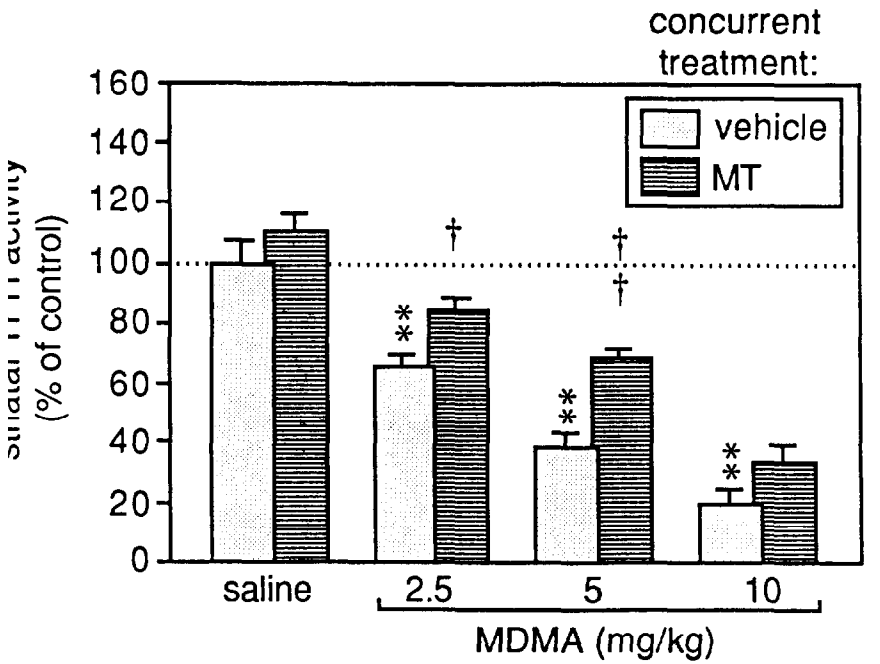

FIGURE 6. *Effect of concurrent MT on the neostriatal TPH deficit induced by multiple doses of MDMA*

**p<0.01 vs. vehicle-saline.

†p<0.05.

††p<0.01 vs. corresponding vehicle-MDMA group, by two-way ANOVA and Newman-Keuls multiple comparisons test.

NOTE: Rats were administered multiple doses of MDMA (2.5, 5, or 10 mg/kg, SC, five doses, one every 6 hr). Concurrent with each MDMA dose, MT (60 mg/kg) or saline vehicle was administered IP. Rats were killed 18 hours after the last dole. Results are the means of ± SEM (n=6 to 8). expressed as a percent of control (vehicle-saline).

It was previously demonstrated that amfonelic acid, a DA-uptake blocker, partially prevented the METH-induced decrease in TPH activity (Schmidt et al. 1985). Recently, effects were investigated of a specific DA-uptake blocker, GBR 12909, on the MDMA-induced response in the serotonergic

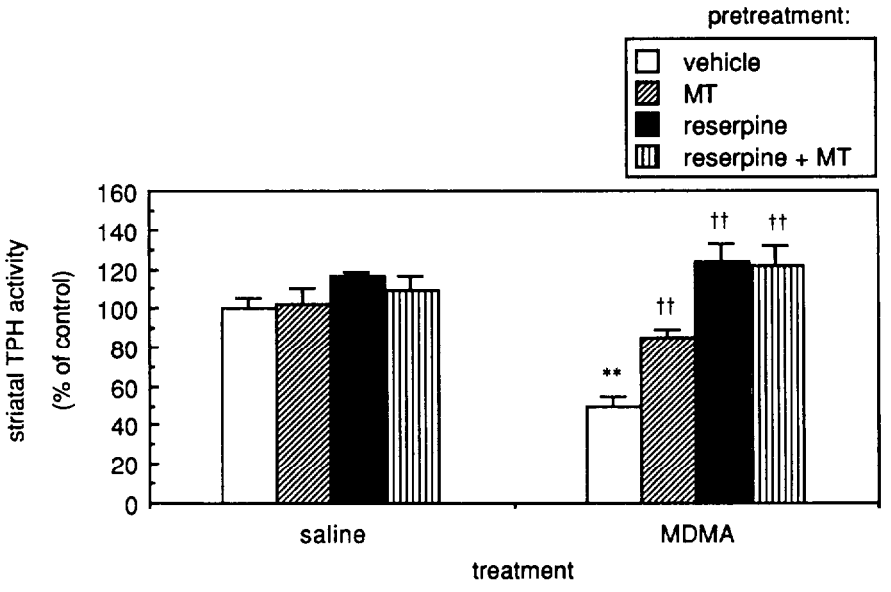

FIGURE 7. *Effect prior DA depletion on the neostriatal TPH deficit induced by a single high dose of MDMA*

**p<0.01 vs. vehicle-saline

†p<0.05.

††p<0.01 vs. vehicle-MDMA by two-way ANOVA and Newman-Keuls multiple comparisons test.

NOTE: Rats were pretreated IP with MT (120 mg/kg. 90 min before), reserpine (5 mg/kg. 12 hr before) or a combination (60 mg/kg MT + 5 mg/kg reserpine, 90 min and 12 hr before, respectiely). A single dose of MDMA (20 mg/kg) was administered after the specified time following pretreatment, and rats killed 3 days later. Results are the means ± SEM (n=6 to 7), expressed as a percent of control (vehicle-saline).

system. GBR 12909 (20 mg/kg) was administered 15 minutes prior to MDMA (20 mg/kg), and rats were killed 3 days later. The DA-uptake blocker significantly attenuated the usual MDMA-induced decrease in striatal TPH activity (figure 9) as well as the decrease in neostriatal 5-HT and 5-HIAA content

These experiments provide evidence that DA and/or its reactive metabolites are likely involved in MDMA-induced changes in the serotonergic system. When DA synthesis was inhibited with MT, or when DA innervation was interrupted by 6-OHDA lesions, the effects of MDMA were prevented or attenuated. Depletion of DA with reserpine, or inhibition of DA uptake with GBR 12909, also attenuated the effects of MDMA on the serotonergic system.

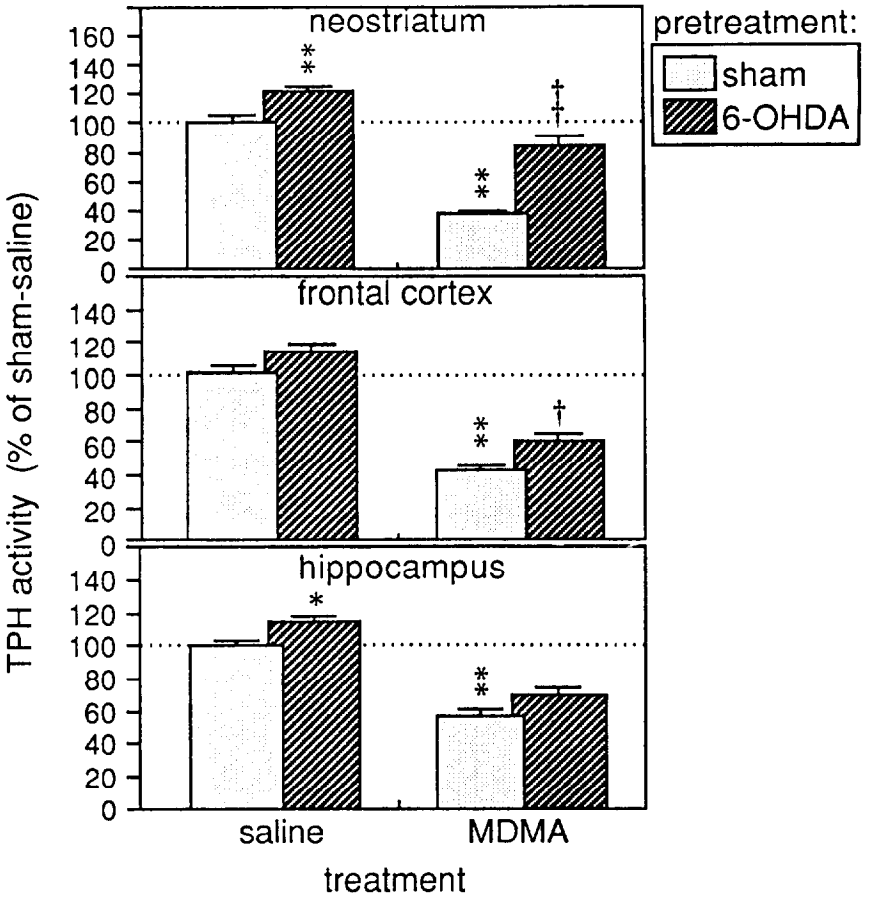

FIGURE 8. *Effect of prior substantia nigral lesions on the immediate MDMA-induced decreases in regional TPH activity*

**p<0.01 vs. sham-saline.

†p<0.05.

††p<0.01 vs. sham-MDMA by two-way ANOVA and Newman-Keuls multiple comparisons test.

NOTE: Lesions were induced bilaterally by local injection of 4 µg 6-OHDA/ 8 µL 0.1% ascorbate. vechile/side. Control rats received sham lesions of ascorbate vehicle alone. After an 11-day recovery period, acute MDMA (10 mg/kg) was adminstered SC and rats killed 3 hours later. Results are the means ± SEM, expressed as a percent of sham-saline (n=22 for sham-saline group, n=14 for 6-OHDA-saline group, n=6 to 8 for MDMA-treated groups). Because 6-OHDA itself significantly elevated TPH activity, values from MDMA-treated rats were expressed as a percentage ± SEM of their respective saline-treated control mean: in the neostriatum, TPH activity for the 6-OHDA-MDMA group was 67.6±5.1% vs. 37.5±2.3% for sham-MDMA, p<0.01 by Student's *t*-test. When similarly expressed, no significant differences were found between sham-MDMA and 6-OHDA-MDMA groups in the hippocampus or frontal cortex.

It is premature to define the exact mechanism by which DA is involved in the response to METH or MDMA. It is known- that these drugs release large quantities of DA and that DA can be readily oxidized to reactive metabolites, which could possibly cause destruction of nerve terminals (Graham 1978; Maker et al. 1986). Moreover, these effects could be enhanced by inhibition of monoamine oxidase, which is known to occur with these drugs (Susuki et al. 1980). The possibility that 6-DOHA is formed and subsequently destroys the nerve terminals, as suggested by Seiden and Vosmer (1984), also requires investigation.

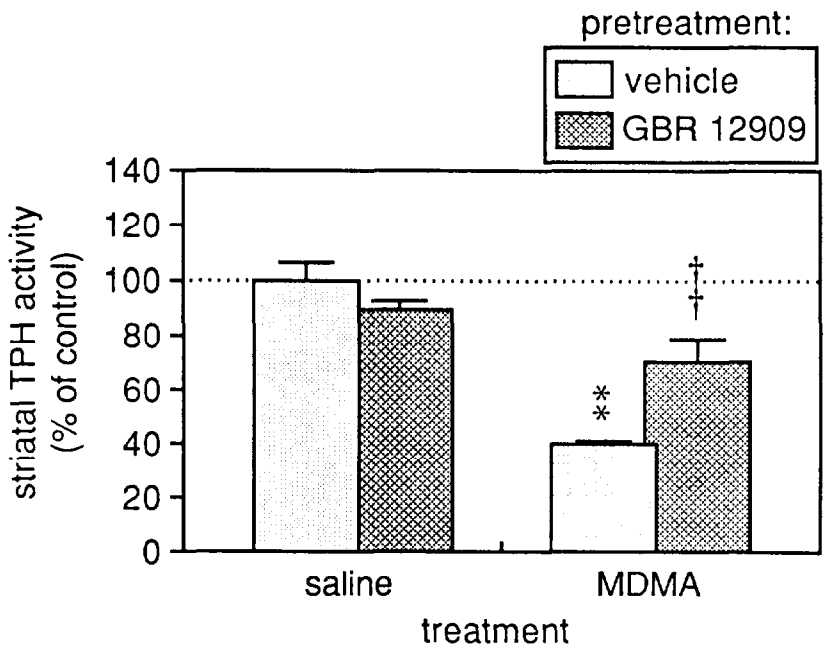

FIGURE 9. *Effect of DA-uptake inhibition on the neostriatal TPH deficit induced by a high single dose of MDMA*

**p<0.01 vs. vehicle-saline.

††p<0.01 vs. vehicle-MDMA by two-way ANOVA and Newman-Keuls multiple comparisons test.

NOTE: GBR 12909 (20 mg/kg, IP) or vehicle was administered 15 min prior to a single dose of MDMA (20 mg/kg. SC); rats were killed 3 days later. Results are the means ± SEM (n=5 to 6). expressed as a percent of control (vehicle-saline).

CONCLUSIONS

Observations made over the last 18 years concerning the effects of METH on the dopaminergic and serotonergic systems, comparisons of the monoaminergic responses to METH and MDMA, and the studies of the possible role of DA and/or its reactive metabolites as mediators in the alterations observed with these drugs provide evidence that DA is necessary for the effects to occur. Further studies are indicated to define more precisely the mechanism(s) responsible for the neurotoxic effects of these drugs. These studies may help to elucidate the potential neurotoxic effects of amphetamine and its related congeners in persons who ingest these agents, and may also have important implications in understanding the etiology of Parkinsonism and mental psychoses.

DISCUSSION

QUESTION: You have dopamine reuptake blockade, you have the SCH 23390 blocking, specifically blocking this tryptophan hydroxylase effect. Do you have a mechanism? How would you see that interaction taking place?

ANSWER: We have thought about that and I think one of the major challenges we have as a group is to determine the mechanism by which this occurs.

I think the results are compatible with the idea that Dr. Seiden has, that it is 6-hydroxydopamine. I think that it could be a 6-hydroxydopamine or some other reactive metabolite of dopamine that is causing the effect.

I think that probably dopamine is taken up and in some way oxidized and therefore may cause the destruction of the nerve terminals. But there are other possibilities as well that need to be explored to find out whether that indeed is the case. I think the jury is still out as to the mechanism that might occur.

COMMENT: That is the reason for mentioning the SCH 23390 being specific for blocking it and the sulpiride is not blocking it--

RESPONSE: In the serotonergic system.

COMMENT: Right, and it wouldn't explain a 6-hydroxydopamine mechanism. It is fascinating that there is that D_1, D_2 relationship.

RESPONSE: Yes, those data are a bit confusing.

I think the fact that haloperidol and all the dopamine antagonists work doesn't bring much clarity to the situation. I think it muddies the water.

But it is there, and I think it is important that we show it here so that the total picture is presented.

COMMENT: I totally agree with the AMT. We have also found that AMT blocks the serotonergic depletion, so we are in agreement.

Furthermore, and we haven't reported it yet, PCPA would also be expected to block the serotonergic depletion. It does nothing to the serotonin toxicity. So you are right, it is somewhat of a mystery as to what is going on.

COMMENT: You have presented some evidence that dopamine may be involved in the neurotoxic action of methamphetamine in terms of dopaminergic neurons, and you presented evidence suggesting that it may be involved in not only the dopamine system but also the serotonin system.

I think one has to be very careful, and it goes back to something that Dr. Seiden raised earlier, that if you are going to speak of the ncurotoxicity, I really think you have to look at a wide array of neurochemical changes, not only at 1 or 2 days but at 2, 3, or 4 weeks. Then the changes should be correlated with morphological changes.

I have no doubt that the bearing on these data is that you have shown the pharmacological effects on tyrosine and tryptophan hydroxylase activity. I am not sure that I can equate those with effects on actual neurotoxicity.

The main reason for my suspicion is that in the experiment with AMT where the effect of methamphetamine on tyrosine hydroxylase activity can be blocked and then reinstituted by coadministering *l*-dopa, one would predict that if one is really talking about neurotoxic effects, then one ought to be able to observe the same changes 2 weeks later.

We have attempted that experiment, And while it is true that on an acute basis we can indeed restore an effect on neurochemical parameters at a day, that is not the case at 2 weeks. This leads me to suspect that one is really dealing with pharmacological effects of methamphetamine, reinstitution of these pharmacological effects with *l*-dopa. But that may not equate with a neurotoxic action of the drug. I think it is important because it opens up the issue of whether dopamine does in fact mediate a neurotoxic action of MDMA and these other compounds rather than some acute pharmacological effects of these drugs.

RESPONSE/QUESTION: I think that your point is well taken, but I would ask: Have you and Dr. Seiden demonstrated your uptake blockade after 2 weeks or so as well? And then I would address the question to you or him--have you done the Fink-Heimer work at longer periods of time?

ANSWER: No, we haven't done the Fink-Heimer work at the longer periods with the blocking agents. We have done the AMT protection experiment 2 weeks later. That seems to work on the 5-HT neurons.

QUESTION: What we really need is to do some morphological studies with something that is very selective for those particular neurons, and I agree with you that that is something we need to strive for. Are you doing that at the Addiction Research Center?

ANSWER: We are trying to look at it using some of the neuroanatomical techniques that you described in terms of localizing uptake sites.

COMMENT: Let me make something clear. We don't need any new techniques. The experiments are very simple; they just have to be done at 2 weeks rather than at 18 hours. And it may be that you are entirely correct, But until those experiments are performed at longer timcpoints, I don't think we know if we are dealing with pharmacology or toxicology.

QUESTION: In your experiments, have you ever looked at the hippocampus? It is interesting to note that there is very little dopamine in the hippocampus. And if the theory of the necessity for dopamine is correct, then you should not see the depletion of serotonin in the hippocampus.

ANSWER: Good point. We have looked at the hippocampus and have found that it is protected. We think that not only dopamine is involved, but probably other catecholamines as well.

QUESTION: How do you imagine that both a receptor antagonist and an uptake inhibitor would block the effects? It would seem that if dopamine is involved, it would either be acting on a membrane receptor or inside, but not both. I would also like to ask a more specific question. You showed that the alpha MT protected effect could be reversed by dopa. And I think you imagined that that was because of dopamine formation. But have you tried dopamine agonists to see if they would antagonize either the protective effect of alpha methyltyrosine or, particularly, the protective effect of the dopamine antagonists to try to verify that those protective effects really have to do with blockade of a dopamine receptor as opposed to some other possibility?

ANSWER: That is a good question. I haven't.

COMMENT: I think that one thing that we are not dealing with effectively is the degeneration in the cortex, which, in our hands, is quite extensive.

Somatosensory cortical, pyramidal cells die at a very high rate with chronic administration. It seems to me that the involvement of dopaminc in that is

less likely, and the postulation that a 6-hydroxydopamine mechanism that doesn't account for the sparing that one sees in certain regions.

RESPONSE: We do have experiments in progress that will address that cortex issue because it is one that needs to be resolved.

COMMENT: We feel that it is due to the formation of 5,6-DHT in the cortex. These cells are indeed innervated by serotonin cells and, as a matter of fact, we have an experiment that is being published in *Brain Research* where we show that if we inject 5,6-DHT into the ventricles, we can produce exactly the same type of degeneration in the pyramidal cells, due to the formation of the 5,6 from the 5-hydroxytryptamine. We are exploring the possibility of it being another catecholamine in addition to dopamine, so I think both of those may be helpful in answering your question.

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AUTHORS

James W. Gibb, Ph.D.
Donna M. Stone, Ph.D.
Michel Johnson, Ph.D.
Glen R. Hanson, D.D.S, Ph.D.

Department of Pharmacology and Toxicology
University of Utah
Salt Lake City, UT 84112

Acute and Long-Term Neurochemical Effects of Methylenedioxymethamphetamine in the Rat

Christopher J. Schmidt

INTRODUCTION

Administration of a single dose of methylenedioxymethamphetamine (MDMA) to rats at doses above 10 mg/kg produces a biphasic pattern of serotonin (5-HT) depletion in the central nervous system (CNS) shown for the cerebral cortex in figure 1. Much of the work on MDMA in our laboratory has involved the characterization of these two phases of transmitter depletion following MDMA. Our results indicate that these two periods of depletion are unique with respect to their mechanism, timecourse, and stereochemical requirements. The acute effect of MDMA, which is maximal between 3 and 6 hours following drug administration, involves a disruption of 5-HT synthesis coupled with an increase in transmitter turnover. These early effects of MDMA on the serotonergic neuron appear to be ultimately reversible. The second phase of depletion develops several days after the administration of MDMA and is associated with a decrease in the number of serotonergic nerve terminals. It is the latter decrease in transmitter concentrations that corresponds to the neurotoxic effect of MDMA. Attempts have been made to compare these *in vivo* effects of MDMA with some of its in vitro activities to gain insight into the mechanism(s) responsible for the complex neurochemical response elicited by this drug.

ACUTE EFFECTS

Characteristic of its amphetamine-like structure, MDMA is a potent monoamine-releasing agent as demonstrated both *in vitro* (Nichols et al. 1982; Johnson et al. 1986; Schmidt et al. 1987) and *in vivo* (Yamamoto and Spanos 1988). This release occurs through a carrier-mediated, Ca^{2+}-independent mechanism typical of the phenethylamines (Schmidt et al. 1987). Figure 2 shows the concentration-dependent, MDMA-induced transmitter release from preloaded rat striatal slices superfused *in vitro*. From the figure, it is apparent that MDMA behaves similarly to the selective

serotonergic neurotoxin *p*-chloroamphetamine (PCA) as a releasing agent. Both PCA and MDMA show a greater potency for [^{3}H]5-HT release as compared to another neurotoxic amphetamine, methamphetamine. However, methamphetamine is a more potent releasing agent for [^{3}H]dopamine (DA) than either MDMA or PCA, which again appear very similar. These results are interesting in that, in contrast to the selective serotonergic neurotoxicity of PCA and MDMA, methamphetamine has been shown to be neurotoxic to both dopaminergic and serotonergic neurons in the rat brain (Gibb et al., this volume).

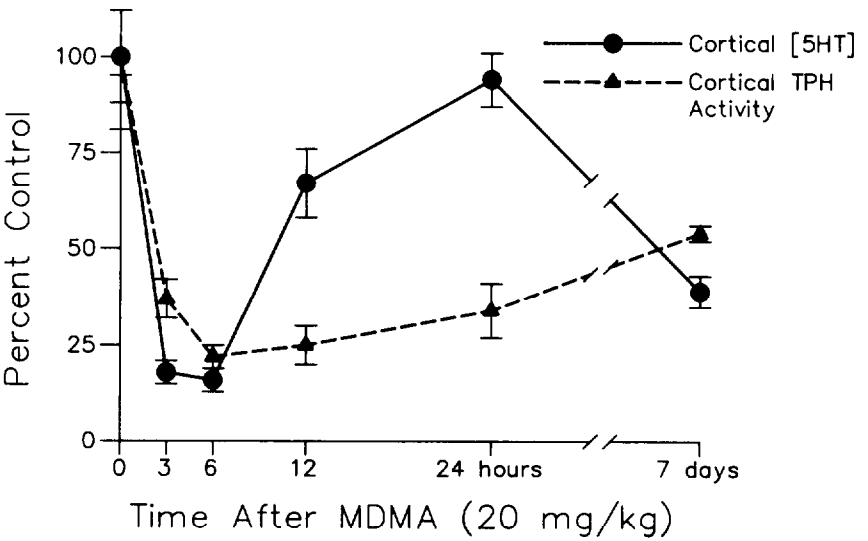

FIGURE 1. *Timecourse of changes in 5-HT concentrations and TPH activity following a single dose of MDMA*

NOTE: All data presented as mean ± SEM.

This similarity between MDMA and PCA is also observed *in vivo* in that PCA produces both an acute and long-term depletion of 5-HT (Fuller et al. 1975; Steranka et al. 1977). Like PCA, the acute decrease in 5-HT concentrations produced by MDMA is associated with a decrease in the activity of the rate-limiting enzyme for 5-HT synthesis, tryptophan hydroxylase (TPH). The timecourse of this change in cortical enzyme activity is also shown in figure 1. More detailed analysis of this acute effect of MDMA and kinetic analysis of TPH activity reveals that the decrease in enzyme activity actually precedes the decline in transmitter levels and is due to a reduction in the V_{max} activity of the enzyme (Schmidt and Taylor 1987; Schmidt and Taylor 1988). As shown for the cortex in figure 3, the decrease in 5-HT

also follows a transient spike in the concentration of 5-HIAA before the metabolite concentrations also begin to decline. These data indicate that an increase in transmitter release and a decrease in TPH activity are both required to produce the large depletion produced acutely by MDMA.

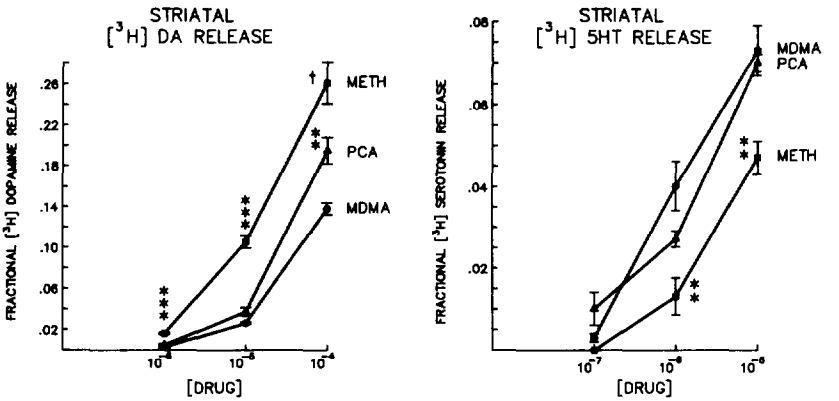

FIGURE 2. *Comparison of the MDMA-, PCA-, and methamphetamine-induced release of tritiated monoamines from superfused striatal slices in vitro*

**p<0.01

***p<0.001 compared to MDMA.

†p<0.001 compared to MDMA and p<0.02 compared to PCA.

NOTE: All data presented as mean ± SEM.

The above conclusion is supported by the results shown in figure 4. Just as inhibitors of the 5-HT uptake carrier can antagonize MDMA-induced [^{3}H]5-HT release *in vitro,* coadministration of MDMA with an uptake inhibitor such as citalopram can completely block the acute depletion of 5-HT. Although citalopram also antagonized the MDMA-induced decrease in TPH activity, there was still a significant loss of enzyme activity when compared to control. This implies that if MDMA requires access to the interior of the nerve terminals to affect TPH activity, it does not require the activity of the uptake carrier to gain entrance. Hence, these results are consistent with the outcome of synaptosomal uptake experiments with [^{3}H]MDMA (Schmidt et al. 1987), which show that MDMA is not actively concentrated by a carrier system. Furthermore, it is apparent that the loss of enzyme activity alone is not sufficient to reduce 5-HT concentrations, but that release via the carrier must occur simultaneously, to deplete the terminal once synthetic capacity is reduced.

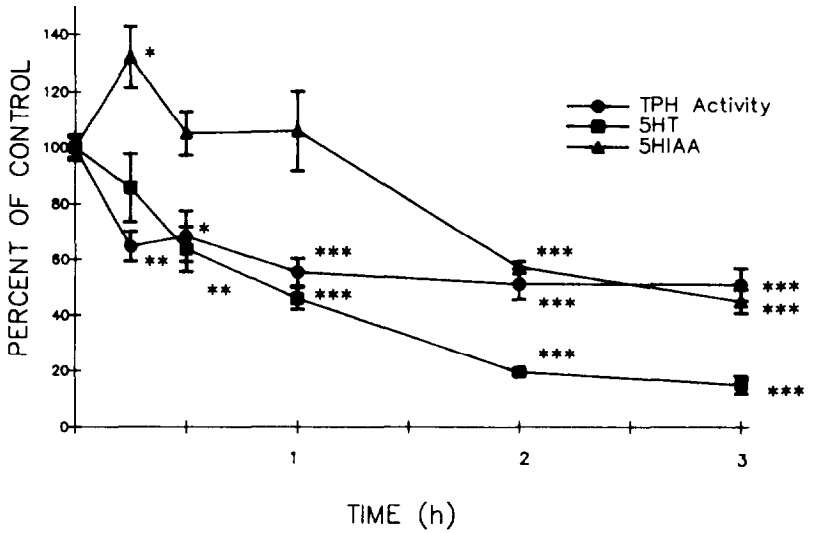

FIGURE 3. *Detailed timecourse of serotonergic changes in the cerebral cortex after the administration of 10 mg/kg MDMA*

*p<0.05 compared to control.

**p<0.01 compared to control.

***p<0.001 compared to control.

NOTE: All data presented as mean ±SEM.

In attempting to determine the mechanism responsible for this loss of enzyme activity, a number of possibilities have been examined. Direct addition of MDMA to brain homogenates was without effect on TPH activity (Schmidt and Taylor 1987) as has been demonstrated previously for a number of amphetamine analogs, including PCA (Knapp et al. 1974). The results of attempts to incubate P2 synaptosomes with MDMA were deemed unreliable due to a large decrease in the activity of synaptosomal TPH upon incubation. The activity of the control synaptosomes incubated for 2 hours at 37 °C was consistently 40 to 50 percent of the activity measured in unincubated synaptosomes. Although, under these conditions, MDMA increased 5-HT release, as evidenced by a decrease in synaptosomal 5-HT concentrations, there was no further effect on TPH activity (table 1). The use of superfused cortical slices was found to stabilize TPH activity in prolonged incubations compared to synaptosomes; however, MDMA was still without effect on enzyme activity even after exposure to concentrations as high as 250 μM for 2 hours (Schmidt and Taylor 1988). In a final attempt to reproduce the effect of MDMA on TPH activity *in vitro,* we selected a

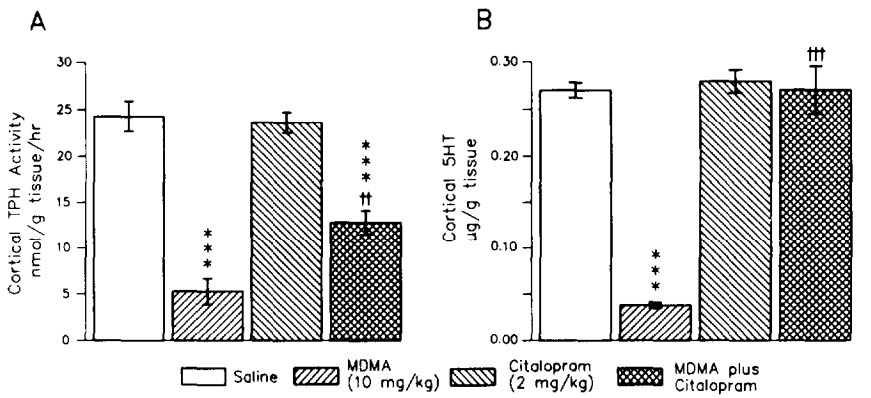

FIGURE 4. *Effect of inhibition of the 5-HT uptake carrier by citalopram on the MDMA-induced changes in cortical TPH activity and 5-HT concentrations 3 hours post-MDMA*

***p<0.001 compared to control.

††p<0.01 compared to MDMA.

†††p<0.001 compared to MDMA.

NOTE: All data presented as mean ± SEM.

mouse mast cell line, P815, to test the effects of MDMA in a cell culture system. These cells have been characterized as containing both 5-HT and a high level of TPH activity. The inset of figure 5 shows this activity was easily measured in our assay. However, as also shown in the figure, incubation of P815s with a high concentration of MDMA (250 µM) for 18 hours had no measurable effect on TPH activity in the cells (Schmidt and Taylor 1988).

The inability to demonstrate an effect of MDMA on TPH activity *in vitro* seemed to point to a requirement for intact neuronal circuitry or *in vivo* metabolism of the drug. We therefore attempted to determine if a direct effect of MDMA in the rat CNS could be achieved by local administration of the drug directly into the brain. The injection sites were selected to include the most likely sites of action in the brain, Figure 6 shows enzyme activity was not altered in the right cerebral cortex 3 hours after 300 µg of MDMA were stereotaxically injected into the right cerebral ventricle. Cortical 5-HT and 5-HIAA concentrations were also unaffected by this treatment, as were transmitter concentrations and enzyme activity in the striatum and hippocampus. Injections of the same dose of MDMA into the substantia nigra and near the serotonergic cell bodies of the dorsal raphe yielded similar results (Schmidt and Taylor 1988). These injections were performed

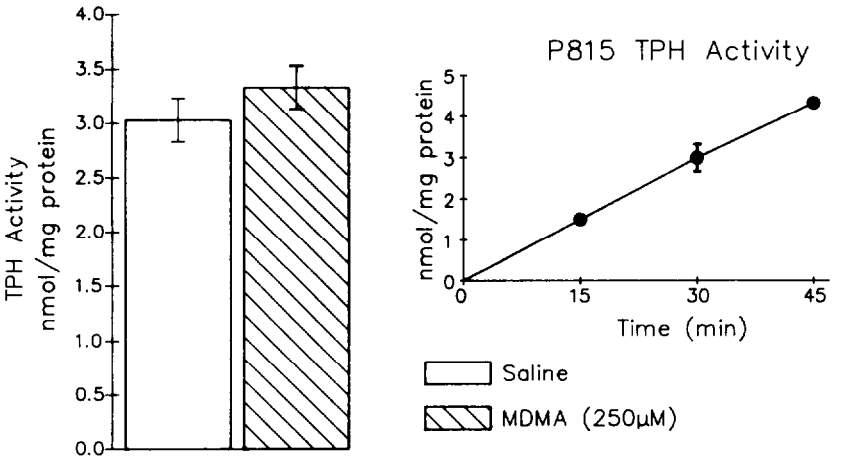

FIGURE 5. *Lack of effect of MDMA on TPH activity in the mouse mastocytoma cell line, P815*

NOTE: Cells were incubated for 18 hours in 250 μM MDMA prior to assay. Enzyme activity was linear with respect to time under the culture conditions used, as shown at the right. Data presented as mean ± SEM.

under halothane to allow rapid recovery from the anesthesia and observation of the animals. Surprisingly, there were no obvious behavioral differences between saline- and MDMA-injected rats. In the absence of any behavioral effect of MDMA, the results from these experiments were considered inconclusive as evidence for or against a direct central effect of MDMA. This prompted us to set up experiments using a constant intracerebroventricular infusion of MDMA to insure that brain concentrations of the drug were maintained for a behaviorally relevant period of time. In the established design, conscious rats were continuously infused with either MDMA or saline for a l-hour period, after which they were observed for an additional 2 hours prior to sacrifice. Using this approach, doses as low as 300 μg produced significant changes in regional TPH activity (figure 7). The latter quantity, corresponding to a dose of approximately 1 mg/kg, was without effect on TPH activity in any of the three brain regions examined, when given peripherally by the subcutaneous route (Schmidt and Taylor 1988). Although this seems a high dose for direct central administration, it is consistent with data reported by Marquardt et al. (1978) showing that 10 percent of a peripherally administered dose of methylenedioxyamphetamine (MDA) was present in the rat brain within 30 minutes of injection. Assuming that the distribution of MDA and MDMA are similar, this means

TABLE 1. *Effect of MDMA on TPH, 5-HT, and 5-HIAA*

	TPH (nmol oxidized/mg/h)	5-HT (ng/mg)	5-HIAA (ng/mg)
Frozen control	0.530		
Incubated control	(0.218± 0.052) 100 ± 23.9	(2.36 ± 0.02) 100 ± 1.0	(2.03 ± 0.14) 100 ± 6.9
1 μM MDMA	85.8 ± 7.3	49.6 ± 5.9	103 ± 12.8
10 μM MDMA	88.1 ± 2.3	25.0 ± 1.7	59.1 ± 3.9

NOTE: P2 synaptosomes were incubated in a Krebs Ringer bicarbonate buffer for 2 hours at 37 °C. The frozen control was not incubated. Values for MDMA-treated samples are given as a percent of the incubated control ± SEM.

that peripheral administration of 10 mg/kg of MDMA could be expected to yield even higher brain concentrations of the drug than were achieved with the infusion of 300 μg over 1 hour. These results therefore indicate that the acute effect of MDMA on TPH activity in the rat is a centrally mediated event requiring sustained, high brain concentrations of the drug. The lipophilicity of MDMA apparently precludes maintaining such concentrations when the drug is rapidly administered directly into the brain. Although these results exclude a peripheral metabolite of MDMA as the causative agent in its acute effect on TPH activity, they do not eliminate a role for a central metabolite. The ultimate cause of this effect of MDMA and related drugs therefore remains to be determined.

LONG-TERM EFFECTS

A clear differentiation of the acute and long-term effects of MDMA was first accomplished by comparing the neurochemical effects of the optical isomers of MDMA at 3 hours and 1 week. As shown in figure 8, either enantiomer of MDMA produced the acute depletion of 5-HT. but only rats treated with the (+)isomer still showed depletion 1 week later. There was also a significant reduction in the uptake of [^{3}H]5-HT into synaptosomes prepared from the latter group of animals (Schmidt 1987a). Hence, the neurotoxic effect of MDMA is primarily a property of the (+)stereoisomer, while the acute effect of MDMA has less stringent stereochemical requirements. In addition, the results with (-)MDMA indicate that the acute effect of the drug on 5-HT concentrations is not permanent, since in the absence of neurotoxicity the depletion of the transmitter produced by (-)MDMA is reversed by 1 week. In addition to comparing the enantiomers *in vivo,* their effects on neurotransmitter release *in vitro* were also compared. As shown in figure 9, (+)MDMA was more potent than (-)MDMA at releasing either

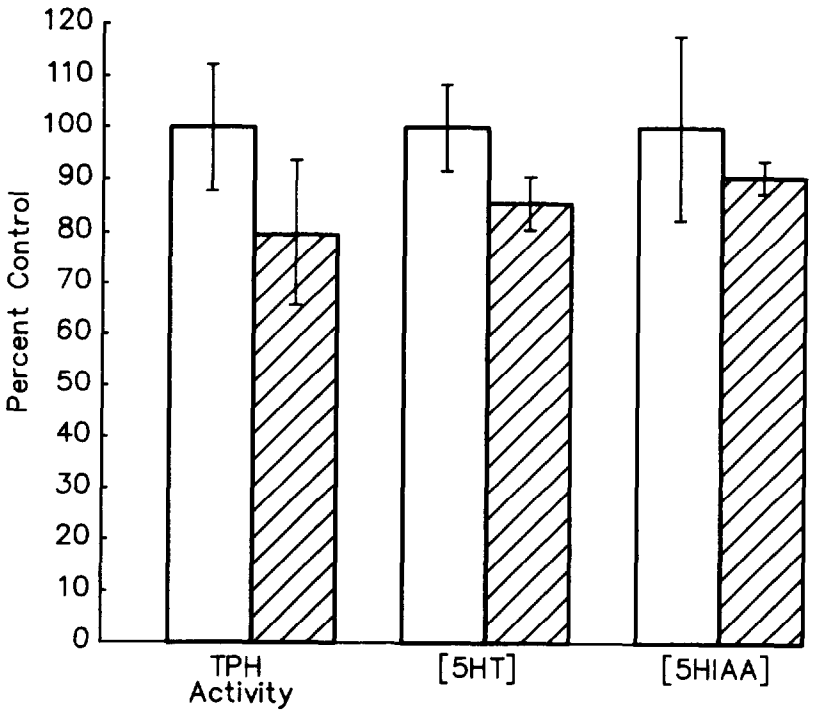

FIGURE 6. *Lack of effect of intracerebroventricular MDMA (300 μg) on serotonergic parameters in the cerebral cortex 3 hours after administration by bolus injection*

NOTE: Data presented as mean ± SEM.

[^{3}H]DA or [^{3}H]5-HT, although the difference between the enantiomers was less marked for the release of [^{3}H]5-HT.

A comparison of the acute and long-term effects of MDMA with those of its homologs MDA and N-ethyl-methylenedioxyamphetamine (MDE) also contrasts the acute and neurotoxic effects of these compounds. It has previously been demonstrated that all three drugs produce the acute depletion of 5-HT measured at 3 hours (Schmidt 1987b). However, as shown in figure 10, if the animals are allowed to survive for 1 week after drug administration, only MDA- and MDMA-treated rats show the reduction in 5-HT concentrations and [^{3}H]5-HT uptake indicative of neurotoxicity. Therefore, the depletion of 5-HT produced at 3 hours by MDE was completely reversible. These results are similar to our observations with the

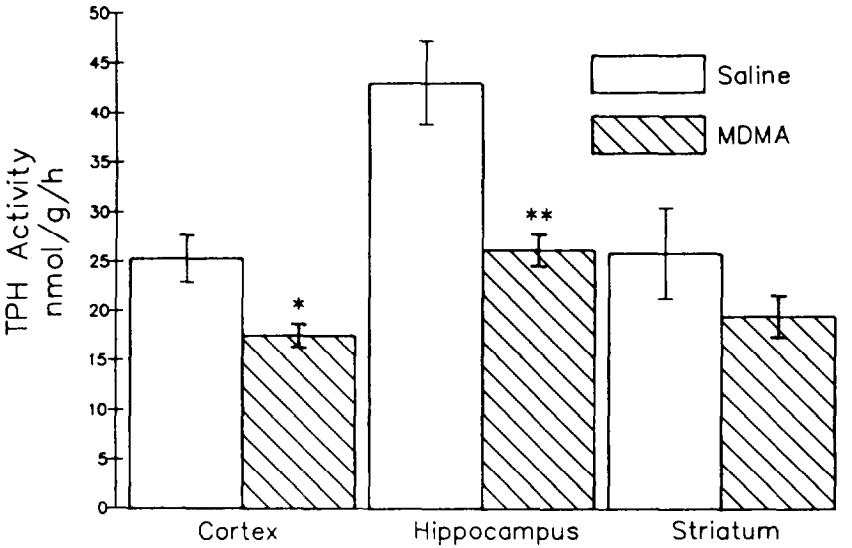

FIGURE 7. *Reduction in regional TPH activity 3 hours after the start of a 1-hour intracerebroventricular infusion of MDMA (300 μg)*

*p<0.05 compared to control.

**p<0.01 compared to control.

NOTE: Data presented as mean ± SEM.

(-)stereoisomer of MDMA. *In vitro,* the three methylenedioxy analogs were very similar in terms of [^{3}H]5-HT release, but differed in their potency for releasing [^{3}H]DA. Here, the order from most to least potent was MDA>MDMA>MDE (figure 11).

A final experiment demonstrating the distinction between the acute and neurotoxic effects of MDMA is shown in figure 12. In this case, the 5-HT uptake inhibitor fluoxetine was administered at various times after MDMA, with all animals being sacrificed 1 week later. The results are shown as a percentage of control cortical 5-HT concentrations. Simultaneous administration of an uptake inhibitor with MDMA completely blocked the decrease in 5-HT concentrations measured 1 week later. However, administration of the inhibitor 3 hours after MDMA still resulted in complete protection from the neurotoxicity. Approximately 50 percent of the depletion could still be blocked 6 hours after MDMA; by 12 hours, the administration of fluoxetine no longer had any effect. Blockade of the neurotoxicity by an uptake inhibitor 3 hours after MDMA clearly differentiates the acute and long-term effects of MDMA, since at this point the acute depletion of 5-HT is already at a maximum. The administration of fluoxetine to MDMA-treated animals

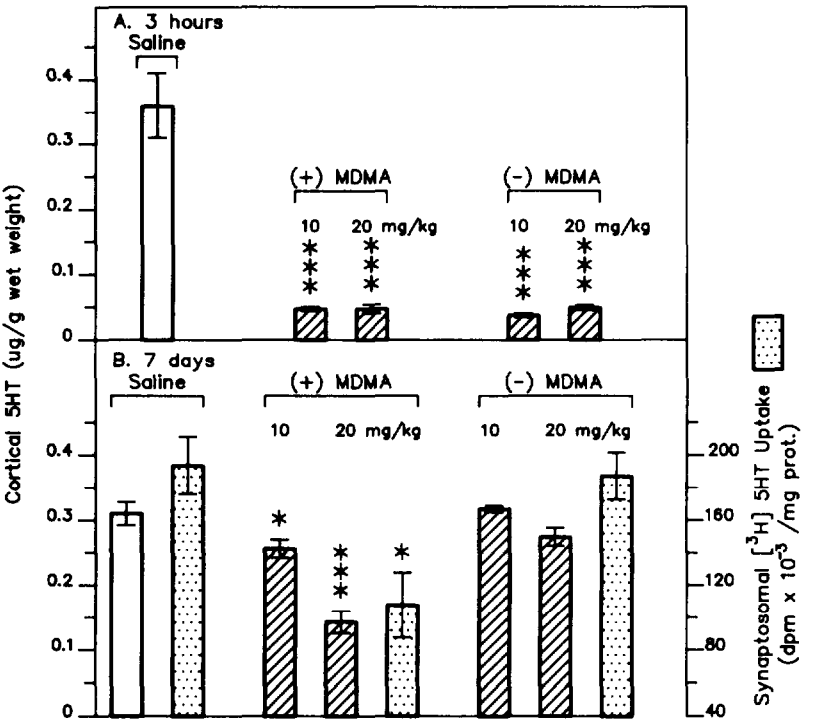

FIGURE 8. *Comparison of the optical enantiomers of MDMA for the acute (3 hours) and long-term (7 days) effects on the serotonergic system*

*p<0.05 compared to saline.

***p<0.001 compared to saline.

NOTE: Data presented as mean ± SEM.

at 3 hours could be considered analogous to the administration of MDE alone, where an acute effect on 5-HT is observed without the subsequent development of neurotoxicity. It is also apparent from these data that a second or later carrier-mediated event is important in the production of the neurotoxicity of MDMA. The critical phase of activity on the part of the carrier leading to the neurotoxic response is occurring some time between 3 and 12 hours post-MDMA.

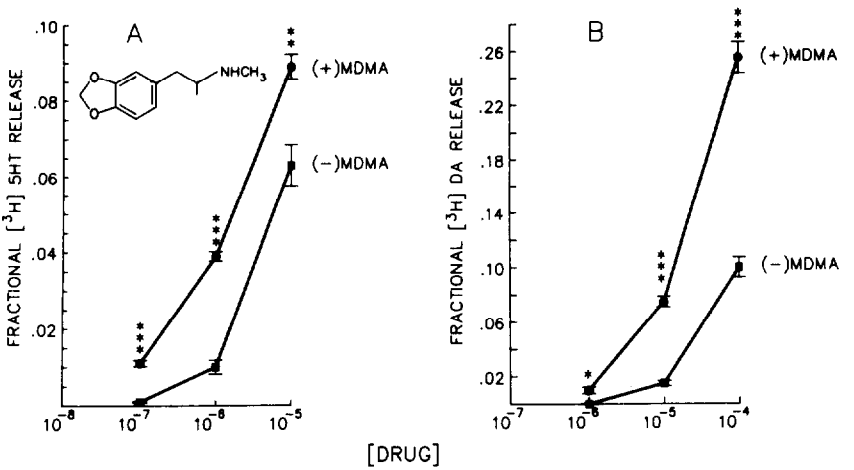

FIGURE 9. *Comparison of the optical enantiomers of MDMA for the release of tritiated monoamines from preloaded striatal slices superfused* in vitro

*p<0.05.

**p<0.01

***p<0.001.

NOTE: Data presented as mean ± SEM.

DISCUSSION

The results of the preceding set of experiments may identify several features of the underlying mechanisms responsible for the acute and long-term effects of MDMA. For example, it is apparent that the production of the acute effect of MDMA has less stringent stereochemical requirements than does the production of neurotoxicity. While both enantiomers of MDMA cause the rapid depletion of transmitter concentrations as well as the depression in TPH activity (Schmidt and Taylor 1988). only the (+)stereoisomer produces neurotoxicity at the doses used in these experiments. In a similar manner, all three methylenedioxy compounds produce the acute interruption of serotonin synthesis, yet only the two lower n-alkyl homologs caused the long-term effect. Therefore, in selecting what ultimately leads to the acute effect of the drug(s) on serotonergic neurons, those activities least influenced by stereochemistry and affected equally by either the desmethyl or N-ethyl homolog of MDMA would be the most likely candidates. The lack of structural stringency characteristic of the acute effect is also observed for the release of 5-HT *in vitro.* The role of carrier-mediated

5-HT release in the acute depletion of 5-HT has already been discussed. It may be that a rapid increase in 5-HT elicited by MDMA and its analogs is also involved in the inactivation of TPH. In contrast to the acute effect of MDMA on 5-HT synthesis, the reduction in 5-HT concentrations and the uptake of [^{3}H]5-HT measured at 1 week after drug administration is less

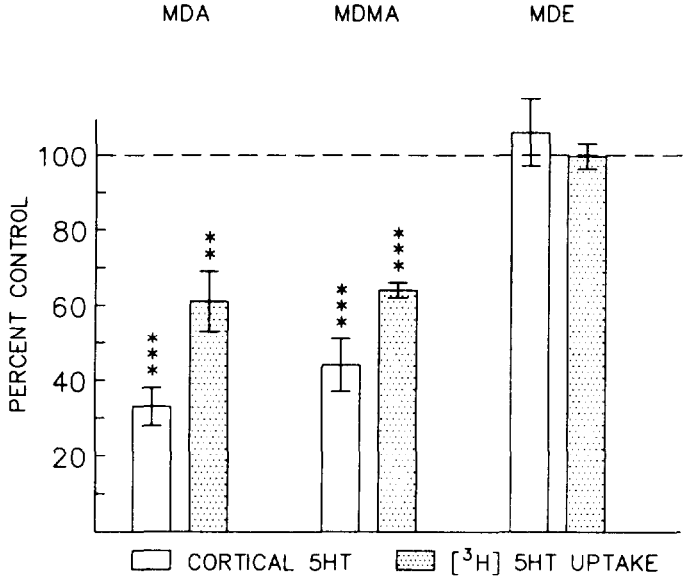

FIGURE 10. *Comparison of the neurochemical effects of the three MDA homologs 7 days after the administration of 20 mg/kg of each drug to rats*

**p<0.01 compared to saline.

**p<0.001 compared to saline

NOTE: Data presented as mean ± SEM.

likely to be a direct result of 5-HT release. since there is little difference in the ability of either (+) or (-)MDMA to produce such release and virtually no difference between the three methylenedioxy homologs. The long-term effects of the three drugs do actually correspond to their potency for producing DA release, however. Both the release of [^{3}H]DA and neurotoxicity follow the same rank order. Similarly, in comparing the enantiomers of MDMA, the stereochemical specificity of the neurotoxicity is the same as that of DA release.

Based upon the above considerations, it is hypothesized that the acute effect of MDMA and its analogs is due to a rapid and sustained increase in the

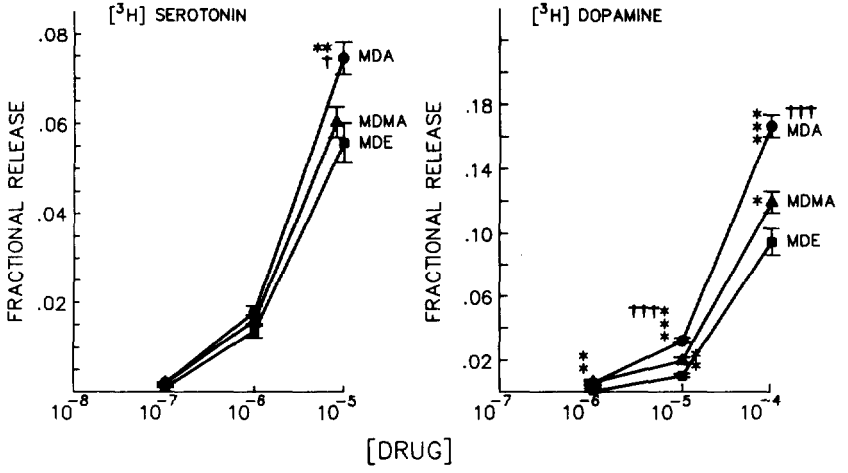

FIGURE 11. *Comparison of the three MDA homologs for the release of tritiated monoamines from preloaded striatal slices super-fused* in vitro

*p<0.05 compared to MDE.

**p<0.01 compared to MDE.

**p<0.001 compared to MDE

†p<0.05 compared to MDMA.

†††pc<0.001 compared to MDMA.

NOTE: Data presented as mean ± SEM.

carrier-mediated release of 5-HT. In contrast, the neurotoxic effect of MDMA and its analog, MDA, may be due to a sustained elevation in the release of DA. In this regard, Sharp et al. (1986) have recently compared the increase in extracellular DA and 5-HT measured by intrastriatal dialysis after PCA administration. Their results show that the release of DA is much more pronounced than that of 5-HT and has a much longer duration. Extracellular DA concentrations 3 hours after injection were approximately tenfold higher in PCA-treated (5 mg/kg) rats when compared to saline-injected controls. In contrast, by 3 hours, extracellular 5-HT concentrations were only two to three times greater than control. Extrapolation of these data to MDMA suggests that, during the period in which the neurotoxicity of MDMA is developing, i.e., 3 to 12 hours after MDMA, extracellular DA is still abnormally elevated. Yamamoto and Spanos (1988) have recently demonstrated that DA release after a neurotoxic dose of MDMA (10 mg/kg) is still elevated severalfold, 3 hours after drug administration.

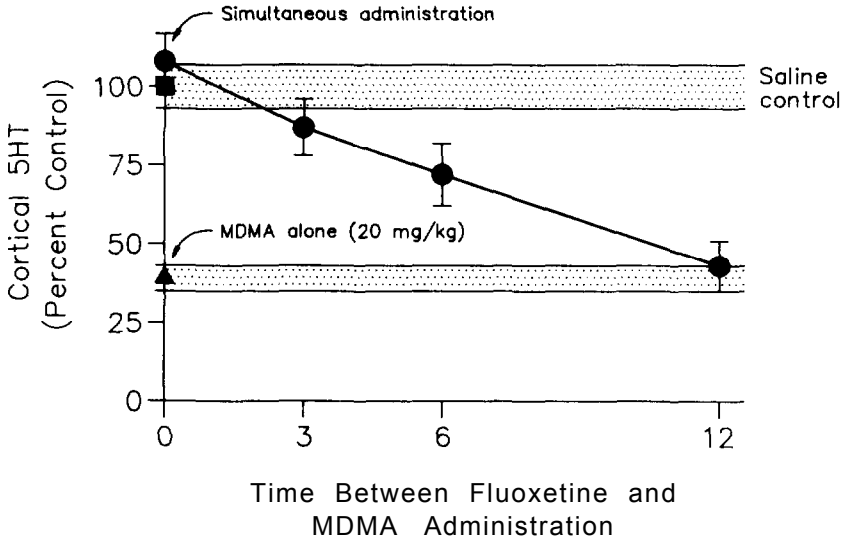

FIGURE 12. *Timecourse for the antagonism of MDMA-induced neurotoxicity by the 5-HT uptake inhibitor fluoxetine*

NOTE: The inhibitor (5 mg/kg) was administered at the indicated times after MDMA, and all animals were sacrificed 7 days later. Data are presented as a percentage of the appropriate control (saline or fluoxetine alone), mean ± SEM.

CONCLUSION

These studies have characterized both the acute and long-term neurochemical effects of a single administration of MDMA in the rat. The acute depletion of 5-HT concentrations results from an as yet unexplained loss of TPH activity in the serotonergic nerve terminals, coupled with a massive carrier-mediated efflux of transmitter. The long-term depression of 5-HT concentrations by MDMA is due to an apparent degeneration of serotonergic nerve terminals. In its pattern of neurochemical effects, MDMA resembles the selective serotonergic neurotoxin PCA, which may suggest a common mechanism. Unfortunately, the mechanism responsible for the neurotoxicity of PCA has been difficult to elucidate in spite of the number of studies that have addressed this issue. MDMA therefore joins a well-studied group of amphetamine analogs including amphetamine itself, methamphetamine, PCA, and fenfluramine, which have in common an unexplained neurotoxic effect on monoaminergic neurons in laboratory animals. It is hoped that the increased interest in this area generated by MDMA and its well-publicized abuse will provide the impetus to resolve the question of amphetamine neurotoxicity.

DISCUSSION

QUESTION: Did you try infusion for less than 1 hour? The acute TPH changes occur as soon as 10 or 15 minutes after single administration, so wouldn't you expect to see changes following a 10- or 15-minute infusion?

ANSWER: You might, but I am not absolutely sure of that because the timecourse of the levels of the drug in brain is going to differ from those infusion paradigms, what you get with peripheral administration of the drug.

Based on the fact that at 3 hours with a very low dose of MDMA, I saw an effect on the enzyme and no effect on the transmitter levels, we probably can't pay too much attention to that timecourse and expect to see changes that are identical to what you see in the whole animal with peripheral administration. That is what I would imagine. The experiments could be done, though. They are not that difficult. You just stick the cannulas in and infuse.

QUESTION/COMMENT: Is there any information on the half-lives of the two enantiomers of MDMA in the brain? What about the half-lives of MDA versus MDMA versus MDE?

My point is whether differing potencies, in regard to long-term effects, might be accountable on the basis of the duration that the compounds persist in the brain rather than intrinsic activities involving release of one or another neurotransmitter.

ANSWER: Yes, that is a very good possibility. The difference between PCA and amphetamine comes down to the fact that parachlorination of that compound makes it persist in the brain longer and you go from something like amphetamine, which has small neurotoxic effects on serotonin, to parachloroamphetamine, which is very neurotoxic. So your point is well taken and that is a possibility, I think.

QUESTION: To follow up on what you said about parachloramphetamine, I think that the importance of that metabolism is even clearer if you compare 4-chloroamphetamine to 3-chloroamphetamine, where those compounds are very different in their long-term effects, but become identical in rats that are pretreated with drugs to block the ring hydroxylation. If you make them equal metabolically, you make them equal in terms of their long-term effects on serotonin. You can do that also by going to the guinea pig, which doesn't parahydroxylate. 3-chloroamphetamine and 4-chloroamphetamine are already equal metabolically and they are equal in terms of their long-term effects, Does the same thing apply to the enantiomers of MDMA and also the analogs of MDMA?

ANSWER: I think that is a very good possibility. It was that sort of thinking that convinced us we really had to do infusion experiments. The drug has to be there for a sufficient amount of time to have these effects.

QUESTION: Did you do those experiments with PCA?

ANSWER: Elaine Sanders-Bush has done those, and the enantiomers do differ, but the enantiomers also differ in half-life, and it is probable that the difference in long-term toxicity is simple because of that difference in half-life.

COMMENT: I know Larry Steranka did that comparison with amphetamine, but I don't recall the results.

COMMENT: We have looked at tritiated MDA and MDMA clearance from brain, and we haven't seen much of a difference over a 24-hour period. The tritium concentrations peaked in brain at about 45 minutes, leveled off for a few hours, and then were gone by 24 hours. There was some indication that more MDA got into the brain than MDMA, but these are very preliminary experiments.

QUESTION: Where is your label?

ANSWER: The label for MDA was on the ring, and that was an important point in terms of assuring that it was not just demethylation.

QUESTION: Were you measuring label or were you measuring specific MDMA? Were you measuring radioactivity or MDMA itself?

ANSWER: We were measuring radioactivity. So far, we have looked at MDA. We haven't seen much metabolism of MDA to any other metabolite. We haven't looked at those experiments with MDMA yet.

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AUTHOR

Christopher J. Schmidt, Ph.D.
Merrell Dow Research Institute
2110 East Galbraith Road
Cincinnati, OH 45215

Effects of MDMA and MDA on Brain Serotonin Neurons: Evidence from Neurochemical and Autoradiographic Studies

Errol B. De Souza and George Battaglia

INTRODUCTION

The drugs 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) are ring-substituted derivatives of methamphetamine and amphetamine, respectively. These methylenedioxy-substituted amphetamines have been reported to exhibit both stimulant and psychotomimetic properties (Anderson et al. 1978; Braun et al. 1980; Shulgin 1986). MDMA has received a great deal of attention recently, since it represents one of a number of "designer drugs" that have been increasingly abused among certain segments of the population, especially college students. MDMA has been the subject of a recent scientific and legal debate, as several psychiatrists have reported that MDMA may "enhance emotions" and "feelings of empathy" and thus serve as an adjunct in psychotherapy (Greer and Tolbert 1986). Recent data demonstrating that MDMA is self-administered in nonhuman primates (Beardsley et al. 1986; Lamb and Griffiths 1987) suggest that the drug may have high abuse potential in man. These reports are particularly disturbing, as the authors and others have recently demonstrated that MDMA is a potent neurotoxin that appears to cause selective degeneration of brain serotonin neurons (Battaglia et al. 1987; Battaglia et al. 1988; Commins et al. 1987; Schmidt 1986; O'Hearn et al. 1988) comparable to that reported for its structural analog MDA (Battaglia et al. 1987; Ricaurte et al. 1985; Stone et al. 1986).

This chapter describes data on the neurotoxic effects of MDMA on brain monoamine systems in rodents. Specifically, studies are described examining the effects of *in vivo* administration of MDMA on brain monoamine systems with respect to:

(1) the selective neurodegenerative effects on brain serotonin systems;

(2) the effects of dose and frequency of drug administration;

(3) the potential neurochemical mechanisms involved in the neurotoxic effects of the drug;

(4) the characteristics and timecourse of recovery following destruction of serotonin neurons;

(5) the relative sensitivity of various animal species; and

(6) the neuroanatomical and morphological specificity of MDMA- and MDA-induced neurotoxicity.

MARKERS OF NEUROTOXICITY

Typically, neurotoxic effects of drugs on monoamine neurons have been assessed from reductions in brain levels of monoamines and their metabolites, decreases in the maximal activity of synthetic enzymes activity, and decreases in the active uptake carrier. In the present study, the traditional markers described above have been used, including the measurement of the content of monoamines and their metabolites in brain at several different timepoints following drug administration. Since reports in the literature have documented that MDMA and MDA can inhibit the activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in serotonin synthesis (Stone et al. 1986; Stone et al. 1987). it is unclear whether MDMA-induced reductions in the content of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) may be due to suppressed neurotransmission in otherwise structurally intact serotonin neurons or may represent the consequence of the destruction of serotonin neurons and terminals.

Since monoamine uptake sites are highly concentrated on their respective nerve terminals (Kuhar and Aghajanian 1973), the authors' approach has been to use selective radioligands to directly label these uptake sites in brain and to assess the neurodegeneration of specific monoamine neurons, by measuring the reductions in the density of their respective uptake sites. For example, the authors have recently reported the feasibility of using the measurement of [^{3}H]paroxetine-labeled serotonin uptake sites to quantify the neurotoxic effects of MDA (Battaglia et al. 1987) and MDMA (Battaglia et al. 1987; Battaglia et al. 1988) on serotonin neurons in homogenates of various regions of rat brain. Visualization of MDMA- and MDA-induced destruction of serotonin axons and terminals using antibodies directed against serotonin (O'Hearn et al. 1988) and autoradiographic studies demonstrating corresponding decreases in [^{3}H]paroxetine-labeled scrotonin uptake sites in slide-mounted brain sections of MDA-treated rats (De Souza and Kuyatt 1987) further validate use of changes in the density of serotonin uptake sites as an appropriate index of serotonin neurodegeneration.

IN VIVO EFFECTS OF MDMA: NEUROCHEMICAL STUDIES

The following studies were designed to assess and quantify both the neuro chemical and neurodegenerative effects of short-term administration of MDMA on monoamine neurons in rat brain.

Dose Dependence

A repetitive treatment regimen (sc injections twice daily for 4 consecutive days) of MDMA at various doses up to 20 mg/kg resulted in dose-dependent decreases in a variety of serotonergic markers in rat frontal cerebral cortex, including serotonin, 5-HIAA, and the density of serotonin uptake sites at 18 hours following the last injection (figure 1). At the lowest dose of MDMA tested (5 mg/kg), serotonin content was markedly reduced (45 percent), while only a small (14 percent), but statistically significant, decrease in the density of serotonin uptake sites was observed; a small decrease in 5-HIAA content was also observed at this dose, although this change was not statistically significant. Higher doses of MDMA (10 and 20 mg/kg) resulted in comparable reductions in 5-HIAA levels (60 to 70 percent), while the decrease in serotonin was significantly greater at 20 mg/kg (90 percent) than at 10 mg/kg (80 percent). The density of serotonin uptake sites decreased progressively as the dose of MDMA was increased, with a maximal reduction of 90 percent observed at 18 hours following administration of 20 mg/kg MDMA.

In contrast, following a treatment regimen of 20 mg/kg MDMA, there were no significant differences in the density of [3H]mazindol-labeled norepine-phrine (NE) uptake sites (fmol/mg protein) in the frontal cerebral cortex between saline-treated (159±17) and MDMA-treated (152±5) animals. With respect to the dose of MDMA, serotonin levels appeared to be more readily decreased (45 percent reduction at 5 mg/kg), while comparable reductions in 5-HIAA levels and serotonin uptake sites were noted only at 10 or 20 mg/kg MDMA. This apparent discrepancy among the three serotonergic markers measured in the present study may relate to effects of lower doses of MDMA on synthetic enzyme activity (i.e., TPH), whereas the effects of higher doses of MDMA in reducing all three markers may relate in part to effects on TPH activity and in part to destruction of serotonin neurons as evidenced by decreases in serotonin uptake sites.

The Effects of Single Versus Multiple Injections of MDMA

Since repeated systemic administration of 10 mg/kg MDMA caused marked neurodegeneration of frontal cerebral cortex serotonin neurons, the authors chose to investigate the neurodegenerative effects of single versus multiple injections of MDMA at this dose. As shown in figure 2, increasing the number of injections of MDMA (10 mg/kg, sc) resulted in significant and progressively greater reductions in serotonin and 5-HIAA content. While

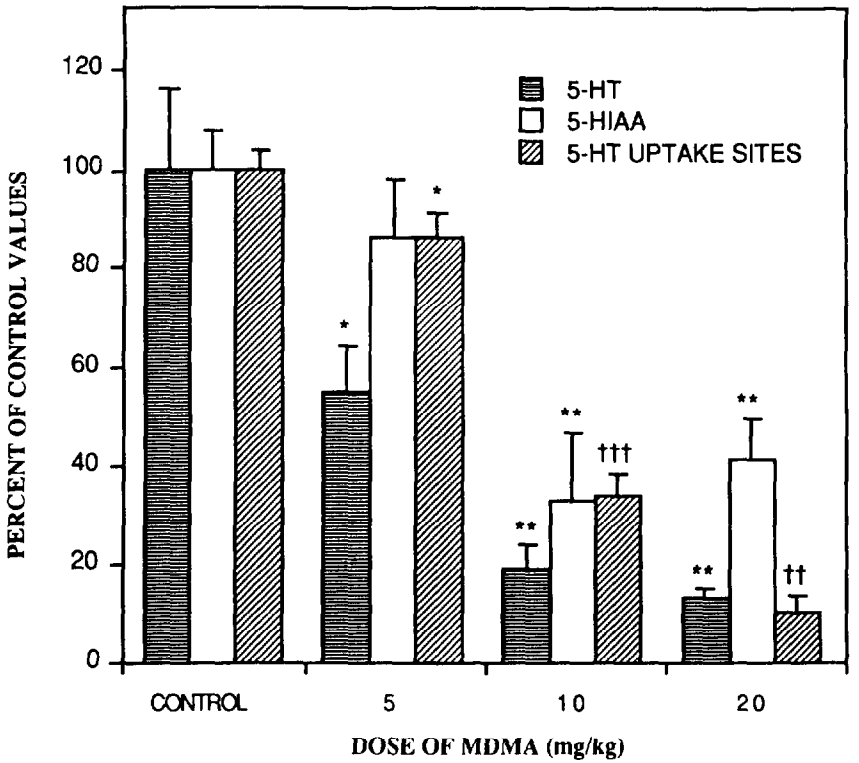


FIGURE 1. *The effect of repeated systemic administration of various doses of MDMA on the content of serotonin (5-HT) and S-HIAA and the density of 5-HT uptake sites in rat frontal cerebral cortex*

*Significantly different from control. $p<0.05$.

**Significantly different from control, $p<0.01$.

††Significantly different from all other MDMA-treated groups. $p<0.01$.

†††Significantly different from all other MDMA-treated groups, $p<0.001$.

NOTE: Rats were administered either saline or MDMA twice a day for 4 consecutive days and sacrificed 18 hours after the last injection. Data represent the mean SEM from three to live rats, expressed as a percent of values in control, saline-injected rats. Control values for 5-HT and 5-HIAA levels were 387±61 and 251±20 pg/mg tissue, respectively. The density of 5-HT uptake sites in the frontal cerebral cortex in controls was 396±15 fmol/mg protein. Data were analyzed by one-way ANOVA and Duncan's multiple range test.

SOURCE: Battaglia et al. 1988.

one injection of MDMA was without effect on any of the serotonergic parameters examined, two doses were sufficient to elicit a significant reduction in serotonin content. As described above, these early effects of MDMA on serotonin content may relate to MDMA suppression of TPH activity. A significant reduction in 5-HIAA content (approximately 34 percent) was observed only after four injections of MDMA. Marked reductions of 84 percent and 75 percent in serotonin and 5-HIAA,

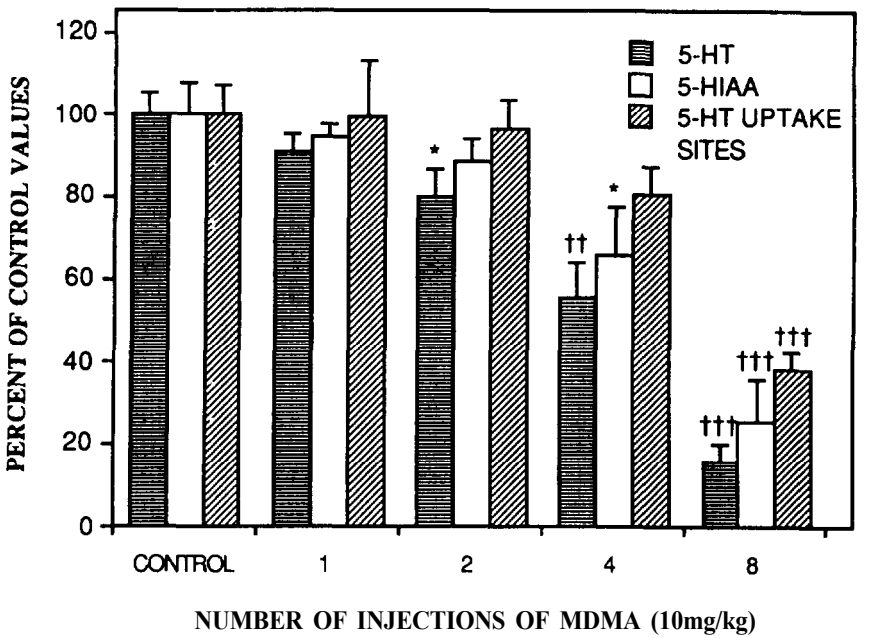


FIGURE 2. *The effects of single and multiple injections of MDMA on the content of serotonin (5-HT) and 5-HIAA and the density of 5-HT uptake sites in rat frontal cerebral cortex*

*Significantly different from control, $p<0.05$.

††Significantly different from all other groups, $p<0.01$.

†††Signiftcantly different from all other groups, $p<0.001$.

NOTE: Rats were injected the specified number of times with either saline or 10 mg/kg MDMA and sacrificed 18 hours after the last injection. Data represent the mean and SEN from three to five animals, plotted as percent of respective values for each marker in control, saline-injected rats. Control levels of 5-HT and 5-HIAA were 475±24 and 332±24 pg/mg tissue, respectively. The density of 5-HT uptake sites was 349±24 fmol/mg protein in control. Data were analyzed by one-way ANOVA and Duncan's multiple range test.

SOURCE: Battaglia et al. 1988.

respectively, were observed following an eight-injection regimen of 10 mg/kg MDMA; the density of [^{3}H]paroxetine-labeled serotonin uptake sites was also significantly decreased (approximately 64 percent) following eight injections of MDMA at this dose. These data suggest that a longer treatment regimen may be required for destruction of serotonin neurons, while effects on the content of serotonin and 5-HIAA may occur following fewer injections.

There was no change in the content of dopamine (DA) in any of the experimental groups; however, a small, inconsistent decrease in NE content (approximately 20 percent) was observed in all MDMA-treated rats. This small change in NE following MDMA treatment was not accompanied by a reduction in the density of [^{3}H]mazindol-labeled NE uptake sites.

Species Differences

Since amphetamines have been shown to be metabolized by different pathways in rat, mouse, and guinea pig (Caldwell 1980). studies were carried out to investigate whether MDMA induced neurotoxicity could be demonstrated in other species such as mouse and guinea pig. Animals in these studies were treated twice daily for 4 consecutive days with 20 mg/kg MDMA. and levels of serotonin, 5-HIAA, and serotonin uptake sites were measured at 7 days following the last injection, to assess the long-term effects of the treatment. As shown in figure 3, MDMA caused comparable and marked decreases in serotonin and 5-HIAA content and in the density of serotonin uptake sites in rat and guinea pig cerebral cortex, but appeared to be without effect on any of these serotonergic markers in the mouse. Other studies (Stone et al. 1987) have also suggested that mice are less susceptible to the neurotoxic effects of MDMA. Similar differences in the sensitivity to the neurotoxic effects of parachloroamphetamine on serotonin neurons have been observed in mouse when compared to its effects in rat and guinea pig (Fuller 1978; Kohler et al. 1978; Sanders-Bush and Steranka 1978). The differential sensitivity may be due, in part, to species-dependent differences in the half-life of the drug (Steranka and Sanders-Bush 1978). Active neurotoxic metabolites or metabolic intermediates of parachloro-amphetamine have been postulated previously as being responsible for its neurotoxic effects on serotonin neurons (Gal and Sherman 1978; Sanders-Bush and Steranka 1978), although to date no active neurotoxic species has been identified. Although there has been no direct demonstration of a neurotoxic metabolite of MDMA, some preliminary data suggest that an active metabolite of MDMA may be responsible for eliciting its ncurotoxic effects. The authors have previously reported that, in contrast to the marked neurodegenerative effects on brain serotonin neurons following systemic administration of MDMA or MDA, single, direct intracerebral injections of MDMA or MDA were without effect on cerebral cortical serotonin neurons, as visualized using immunohistochemistry (Molliver et al. 1986). This observation of marked species differences and sensitivity

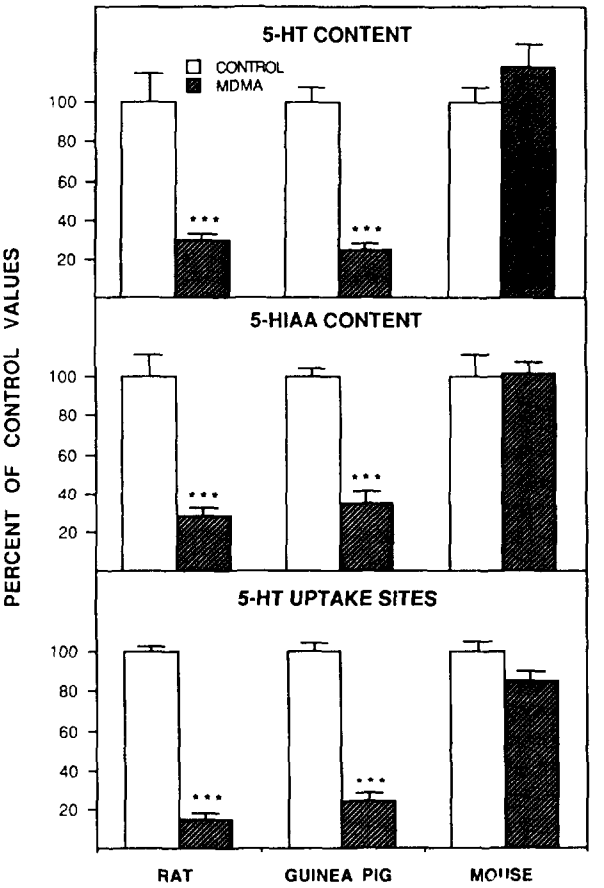


FIGURE 3. *The effects of repealed systemic adminisration of MDMA on content of serotonin {5-HT) and 5-HIAA, and density of 5-HT uptake sites in rat, guinea pig, and mouse frontal cerebral cortex*

**Signifiantly different from control, p<0.001.

NOTE: Animals were treated with saline or 20 mg/kg MDMA twice a day for 4 consecutive days and sacrificed 7 days after the last injection. Data represent ± SEM of five animals per group, expressed as percent of saline-injected control values in respective species. In rat, guinea pig, and mouse, control values of 5-HT were 275±41, 296±14, and 449±36 pg/mg tissue, respectively; control values of 5-HIAA were 345±40, 92±4. and 319±34 pg/mg tissue, respectively; control values of 5-HT uptake site were 397±10, 216±6, and 233±12 fmol/mg protein, respectively. Data were analyzed by Student's *t*-test.

SOURCE: Battaglia et al. 1988.

to MDMA-induced serotonin neurotoxicity would be consistent with the hypothesis of a peripherally produced neurotoxic metabolite of MDMA.

In more recent studies, it has also demonstrated that administration of 2.5 or 10 mg/kg MDMA twice daily for 4 consecutive days resulted in neurotoxic effects in rhesus monkeys, with decreases in the density of scrotonin uptake sites occurring at the higher dose (Johannessen et al. 1988). The neurotoxic effects of MDMA observed in primates included reductions in the content of serotonin and 5-HIAA and marked reductions in the ccrebrospinal (CSF) concentrations of 5-HIAA levels that were observed following drug administration. These fmdings and other reports of neurotoxic effects of MDMA in primates (Ricaurte et al. 1988) raise serious concerns for its potential hazard in humans.

Potential Mechanisms

Since the neurotoxic effects of drugs such as parachloroamphetamine on serotonin neurons can be prevented by serotonin uptake blockers (Ross 1976; Sanders-Bush and Steranka 1978). the possibility that serotonin uptake carrier protein was likewise involved in the neurotoxic effects of MDMA was investigated. As shown in figure 4, pretreatment of rats with the selective serotonin uptake blocker citalopram (10 ml/kg), prior to each injection of 10 mg/kg MDMA, resulted in nearly complete protection against the neurotoxic effects of MDMA. Citalopram-pretreated rats exhibited only a 15 percent decrease in serotonin uptake sites. No significant alterations in the content of serotonin and 5-HIAA were observed following MDMA treatment, in comparison with 60 to 80 percent reductions in the serotonergic parameters observed in rats treated with an identical dose of MDMA alone.

The data described above demonstrate that destruction of serotonin axons by MDMA involves the serotonin active uptake carrier and that administration of citalopram, a selective serotonin uptake blocker, prior to administration of MDMA, can prevent the decreases in serotonin markers elicited by MDMA alone. These data are consistent with previous reports for other potent serotonin neurotoxins, demonstrating that pretreatment with serotonin uptake blockers can prevent the neurotoxic effects of parachloroamphetamine (Ross 1976; Sanders-Bush and Steranka 1978). Furthermore, it has been shown that MDMA-induced neurotoxicity can be prevented or reversed if a serotonin uptake blocker such as fluoxetine is administered no later than 12 hours after MDMA treatment (Schmidt 1986).

In previous studies, it has been observed that, in contrast to the marked serotonin neurodegenerative effects following systemic administration of MDMA or MDA, single, direct intracerebral injections of MDMA or MDA are without effect on cerebral cortical serotonin neurons, as visualized using serotonin immunocytochemistry (Molliver et al. 1986). In addition, as

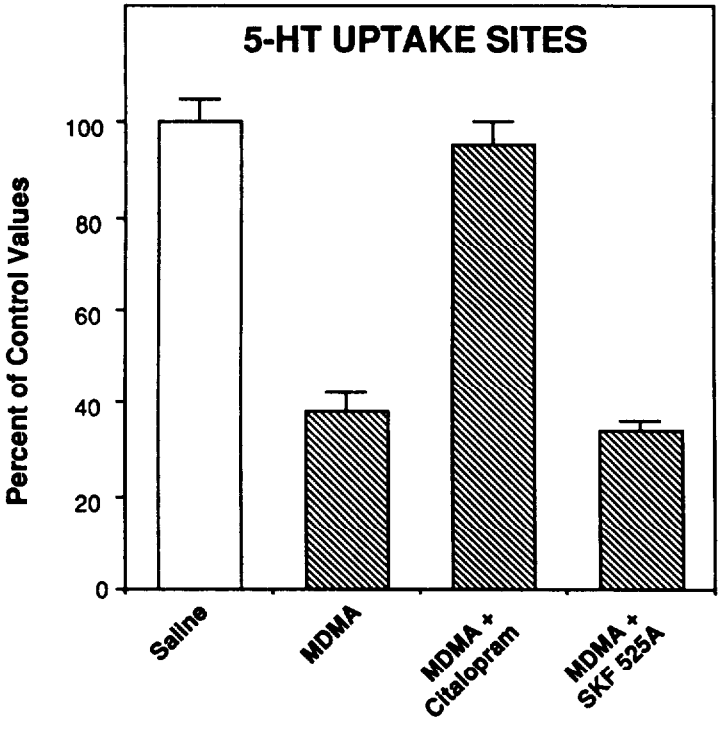


FIGURE 4. *The effect of repeated systemic administration of 10 mg/kg MDMA, MDMA plus 10 mg/kg citalopram, and MDMA plus 25 mg/kg SKF 525A on the density of serotonin (5-HT) uptake sites in homogenates of rat frontal cerebral cortex*

NOTE: Data are expressed as percent of values in control saline-treated rats and represent the mean ± SEM from four to six animals. Control levels of 5-HT uptake sites were 356±15 fmol/mg protein.

described above, there appears to be a differential sensitivity to the neurotoxic effects of MDMA in different species, which metabolize amphetamines by different pathways. These observations of marked differences in serotonin neurotoxicity to-centrally versus systemically administered MDMA and differences in various species would be consistent with the hypothesis of a peripherally produced neurotoxic metabolite of MDMA or MDA. Since MDMA has been reported to interact with the hepatic microsomal enzyme cytochrome P450 (Brady et al. 1986), the authors investigated whether inhibition of this enzyme would alter the neurotoxic effects of MDMA was investigated. In preliminary studies, it was found that, in rats pretreated with the cytochrome P450 enzyme inhibitor SKF 525A, 45 minutes prior to

each administration of MDMA, there was neither potentiation nor attenuation of the neurodegeneration found following repeated administration of 10 mg/kg MDMA. As shown in figure 4, no changes in the density of serotonin uptake sites were observed between rats treated with MDMA alone and those treated with MDMA plus SKF 525A. Although these data suggest that it is unlikely that the putative neurotoxic species is a cytochrome P450-dependent metabolite of MDMA, the involvement of some other peripheral and/or central metabolite of MDMA or the formation of an MDMA-induced endogenous neurotoxin cannot be ruled out. Additional studies are required to identify the mechanisms responsible for MDMA-induced neurotoxicity.

Regeneration of Serotonin Neurons

A detailed timecourse of recovery of affected serotonin neurons was carried out to investigate whether serotonin neurons regenerate subsequent to their destruction following MDMA treatment. As shown in figure 5, the timecourse of neuronal regeneration was investigated by measuring the recovery of serotonin uptake sites and serotonin levels in rat frontal cerebral cortex at various timepoints up to 12 months following repeated systemic administration (i.e., twice daily sc injections for 4 days) of 20 mg/kg MDMA. At all timepoints up to 6 months during the recovery timecourse, the density of serotonin uptake sites was significantly below the corresponding values in age-matched, saline-treated control rats. At the 6-month timepoint, the density of serotonin uptake sites was only 75 percent of the values of saline-treated controls, whereas by 12 months after MDMA treatment, the density of serotonin uptake sites returned to control levels. The shape of the recovery curve suggests that there may be a faster initial rate of recovery of serotonin uptake sites occurring between 18 hours and 4 weeks, which is followed by a slower rate of recovery between 4 weeks and 12 months. These data indicate that more than 6 months are required for a complete recovery of serotonin uptake sites to control levels.

It was of interest that, in spite of the recovery of serotonin uptake sites to control levels, the content of serotonin in the same brain region remained markedly (40 to 50 percent) below age-matched controls for as long as 1 year after MDMA administration. It is unclear from these data whether there is a regeneration of axons that have previously undergone degeneration or whether the increased density of uptake sites is a consequence of increased collateral sprouting of neurons unaffected by the drug treatment. It is also possible that axonal regeneration and collateral sprouting arc associated with considerably greater densities of uptake sites per neuron, thereby making it more difficult to assess neuronal recovery from this index. The fact that serotonin levels remain 40 to 50 percent below age-matched controls for up to 1 year in spite of normal levels of serotonin uptake sites indicates that, following lesion by MDMA, the serotonin

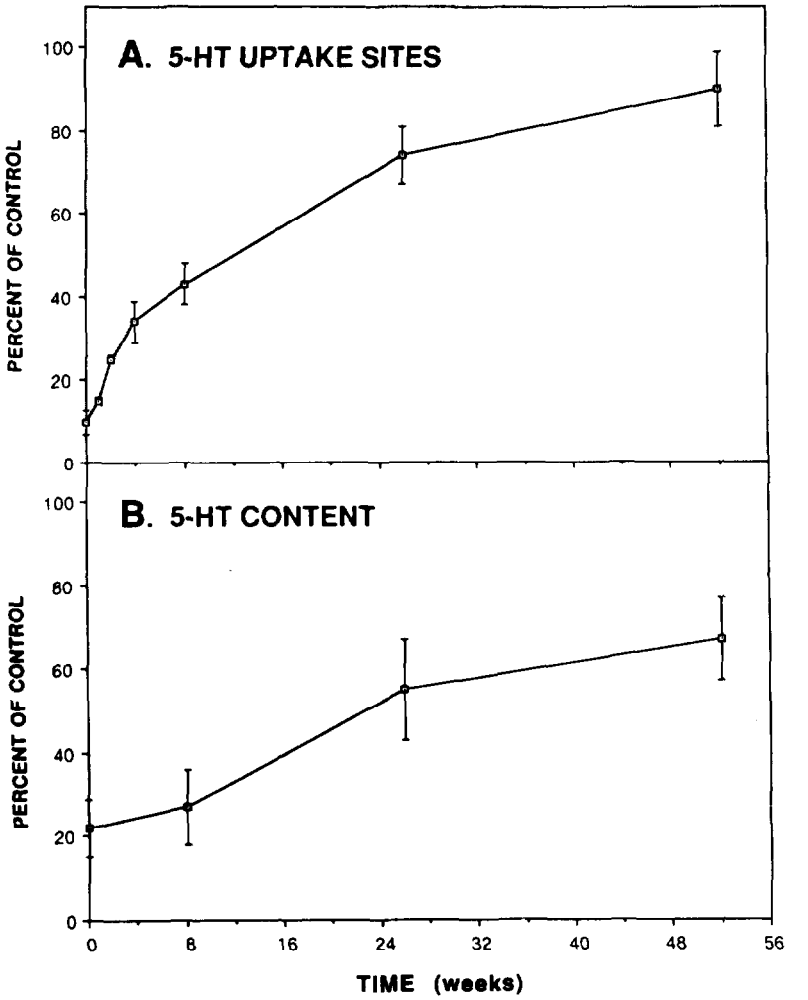


FIGURE 5. *Timecourse of recovery of (A.) serotonin (5-HT) uptake sites and (B.) 5-HT conten in rat cerebral cortex following repeated systemic administration of MDMA*

NOTE: Rats were treated with either saline or 20 mg/kg MDMA twice a day for 4 consecutive days, then sacrificed at various times up to 12 months after the last injection of the drug. Saline-injected control rats were killed at each of the timepoints; data represent the mean ± SEM of five rats per group, plotted as percent of the value of age-matched saline-injected control rats.

SOURCE: Adapted from Battaglia et al. 1988.

neurons that "recover" may not be functionally identical to those present in age-matched control brains.

The persistent neurotoxic effects of MDMA on serotonin neurons is similar to that observed following parachloroamphetamine administration, in which marked reductions in serotonin have been observed up to 4 months after a single injection of parachloroamphetamine (Fuller 1978; Kohler et al. 1978; Sanders-Bush and Steranka 1978). Since neurochemical recovery of serotonin uptake sites and serotonin content have been used, rather than neuroanatomical indices of neuronal regeneration, it is unclear from the present data whether there is actual regeneration of neurons that have previously undergone axon or terminal degeneration or whether the increased density of uptake sites is a consequence of increased collateral sprouting of neurons unaffected by the drug treatment, It has previously been reported that following 5,6-dihydroxytryptamine-induced axotomy, axonal sprouting occurs within 4 to 5 days, and the appearance of new axonal sprouts correlates with the recovery of [^{3}H]serotonin uptake (Bjorklund et al. 1973). Evidence from both immunocytochemical data (O'Hearn et al. 1988) and autoradiographic studies quantifying changes in the density and distribution of [^{3}H]paroxetine-labeled serotonin uptake sites (see figure 10) indicates that serotonin cell bodies appear to be insensitive to the neurotoxic effects of repeated systemic administration of MDMA in rats. The fact that serotonin cell bodies are unaffected by MDMA treatment provides a mechanism by which terminal regeneration of MDMA-affected neurons may occur in rats.

NEUROANATOMICAL AND MORPHOLOGICAL SPECIFICITY OF THE EFFECTS OF MDMA AND MDA

The data described above clearly demonstrate the specific and marked neurodegenerative effects of MDMA on serotonin axons and terminals in the cerebral cortex. Two approaches have been taken to investigate whether the effects of MDMA on brain serotonin neurons are ubiquitous or whether the effects of MDMA show neuroanatomical specificity. The first approach involved the measurements of various monoamines, their metabolites, and monoamine uptake sites in homogenates of discrete areas of rat brain following treatment with MDMA or MDA. Second, autoradiographic techniques were used to visualize the effects of MDMA treatment on the localization and density of [^{3}H]paroxetine-labeled serotonin uptake sites and [^{3}H]mazindol-labeled NE and DA uptake sites in slide-mounted sections of rat brain. In addition, to address further the neurochemical specificity of the effects of MDMA on brain serotonin neurons, effects of MDMA and MDA treatment on the content of DA, NE, and their respective mctabolites and DA and NE uptake sites in homogenates of various brain regions will be described. In some studies, the changes induced by the N-ethyl derivative of MDA (MDE) have been investigated.

Effects of MDMA on Serotonin and 5-HIAA Content and [^{3}H]Paroxetine-Labeled Serotonin Uptake Sites in Discrete Regions of Rat Brain

The effects of repeated systemic administration of MDMA and MDA on various serotonergic parameters were investigated at 2 weeks following the last injection of the treatment regimen previously described (i.e., 20 mg/kg, sc, twice daily for 4 days). As shown in figure 6, MDMA and MDA produced marked decreases in the content of serotonin and 5-HIAA in various brain regions. Both MDMA and MDA caused dramatic decreases in 5-HIAA levels in cerebral cortex, hippocampus, striatum, and hypothalamus (figure 6B). In hypothalamus, the reduction in 5-HIAA levels elicited by MDA was significantly greater ($p<0.05$) than that observed with MDMA (figure 6B). When plotted as a percent of control values in the respective brain regions, it was apparent that, while decreases in 5-HIAA content were observed in all the brain regions examined. the reductions in cerebral cortex and in hippocampus (40 to 60 percent) were greater than those observed in striatum and in hypothalamus (30 to 40 percent). With respect to serotonin levels, marked decreases were observed in cerebral cortex and hypothalamus in both MDMA- and MDA-treated rats (figure 6A). While small decreases were observed in hippocampal and striatal serotonin content following either MDA or MDMA treatment, these reductions were found to be statistically significant only in striatum ($p<0.01$) of MDMA-treated rats. Data calculated as a percent of control serotonin levels in their respective brain regions (figure 6A) indicate a more marked reduction in serotonin in cerebral cortex (40 to 60 percent) than in hypothalamus (18 to 33 percent).

To determine whether changes in serotonin and/or 5-HIAA were a consequence of long-term suppression of serotonergic function in structurally intact neurons or whether MDMA and MDA may be affecting a neurodegenerative process in each of the brain regions, we measured the density of serotonin uptake sites in these brain regions. Both MDMA and MDA caused substantial reductions in the densities of [^{3}H]paroxetine-labeled serotonin uptake sites in all the brain regions examined. The densities of serotonin uptake sites were calculated as a percent of the respective control levels in cerebral cortex, hippocampus, striatum, hypothalamus, and midbrain and are shown in figure 7. Significant reductions (all $p<0.001$) were observed in cerebral cortex (60 to 70 percent), hippocampus (70 to 75 percent), striatum (50 percent), hypothalamus (40 to 50 percent) and midbrain (50 to 60 percent). Interestingly, MDA produced a significantly greater reduction in the density of serotonin uptake sites in cerebral cortex than did MDMA. It has also been observed that MDE causes 40 percent reductions in serotonin uptake sites in cerebral cortex with a comparable treatment regimen, suggesting that this compound may be less toxic than either MDA or MDMA. Scatchard analysis of [^{3}H]paroxetine saturation

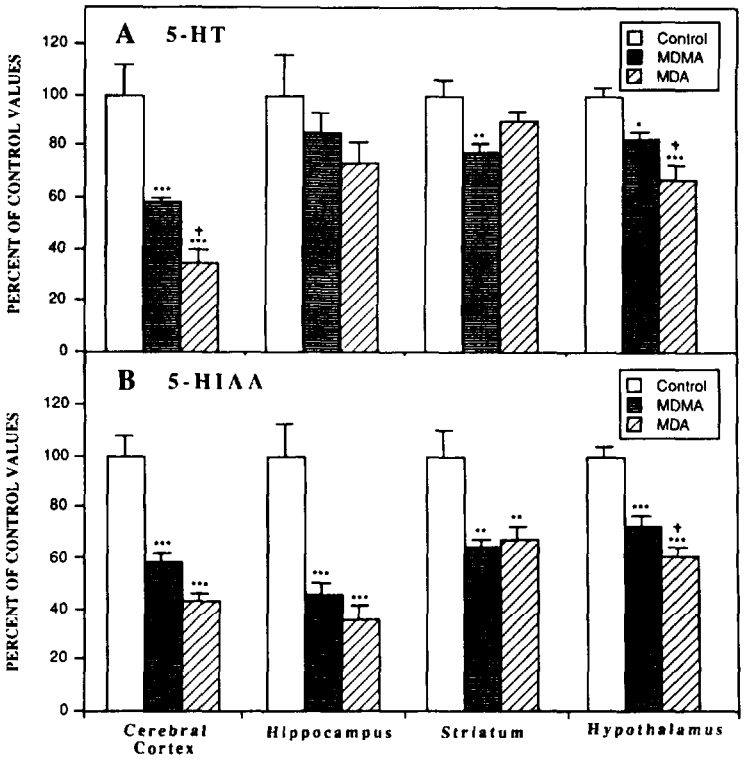


FIGURE 6. *Effect of repeated systemic administration of MDMA and MDA on the concentration of (A) serotonin (5-HT) and (B) 5-HIAA in various brain regions*

*Significantly different from control, $p<0.05$.

**Significantly different from control, $p<0.01$.

***Significantly different from control, $p<0.001$.

†Significantly different from MDMA-treated rats, $p<0.05$.

NOTE: Rats were injected subcutaneously twice daily for 4 days with drug (20 mg/kg) or saline vehicle (1 mL/kg) and sacrificed 2 weeks after the last injection. 5-HT and 5-IIIAA levels were measured using reversed phase HPLC. Data, plotted as percent of control values in each brain region, represent the mean and SEM from four to six control and drug-treated rats. Control values for 5-HT and 5-HIAA in each region were: cerebal cortex, 504±58 and 422±32; hippocampus, 4l0±67 and 684±89; striatum, 363±22 and 492±50; hypothalamus, 1605±55 and 997±42 pg/mg tissue, respectively. Data were analyzed by one-way ANOVA and Duncan's multiple range test.

SOURCE: Battaglia et al. 1987.

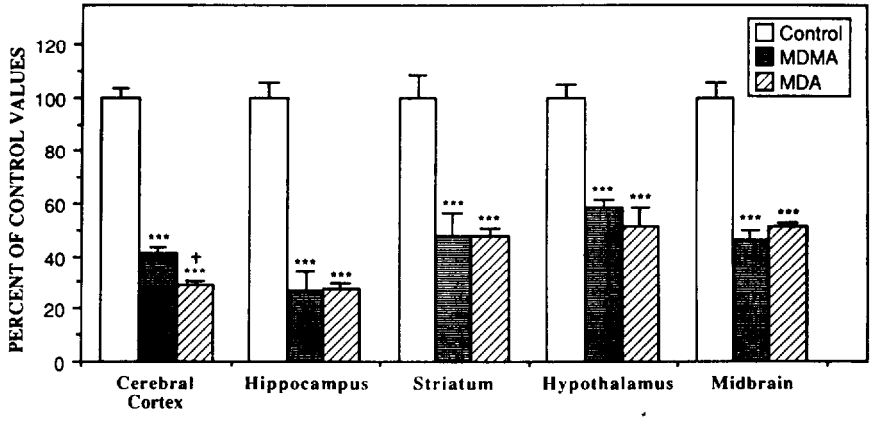


FIGURE 7. *Effect of repeated systemic administration of MDMA and MDA on the density of serotonin (5-HT) uptake sites in various brain regions*

***Significantly different from control. $p<0.001$.

†Significant difference between MDA and MDMA treatments, $p<0.001$.

NOTE: Rats were injected subcutaneously twice daily for 4 days with MDMA or MDA (20 mL/kg) or saline vehicle (1 mL/kg) and sacrificed at 2 weeks a fter the last injection. Values were determincd from saturation studies in each region except striatum and hypothalamus, where the density of 5-HT uptake sites was assessed using a saturating concentration (0.25 nM) of [^{3}H]paroxetine. No significant different from control K_D values (10-20 pM) were observed in either MDMA- or MDA-treated rats. Data, plotted as percent of the 5-HT uptake site density observed in controls in each brain region, represent the mean and SEM from three to six rats per group. Control values were: cerebral cortex, 338±10; hippocampus, 360±17; striatum, 344±30; hypothalamus, 775±36; and midbrain, 570±16 fmol/mg protein. Data were analyzed by one-way ANOVA and Duncan's multiple range test

SOURCE: Battaglia et al. 1987.

data in control and drug-treated rats indicated that, in cerebral cortex, [^{3}H]paroxetine binding was through a single population of binding sites as indicated by the Hill coefficient values (1.02, 1.01, and 1.03 in control, MDMA-. and MDA-treated rats, respectively). Furthermore, there were no significant differences in the affimity of [^{3}H]paroxetine for the serotonin uptake site (i.e., K_D) between control and drug-treated rats (18.8, 20.8, and 17.9 pM in control, MDMA-, and MDA-treated rats, respectively). Differences in the sensitivity in various brain regions to the effects of MDMA and MDA are not unique, as previous data have demonstrated differential sensitivity to the effects of methamphetamine (Ricaurte et al. 1980) and parachloroamphetamine (Kohler et al. 1978; Fuller 1978) on serotonergic systems in various brain regions. Biochemical and

histochemical data suggest that parachloroamphetamine primarily affects the ascending serotonin systems, whereas the descending pathways are left intact (Kohler et al. 1978; Fuller 1978).

Effects of MDA and MDMA on Catecholamine Neurons

In contrast with the marked and consistent effects of MDMA and MDA on serotonergic systems, neither drug produced any widespread or consistent changes in the levels of NE, DA, or their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) in the various brain regions examined (table 1). Small changes, however, were observed in some brain regions. Both MDMA and MDA produced statistically significant increases in striatal DOPAC and cerebral cortical HVA content, whereas only MDMA treatment resulted in an increase in hippocampal DOPAC levels (table 1). Furthermore. neither MDMA nor MDA treatment caused any significant reduction in the levels of [^{3}H]mazindol-labeled NE uptake sites in cerebral cortex, hippocampus, or midbrain when compared with the respective saline-treated controls (figure 8). Although a small reduction was noted in NE uptake sites in hippocampus, this change was not statistically significant. Similarly, no significant decreases were observed in the number of [^{3}H]mazindol-labeled DA uptake sites in cerebral cortex, hippocampus, striatum, and midbrain following treatment with MDA. MDMA caused a statistically significant reduction (37 percent) in the density of DA uptake sites only in midbrain.

The neurotoxic effects of MDMA and MDA appeared to be exerted preferentially on serotonergic neurons, as no widespread changes in a variety of catecholamine markers were seen after chronic administration of these drugs. The small increases in DOPAC and/or HVA that were seen in the cerebral cortex, hippocampus, and striatum after chronic administration of these MDA derivatives are comparable to similar increases in DA metabolite levels that have been previously reported following both acute (Schmidt et al. 1986) and chronic (Stone et al. 1986) administration of MDMA and MDA. These alterations may reflect increases in DA turnover. Because serotonin-containing terminals are present in high concentrations in midbrain areas (substantia nigra and ventral tegmental area) and DA cell bodies (Steinbusch 1983). the degeneration of serotonin terminals in these regions after MDMA or MDA treatment may be responsible for the observed changes in DA metabolites. Despite the small effects on DA turnover, MDMA and MDA do not appear to produce any widespread destruction of catecholaminergic terminals, as the only significant change observed was a reduction in DA uptake sites in midbrain after administration of MDMA. The reasons for the decrease in DA uptake sites are not clear at present. Preliminary immunocytochemical data indicate that there are no changes in the density or morphology of catecholamine axons after chronic administration of MDMA or MDA (O'Hearn et al. 1988).

TABLE 1. *Effect of repeated systemic administration of MDMA and MDA on NE, DA, and DA metabolite levels in various regions of rat brain*

Brain Region	Concentration (pg/mg tissue) NE	DA	DOPAC	HVA
Cerebral Cortex				
Control	447 ± 53	59 ± 12	96 ± 14	19 ± 4
MDMA	424 ± 13	72 ± 3	73 ± 5	32 ± 4*
MDA	404 ± 26	63 ± 4	94 ± 19	36 ± 5*
Hippocampus				
Control	528 ± 62	31 ± 9	39 ± 6	6 ± 2
MDMA	573 ± 34	13 ± 5	65 ± 12*	15 ± 5
MDA	608 ± 46	16 ± 4	32 ± 13	8 ± 52
Striatum				
Control	ND	6,091 ± 596	3,212 ± 159	788 ± 58
MDMA	ND	6,974 ± 228	3,954 ± 320*	767 ± 46
MDA	ND	6,168 ± 569	3,669 ± 189*	890 ± 48
Hypothalamus				
Control	3,320 ± 209	569 ± 40	228 ± 43	54 ± 4
MDMA	3,052 ± 159	475 ± 38	200 ± 30	54 ± 5
MDA	3,577 ± 148	585 ± 67	229 ± 26	58 ± 3

*Significant difference from saline-treated control rats at $p<0.05$.

KEY: ND=levels below the sensitivity of the assay. Data were analyzed by one-way ANOVA and Duncan's multiple range test.

NOTE: NE, DA, DOPAC, and HVA measured 2 weeks after administration of 20 mg/kg MDMA or MDA, subcutaneously. every 12 hours for 4 consecutive days. Values represent the mean ± SEM. n=4 to 6 rats.

SOURCE: Battaglia et al. 1981.

Autoradiographic Studies on [^{3}H]Paroxetine-Labeled Serotonin Uptake Sites

In vitro autoradiographic studies of [^{3}H]paroxetine-labeled serotonin uptake sites in control and MDMA-treated brains were carried out as previously described (De Souza and Kuyatt 1987) to assess the neuroanatomic localization of lesions induced by MDMA. Substantial reductions in serotonin uptake sites (50 to 100 percent decreases) were observed in all areas of cerebral cortex as early as 18 hours after a 4-day treatment

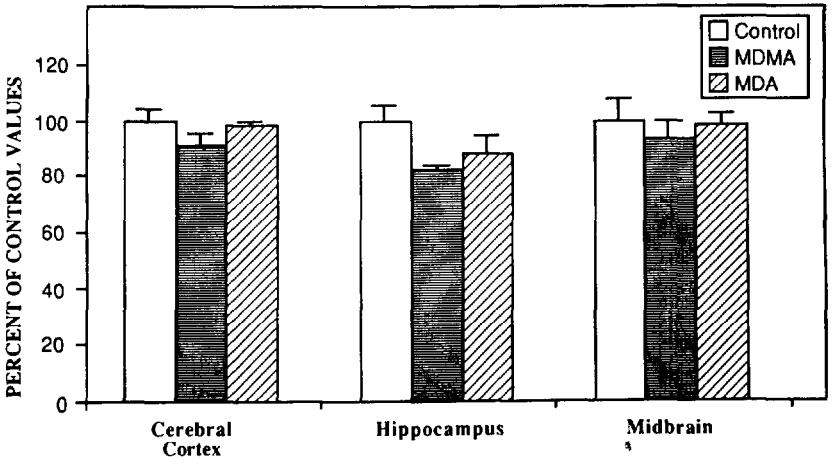


FIGURE 8. *Effect of repealed systemic administration of MDMA and MDA on the density of NE uptake sites in various brain regions*

NOTE: Rats were injected subcutaneously twice daily for 4 days with MDMA or MDA (20 mg/kg) or saline vehicle (1 mL/kg) and sacrificed 2 weeks after the last injection. NE uptake sites were measured using 6 nM [^{3}H]mazindol in the presence of selective blockers as previously described (Javitch et al. 1984). Data, plotted as percent of control values in each brian region, represent the mean and SEM from six control. MDMA-, and MDA-treated animals. Control values of NE uptake sites were: cerebral cortex. 164±6; hippocampus, 176±9; midbrain, 157±13 fmol/mg protein.

SOURCE: Battaglia et al. 1987.

regimen (20 mg/kg MDMA twice daily); the reductions were maintained for at least 2 weeks. As shown in table 2, cerebral cortical regions that showed the most extensive destruction of serotonin uptake sites (i.e., more than 90 percent) were the prefrontal (area 32), anterior cingulate (area 24). entorhinal, and parietal cortex. Comparable decreases in serotonin uptake sites were observed between day 0 (18 hours after last injection) and day 14 (14 days after last injection) in several regions of cerebral cortex such as prefrontal, pyriform, frontal areas 8 and 10, entorhinal, and primary auditory regions. In other areas of cerebral cortex, such as the sensory motor regions, significant reductions in serotonin uptake sites were observed only 2 weeks after the treatment.

As shown in table 2 and figure 9, marked decreases in serotonin uptake sites were observed following MDMA administration in all regions of caudate putamen, olfactory tubercle, endopiriform nucleus, islands of Calleja, and nucleus accumbens. Within the caudate putamen, some time-dependent

TABLE 2. *Effects of repeated systemic administration of MDMA on the regional decreases in [^{3}H]paroxetine-labeled serotonin uptake sites*

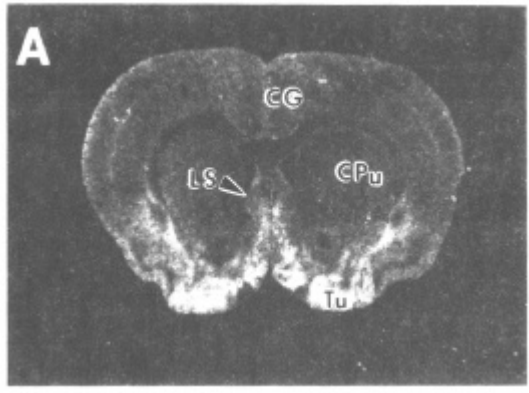
Brain Region	Control	MDMA
Cerebral Cortex		
Prefrontal area 32	2-	1
Cingulate area 24	2	1
Indusium griseum	1	2
Piriform	2	1
Frontal area 8	2	1+
Frontal area 10	1+	1-
Sensorymotor	2	1-
Parietal	1+	0
Entorhinal	4	1+
Primary auditory	1	1-
Primary visual	2+	2
Olfactory tubercle	4	2
Endopiriform nucleus	3	2
Islands of Calleja	3+	1+
Basal Ganglia		
Caudate putamen		
dorsolateral	2	1-
dorsomedial	1+	1-
ventrolateral	3	2
ventromedial	2+	1+
Nucleus accumbens	2	1-
Septal Area		
Medial septal nucleus	4-	3+
Lateral septal nucleus	3	2
Amygdala basolateral nucleus	4-	3+
Thalamus and Epithalamus		
Anteroventral nucleus	3-	0
Anteromedial nucleus	3	0
Anteroventral dorsomedial nucleus	3-	0
Reuniens	4+	1
Lateroposterior nucleus	3	1
Posterior nucleus	1	1
Posterioventromedial nucleus	1	1
Parafascicular nucleus	2	1+
Lateral geniculate body	5-	1+
Medial geniculate body	2	1
Lateral habenula	3	1-

TABLE 2. (Continued)

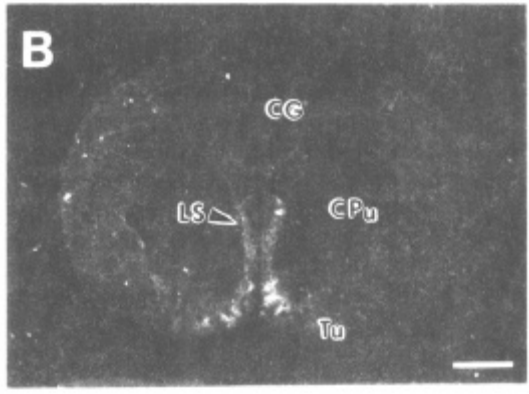
Brain Region	Control	MDMA
Hypothalamus		
Lateral nucleus	4	4-
Hippocampus		
CA3 region	2+	1
Dentate gyrus	2	1
Molecular layer	2+	1
Parasubiculum	3+	2
Presubiculum	3	2
Midbrain		
Inferior colliculus	3	1-
Interpeduncular nucleus	3+	3
Central gray	5+	5+
Superior colliculus		
superficial layers	3	1
profundum	2+	1
Substantia nigra		
pars compacta	3+	2
pars reticulata	3	2+
paranigral nucleus	2	3
Ventral tegmental area	2+	2
Dorsal raphe nuclei	5+	5+
Median raphe nuclei	5+	5+
Pons-Medulla		
Locus coeruleus	5+	5
Pontine reticular formation	2	2
Cerebellum (all lobules)	2	2

KEY: Relative density of [^{3}H]paroxetine binding sites: 1=0-50 fmol/mg tissue; 2=50-150 fmol/mg tissue; 3=150-250 fmol/mg tissue; 4=250-400 fmol/mg tissue and 5=>400 fmol/mg tissue; + and - values indicate the upper and lower limits, respectively, of each range.

NOTE: Data are based on observations from three animals per group. Rats were injected subcutaneously twice daily for 4 days with MDMA (20 mg/kg) or saline (1 mL/kg) (control) and sacrificed 14 days after the last injection. Anatomical terminology is derived from Paxinos and Watson (1982). [^{3}H]Paroxetine binding sites were visualized by using a saturating concentration (0.25 nM) of [^{3}H]paroxetine. Autoradiograms of rat brain were generated using [^{3}H]Ultrofilm. Analysis of [^{3}H]paroxetine-labeled serotonin uptake site densities in the various brain regions was performed by computerized image analysis densitometry. No correction for "grey-white" quenching of tritium was used.



SALINE



MDMA

FIGURE 9. *Autoradiographic distribution of [^{3}H]paroxetine-labeled serotonin uptake sites in coronal sections at the level of the caudate putamen from (A) saline-treated and (B) MDMA-treated rats*

NOTE: In these darkfield photomicrographs (tritium-sensitive Ultrofilm), autoradiographic grains (i.e., binding sites) appear as white spots; the tissue is not visible. The degree of nonspecific binding defined in the presence of 2 μM citalopram was comparable for both treatments. In (A), note the high density of serotonin uptake sites in cingulate cortex (CG), caudate putamen (CPu), olfactory tubercle (Tu), islands of Calleja, and lateral septal nuclei (LS) in control brains. In MDMA-treated animals (B), marked reductions were observed in most regions except for the septal nuclei, which were relatively unaffected.

reductions in serotonin uptake sites were observed following MDMA treatment. For example, equivalent decreases in serotonin uptake sites were observed between day 0 and day 14 groups in the ventrolateral region, while in dorsolateral and dorsomedial areas a significantly greater reduction in serotonin uptake sites was observed only at the later timepoint. Other brain regions that were sensitive to the neurodegenerative effects of MDMA included various thalamic nuclei and regions of hippocampus. In contrast, the dorsal and medial septal nuclei appeared to be less sensitive to the neurotoxic effects of MDMA as the reductions (approximately 25 percent) in serotonin uptake sites in these brain regions were not statistically significant. Likewise, no significant reductions were observed in the indusium griseum, which contains primarily serotonin axons of passage.

Within midbrain structures, regions containing serotonin projections appeared to be more dramatically affected by MDMA than those containing scrotonin cell bodies (figure 10). For instance, in both the superficial layers of superior colliculus and profundum, serotonin uptake sites were reduced 85 to 90 percent, while in dorsal median raphe, central grey, and the ventral tegmental region, there was little or no change after MDMA treatment. Likewise, serotonin projections to substantia nigra pars compacta and reticulata were markedly affected, while no changes in serotonin uptake sites were observed in the interpeduncular nucleus and pontine reticular formation up to 14 days following MDMA administration.

To assess the serotonergic selectivity of the neurodegenerative effects of MDMA in brain, additional autoradiographic studies of NE and DA uptake sites in brain regions containing catecholamine terminals and cell bodies were carried out. NE and DA uptake sites were labeled using [^{3}H]mazindol in the presence of specific blockers as previously published (Javitch et al. 1985). With respect to NE uptake sites, no change was observed from control levels of [^{3}H]mazindol binding sites in midbrain regions such as locus coeruleus, interpeduncular nucleus, substantia nigra pars compacta, or reticulata up to 14 days following MDMA treatment. In a number of cerebral cortical regions that receive NE projections, [^{3}H]mazindol-labeled NE uptake sites were not decreased 18 hours after treatment (i.e., day 0) but rather appeared to be slightly increased at day 14. Consistent with the minimal effects of MDMA on metabolic parameters associated with catecholamine neurons, there were no changes in the density of DA uptake sites, when compared to levels in saline-treated rats, in either cell body regions such as substantia nigra pars compacta and reticulata or terminal regions such as caudate putamen, nucleus accumbens, and olfactory tubercle. These results are therefore consistent with what has been observed in neurochemical studies in brain homogenates and indicate that the neurodegenerative effects of MDMA appear to be confined primarily to serotonergic pathways, since this treatment regimen did not reduce the density of uptake sites associated with catecholamine-containing neurons. Additional studies are necessary to assess further the neuroanatomic localization of any long-term

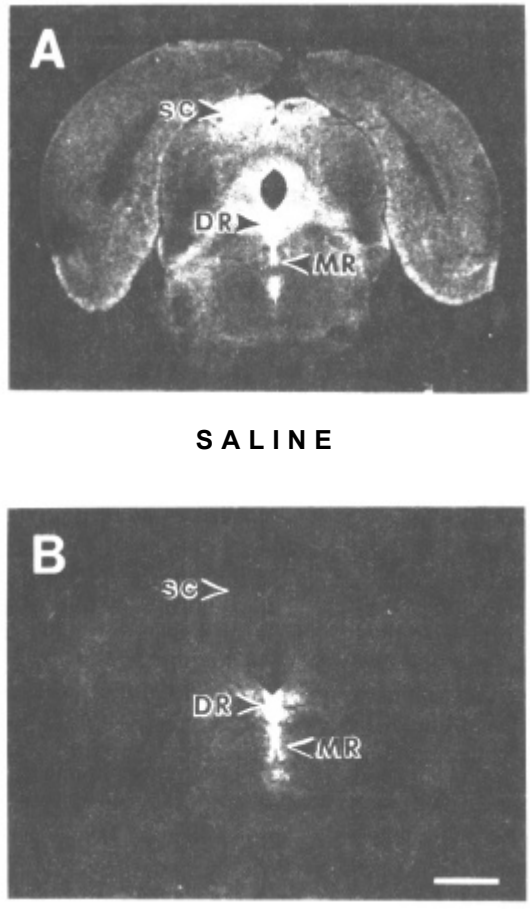


FIGURE 10. *Autoradiographic distribution of [^{3}H]paroxetine-labeled serotonin uptake sites in coronal sections at the level of midbrain in (A) saline-treated and (B) MDMA-treated rats*

NOTE: In (A), note the high density of serotonin uptake sites in control brain in regions containing serotonin projections such as entorhinal cortex, superior colliculus (sc), presubiculum and parasubiculum, as well as cell body regions such as dorsal (DR) and median (MR) raphe and central grey. MDMA-treated animals exhibited marked reductions in [^{3}H]paroxetine binding rites in presubiculum and parasubiculum, entorhinal cortex, and superior colliculus (sc), while no changes in densities were observed in areas containing primarily serotonin perikarya such as the raphe nuclei (DR and MR).

compensatory changes in NE projections or other neurotransmitter recognition sites that may occur as a consequence of MDMA lesion of scrotonin pathways.

SUMMARY AND CONCLUSIONS

The data presented in this chapter provide strong evidence, from both neurochemical and neuroanatomical studies, demonstrating that, following *in vivo* administration of a number of methylenedioxy-substituted amphetamine derivatives, there is widespread and long-lasting degeneration of scrotonin neurons in brain, without any major or consistent effects on catecholamine neurons. A detailed examination of the parameters involved in the neurotoxic and neurodegencrative effects of MDMA on brain scrotonin neurons indicates that:

(1) the severity of the lesion by MDMA is dependent on both the dose and frequency of drug administration;

(2) the neurodegenerative effects of MDMA can be elicited in a number of animal species including primates;

(3) the neurodegenerative effects on brain serotonin neurons can be prevented by the serotonin uptake blocker, suggesting a role for the active uptake of MDMA, a neurotoxic metabolite of MDMA, or an unidentified endogenous neurotoxin; and

(4) the neurodegenerative effects of the drug are long-lasting (up to 1 year) with respect to neuronal recovery, while functional recovery may be permanently impaired.

In addition, the neurochemical and autoradiographic data suggest that there is some neuroanatomical and morphological specificity to the neurodegenerative effects of MDMA and MDA, as evidenced by predominant reductions in serotonin uptake sites in brain regions containing primarily serotonin terminals, while regions containing serotonin axons of passage and cell bodies are relatively unaffected.

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AUTHORS

Errol B. De Souza, Ph.D.
Chief, Laboratory of Neurobiology

George Battaglia. Ph.D.
Assistant Professor

Department of Pharmacology
Loyola University Medical Center
Stritch School of Medicine
2160 South First Avenue
Maywood, IL 60153

Characterization of Brain Interactions With Methylenedioxyamphetamine and Methylenedioxymethamphetamine

Robert Zaczek, Stephen Hurt, Steven Culp, and Errol B. De Souza

INTRODUCTION

Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA), like other amphetamine analogs, affect multiple *in vitro* monoamine parameters. These effects include stimulation of [^{3}H]serotonin and [^{3}H]dopamine release (Johnson et al. 1986) and inhibition of serotonin, dopamine, and norepinephrine uptake (Steele et al. 1987). In addition, recent radioligand binding studies have demonstrated interactions of MDA and MDMA at a variety of established postsynaptic brain recognition sites, including serotonergic, adrenergic, and cholinergic receptors (Battaglia et al. 1988).

Several studies have identified specific sites of interaction in brain that may mediate the actions of amphetamine and its substituted analogs. Binding sites for [^{3}H]amphetamine (Hauger et al. 1984) and [^{3}H]fenfluramine (Garattini et al. 1987) have been identified in discrete areas of rat brain. These sites have similar characteristics in that both have high binding capacities (amphetamine, B_{max}=60 pmol/mg protein; fenfluramine, B_{max}=63 pmol/g tissue), and binding to both sites is inhibited by sodium ions. To date, two groups have studied interactions of [^{3}H]MDMA with rat brain. Gehlert et al. (1985) reported high affinity specific binding (K_d=99 nM, B_{max}=31 fmol/mg protein) of [^{3}H]MDMA to rat brain membranes. However, a subsequent study (Wang et al. 1987) suggested that these apparent binding sites represent [^{3}H]MDMA association with glass fiber filter paper. The present study reexamines the possibility of [^{3}H]MDMA as well as [^{3}H]MDA association with rat brain membranes. Also characterized is the nature of [^{3}H]MDA association with brain membranes to evaluate the possible importance of the binding site in mediating MDA's neurochemical and behavioral effects and to examine similarities between apparent [^{3}H]MDA binding sites and those labeled by [^{3}H]amphetamine and [^{3}H]fenfluramine.

Centrifugation assays were employed in all our studies to circumvent the problem of [^{3}H]MDA and [^{3}H]MDMA absorbing onto glass filters.

ASSOCIATION TO RAT BRAIN MEMBRANES

Assay for [^{3}H]MDA and [^{3}H]MDMA Association

Assays were performed using a crude synaptosomal preparation of rat brain. Male Sprague-Dawley rats were sacrificed by decapitation, and brains were immediately dissected on ice for membrane preparation. Telencephalon containing cerebral cortex, striatum, and hippocampus was used in all experiments except in studies examining the regional distribution of the binding sites. Brain areas were homogenized in 20 volumes of ice-cold 0.32 M sucrose using a smooth glass homogenizer equipped with a motor-driven teflon pestle. The homogenate was centrifuged at 800 x g for 10 minutes at 4 °C to remove large particulate material, and the supematant was removed and centrifuged at 20,080 x g. The resultant pellet was resuspended in 20 volumes of the original wet weight in either ice-cold 50 mM Tris-HCl buffer (pH 7.1) alone or in the same buffer containing 0.27 M sucrose. Binding assays were performed in Beckman minivials containing either 0.9 mL of 50 mM Tris-HCl (pH 7.1) or the same buffer containing 0.27 M sucrose. The vials also contained 2 nM [^{3}H]MDA (54 Ci/mmol) or [^{3}H]MDMA (74 Ci/mmol), competing drugs at various concentrations, and 100 µL of each of the homogenates as indicated above. Nonspecific binding was assessed by measuring the [^{3}H]MDA or [^{3}H]MDMA incorporated into boiled tissue. All incubations were performed for 90 minutes at 4 °C: unless indicated otherwise. Assays were terminated by centrifugation at 32,008 x g at 4 °C. The vials were removed and the supernatant fluid discarded. The pellets were superficially washed with ice-cold water (7 mL three times), after which the excess water was wiped from the inside of the vial and 5.0 mL of scintillation fluid was added to each vial. The pellets were allowed to solubilize overnight, and the vials were assessed for radioactivity by scintillation counting. Data from saturation isotherms were analyzed by the nonlinear curve-fitting program LIGAND (Munson and Rodbard 1980). Protein was measured by the method of Lowry et al. (1951).

[^{3}H]MDA and [^{3}H]MDMA Association to Rat Brain Membranes

Preliminary experiments to delineate the optimum conditions for [^{3}H]MDA and [^{3}H]MDMA binding to rat brain membranes indicated that the best signal-to-noise ratio was found using a crude synaptosomal preparation incubated at 4 °C. While most experiments employed 50 mM Tris-HCl (pH 7.1) as the incubation medium, we found that the addition of 0.27 M sucrose to the incubation medium increased the specific binding approxi-mately fivefold. Subsequent experiments were carried out under both conditions. Other preliminary experiments suggested that [^{3}H]MDA and

[^{3}H]MDMA interacted with multiple sites in rat brain. A low affinity [^{3}H]MDA binding site (apparent K_d>1.0 mM) was found to be resistant to boiling of the synaptosomal preparation for 15 minutes. This site was saturable, as indicated by a 30 percent inhibition of [^{3}H]MDA binding to boiled synaptosomes by 1.0 mM MDA and a 56 percent inhibition of the binding by 0.1 mM of the serotonin uptake blocker paroxetine. The indication of a saturable, nonspecific binding site for [^{3}H]MDA in boiled membranes necessitated that we use boiled tissue to assess nonspecific binding in all subsequent experiments.

The saturation profiles of [^{3}H]MDA and [^{3}H]MDMA recognition sites in rat brain synaptosomes in the presence of 0.27 M sucrose are shown in figure 1. Both ligands exhibited shallow saturation curves, which extended over 4 log units from approximately 100 nM to 1.0 mM MDA, suggesting the presence of multiple binding sites. Data from [^{3}H]MDA saturation experiments fit significantly ($p<0.015$) better to a two-site model, indicating both high (K_d=887 nM, B_{max}=23 pmol/mg protein) and low (K_d=45 μM, B_{max}=3.2 nmol/mg protein) affinity sites upon iterative nonlinear curve-fitting analysis. Analysis of saturation data of apparent [^{3}H]MDMA binding in the presence of sucrose also fit significantly better to a two-site model ($p<0.02$; high affinity K_d=2.9 μM, B_{max}=79 pmol/mg protein; low affinity K_d=128 μM, B_{max}=7.4 nmol/mg protein).

The effect of eliminating sucrose from the incubation medium of [^{3}H]MDA binding assays is shown in figure 2. While the Eadee-Scatchard plot of the data from [^{3}H]MDA saturation experiments performed in the absence of sucrose was linear, suggesting [^{3}H]MDA binding to one population of sites, the plot representing the data from experiments performed in the presence of 0.27 M sucrose was curvilinear, consistent with [^{3}H]MDA binding to multiple populations of sites (see above). Nonlinear curve-fitting analysis of the data suggested a single apparent binding site for [^{3}H]MDA, when incubated with synaptosomes in the absence of sucrose (K_d=2.8 μM, B_{max}pmol/mg protein). Thus, removal of sucrose from the incubation medium led to the elimination of low-affinity specific [^{3}H]MDA binding. Similar to observations of [^{3}H]MDA binding, removal of sucrose from the incubation medium led to a 61 percent decrease in the apparent specific binding at 100 nM [^{3}H]MDMA.

The affinity (K_d values) observed for [^{3}H]MDA and [^{3}H]MDMA binding were similar to the effective doses (i.e., ED_{50} or K_1 values) of MDA and MDMA reported for various pre- and postsynaptic monoamine markers, such as serotonin and dopamine release (Johnson et al. 1986), monoamine transport (Steele et al. 1987), and multiple brain, ligand binding sites (Battaglia et al. 1988).

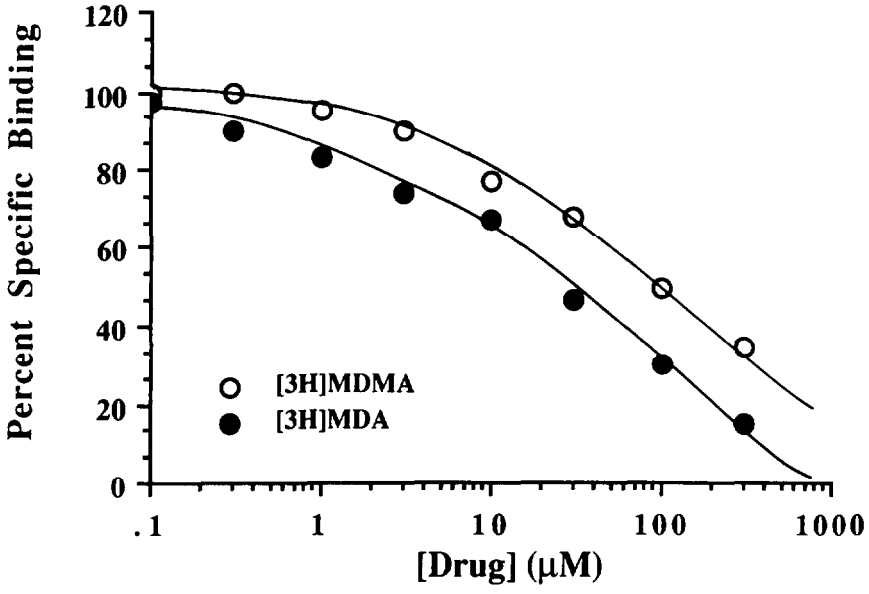

FIGURE 1. *Saturation of [^{3}H]MDA and [^{3}H]MDMA binding in rat brain synaptosomes*

NOTE: lncreasing amounts of unlabeled MDA or MDMA were added to 1.0 mL of incubation buffer containing 2 nM [^{3}H]MDA or 2 nM [^{3}H]MDMA, respectively. Experiments were performed on tissue in the presence of 0.27 M sucrose. Data are expressed as percent of [^{3}H]MDA or [^{3}H]MDMA bound to tissue in the absence of added nonradioactive drug. Nonspecific binding was assessed by measuring the amount of [^{3}H]ligand bound to boiled synaptosomes incubated in the presence of 0.27 M sucrose.

The high capacities (i.e., B_{max} value) of [^{3}H]MDA and [^{3}H]MDMA binding sites, as well as those that have been reported for [^{3}H]amphetamine binding sites (60 pmol/mg protein) (Hauger et al. 1984) and [^{3}H]fenfluramine binding sites (63 pmol/g tissue) (Garattini et al. 1987), argue against bimolecular interactions of these drugs with monovalent protein-binding sites. Although the mechanism by which sucrose acts to preserve low affinity [^{3}H]MDA binding is yet to be determined, a similar phenomenon has been observed for [^{3}H]amphetamine binding (Hauger et al. 1984). In the latter study. a wash of tissue in isotonic sucrose prior to incubation was reported to increase nearly threefold the specific binding over a wash with 50 mM Tris-HCl alone (Hauger et al. 1984).

Pharmacology of Specific [^{3}H]MDA Binding in Rat Brain

The pharmacology of [^{3}H]MDA binding was determined by examining the effects of other monoamine reuptake blockers and related amphetamine

analogs on the inhibition of [^{3}H]MDA binding. The pattern of paroxetine, desipramine (DMI), dimethoxymethamphetamine (DOM), and MDA inhibition of specific [^{3}H]MDA binding is shown in figure 3. Experiments were performed in the presence of 0.27 M sucrose using 2 nM [^{3}H]MDA, conditions under which both high- and low-affinity [^{3}H]MDA binding sites are labeled. Paroxetine was the most potent inhibitor (IC_{50}= 1.6 μM) followed by DMI (IC_{50}= 5.9 μM), DOM (IC_{50} = 17 μM), and MDA (IC_{50} = 43 μM). Analysis of paroxetine and DMI inhibition curves revealed Hill coefficient values (n_H) close to 1.00 (paroxetine, n_H = 0.85; DMI, n_H = 0.91). MDA and DOM gave rise to much shallower inhibition curves (MDA, n_H = 0.56; DOM, n_H = 0.53), providing additional evidence for the existence of multiple apparent [^{3}H]MDA binding sites.

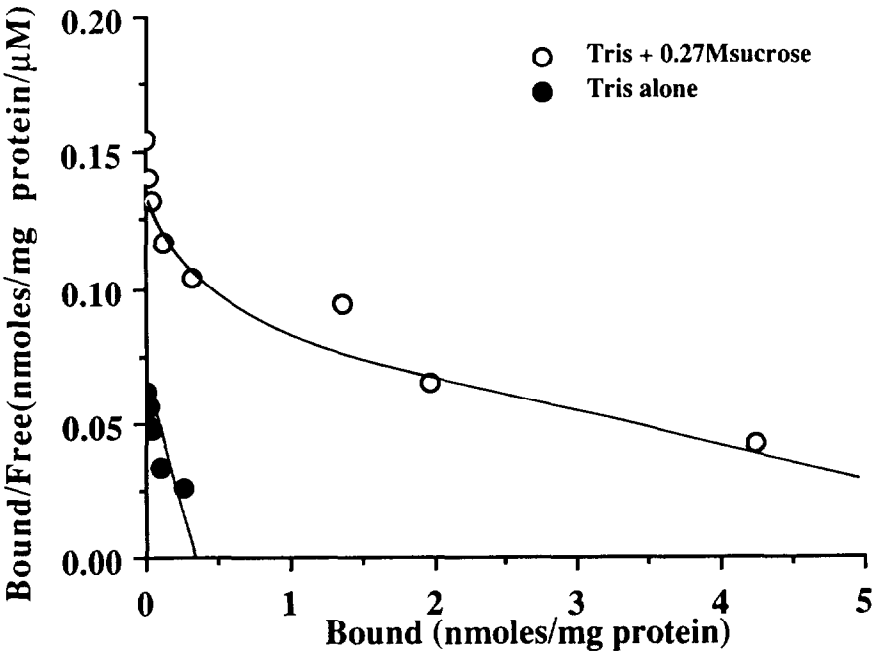

FIGURE 2. *Eadee-Scatchard transformation of saturation data of [^{3}H]MDA binding in rat brain synaptosomes*

NOTE: Increasing amounts of unlabeled MDA were added to 1.0 mL of incubation buffer containing 2 nM [^{3}H]MDA. Experiments were performed on tissue in the presence (open circles) and absence (closed circles) of 0.27 M sucrose. Nonspecific binding was assesed by measuring the amouut of [^{3}H]MDA bound to boiled synaptosomes incubated in the presence of 0.27 M sucrose.

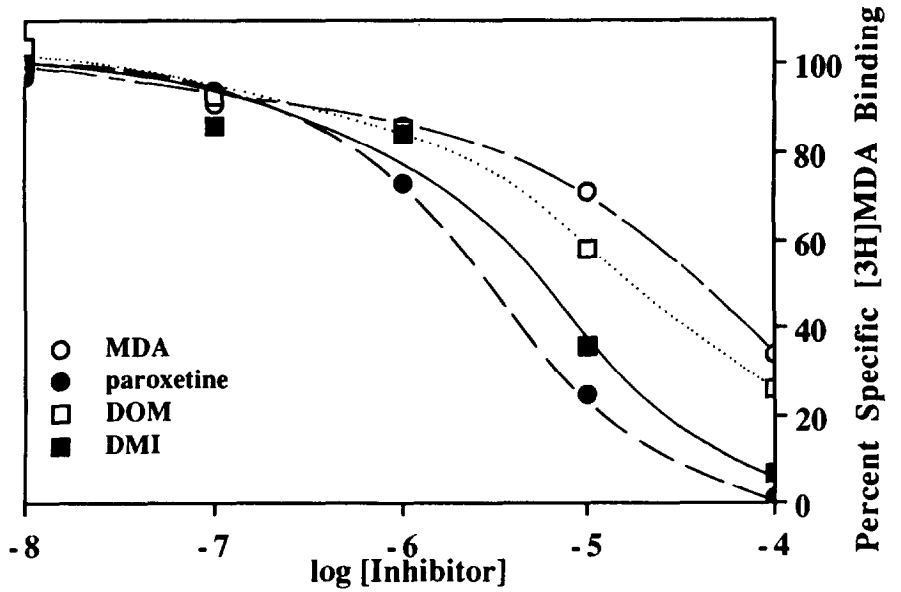

FIGURE 3. *Inhibition of [^{3}H]MDA incorporation into synaptosomes*

NOTE: [^{3}H]MDA binding assays were performed in 50 mM Tris-HCl (ph 7.1) containiag 0.27 M sucrose and 2.0 nM [^{3}H]MDA as described in the text. Results are expressed as percent inhibition of specific [^{3}H]MDA incorporation in the absence of inhibitors. Boiled tissue blanks were performed at each concentration of drug.

The inhibition of [^{3}H]MDA binding by several other related compounds is seen in table 1. All compounds were tested at a concentration of 10 μM under conditions that favored the labeling of the high-affinity [^{3}H]MDA binding site (zero sucrose, 2 nM [^{3}H]MDA) and at 100 μM concentration under conditions designed to favor the study of the low-affmity [^{3}H]MDA binding site (0.27 M sucrose, 3 μM [^{3}H]MDA). A significant positive correlation (r^2=0.80, $p<0.01$) between the relative inhibition potencies of the test drugs at the high- and low-affinity [^{3}H]MDA binding sites was observed upon linear regression analysis (figure 4A).

EFFECTS OF OSMOLARITY AND DETERGENTS

A possible explanation for the large capacity (i.e., high B_{max} values) of [^{3}H]MDA binding sites and stimulation of [^{3}H]MDA binding by isotonic sucrose is intrasynaptosomal internalization and sequestration of [^{3}H]MDA. Studies of apparent chloride-dependent [^{3}H]glutamic acid binding (Pin et al. 1984; Zaczek et al. 1987) have demonstrated this type of phenomenon for labeled glutamate. This possibility was examined by measuring [^{3}H]MDA binding in the presence of varying concentrations of sucrose and

estimating the relative synaptosomal volume by measuring [^{3}H]H_2O incorporation into synaptosomes in parallel experiments. As shown in figure 5, decreasing the concentration of sucrose in the incubation medium led to a decrease in the level of [^{3}H]MDA binding. This contrasted with an increase in intrasynaptic volume, as indicated by an increase in the capacity of the synaptosomes to retain [^{3}H]H_2O. A significant negative correlation (r^2=0.84; $p<0.02$) was obtained when [^{3}H]H_2O incorporation was correlated with [^{3}H]MDA incorporation by linear regression analysis. These data argue against a sequestration phenomenon, since the amount of [^{3}H]MDA binding should increase with increasing vesicular volume, if intrasynaptosomal internalization and sequestration was occurring.

TABLE 1. *Pharmacology of inhibition of [^{3}H]MDA binding*

Drug	High-Affinity Binding	Low-Affinity Binding
Amphetamine	47 ± 21	31 ± 13
Mescaline	36 ± 4	12 ± 6
MDMA	48 ± 12	32 ± 3
N,N-DMT	59 ± 11	56 ± 20
PCA	84 ± 7	56 ± 8
DOM	70 ± 3	35 ± 8
Fenfluramine	63 ± 6	34 ± 9
Paroxetine	100 ± 1	91 ± 8
Desipramine	96 ± 5	83 ± 9
Imipramine	96 ± 3	61 ± 15

NOTE: Inhibition of high-affinity [^{3}H]MDA binding was performed in 50 mM Tris-HCl (pH 7.1) in the presence of 2 nM [^{3}H]MDA to preferentially label the high-affinity site. Drugs were tested at 10 μM concentrations for inhibition of high-affinity binding. Low-affinity [^{3}H]MDA binding inhibition was performed in 50 mM Tris-HCl (pH 7.1) containing 0.27 M sucrose in the presence of 3.0 μM [^{3}H]MDA to preferentially label the low-affinity site. Inhibition was peformed using 100 μM concentrations of the drugs tested. Values represent percent inhibition of specific [^{3}H]MDA binding (mean ± SEM) performed in the absence of inhibiting drugs. Boiled tissue was no in simultaneous assays to assess nonspecific binding.

Another approach used to examine the possible existence of [^{3}H]MDA sequestration into synaptosomes was to investigate the effects of the detergents Triton X-100 and digitonin on the level of [^{3}H]MDA incorporation into rat brain synaptosomes (table 2). Concentrations of detergents lower than 0.01 percent did not affect specific [^{3}H]MDA binding. Digitonin. at a concentration of 0.01 percent, caused a 30 percent decrease ($p<0.05$) in the level of apparent [^{3}H]MDA binding as compared to control, and 0.01 percent Triton caused a 71 percent decrease ($p<0.01$). These data provide additional evidence against intrasynaptosomal internalization and sequestration of [^{3}H]MDA since relatively high concentrations (0.01 percent)

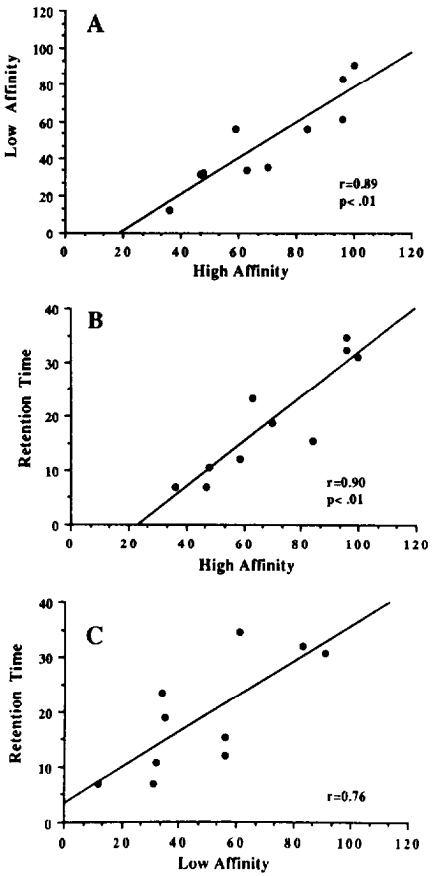

FIGURE 4. *Correlation between the relative inhibitory potencies of various drugs at high- and low-affinity [^{3}H]MDA binding and between drug lipophilicities and inhibition potencies of [^{3}H]MDA binding*

NOTE: Panel A represents the relationship between the relative inhibitory potencies of various drugs at high- and low-affinity [^{3}H]MDA binding. Percent inhibition by test drugs of low-affinity [^{3}H]MDA binding is plotted vs. the inhibition of high-affinity binding. Panels B and C represent the relation between the lipophilicity of test drugs and their ability to inhibit high and low [^{3}H]MDA binding, respectively. In both cases, the retention times of test drugs on reverse-phase HPLC are plotted vs. percent inhibition of [^{3}H]MDA binding. Pearson's r values and levels of significance are derived from linear regression analysis of the data.

of the detergents were required to cause significant decreases in [^{3}H]MDA incorporation into the tissue. Furthermore, these decreases were only partial, which is in contrast to what is generally observed when labeled

substances am released from a membrane-intemalized pool by pore-forming detergents, which abruptly release the total contents of membrane-sequestered compounds.

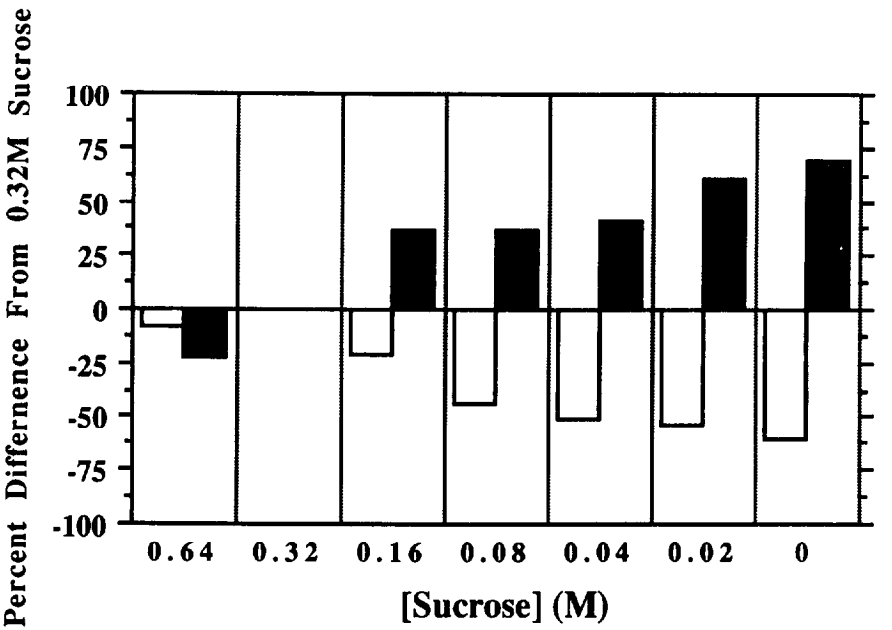

FIGURE 5. *Effects of varying sucrose concentration on [^{3}H]MDA and [^{3}H]water incorporation into rat brain synaptosomes*

NOTE: Synaptosomes were prepared and incubated under standard procedures for [^{3}H]MDA incorporation. The osmolarity of the incubation buffer was changed by the addition of various concentrations of sucrose to 50 mM Ttis-HCl (pH 7.1). The incubation medium contained either 100 nM [^{3}H]MDA or 2 million cpm of [^{3}H]water. After a 90-min incubation at 4 °C, the assays were terminated and assessed for radioactivity. Bars represent the percent change in the level of radioactive H_2O (shaded bars) end [^{3}H]MDA (open bars) incorporated from the respective incorporation at 0.32 M surcrose.

The results of the osmolarity and detergent experiments indicate that MDA is not internalized into synaptosomes to any appreciable degree. In addition, the [^{3}H]MDA binding assays were performed at 4 °C, demonstrating a lack of dependence on physiological temperatures; this lack is characteristic of membrane internalization phenomena. Since other investigators have shown that [^{3}H]MDMA is not taken up into synaptosomes by a sodium-dependent mechanism (Wang et al. 1987), the internalization of MDA, MDMA, and related compounds does not appear to be necessary for their presynaptic actions to enhance the release and to inhibit the reuptake of monoamines. Furthermore, the intraneuronal internalization of MDA or

MDMA is not likely to be involved in the ability of these compounds to cause serotonin terminal degeneration. The preponderance of the evidence supports the hypothesis that the association of [^{3}H]MDA with synaptosomes is primarily with membrane elements.

TABLE 2. *Effects of detergents on apparent [^{3}H]MDA binding*

Detergent	Weight/Vol (Percent)	Percent Control
Triton X-100	.0001	95 ± 9
	.001	98 ± 8
	.01	37 ± 9**
Digitonin	.0001	89 ± 9
	.001	85 ± 11
	.01	70 ± 4*

Difference significant at $p<0.05$ by ANOVA and Duncan's multiple range test.

**Difference significant at $p<0.01$ by ANOVA and Duncan's multiple range test.

NOTE: Synaptosomes were incubated for 90 min at 25 °C in 50 mM Tris-HCl (pH 7.1) containing 0.27 M sucrose and 100 nM [^{3}H]MDA. Results are expressed as percent of specific [^{3}H]MDA binding in the absence of detergent. Results are the means ± SEM from three seperate experiments done in duplicate.

Role of Lipophilicity in the Incorporation of [^{3}H]MDA Into Rat Brain Synaptosomes

Compounds that were included in the pharmacologic profile of [^{3}H]MDA binding were subjected to reverse-phase HPLC analysis to assess their relative lipophilicity. Briefly, each compound (10 µg) was injected onto a Waters Nova-Pak C18 column and eluted with a linear gradient from 95 percent buffer A:5 percent buffer B to 20 percent buffer A:80 percent buffer B (buffer A=95 percent water, 5 percent acetonitrile, 0.1 percent ammonium acetate; buffer B=20 percent water, 80 percent acetonitrile, 0.1 percent ammonium acetate). Detection was performed using a Waters Model 441 UV detector at 214 nm. Figure 6 shows the reverse-phase chromatographic elution pattern of MDA and related compounds, which are listed in table 1. Since separation of compounds by reverse-phase chromatography is based upon the aqueous/organic partition coefficients of the substances separated, this method gives an index of the relative lipophilicity of various compounds. The more lipophilic a compound is, the greater its retention time on C18 material. The retention times, in minutes, of the compounds tested in the present study were: amphetamine, 6.93; mescaline, 6.93; MDMA, 10.6; N,N-dimethyltryptamine (N,N-DMT), 12.1;

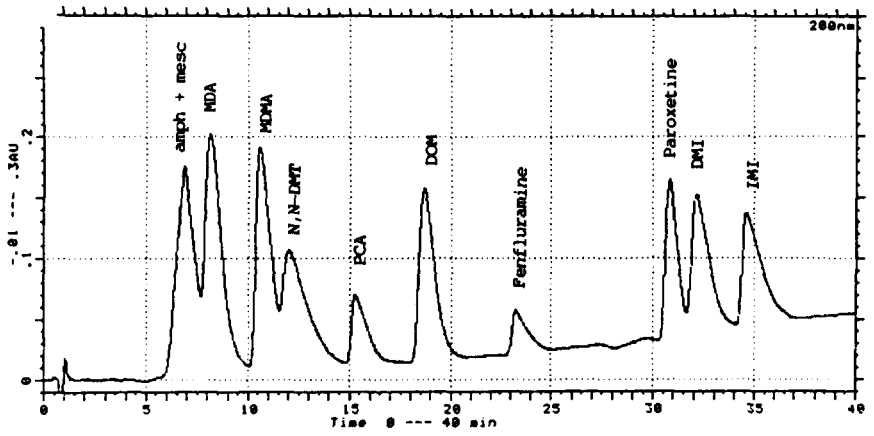

FIGURE 6. *Elution pattern of monoamine uptake blockers and amphetamine analogs from reverse-phase HPLC*

KEY: amph=amphetamine; mesc=mescaline; N,N-DMT=N,N-dimethyltryptamine
PCA=parachloroamphetamine; DOM=dimethoxymethamphetamine; DMI = desipramine; IMI=imipramine.

NOTE: HPLC detection of test drugs at 200 nm UV. Peak are named for the drugs they represent. Peak identity was discerned by analyzing each drug individually and observing its retention time and UV spectrum by photodiode array detection between 190 and 360 nm using a Waters Model 990 PDA detector. Retention times are listed in the text.

parachloroamphetamine (PCA), 15.4; DOM, 18.8; fenfluramine, 23.4; paroxetine. 30.9; DMI, 32.2; and imipramine, 34.7. Figure 4B and 4C show the correlations that were found between the retention times of the tested drugs and their levels of inhibition of high- and low-affinity [^{3}H]MDA binding, respectively (table 1). A significant positive correlation (r^2=0.81, $p<0.01$) was observed between the HPLC retention times of the tested drugs and their respective levels of inhibition of high-affinity [^{3}H]MDA binding. Although a positive correlation was found between the lipophilicity and the ability of test compounds to inhibit low-affinity [^{3}H]MDA binding (r^2=0.56), this correlation did not reach the level of statistical significance.

As stated earlier, the primary site of association of [^{3}H]MDA with brain synaptosomes is with membrane components, not with the intrasynaptic space. While the phenolic ends of these compounds may enable them to interact with hydrophobic environments of brain membrane components, their polar side chains may inhibit the ability of these compounds to move freely across the membranes, thus inhibiting internalization. The pKa of

amphetamine is 9.9, which indicates that over 99 percent of this drug and, most likely, its structural analogs will be ionized at pH 7.4. The ability of these compounds to enter the brain readily, which has been ascribed to their supposed lipophilicity, may be due to the existence of a saturable transport process for these drugs across the blood-brain barrier (Pardridge and Connor 1973). The high capacity for MDA and MDMA incorporation into brain membranes could be explained by their absorption into a hydrophobic membrane environment. We have shown positive correlations between the lipophilicity of several monoamine uptake blockers and amphetamine analogs and their relative inhibition potencies of both high- and low-affinity [^{3}H]MDA binding (figure 4); however, this correlation is not perfect. For instance, MDMA, which has a lower affinity (higher IQ for these sites of interaction, is more lipophilic than MDA; fenfluramine, which is less potent at inhibiting [^{3}H]MDA binding than PCA, is more lipophilic than the latter compound. These data suggest that there may exist some level of structural specificity beyond lipophilicity.

REGIONAL DISTRIBUTION OF APPARENT [^{3}H]MDA BINDING

The relative distribution of [^{3}H]MDA incorporation into p_2 preparations from various regions of rat brain, liver, and kidney is shown in table 3. Apparent [^{3}H]MDA binding had a heterogeneous regional distribution in brain, being highest in neocortex and midbrain followed by medulla-pons, striatum, and diencephalon. Brain levels of [^{3}H]MDA incorporation were lowest in cerebellum, which had 50 percent fewer binding sites than neocortex. Although [^{3}H]MDA incorporation was detected outside the brain, levels were much lower than those found in most of the brain structures studied. The level of [^{3}H]MDA binding in liver was 40 percent and that in kidney 20 percent of that found in neocortex. The regional distribution of [^{3}H]MDA incorporation in the absence of sucrose had a profile similar to that performed in the presence of 0.27 M sucrose (data not shown). There was a significant positive correlation between the regional levels of apparent binding studied under the two conditions (r^2=0.84, $p<0.61$). These data, together with those indicating a significant correlation between the relative inhibition potencies of MDA analogs at high- and low-affinity [^{3}H]MDA binding, suggest an intimate relationship between the high- and low-affinity [^{3}H]MDA binding sites. Although there exist some differences in the pattern of regional distribution between [^{3}H]MDA binding found in the present study and that of [^{3}H]amphetamine binding found by Hauger et al. (1984). similarities also exist. For example, the lowest level of binding in brain for both ligands is found in the cerebellum and low levels of binding are found for both ligands in the periphery. The differences among the regional distributions of [^{3}H]amphetamine, [^{3}H]MDA, and [^{3}H]fenfluramine (Garattini et al. 1987) binding may be attributed to the various membrane preparation and assay procedures used in each study.

TABLE 3. *Regional distribution of apparent [^{3}H]MDA binding*

Region	[^{3}H]MDA Bound (pmol/mg protein)
Neocortex	31 ± 4
Striatum	2 4 ± 3
Diencephalon	2 4 ± 5
Midbrain	29 ± 5
Medulla-Pons	2 6 ± 5
Cerebellum	15 ± 3
Liver	10 ± 1
Kidney	6 ± 2

NOTE: A p_2 preparation was prepared from each dissected region and assayed for [^{3}H]MDA incorporation as described in the text. Incubation was performed in 50 mM Tris-HCI (pH 7.1) containing 0.27 M sucrose and 2.0 nM [^{3}H]MDA. [^{3}H]MDA incorporation into boiled tissue served as the measure of nonspecific binding. Values are expressed as specific [^{3}H]MDA bound (mean ± SEM; pmol/mg protein) and are the results of three experiments performed in triplicate.

Concentration of MDA in Brain After Systemic Administratian

To elucidate further the relevance of the high-nanomolar to low-micromolar affinities of tbe [^{3}H]MDA binding sites, we determined the brain concentrations of MDA following systemic administration of behaviorally active doses (20 mg/kg) of the drug to rats. Rats were injected subcutaneously with 20 mg/kg MDA containing 0.5 μCi of [^{3}H]MDA. Rats were sacrificed at various times after injection, and the hippocampus was removed, weighed, and solubilized overnight in Soluene 100 (Packard). Econoflour (5 mL) (NEN) was then added to the solution, and radioactivity was assessed by liquid scintillation counting. To evaluate the identity of the radioactivity, rats were sacrificed 45 minutes after injection, and brains were removed and homogenized in 0.1 M sodium acetate using a polytron (10 seconds, position 6). After centrifugation at 30,000 x g, the supernatant fluid was collected and 4.0 mL was applied to a C18 Sep Pak cartridge (Waters). The cartridge was washed with 3.0 mL of water, and radioactivity was eluted in 1.0 mL of acetonitrile containing 0.1 percent trifluoroacetic acid. After drying the organic eluate to approximately 200 μL under nitrogen, 50 μL of the extract was injected onto a Waters Nova Pak C18 column and eluted at 1.0 mL per minute with a linear gradient from 3 percent acetonitrile:97 percent 50 mM potassium phosphate buffer (pH 6.4) to 80 percent acetonitrile: 20 percent water over 30 minutes. Eluted compounds were detected by UV absorbance at 214 nm. Fractions (1.0 mL) of the column eluate were collected and added to vials containing 5.0 mL of formula 963, which were then assessed for radioactivity by scintillation counting.

Accumulation and Clearance Findings

Figure 7A shows the timecourse of [^{3}H]MDA accumulation and clearance from rat brain after a subcutaneous injection. Peak concentration, which was reached at 45 minutes, was equivalent to 165 μM (36 μg/g). To verify the identity of the radioactivity as [^{3}H]MDA, reverse phase chromatographic analysis was performed on brain extract from a rat 45 minutes after MDA (20 mg/kg) injection (figure 7B). Radioactivity eluted as a single peak at 14 minutes, which coincided with a peak having a retention time of 13.8 minutes observed by UV detection. This peak, which was not observed in naive animals, was isochromatographic with standard MDA. Thus, the injected material reaching the brain at 45 minutes was MDA, not a metabolite. The level of MDA found in brain indicates that the K_d values of MDA's interaction with rat brain synaptosomes are within the range of the brain concentrations of MDA reached following administration of behaviorally active doses of the drug.

SUMMARY

Brain recognition sites have been identified for [^{3}H]MDA and [^{3}H]MDMA. The dissociation constants of MDA and MDMA for these sites are similar to the concentrations needed to affect several brain neurochemical parameters and are in keeping with concentrations of MDA in brain (165 μM) following administration of behaviorally active doses (20 mg/kg) of the drug. While the characteristics of these binding sites suggest a possible hydrophobic interaction with brain membranes, this interaction is not without specificity, since it has a unique pharmacology and a heterogeneous distribution in brain.

Similarities have been found between [^{3}H]MDA binding studied in the present report and that of apparent [^{3}H](+)amphetamine binding studied by Hauger et al. (1984). Both have extremely high B_{max} values, are optimal in p_2 preparations, are stabilized by sucrose, and share similar patterns of regional distribution. Measuring the specific binding of [^{3}H]amphetamine, [^{3}H]fenfluramine, [^{3}H]MDA, and related compounds under identical conditions will be required to determine the possible relationships among the interactions of these compounds with brain membranes. Further study is also needed to determine the possible importance such interactions of amphetamine and its substituted analogs may have with brain membranes in relation to the pharmacology of these substances.

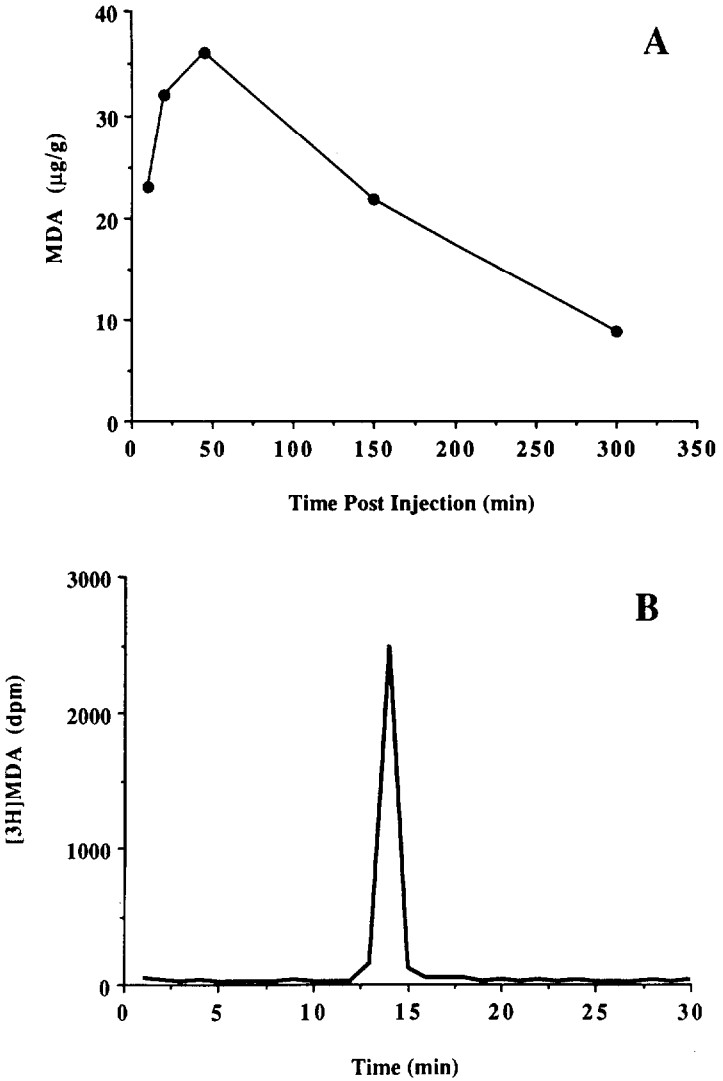

FIGURE 7. *Determination of MDA concentration in rat brain following administration of behaviorally active doses of the drug*

NOTE Panel A shows concentration of hippocampal MDA at various timer after adminitration of 20 mg/kg MDA containing 0.5 uCi of [^{3}H]MDA. Each point represents the average MDA concentration of four hippocampi from two animals. No regional variation was observed in the analysis of MDA concentrations in other brain areas. Panel B shows the reverse-phase chromatographic elution profile of radioactivity extracted from MDA-treated rats.

DISCUSSION

QUESTION: Do you know if there is cell activity at the site?

ANSWER: It has not been tested. We have not done much pharmacology at all.

QUESTION: Do you know if your regional variations in this binding site correlate at all with myelin?

ANSWER: No, I do not know offhand the concentration of myelin in those areas. In fact, I did the profile to prove that it was not lipophilicity, so I was not expecting that. I did not have the foresight to look at the concentration of myelin.

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AUTHORS

Robert Zaczek, Ph.D.
Steven Culp, B.S.
Errol B. De Souza. Ph.D.

Neurobiology Laboratory, Neuroscience Branch
Addiction Research Center
National Institute on Drug Abuse
P.O. Box 5180
Baltimore, MD 21224

Stephen Hurt, Ph.D.
Dupont Company
Boston, MA 02118

Pharmacologic Profile of Amphetamine Derivatives at Various Brain Recognition Sites: Selective Effects on Serotonergic Systems

George Battaglia and Errol B. De Souza

INTRODUCTION

The amphetamines have found widespread use in a number of clinical conditions, including narcolepsy, manic-depressive psychosis, orthostatic hypotension, nasal congestion, migraine, asthma, hyperactivity, and obesity. Although amphetamine (phenylisopropylamine) is structurally a simple molecule, modification of the compound at the aromatic ring, side chain, or terminal amino group can considerably change the pharmacological specificity of the resulting compound. Amphetamine itself is a potent central nervous system (CNS) stimulant and anorectic agent, which acts primarily by blocking catecholamine uptake and causing neurotransmitter release. The addition of a hydroxy group on the beta carbon atom reduces both the stimulant and anorectic effects of the compound, while addition of a second alpha methyl group to amphetamine preferentially attenuates the CNS stimulant properties. Anorectics with reduced stimulant and cardiovascular effects can be created by insertion of groups onto the side chain, terminal amino group, or aromatic ring. Aromatic ring substitution by a number of substituents, including methoxy groups, have been shown to markedly alter the pharmacologic specificity of the drug, from a catecholaminergic agent to one exerting effects primarily on serotonergic systems (Loh and Tseng 1971). For example, paramethoxylation of amphetamine was found to increase greatly the blockade of serotonin uptake and increase the release of [^{3}H]5-HT, while uptake and release of dopamine were found to be attenuated (Loh and Tseng 1971). Since a substantial amount of data have implicated the involvement of brain serotonergic systems in the mechanism of action of hallucinogenic agents (Downing 1964; Brawley and Duffield 1972; Freedman and Halaris 1978; Glennon and Rosecrans 1981; Glennon 1983), it would not be unexpected for a number of ring-substituted psychotomimetic amphetamines to elicit their behavioral and/or subjective effects via their preferential and potent interaction with central serotonin recognition sites.

The psychotomimetic mono- and dimethoxyamphetamines have been reported to produce a number of subjective effects similar to those elicited by agents such as LSD and mescaline (Shulgin et al. 1969; Snyder et al. 1969). Indeed, some of the most potent hallucinogens have been ring-substituted structural analogs of amphetamine. Since the actions of psychotomimetic amphetamines may be mediated at presynaptic serotonin recognition sites, as well as at one or more of the postsynaptic serotonin receptor subtypes (Titeler et al. 1987). it is important to develop a relative pharmacologic profile for these drugs at the various serotonin binding sites.

This chapter will (1) elucidate the serotonergic sites of action of various ring-substituted psychoactive derivatives of amphetamine with an emphasis on derivatives of 2,5-dimethoxyamphetamine (2,5-DMA); (2) describe a detailed pharmacological profile of the newer types of psychoactive methylenedioxy-substituted amphetamine derivatives, the so-called "designer drugs" such as 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA); and (3) elucidate the role of various serotonergic brain recognition sites in mediating some of the behavioral and/or subjective effects of these methylenedioxy derivatives of amphetamine.

INTERACTIONS OF 2,5-DMA DERIVATIVES WITH SEROTONIN RECEPTORS

As mentioned, aromatic ring substituents can greatly enhance the serotonergic activity (Cheng and Long 1973; Cheng et al. 1974; Dyer et al. 1973; Nair 1974) of the amphetamines. Substitution of methoxy groups in the 2,5 position and further substitution of substituents in the para position of the phenyl ring of amphetamines markedly enhances their affinity for serotonin receptors. The advent of the drug discrimination paradigm and its application to the study of such hallucinogenic agents (Hirschhorn and Winter 1971; Silverman and Ho 1980; Glennon et al. 1982; Glennon 1983; Appel et al. 1982) has greatly enhanced our understanding of the putative sites of action of hallucinogenic agents and of the similarities among various hallucinogenic compounds. These studies have demonstrated a significant correlation between the potencies of numerous agents in eliciting intero ceptive hallucinogenic cues in animals and humans (Glennon et al. 1982; Glennon 1983). Drs. Glennon, Titeler, and their collaborators have carried out a series of behavioral and radioligand binding studies to elucidate the serotonin receptor subtype(s) that may be primarily responsible for the actions of these psychoactive agents. Specifically, these studies involved a detailed determination of the affinities of various 2,5-dimethoxy derivatives of amphetamine at 5-HT_1 and 5-HT_2 serotonin receptors (table 1) using radioligand binding techniques to directly label these sites.

The structures of some of these agents are shown in figure 1. Compounds listed in figure 1A are either hallucinogenic in man or produce

TABLE 1. *Affinities of 2,5-DMA derivatives for 5-HT$_1$ and 5-HT$_2$ serotonin receptors*

Agent	5-HT$_2$ Binding[a] K_i(nM)	(Hill Coefficient)	5-HT$_1$ Binding[b] K_i(nM)	Hill Coeficient	K_i(5-HT$_1$)/ K_i(5-HT$_2$)
R(-)-DOI	9.9	(0.72)	2,290	(0.98)	230
(±)-DOI	18.9	(0.73)	2,240	(0.86)	120
S(+)-DOT	35	(0.66)	920	(0.73)	26
R(-)-DOM		(0.71)	3,550	(0.84)	60
(±)-DOB	63	(0.80)	38,340	(0.77)	50
(±)-DOM	100	(0.71)	2,890	(0.82)	30
α-demethyl DOM	110	(0.77)	350	(0.72)	3
R(-)-DON	210	(0.75)	13,300	(0.85)	60
(±)-DON	300	(0.79)	14,100	(1.0)	45
R(-)-N-methyl DOM	260	(0.91)	4,300	(0.86)	15
(±)-N-methyl DOM	415	(0.83)	3,870	(0.86)	10
(±)-DOF	1,110	(0.76)	3,470	(0.97)	3
(±)-2,4,5-TMA	1,650	(0.68)	46,800	(0.49)	30
(±)-4-OEt2,5-DMA	2,220	(0.77)	35,500	(0.83)	15
(±)-4-Me PIA	3,360	(0.89)	14,800	(0.96)	4
(±)-2,5-DMA	5,200	(0.85)	1,020	(0.75)	< 1
(±)-PMA	33,600	(0.87)	79,400	(0.97)	2
(±)-3,4-DMA	43,300	(0.66)	64,600	(0.94)	1

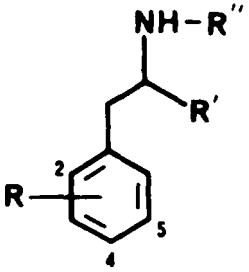
NOTE: [a]K_i values and Hill coefficients determined by competition experiments for 0.4 nM [^{3}H]ketanserin-labeled 5-HT$_2$ serotonin binding sites in rat frontal cortical homogenates. Data from Shannon et al. 1984.

[b]K_i values and Hill coefficents determined by competition experiments for 1.0 nM [^{3}H]LSD(+[10-^{3}M]Ketanserin)-labeled 5-HT^1 sites in rat frontal cortical homogenates. Data from Shannon et al. 1984.

[c]Data from Glennon et al. 1986

hallucinogen-like responding in behavioral studies, while agents listed in figure 1*B* do not generalize to a hallucinogen cue. As shown in table 1, although 2,5-DMA itself exhibits higher affinity for 5-HT$_1$ versus 5-HT$_2$ serotonin receptors, all of the derivatives of 2,5-DMA exhibit substantially higher affinity for 5-HT$_2$ serotonin binding sites and appear to interact more selectively with this site than do tryptamine agonists such as serotonin. The selectivity of 2,5-DMA derivatives for 5-HT$_2$ serotonin receptors is particularly marked for compounds with substituents in the para position. For example, some of the para-halogenated compounds such as the iodinated (DOI) and brominated (DOB) derivatives demonstrate an extremely high affinity and degree of selectivity in their interactions with 5-HT$_2$ serotonin receptors.

Nearly all the derivatives of 2,5-DMA exhibited radioligand binding characteristics at 5-HT$_2$ serotonin receptors that were consistent with those of serotonin and other tryptamine agonists. It has been demonstrated



	R'	R"	R_2	R_4	R_5
(A) 2,5-DMA derivatives					
(±)-2,5-DMA	CH_3	H	OCH_3	H	OCH_3
(±)-2,4,5-TMA	CH_3	H	OCH_3	OCH_3	OCH_3
(±)-4-OEt 2,5-DMA	CH_3	H	OCH_3	OC_2H_5	OCH_3
(±)-DOF	CH_3	H	OCH_3	F	OCH_3
(±)-DOB	CH_3	H	OCH_3	Br	OCH_3
(±)-DOI	CH_3	H	OCH_3	I	OCH_3
R(–)-DOI	CH_3	H	OCH_3	I	OCH_3
(±)-DON	CH_3	H	OCH_3	NO_2	OCH_3
R(–)-DON	CH_3	H	OCH_3	NO_2	OCH_3
(±)-DOM	CH_3	H	OCH_3	CH_3	OCH_3
R(–)-DOM	CH_3	H	OCH_3	CH_3	OCH_3
α-Demethyl DOM	H	H	OCH_3	CH_3	OCH_3
(±)-N-Methyl DOM	CH_3	CH_3	OCH_3	CH_3	OCH_3
R(–)-N-Methyl DOM	CH_3	CH_3	OCH_3	CH_3	OCH_3
(B) Non-2,5-DMA derivatives					
(±)-PMA	CH_3	H	H	OCH_3	H
(±)-3,4-DMA	CH_3	H	H	OCH_3	OCH_3
(±)-4-Me PIA	CH_3	H	H	CH_3	H

FIGURE 1. *Structures of a series of 2,5 DMA and non-2,5 DMA derivatives*

SURCE: Shannon el at. 1984.

previously that classical serotonergic agonists of the hyptamine class interact with high- and low-affinity states of the 5-HT_2 serotonin receptor (Battaglia et al. 1984). Agonist-like properties of serotonin-related compounds were

initially revealed by Hill coefficient (n_H) values of less than one in radioligand binding studies. Values of less than one for n_H suggest interaction of the compound with multiple binding sites or multiple states of the receptor. As shown in table 1, nearly all the derivatives of 2,5-DMA exhibited n_H values of less than one at 5-HT_2, serotonin receptors, suggesting an agonist-like activity at this receptor. Data from competition experiments can be further quantitated using a computer-assisted two-site analysis program (Munson and Rodbard 1980). Computer-assisted two-site analysis for the interactions of a number of the 2,5-DMA derivatives with 5-HT_2 serotonin receptors indicates that these compounds do indeed interact with high- and low-affinity states of 5-HT_2 serotonin receptors, with the percentage of binding sites in the high-affinity state for 2,5-DMA derivatives being comparable to that observed for tryptamine agonists (table 2) (Battaglia et al. 1984). The agonist high-affinity state of 5-HT_2 serotonin receptors has also been shown to be sensitive to divalent cations and guanine nucleotides (Battaglia et al. 1984; Titeler et al. 1985), as previously demonstrated for agonists interacting with receptors coupled to a guanine nucleotide regulatory protein. Consistent with other agonist characteristics and, as shown in figure 2, DOI, the 4-iodo-DMA derivative, exhibited guanine nucleotide sensitivity. This is revealed by the decrease in overall affinity (K_I value) and increase in n_H closer to one in the presence of 5'-gyanylimidodiphosphate (Gpp(NH)p) (figure 2). Furthermore, derivatives such as DOI, DOB, and DOM exhibited substantially higher overall affinities (K_I) and higher affinities at the high-affinity component (K_H of 5-HT_2 serotonin receptors than did a number of tryptamine agonists at this site (table 2) (Battaglia et al. 1984; Shannon et al. 1984). With respect to stereospecificity, the *R*(-) isomers of DOI and other 2,5-DMA derivatives were the more potent isomers at 5-HT_2 serotonin receptors, while the *S*(+) isomers of methoxyamphetamines were more potent at presynaptic serotonin recognition sites. In the last few years, [^{125}I]-DOI (Glennon et al. 1988) and [^{3}H]-DOB (Titeler et al. 1985; Lyon et al. 1987) have proven to be highly selective agonist radiolabels for the high-affinity component of 5-HT_2 serotonin receptors.

Subsequent studies investigating the affinities of these and additional hallucinogenic phenylisopropylamines at 5-HT_2 serotonin receptors have clearly established a prominent role for 5-HT_2 serotonin receptors in the hallucinogenic process. Significant correlations were demonstrated between the *in vitro* affinities of a series of amphetamine derivatives at 5-HT_2 serotonin receptors and both their human hallucinogenic dose and their ED_{50} values in behavioral generalization to a hallucinogen cue (Glennon et al. 1984; Titeler et al. 1988).

Although initial studies indicated that the various derivatives of 2,5-DMA exhibited low affinity for 5-HT_1 serotonin receptors (Shannon et al. 1984). it was unclear from these studies what the affinities of the drugs were for the respective subtypes of 5-HT_1serotonin sites (i.e., 5-HT_{1A}, 5-HT_{IB}, and 5-HT_{1C} receptors). In subsequent studies (Titeler et al. 1988), the affinities

TABLE 2. *Two-site analysis of the interaction of tryptamine agonists and 2,5-DMA derivatives with 5-HT$_2$ serotonin receptors*

	K_H	K_L	$\%R_H$	K_I/K_H
2,5 DMA Derivatives[a]				
R(-)-DOI	1.5 ± 0.5	30.0 ± 5.1	40 ± 5	20
(±)-DOI	2.3 ± 1.0	47.4 ± 16.8	34 ± 9	21
R(-)-DOM	2.7 ± 1.6	190 ± 30	22 ± 5	71
		245 ± 90	50 ± 9	17
(±)-DOB	2.4 ± 0.7	100 ± 25	19 ± 1	42
a-demethyl DOM	35 ± 11	400 ± 110	52 ± 2	12
R(-)-DON	68 ± 29	900 ± 400	55 ± 18	13
(±)-DON	137 ± 49	1,500 ± 730	65 ± 19	11
(±)-2,4,5-TMA	200 ± 60	6,250 ± 1,200	41 ± 6	31
(±)3,4-DMA	3,100 ± 950	80,600 ± 19,000	25 ± 5	26
Tryptamine Derivatives[b]				
Serotonin	30 ± 3	1,173 ± 66	25 ± 4	39
5-Methoxytryptamine	130 ± 26	2,659 ± 550	45 ± 7	20
Bufotenine	96 ± 17	1,043 ± 220	35 ± 8	11
Tryptamine	302 ± 48	4,193 ± 570	15 ± 4	14

NOTE: Data were computer-analyzed with a two-site model (Munson and Rodbard 1980). K_H represents the dissociation constant of agonists clculated for the high-affinity component of [$_3$H]ketanserin binding. K_L is the dissociation constant calculated for the low-affinity component of [$_3$H]ketanserin competition curves. K_I/K_H is the ratio of the two dissociation constants. $\%R_H$ represents the percentage of sites in a high-form for the agonist.

SOURCE: Shannon et al. 1984; Battaglia et al. 1984.

of a comparable series of psychoactive amphetamine derivatives were compared at 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ serotonin receptors. As shown in table 3, all derivatives exhibited relatively low affinities at 5-HT$_{1A}$ and 5-HT$_{1B}$ serotonin receptors but markedly higher affinities at the 5-HT$_{1C}$ serotonin receptor. Although these data suggest that 5-HT$_{1C}$ sites may contribute to some of the effects of these psychoactive amphetamines, the precise role of the 5-HT$_{1C}$ serotonin receptors in the hallucinogenic process or other effects of these drugs remains unclear at the present time.

PHARMACOLOGIC PROFILE OF MDMA AT VARIOUS BRAIN RECOGNITION SITES

Psychotomimetic amphetamines such as mescaline and DOM (STP) have experienced periods of popularity during the last two decades. In recent

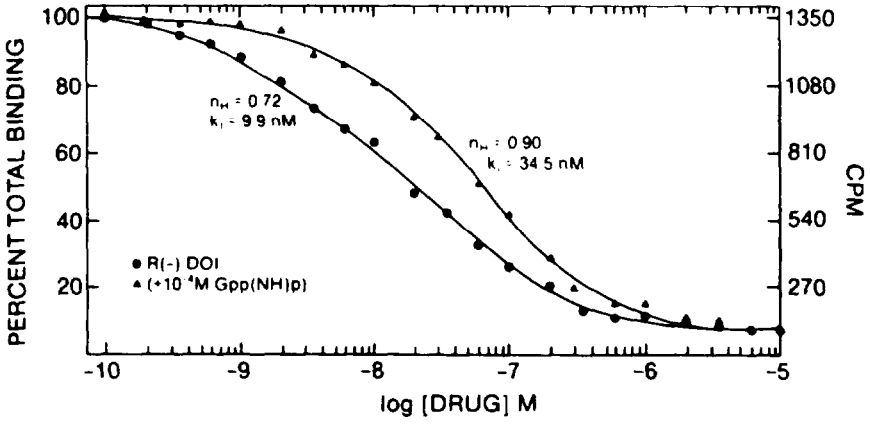


FIGURE 2. *Competition curves of R(-)-DOI for [^{3}H]ketanserin binding to 5-HT$_2$ serotonin receptors in rat frontal cortex membranes in the presence and absence of the guanine nucleotide analog Gpp(NH)p*

NOTE: Lines represent the best fit of the data acording to a model for tow biding sites [in the absence of Gpp(NH)p] and a model for one binding site [in the presence of Gpp(NH)p].

SOURCE: Shannon et al. 1984

years, a new class of designer drug, the methylenedioxyamphetamine derivatives, has received a great deal of attention. These compounds, which include MDMA, MDA, and MDA's N-ethyl derivative MDE, have been reported to elicit both moderate "amphetamine-like" stimulant and weak "LSD-like" hallucinogenic effects.

To elucidate the brain recognition sites through which MDMA might elicit its various behavioral, psychotomimetic, and neurotoxic effects. an extensive *in vitro* pharmacologic screening of MDMA was carried out at various brain neurotransmitter receptors and recognition sites. The relative potencies of MDMA at the various brain recognition sites were assessed from competition data in which affinities (K_i values) were determined using the nonlinear curve-fitting program LIGAND (Munson and Rodbard 1980). Details of the assay conditions and affinities of MDMA at the various recognition sites are reported in table 4. The pharmacologic profile of MDMA demonstrates a broad range of affinities of the drug for various brain recognition sites (Battaglia et al. 1988a). MDMA had the highest affinity for serotonin uptake sites (<1 μM) with lower but comparable affinities at 5-HT$_2$ serotonin, α_2-adrenergic, M-1 muscarinic cholinergic and H-1 histamine receptors (K_i values $\leq$ 5μM). The rank order of affinities of MDMA at

TABLE 3. *Affinities of 2,5-DMA derivatives at 5-HT$_1$ serotonin receptor subtypes*

Agent	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1C}$
R(-)-DOB	2,332 ± 188	683 ± 46	47 ± 10
DOI	2,355 ± 77	1,261 ± 105	30 ± 4
DOB	3,770 ± 188	831 ± 37	69 ± 16
DOPR	2,849 ± 170	2,330 ± 101	14 ± 1
R(-)-DOM	4,004 ± 107	1,840 ± 172	94 ± 17
DOET	3,930 ± 115	2,451 ± 226	101 ± 20
DOM	5,122 ± 140	2,063 ± 112	193 ± 20
S(+)-DOB	4,041 ± 156	883 ± 49	81 ± 7
DOBU	4,178 ± 165	1,211 ± 86	26 ± 5
2,4,5-TMA	>10,000	>10,000	2,666 ± 76
MEM	>10,000	>10,000	2,278 ± 90
2,5-DMA	1,131 ± 55	8,435 ± 668	1,217 ± 89
2,4-DMA	>10,000	>10,000	3,152 ± 83
3,4,5-TMA	>10,000	>10,000	5,710 ± 150

NOTE: The 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ receptors were labeled with ^{3}H-OH-DPAT, ^{3}H-5-HT, and ^{3}H-mesulergine respectively.

SOURCE: Titeler et al. 1988.

various brain receptors and uptake sites were as follows: serotonin uptake > α_2-adrenergic = 5-HT$_2$ serotonin = M-1 muscarinic = H-1 histamine > norepinephrine uptake = M-2 muscarinic = α_1-adrenergic = β-adrenergic ≥ dopamine uptake = 5-HT$_1$ serotonin >> D-2 dopamine > D-1 dopamine. MDMA exhibited negligible affinities (>500 µM) at mu, delta, and kappa opioid, central-type benzodiazepine, and corticotropin-releasing factor receptors, as well as at choline uptake sites and at calcium channels. Although not shown here, the affinities of MDA were comparable (< two-fold difference) to those of MDMA at each of the respective brain recognition sites investigated. In general, the affinities of MDMA at the receptor sites investigated could be classified as high-, moderate-, and low-affinity interactions. These are summarized in table 5. MDMA appears to be most potent at a number of serotonin recognition sites as well as α_2-adrenergic and M-1 muscarinic receptors with affinity constants (K_i values) in the high nanomolar to low micromolar range. Affinities of MDMA in the micromolar range at the various recognition sites appear to be pharmacologically relevant, since similar brain concentrations of the drug have been detected in rats following systemic administration of a single dose of MDMA (20 mg/kg), which elicits behavioral as well as neurotoxic effects (Zaczek et al., unpublished observation).

TABLE 4. *Pharmacologic profile of MDMA at various brain recognition sites*

Brain Recognition Site	Affinity K_i(μM)	Radioligand/Displacer	Brain Region	Assay Time, Tempeature	Buffer
Uptake Sites					
Serotonin	0.61 ± .05	0.55nM[^{3}H]paroxetinel 1μM citalopram	1	120 min, Rm T	A
Norepinephrine	15.8 ± 1.7	4.OnM[^{3}H]mazindol/ 0.3μM desipramine	1	90 min, 4 °C	A
Dopamine	24.4 ± 1.9	1.0nM[^{3}H]GBR 12935/ 1μM mazindol	2	60 min, Rm T	A
Choline	>500	10nM[^{3}H]hemicholinium-3/ 10μM hemicholinium-3	2	30 min, 25 °C	B
Adrenergic Receptors					
α_1	18.4 ± 1.2	0.5nM[^{3}H]prazosin/ 10μM phentolamine	1	30 min, 37 °C	C
α_2	3.6 ± 0.8	0.5nM[^{3}H]para-aminocloni- dine/10μM phentolamine	1	30 min, 37 °C	
β	191 ± 21	0.5nM[^{3}H]dihydroalprenalol/ 1μM propranalol	1	30 min, 37 °C	C
Dopamine Receptior					
D-1	148 ± 14	0.2nM[^{3}H]SCH 23390/ 0.1μM flupenthixol	2	30 min, 37 °C	C
D-2	95 ± 15	0.2nM[^{3}H]spiperone/ 1μM (+)butaclamol	2	30 min, 37 °C	C
Serotonin Receptors					
5-HT_1	23 ± 1.5	2.5nM[^{3}H]serotonin/ 10μM serotonin	1	30 min, 37 °C	C
5-HT_2	5.1 ± 0.3	0.4nM[^{3}H]ketanserin/ 0.5μM cinanserin	1	30 min, 37 °C	C
Cholinergic Receptors					
M-1 muscarinic	5.8 ± 0.3	0.1nM[^{3}H](-)QNB/ 1μM atropone	1	90 min, Rm T	D
M-2 muscarinic	15.1 ± 0.1	0.1nM[^{3}H](-)QNB/ 1μM atropine	3	90 min, Rm T	D
Opioid Receptors					
Mu	>500	2nM[^{3}H]dihydromorphine/ 1μM levallorphan	4	45 min, 25 °C	E
Delta	>500	4nM[^{3}H]D-ala^2-D-leu^5- enkephalin (30nM morphine)/ 1μM levallorphan	4	45 min, 25 °C	E
Kappa	>500	1.6nM[^{3}H]ethylketazocine (30nM morphine + 100nM D-ala^2-D-leu^5-enkephalin)/ 1μM levallorphan	4	45 min, 25 °C	E

TABLE 4. (Continued)

Bran Recongnition Site	Affinity K_i(μM)	Radioligand/Displacer	Brain Region	Assay Time, Temperature	Buffer
Other Sites					
H-1 histamine Receptors	5.7 ± 2.4	2nM[^{3}H]mepyramine/ 1μM doxepin	1	60 min, Rm T	F
Benzodiazepin receptors	>500	0.2nM[^{3}H]flunitrazepam/ 1μM clonazepam	1	60 min, Rm T	G
Corticotropin-releasing factors (CFR) receptors	>500	0.1nM ^{125}I-Tyr^0-rat CRF/ 1μM ovine CCRF	5	120 min, Rm T	H
Calcium channels	>500	0.2nM[^{3}H]nitredipine/ 0.1μM nifedipine	1	60 min, Rm T	G

KEY: Assay buffers: A = 50 mM TRIS-HCl, 120 mM NaCl, 5 mM KCl (pH 7.4 at Rm T); B = 50 mM glycylglycine, 200 nM NaCl (pH 7.8 at 25 °); C = 50 mM TRIS-HCl, 10 mM MSO_4, 0.5 mM K_3HDTA (pH 7.4 aat 37 °C); D = 50 nM TRIS-HCl, 10 mM $MGSO_4$ (pH 7.7 at Rm T) E = 0.17 M TRIS HCl (pH 7.6 at 25 °C); G = 50 mM TRIS-HCl (pH 7.7 at Rm T); F = 50 nM Na^+K^+ phospate (pH 7.4 at Rm T); H = 50 mM TRIS-CHl, 10 mM $MgCl_2$, 2 mM EGTA 0.1% bovine serum albumin, 0.1 mM bacitracin sprotinin (100 KIU/mL) (pH 7.2 at 22 °C). Brain regions: 1 = frontal contex; 2 = striatum; 3 = brain stem; 4 = whole brain; and 5 = olfactory bulb.

NOTE: Data represent the mean ± SEM from three to five competition curves at each of the sites. K_i values were determined using the nonlinear least-sqares curve-fitting program LIGAND.

SOURCE: Battaglis et al. 1988.

TABLE 5. *Relative potencies of MDMA at various brain recognition sites*

High Affinity (0.6 to 6μM)	Moderate Affinity (10 to 100 μM)	Low Affinity (< 100 μM)
Serotonin uptake sites	Norepinephrine uptake sites	D-1 dopamine receptors
5-HT_2 serotonin receptors sites	Dopamine uptake sites	Choline uptake
α_2-adrenergic receptors	5-HT_1 serotonin receptors	Mu, delta, and kappa opioid receptors
M-1 muscarinic receptors	β-adrenergic receptors	Benzodiaepine receptors
		D-2 dopamine receptors

As shown in table 6, we have compared the affinities of a series of methylenedioxy derivatives with those of the parent compounds (amphetamine and methamphetamine) at some of the recognition sites in brain at which MDMA exhibited the highest affinities. These comparative studies indicate that addition of the methylenedioxy substituent in the 3,4 position increases their affinity at serotonin uptake, 5-HT_2 serotonin, and M-1 muscarinic receptors, while the unsubstituted parent compounds appear to be more potent at α_2-adrenergic receptors.

TABLE 6. *Relative potencies of amphetamine derivatives at selected brain recognition sites*

Compound	5-HT Uptake	5-HT_2 Serotonin	α_2 Adrenergic	M-1 Muscarinic
MDMA	1.0	1.0	1.0	1.0
MDA	1.8	0.5	0.5	1.4
MDE	0.4	3.5	3.3	1.8
Amphetamine	4.8	2.6	0.09	4.8
Meth-amphetamine	3.4	2.4	0.61	3.6

NOTE: Comparison of the affinities (K1 values) of amphetamine derivates at serotonin(5-HT) uptake sites, 5-HT_2serotonin, α_2 adrenergic, and M-1 muscarinic receptors with respect to the affinity of MDMA at these sites. Values smaller or larger than 1.0 indicate affinities higher or lower, respectively, than those of MDMA.

SOURCE: Battaglia et al. 1988a.

Interestingly, the anxiolytic-like effects of MDMA do not appear to be mediated through agonist actions at benzodiazepine receptors or antagonist effects at corticotropin-releasing factor receptors as evidenced by the low affinity of MDMA (>500 μM) at each of these receptors. In addition, the reinforcing, analgesic, and mood-altering properties of the drug do not appear to be mediated through interactions with any of the opioid receptor subtypes, since MDMA has relatively low affinities for these receptors.

INTERACTIONS OF MDMA WITH SEROTONIN RECOGNITION SITES

The previous data suggest that a number of the behavioral, psychotomimetic, and neurochemical effects of MDMA and other methylenedioxy derivatives of amphetamine may be explained by interactions of MDMA at multiple serotonin recognition sites in brain. MDMA may alter serotonergic transmission in brain through direct actions at postsynaptic as well as presynaptic serotonin recognition sites. As mentioned above, a number of hallucinogenic phenylisopropylamine derivatives exhibit potent agonist-like activity at brain 5-HT_2 serotonin receptors (Shannon et al. 1984) and the *in vitro* affinities of these hallucinogens at 5-HT_2 serotonin receptors significantly correlate with both their behavioral potencies in animals in generalization to other hallucinogens and with their human hallucinogenic potencies (Glennon et al. 1984; Titeler et al. 1988). Similar to previous observations for other ring-substituted amphetamines such as the derivatives at 2,5-DMA, we found that MDMA and other methylenedioxy derivatives of amphetamine also exhibited high-affinity agonist-like binding characteristics at 5-HT_2 serotonin receptors. The stereospecificity observed for methylene dioxy derivatives at 5-HT_2 receptors was consistent with that observed for other hallucinogenic compounds at this receptor (Lyon et al. 1986; Battaglia

et al. 1986). In addition, it was reported that MDMA interactions with the high-affinity state of 5-HT_2 serotonin receptors were sensitive to the effects of guanine nucleotides, similar to that observed for serotonin and other classical tryptamine agonists at this site (Battaglia et al. 1984) (figure 3). While the overall apparent affinity (K_1 value) of MDMA for [^{3}H]ketanserin-labeled 5-HT_2 serotonin receptors is in the low micromolar range, the authors have observed that the interactions of MDMA and other methylene-dioxyamphetamines with the high-affinity state of 5-HT_2 serotonin receptors labeled directly by [$_3$H]DGB (Lyon et al. 1987) are much more potent (K_1<300 nM). Since this high-affinity component of 5-HT_2 serotonin receptors represents the most potent site of action for MDMA in brain, it is likely that some of the "mood-altering" effects of MDMA may be mediated by direct agonist actions at 5-HT_2 serotonin receptors. A recent study demonstrating that the serotonin receptor antagonist methysergide can potentiate the MDMA-induced increases in locomotor activity (Gold and Koob 1988) further supports the claim for direct actions of MDMA at postsynaptic 5-HT_2 serotonin receptors. A comparison of the relative affinities of MDMA and MDA at postsynaptic 5-HT_2 serotonin receptors with those of other ring-substituted amphetamine hallucinogens suggests that MDMA and MDA would be much weaker hallucinogens at this site than would compounds such as DOM (STP) or DOI. This is not surprising, as the methylenedioxy class of designer drugs has been reported to have unique and subtle mood-enhancing subjective effects, rather than having the more vivid and disorienting sensations commonly attributed to very potent hallucinogens such as DOM or LSD.

In addition to the actions of MDMA and other derivatives at 5-HT_2 serotonin receptors. some of the effects on serotonergic systems could be mediated via 5-HT_{1A} receptors, at which MDMA has a moderate affinity. Direct agonist effects at this site might contribute to the mood-altering and calming effects of the drug, since similar effects have been reported for novel anxiolytics such as ipsaperone and buspirone, which interact with 5-HT_{1A} serotonin receptors.

In addition to its relatively high affinity at postsynaptic 5-HT receptors, MDMA exhibited high affinity for 5-HT uptake sites and has been shown to increase the release of [^{3}H]5-HT and block [^{3}H]5-HT uptake *in vitro.* These data suggest that some of the actions of MDMA may be mediated at presynaptic binding sites. With respect to [^{3}H]5-HT release, MDMA has been reported to increase the release of [^{3}H]5-HT from brain synaptosomes (Nichols et al. 1982) and hippocampal slices (Johnson et al. 1986). With respect to uptake blockade, MDMA has been reported to competitively inhibit ^{3}H-5-HT uptake *in vitro* (Shulgin 1986). Furthermore, the neurotoxic effects of *in vivo* administration of MDMA on serotonin terminals can be blocked by concomitant administration of the 5-HT uptake blocker citalo-pram (Battaglia et al. 1988b; Schmidt and Taylor 1987). Additional evidence in support of the hypothesis that MDMA produces some of its

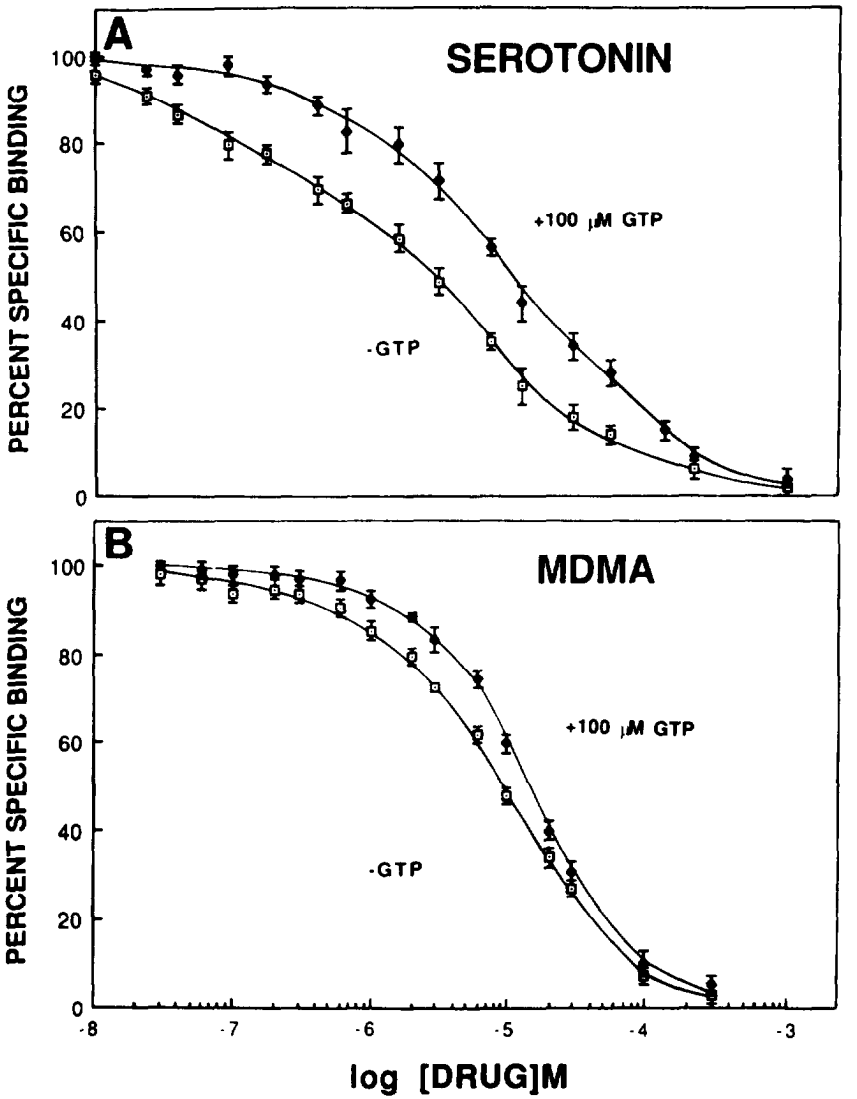


FIGURE 3. *Competition curves of (A) serotonin and (B) MDMA for [^{3}H] ketanserin binding to 5-HT^2 serotonin receptors in rat frontal cortex membranes in the presence and absence of the guanine nucleotide quanosine-5-triphosphate (GTP)*

effects through presynaptic serotonergic mechanisms is provided by data demonstrating that MDMA can generalize to a fenfluramine cue in stimulus discrimination studies (Schechter 1986).

Classic a-adrenergic receptor antagonists such as phentolamine have been reported to increase the release of [^{3}H]5-HT via effects on α_2-adrenergic receptors (Timmermans and Van Zwieten 1982). Thus, one might speculate that the serotonin-releasing effects of MDMA may be mediated, in part, by high-affinity antagonist-like effects at α_2-adrenergic receptors localized to presynaptic serotonin terminals. The relatively high affinity of MDMA at the serotonin uptake site and α_2-adrenergic receptor may contribute, in part, to the neurochemical, neurotoxic, and behavioral effects mediated at presynaptic serotonin terminals.

While brain serotonin systems may play a key role in mediating some of the effects of MDMA on analgesia and body temperature as well as in the reported anxiolytic-like and mood-altering subjective effects of the drug, additional neurotransmitter systems may contribute to some of the unique subjective experiences reported for MDMA and other drugs in this class.

SUMMARY AND CONCLUSIONS

Ring-substituted psychoactive derivatives of amphetamine exhibited high affinities for a number of serotonin recognition sites. Derivatives of 2,5-DMA exhibited preferential high affinity at 5-HT$_2$ serotonin receptors when compared to their relative affmities at 5-HT$_1$ serotonin receptors. Furthermore, 2,5-DMA derivatives exhibited agonist-like binding characteristics at 5-HT$_2$ serotonin receptors with the *R*(-) isomer being the more potent isomer. There were significant correlations between the *in vitro* affinities of 2,5-DMA derivatives at 5-HT$_2$ serotonin receptors and their human hallucinogenic potencies as well as with their potencies in behavioral generalization studies, suggesting the importance of 5-HT$_2$ serotonin receptors in mediating the hallucinogenic effects of the various 2,5-DMA derivatives.

A pharmacological profile of the methylenedioxy-substituted amphetamine derivatives indicates that MDMA and MDA exhibited highest affinity for serotonin uptake sites, 5-HT$_2$ serotonin, α_2-adrenergic and M-1 muscarinic receptors. The methylenedioxy amphetamine derivatives exhibited an inverse stereospecificity with respect to serotonin uptake sites versus postsynaptic 5-HT receptors with the *S*(+) isomer being more potent at the presynaptic recognition site, while the *R*(-) isomer was more potent at the postsynaptic recognition sites. Similar to the 2,5-DMA derivatives, MDMA and MDA exhibited agonist-like binding characteristics at 5-HT$_2$ serotonin receptors. Unlike 2,5-DMA derivatives, MDMA and MDA demonstrated little selectivity for 5-HT$_2$ versus 5HT$_1$ subtypes of receptors. The relatively weak hallucinogenic effects of the methylenedioxy-substituted

amphetamine derivatives (when compared to the 2,5-DMA derivatives) may be mediated through actions at 5-HT_2 serotonin receptors. In addition, the neurotoxic, psychotomimetic, analgesic, temperature regulation, and mood-altering effects of MDMA and other methylenedioxy-substituted derivatives may be mediated, in part, through their actions at other serotonin recognition sites in brain, including serotonin uptake sites and 5-HT_{1A} serotonin receptors. Other behavioral. cardiovascular, and toxic effects of MDMA and related derivatives may be mediated by actions at other central and/or peripheral recognition sites, including muscarinic cholinergic receptors and α_2-adrenergic receptors, for which these compounds exhibit relatively high affinity. The precise mechanisms for the various effects of the amphetamine derivatives remain to be determined.

DISCUSSION

QUESTION: In the absence of serotonin neurons, could MDMA still have a direct agonist action at postsynaptic receptors or is that 5-HT?

ANSWER: Yes. From the present data, one would expect direct agonist effects of MDMA at 5-HT_2 receptors in the absence of serotonin neurons. Based on the data that I showed you today, MDMA and other methylene dioxy amphetamine derivatives exhibit agonist-like binding properties that resemble those observed for 5-HT and other tryptamine agonists as well as for other hallucinogenic amphetamines. We would expect the effects of MDMA at 5-HT_2 receptors to be somewhat weaker compared to those of other amphetamine derivatives, since MDMA-like compounds exhibit substantially lower affinity than the 2,5-DMA derivatives at 5-HT_2 sites. In addition, unlike what we Observe with the 2,5-DMA derivatives, MDMA and the other methylenedioxy compounds do not exhibit the preferential affinity for 5-HT_2 sites over 5-HT_1 subtypes as observed for the more potent hallucinogens. The comparable affinity of MDMA for multiple 5-HT receptors may contribute to the comparatively weak hallucinogen-like properties of this class of compounds. With respect to the second part of the question, it would be expected that, in the presence of an intact serotonergic system, MDMA-induced release of 5-HT via presynaptic sites of action would also have some postsynaptic 5-HT_2 receptor consequences. I inferred that MDMA may have antagonist-like effects at $alpha_2$ adrenergic receptors, and this may be responsible for increased 5-HT release. However, the only recognition site where we have tried to discern agonist versus antagonist characteristics is at the 5-HT_2 serotonin receptors.

QUESTION: Not the presynaptic?

ANSWER: No, only the postsynaptic 5-HT_2 receptors.

COMMENT: It seems to me that, in the absence of the serotonin input, the 5-HT_2 serotonin receptors downregulate instead of upregulate. So if you

took away the serotonin input, you would expect to see a decreased potency.

RESPONSE: Downregulation of 5-HT_2 receptors, which has been observed following treatment with antagonists, may be viewed as due to compensatory changes in response to tbe absence of 5-HT input. In order to assess the hypothesis that there was modulation in the absence of serotonin, we looked at 5-HT_2 serotonin receptors following lesion with MDMA. We chose to look at a time point 2 weeks after treatment in order to allow time for postsynaptic receptor changes to occur. Although we did not see any changes in the density of sites, we have not investigated whether there may have been changes in second messenger systems coupled to these receptors.

QUESTION: Would you expect the direct effects of MDMA on the 5-HT_2 receptor to have any significance in the presence of this massive 5-HT release that it is causing?

ANSWER: When we are dealing with the effects of the methylenedioxy-substituted derivatives on serotonergic systems, we are dealing with a multiplicity of effects.

Our data indicate that the racemates of these compounds most likely mediate effects on both presynaptic as well as postsynaptic 5-HT sites. Furthermore, there is an inverse stereospecificity associated with these actions. For example, the dextro isomers of MDMA and other drugs in this class exhibit higher affinity than the levo isomer for the presynaptic 5-HT uptake and also appear to be more potent in causing 5-HT release. This is the opposite of the isomer affinities at postsynaptic receptors. The levo isomers of MDMA-like compounds exhibit preferentially higher affinity than the dextro isomers for both 5-HT_1 and 5-HT_2 serotonin receptor subtypes. Similar stereospecificity is observed with the parent compounds, amphetamine and methamphetamine, as well for the hallucinogenic 2,5-DMA derivatives. While we can discern the agonist properties of these compounds at 5-HT_2 receptors, it is unclear whether these drugs are acting as agonists or antagonists at the various subtypes of 5-HT_1 receptors. With respect to the original question, if MDMA exhibits simultaneous 5-HT_2 agonist and 5-HT_1 antagonist activity, then one may speculate that these effects can significandy influence the fmal response, even in the presence of massive 5-HT release by these agents.

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AUTHORS

George Battaglia. Ph.D.
Assistant Professor
Department of Pharmacology
Loyola University of Chicago
Stritch School of Medicine
2160 South First Avenue
Maywood, IL 60153

Errol B. De Souza, Ph.D.
Chief, Laboratory of Neurobiology
Neuroscience Branch
Addiction Research Center
National Institute on Drug Abuse
Baltimore, MD 21224

Effects of Amphetamine Analogs on Central Nervous System Neuropeptide Systems

Glen R. Hanson, Patricia Sonsalla Anita Letter, Kalpana M. Merchant, Michel Johnson, Lloyd Bush, and James W. Gibb

INTRODUCTION

Substantial efforts have been devoted to elucidating the effects of amphetamine analogs on central nervous system (CNS) monoaminergic pathways. These agents enhance the activity of such neuronal systems by causing release of their transmitter substances as well as by interfering with transmitter metabolism and reuptake. However, little is known about the consequences of the monoaminergic changes resulting from the administration of these agents, i.e., the eventual effect of these drugs on transmitter systems directly influenced by the monoaminergic pathways. Such effects are important in transmitting the monoamine-initiated messages to those brain regions that eventually mediate the drug-related behavioral changes. In addition, these systems likely have important feedback functions on the amphetamine-sensitive monoaminergic pathways. Consequently, drug-induced changes in these feedback pathways might contribute to phenomena such as tolerance and sensitization.

Of interest to the present work are the neuropeptide neuronal projections associated with extrapyramidal structures and the responses of peptidergic pathways to treatments with amphetamine analogs. These peptide systems were selected for study because of their close association with the mesostriatal dopaminergic neuronal circuitry, a system thought to contribute to the locomotor and mood-altering effects of the amphetamine compounds. For example, neurons containing substance P (SP), which originate within the shiatum and terminate in the substantia nigra, are thought to serve an excitatory feedback function on the mesostriatal dopamine (DA) pathway. Thus, intranigral injections of SP cause striatal release of DA (Reid et al. 1988) and stimulate locomotion (Herrera-Marschitz et al. 1986). Nigral administration of SP has no effect on locomotor activity in animals that have received 6-hydroxydopamine lesions to their mesostriatal DA pathway (Herrera-Marschitz et al. 1986).

The interactions between neurotensin (NT) pathways and the extrapyramidal dopaminergic system are somewhat more complex. As the vast majority of striatal and nigral NT receptors are associated with DA neurons (Quirion et al. 1985). NT pathways certainly contribute to the regulation of extrapyramidal DA activity. The overall CNS pharmacology of NT has been compared to that of neuroleptic drugs (Nemeroff 1986), while intraventricular administration of this peptide is reported to antagonize some of the behavioral activity of amphetamine and cocaine (Skoog et al. 1986). Finally, dynorphin (Dyn) A1-17 is associated with striatal-nigral neurons that, like the SP pathway, have been postulated to be part of a feedback system to the nigral-striatal DA neurons (Herrera-Marschitz et al. 1983). However, such a feedback role for Dyn has been questioned recently, as the locomotor activity induced by nigral Dyn injections is not impaired by elimination of the nigral-striatal DA pathway (HerreraMarschitx et al. 1986).

EVALUATION OF NEUROPEPTIDE RESPONSE TO AMPHETAMINE ANALOGS

This chapter discusses the responses of these extrapyramidal neuropeptide systems to the amphetamine analogs methamphetamine (METH), methylene dioxyamphetamine (MDA), and methylenedioxymethamphetarnine (MDMA). These drugs were selected for this study because they represent somewhat diverse mechanisms of action. While all three agents are able to enhance extrapyramidal serotonergic activity (Schmidt et al. 1987). only METH has been characterized as a substantial stimulant of the DA system. The effects of MDA and MDMA on extrapyramidal DA systems have not been well elucidated. Thus, evaluating and comparing the responses of the SP, NT, and Dyn extrapyramidal systems to these drugs will help to determine the nature of the DA responses to METH, MDA, and MDMA administrations.

Methods

Sprague Dawley rats (180 to 220 g) were treated with METH, MDA, and MDMA generously donated by the National Institute on Drug Abuse. Following drug treatments, rats were sacrificed, brains removed, and the striatal and nigral areas dissected out. Tissue samples were rapidly frozen and stored until analyzed. The responses of these neuropeptide systems to treatments by the amphetamine analogs were assessed with radioimmunoassay techniques by measuring drug-induced changes in the tissue content of neuropeptidelike immunoreactivity. Highly selective and sensitive antibodies were used in the detection of SP (Hanson and Loveberg 1980), NT (Letter et al. 1987). and Dyn (Hanson et al. 1987). The mean nigral contents of SP, NT, and Dyn for the control groups were 12 nanograms (ng), 595 picograms (pg), and 766 pg per mg protein, respectively. The mean striatal contents for SP, NT, and Dyn for the control groups were 1,250,127, and 380 pg/mg protein, respectively. To characterize the

METH-induced changes in neuropeptide levels, selective D_1 (SCH 23390) and D_2 (sulpiride) dopaminergic receptor antagonists were coadministered. The results are expressed as percent of control to facilitate comparisons; each value represents the mean ± SEM of five to seven animals. Data were subjected to either a Student's r-test (figures 4 and 5) or ANOVA analysis followed by a multiple comparisons test (figures 1, 2, and 3). Significance was set at the .05 level.

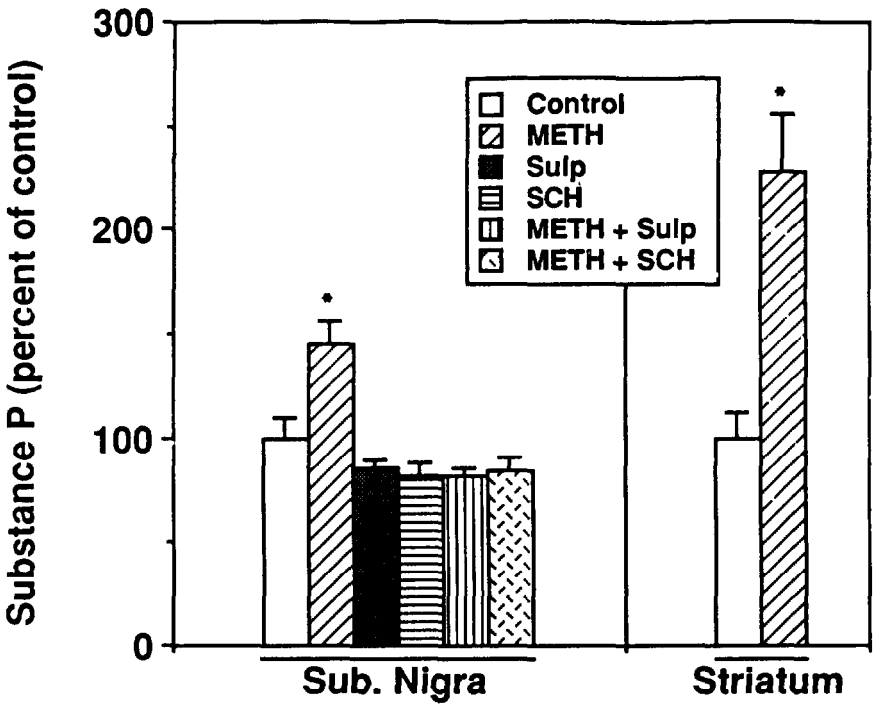

FIGURE 1. *Effects of METH on extrapyramidal SP content*

*p<0.02 compared to corresponding groups.

NOTE: METH was injected alone or concurrently with either SCH 23390 (SCH) (0.5 mg/kg/injection) or sulpiride (sulp) (80 mg/kg/injection).

Results

Administrations of five injections of METH (15 mg/kg/injection; 6-hour intervals between injections) caused substantial increases in the striatal and nigral levels of all three neuropeptides examined in rats sacrificed 18 hours following treatment. Figures 1 to 3 present the effects of blocking the D_1 and D_2 dopaminergic receptors on the responses by these peptide systems

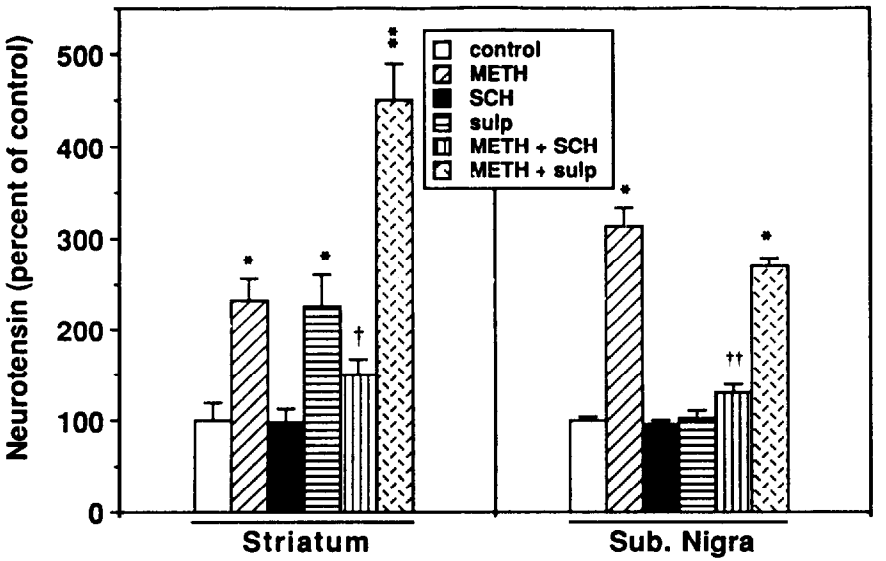

FIGURE 2. *Effects of METH on extrapyramidal NT content*

*p<0.02 compared to corresponding control.

**p<0.01 compared to the corresponding METH- and sulpride (sulp)-treated groups, and p<0.001 compared to the corresponding control.

†p<0.05 compared to the corresponding METH-treated groups.

††p<0.01 compared to the corresponding METH-treated group.

NOTE: Animals were treated as described in figure 1.

to METH treatment. Figures 4 and 5 present the responses of SP, NT, and Dyn extrapyramidal pathways to MDMA and MDA treatments, respectively.

Following METH administration, levels of SP were elevated to 150 percent of control in the substantia nigra and 227 percent of control in the striatum (figure 1). Blockade of either D_1 or D_2 receptors totally prevented the METH-induced rise in nigral SP content.

In rats sacrificed 18 hours following METH treatment, nigral and striatal levels of both NT (figure 2) and Dyn (figure 3) increased dramatically, to 200 to 400 percent of respective controls. However, the effects of D_1 and D_2 receptor antagonism on the METH-induced changes in these peptide systems were somewhat different. Administration of sulpiride alone caused an increase in striatal NT levels. METH administration in the presence of

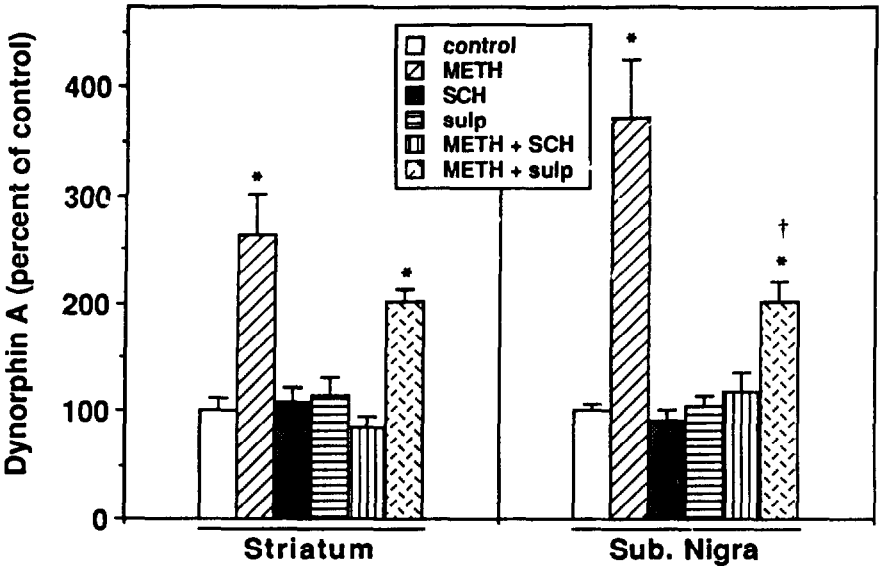

FIGURE 3. *Effects of METH on extrapyramidal Dyn A content*

*p<0.02 compared to corresponding controls.

†p<0.05 compared to the corresponding METH-treated group.

NOTE: Animals were treated as described in figure 1.

this D_2 blocker resulted in increases of striatal NT content approximately equal to the summation of the effects of the two drugs when given individually. In contrast, the D_1 antagonist, SCH 23390, had no effect alone and substantially attenuated the METH-induced striatal changes in NT content. The SCH 23390 compound also completely blocked the METH-mediated elevation of nigral NT levels, while sulpiride had no effect of its own, nor did its presence significantly influence the response of the nigral NT system to METH treatment.

Administration of sulpiride or SCH 23390 alone did not alter the striatal or nigral content of Dyn. Blockade of D_1 receptors substantially interfered with the METH-induced changes in both striatal and nigral Dyn levels. Blockade of D_2 receptors by sulpiride appeared to attenuate the METH-related changes in the Dyn levels, especially in the substantia nigra, but its interference with the METH effects was less than that of the SCH 23390 compound.

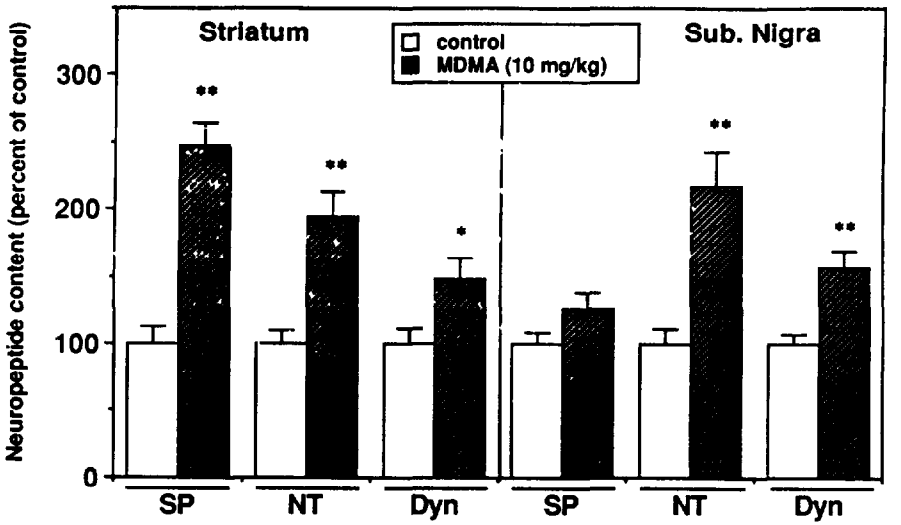

FIGURE 4. *Effects of MDMA on extrapyramidal neuropeptide contents*

*p<0.05 compared to corresponding controls.

**p<0.001 compared to corresponding controls.

NOTE: Animals were given multiple injections of MDMA (10 mg/kg/injection) and sacrificed 18 hours after treatment. Striatal and nigral content of SP, NT, and Dyn were examined

The effects of five injections of MDMA (10 mg/kg/injection) on striatal and nigral neuropeptide content are presented in figure 4. Animals were treated in a manner similar to that used for the METH experiments. Following multiple MDMA administrations, the striatal levels of SP, NT, and Dyn were elevated to 248 percent., 195 percent, and 148 percent, respectively, of corresponding controls. Nigral content of these same peptides were increased to 127 percent, 217 percent, and 157 percent, respectively, compared to their controls. These effects resembled those observed with METH treatment, Similar SP and NT responses were observed following MDA treatment (figure 5).

CONCLUSION

These findings demonstrate that some neuropeptide systems associated with mesostriatal dopaminergic projections are profoundly altered by treatment with each amphetamine analog examined.Although the significance of these drug-induced increases in striatal and nigral contents of SP, NT, and

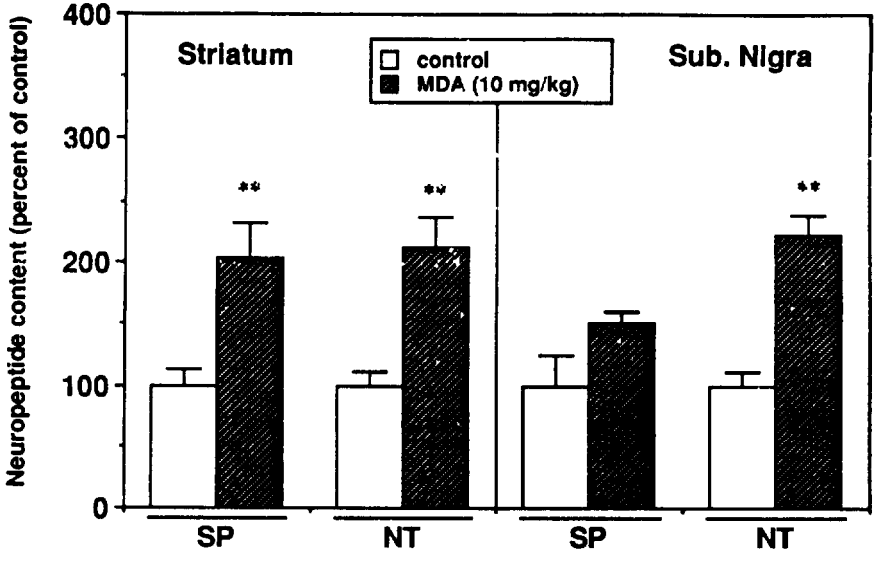

FIGURE 5. *Effects of MDA on extrapyramidal neuropeptide contents*

**p<0.01 compared to corresponding controls.

NOTE: Animals were given multiple injections of MDA (10 mg/kg/injections) and sacrificed 18 hours after treatment. Striatal and nigral levels of SP and NT were determined.

Dyn is not yet known, it is likely that such changes reflect variations in the activity of the associated pathways. One possible explanation is that increases in neuropeptide tissue levels are due to decreased release of the transmitter, which diminishes the extracellular peptide metabolism and results in accumulation of these peptide substances. Another possible contributing factor is a drug-related alteration in neuropeptide synthesis. For example, Bannon et al. (1987) reported that METH administration increased the quantity of striatal messenger RNA for the SP precursor preprotachykinin. Thus, increases in peptide synthesis might contribute to increases in peptide content caused by treatment with METH or the other amphetamine analogs.

The dramatic responses to METH reported herein were most certainly a consequence of drug-mediated changes in postsynaptic dopaminergic activity. It is interesting that each neuropeptide response to METH treatment was subtly unique. The increases in SP content cased by METH appeared to occur primarily by activation of D_2 receptors (figure 1). This conclusion is based on previously reported findings that D_2 agonists also increase nigral SP levels, while D_1 agonists actually cause a decrease in the nigral SP

concentration (Sonsalla et al. 1984). Even so. D_1 receptors appeared to play a facilitatory role in this drug effect, as blockade of this receptor completely prevented the METH effects. The effects of METH on the NT systems appeared to be mediated completely by D_1 receptors, as the presence of SCH 23390 almost entirely blocked the METH-mediated changes in NT levels, while sulpiride did not appear to interfere with the METH effects (figure 2). Finally, these data suggest that the actions of METH on the Dyn systems were mediated primarily by D_1 receptors; even so, D_2 receptors also contributed to these effects as their blockade attenuated, although to a lesser degree than D_1 blockade, the METH-related increases in Dyn levels (figure 3).

The present data demonstrate that the amphetamine analogs MDA and MDMA influence the extrapyramidal neuropeptide systems in a METH-line manner (figures 4 and 5). As already discussed, the METH effects on these peptide systems are dopaminergically mediated, thus, it is likely that the amphetamine designer drugs also influence SP, NT, and Dyn extrapyramidal pathways by enhancing extrapyramidal dopaminergic activity. In support of this conclusion, we have observed that blockade of D_1 receptors with SCH 23390 completely blocks the increases in striatal NT and Dyn induced by MDMA treatment (unpublished observation). This finding is consistent with observations that MDMA and MDA stimulate the release of striatal DA from tissue slices (Schmidt et al. 1987) and intact animals (Yamamoto and Spanos 1988). In addition, Stone et al. (1986) reported that treatments with MDA and MDMA resulted in increases in striatal concentrations of homovanillic acid, a DA metabolite, which reflects the extent of DA release.

While perhaps quantitatively different, each of the amphetamine analogs examined had substantial effects on the extrapyramidal SP, NT, and Dyn pathways. Thus. these peptide pathways likely contribute to the behavioral effect of this group of agents in general; specifically, they might participate in mediating the changes in locomotion or mood or the development of psychotic disorders associated with administration of high doses of the amphetamine analogs. More studies are necessary to identify specific contributions ma& by each of these peptide systems to the pharmacological profiles of these agents. In addition, these neuropeptide changes are of interest as nemochemical markers for the effects of the amphetamine drugs on postsynaptic dopaminergic activity and could be useful in the study of such consequences of these drugs as tolerance and sensitization.

DISCUSSION

QUESTION: The last slide referred to postsynaptic actions of the drugs. Do you mean postsynaptic consequences of their presynaptic actions?

ANSWER: Yes. We all know that the dopamine comes out The question is: What happens after the dopamine comes out? We know that if nothing occurred following the dopamine release as far as other transmitter systems being influenced, them would be no behavioral effect. So downstream systems like these peptides are probably involved in mediating those monoaminergic messages to some other parts of the brain or playing feedback roles and altering the way that the monoamine systems respond So they may play roles in sensitization or tolerance by impacting on the activity of those projections.

QUESTION: Did you mention that 6-hydroxydopamine blocks or elevates neurotensin levels?

ANSWER: Yes, 6-hydroxydopamine by itself elevates neurotensin levels. When you combine it with methamphetamine, you do not get any additivity. It is just a 6-hydroxydopamine action. It is a bit complicated to interpret, but it appears that it is still the nigral striatal dopamine pathway that is mediating the methamphetamine effect.

QUESTlON: Have you had the opportunity to look at substance P, possibly in the spinal cord? I am thinking about some of the work that Dr. Seiden presented and potentially a role in analgesia.

ANSWER: We have not looked in the spinal cord at all for substance P. Everything has been in the extrapyramidal and limbic systems.

COMMENT: Another reason why you should be looking at substance P in the spinal cord is that, in spinal cord, substance P is cocontained in neurons together with tyrosine hydroxylase.

RESPONSE: Right. And we have asked ourselves the question because of the issue of coexistence, not only with substance P but with neurotensin and probably dynorphin. Is this the reason these things are changing? Because if they are coexisting with dopamine projections and there is some alteration in dopamine, then maybe there is an intraneuronal action that results in the peptide changes.

COMMENT: I was thinking about this, but I couldn't remember if substance P had been shown to be colocalized in the striatum.

RESPONSE: No, if there is any, it is very, very small coexistence of substance P and tyrosine hydroxylase in the striatum. That is why we don't feel that that is the explanation for these changes.

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AUTHORS

Glen R. Hanson, Ph.D., D.D.S.
Anita Letter, Ph.D.
Kalpana M. Merchant, Ph.D.
Michel Johnson, Ph.D.
Lloyd Bush, M.S.
James W. Gibb, Ph.D.

University of Utah
Salt Lake City, UT 84112

Patricia Sonsalla, Ph.D.
Robert Wood Johnson Medical School
Piscataway, NJ 08854-5635

Effects of Neurotoxic Amphetamines on Serotonergic Neurons: Immunocytochemical Studies

Mark E. Molliver, Laura A. Mamounas, and Mary Ann Wilson

INTRODUCTION

The goal of this chapter is to review recent morphologic studies in which current anatomic methods have been used to characterize the neurotoxic effects of psychoactive amphetamine derivatives. Several strategies combining anatomic with biochemical data have been employed to analyze the effects of selected drugs in this class. These studies show that psychoactive drugs that have selective neurotoxic effects can be useful experimental tools to study the neural mechanisms of elusive brain functions such as affective state control and perceptual integration.

Serotonergic neurons appear to play an important role in higher mental functions, especially in emotional expression, and in mediating many of the effects of psychotropic or hallucinogenic drugs. The presence of serotonin (5-HT) in the brain was first demonstrated in the 1950s employing assay techniques based on the action of 5-HT on smooth muscle (Twarog and Page 1953; Amin et al. 1954). Marked regional differences in brain levels of 5-HT were later found in the dog and cat brain using spectrophotometry (Bogdanski et al. 1957). High 5-HT levels in limbic areas of the brain led these authors to speculate that 5-HT may be involved in emotional expression. The observation that the hallucinogenic drug LSD antagonized the contractile action of 5-HT on uterine muscle led Gaddum in 1953 and 1958 to propose that 5-HT may act as a central neurotransmitter with a specific role in cerebral function. Based on the behavioral effects of LSD, Woolley and Shaw (1954) postulated that 5-HT may be involved in psychiatric disorders such as schizophrenia. LSD was then shown to decrease 5-HT turnover in the brain and increase 5-HT levels (Freedman 1961), an effect that was presumably due to inhibition of 5-HT cells in the dorsal raphe (DR) nucleus (Aghajanian et al. 1968; Aghajanian et al. 1970). Subsequent physiologic studies have shown that the primary effects of LSD and of phenethylamine hallucinogens (such as mescaline or 2,5-dimethoxy-4-methylamphetamine (DOM) are exerted at serotonergic synapses in

forebrain (Aghajanian et al. 1970; Rasmussen and Aghajanian 1986, Trulson et al. 1981; Jacobs 1984). Receptor binding studies have indicated that the behavioral effects of several of these hallucinogenic drugs are blocked by ketanserin. a 5-HT_2 antagonist (Heym et al. 1984) and that the hallucinogenic potency correlates roughly with the affinity of such compounds for 5-HT_2 binding sites (Glennon et al. 1984; Glennon 1985). The studies cited above strongly implicate serotonergic synapses in mediating hallucinogenic drug effects. Mom recent investigations have supported the view that designer drugs that are substituted amphetamine derivatives with psychotropic properties typically release 5-HT from 5-HT axon terminals and, in some cases, may produce neurotoxic effects.

SURVEY OF SEROTONERGIC NEURONAL SYSTEMS IN THE BRAIN

An overview of the anatomic organization of 5-HT projections in the brain is useful as background for understanding the actions and toxicity of psychotropic amphetamines. Important features of 5-HT neurons are the diversity of cell types in multiple raphe nuclei and the specificity of their organization. Serotonergic neurons, first demonstrated by the histofluorescence method (Falck et al. 1962), are restricted to the brain stem, where they are localized in multiple discrete clusters along the midline, primarily within neuronal cell groups designated as the raphe nuclei (Taber et al. 1960; Dahlstrom and Fuxe 1964). These serotonergic nuclei extend from the midbrain to the caudal medulla and were originally described as nine cell groups, named B1 to B9, by Dahlstrom and Fuxe (1964). Serotonergic axon terminals have been found in widespread areas of the forebrain (including cerebral cortex, striatum, and diencephalon) (Fuxe 1965) and throughout the brain stem and spinal cord. A series of studies employing small intracerebral lesions (Anden et al. 1966; Ungerstedt 1971) indicated that most 5-HT nerve terminals in the forebrain arise from raphe nuclei in the midbrain and that the axons ascend through the lateral hypothalamus within the medial forebrain bundle (Moore and Heller 1967; Azmitia 1978; Conrad et al. 1974).

While most serotonergic cell bodies are located primarily in the midline raphe of the brain stem, some 5-HT cells lie outside the boundaries of the raphe nuclei, and not all raphe cells are serotonergic. Serotonergic axons that innervate the forebrain arise from neurons within the mesencephalic raphe nuclei. These cell groups are found primarily in the midbrain and rostral pons and were originally classified as groups B6 to B9. The largest group of serotonergic neurons is the DR nucleus (B7, DR), which lies within the periaqueductal gray matter. This nucleus extends from a level just caudal to the oculomotor nucleus down to the rostral portion of the fourth ventricle. The DR is continuous caudally with a smaller group of 5-HT cells (B6) that lie along the midline and the floor of the fourth ventricle. The median raphe (MR) nucleus (also designated central superior or B8)

lies within the central portion of the reticular formation in the midbrain tegmentum (figure 1). The B9 cell group consists of a scattered group of 5-HT neurons that lie along the dorsal surface of the medial lemniscus in the ventrolateral tegmentum. The other raphe nuclei, B1 to B5, contain fewer serotonergic cells and are located along the midline in the midpons and medulla. These more caudal cells give rise primarily to connections in brain stem and spinal cord. Several more detailed reviews of the serotonergic cells have been published recently and should be consulted for further information (Moore 1981; Wiklund et al. 1981; Consolazione and Cuello 1982; Jacobs et al. 1984; Molliver 1987). An account of serotonergic pathways and ascending projections in the rat has been published by Azmitia and Segal (1978). and a map of raphe cells and projections in the primate is presented elsewhere (Azmitia and Gannon 1986).

SEROTONERGIC INNERVATION OF CORTEX

While it has been widely believed that 5-HT along with other monoamine (MA) neurons have diffuse and nonspecific projections, numerous pieces of evidence indicate that 5-HT projections, although widely distributed throughout the forebrain, have a high degree of heterogeneity, specificity, and organization. Recent studies have shown that all cortical areas are innervated by 5-HT axons, which form a dense terminal arborization with striking regional differences in the laminar distribution and density of axons. The original histofluorescence studies were limited by weak fluorescence of 5-HT and rapid fading due to photodecomposition of fluorescent molecules. The low sensitivity of histofluorescence did not permit detection of fine axons in the forebrain, so that the density of innervation was initially underestimated. It was not feasible to visualize the full extent of cortical 5-HT innervation until the advent of immunocytochemistry using 5-HT antibodies developed by Steinbusch et al. (1978). which were used to depict the distribution of 5-HT innervation in rat brain (Steinbusch 1981). An antibody to 5-HT produced in this laboratory was used to analyze the 5-HT innervation pattern of cerebral cortex (Lidov et al. 1980). Lidov and colleagues demonstrated a high density of 5-HT-containing axons throughout the cerebral cortex of the rat with marked regional differences in the density of axons and the laminar pattern of innervation. A high density of axons was found in frontal cortex with a gradual decrease in more caudal areas. In that and subsequent studies (Kosofsky 1985; Blue et al. 1988a), a distinct laminar pattern of innervation was found in somatosensory cortex, and a quite different pattern in the cingulate cortex, hippocampus, and dentate gyrus, where there are distinct bands of highly varicose axons. In the primate, the 5-HT innervation of cerebral cortex is denser and more highly differentiated among different architectonic and functional areas (Kosofsky et al. 1984; Morrison et al. 1982; Morrison and Foote 1986; Wilson and Molliver 1986; Wilson et al. 1989). For example, marked differences in the density and distribution of 5-HT axons are found in the macaque on either side of the central sulcus, in primary motor and somatosensory cortex: while

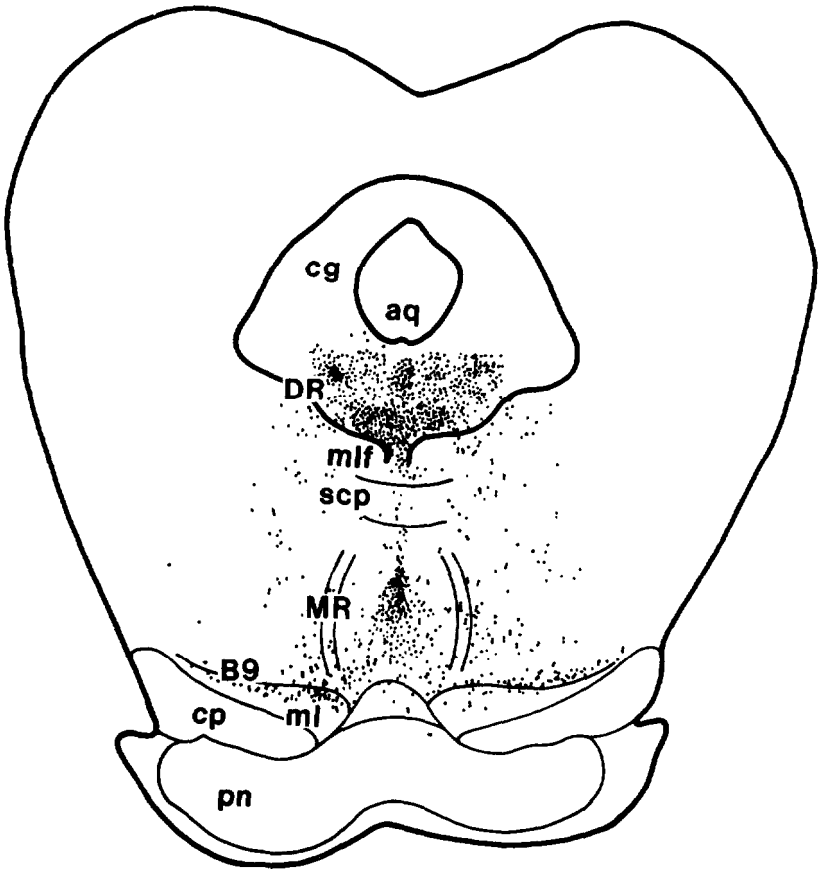

FIGURE 1. *The locations of serotonergic cell bodies in midbrain raphe nuclei*

NOTE: The three raphe cell groups depictcd here are the source of serotonergic projections to most parts of the forebrain. The DR is located in the central gray matter (cg) with many cells between and dorsal to the medial longitudinal fasciculus (mlf). The MR is a more scattered group of 5-HT neurons located in the central portion of the midbrain tegmentum. A small number of cells lies in the B9 cell group along the medial lemniscus (ml) and give rise to a small number of cortical projections. This map was prepared by Dr. L Mamounas based on a section prepared for 5-HT immunocytochemistry.

SOURCE: Mamounas and Molliver 1988, Copyright 1988, Academic Press.

motor cortex is sparsely innervated, somatosensory cortex is characterized by a high density of 5-HT axons extending acres most layers, with subtle changes seen within the subdivisions of somatosensory cortex. Primary visual cortex (Area 17) has an exceptionally dense innervation with a

distinctive laminar distribution of 5-HT axons. In visual cortex, the density in layer IV is particularly high but varies across sublayers, with a decrease in laminae IVC_{β} in the macaque (Kosofsky et al. 1984). Further primate studies from this laboratory have revealed highly intricate, detailed variations in innervation pattern and in the distribution of fine and beaded axons (Wilson, in preparation; Wilson and Molliver 1989; Wilson et al. 1989). In addition to these marked differences in innervation, subtle differences in the laminar pattern of innervation are found between closely related cortical areas, e.g., between the banks of the principal sulcus, the divisions of the hippocampal formation, the anterior and posterior parts of cingulate cortex, and among the subdivisions of somatosensory cortex (Wilson, in preparation). The main point to be emphasized with regard to cortical 5-HT innervation is that the characteristic regional differences may reflect different functional influences of 5-HT neurons upon separate cortical areas and variations in the effects of 5-HT projections upon particular cortical cell types.

The intricacy of the 5-HT innervation of cortex is further emphasized by differential cortical projections from the midbrain raphe nuclei. With the development of new techniques, there has been progressive clarification of the complex pattern of raphecortical innervation. Initial axon transport studies suggested that the DR nucleus projects preferentially upon cerebral neocortex and striatum while the MR innervates primarily hippocampus and hypothalamus. Later studies using more sensitive methods demonstrated that the projections were far more complex and that there is considerable overlap in raphe projections to forebrain (O'Hearn and Molliver 1984; Imai et al. 1986). Azmitia and Segal (1978) showed that the DR and MR nuclei have direct projections to forebrain and give rise to multiple, anatomically distinct ascending fiber bundles. The terminal distributions of the DR and MR ascending projections converge, so that most areas of cerebral cortex are innervated by both nuclei. with regional differences in the relative contribution from each nucleus; evidence for differential but overlapping raphe-cortical projections has been presented elsewhere (O'Hearn and Molliver 1984) and summarized in a recent review (Molliver 1987). Studies employing highly sensitive retrograde transport methods have shown that calls within different regions of the raphe nuclei project topographically to separate areas of cortex (Kohler and Steinbusch 1982; Jacobs et al. 1978; O'Hearn and Molliver 1984; Waterhouse et al. 1986; Wilson and Molliver 1988). The functional significance of this complex topographic order is indicated by evidence that individual zones of the raphe nuclei project to functionally related parts of the brain (Kosofsky 1985; Imai et al. 1986). Initial retrograde transport studies in the monkey reveal that there is a more complex and intricate regional pattern of raphe-cortical projections in the primate than in the rat (Wilson and Molliver 1988; Wilson, in preparation). These topographic findings, although seemingly complex in detail, indicate that the DR is heterogeneously organized and that particular zones of this nucleus project to different cortical areas. The DR and MR nuclei have

overlapping projections with dissimilar patterns of organization, and they terminate predominantly in different cortical layers and upon different cell types.

DUAL 5-HT AXON TYPES

Further evidence for the specificity of 5-HT projections, of particular relevance to amphetamine neurotoxicity, came from anterograde transport studies of the lectin PHA-L conducted by Kosofsky (1985). While it was known from several previous studies that 5-HT axon morphology is heterogeneous, Kosofsky made the unexpected discovery that there are consistent morphologic differences between cortical axon terminals that arise from the DR and MR nuclei, respectively (Kosofsky and Molliver 1987). 5-HT axon terminals arising from the MR nucleus have large, spherical varicosities (typically 2 to 3 μm in diameter), giving these axons a characteristic beaded appearance (figure 2). In contrast, axons that arise from the DR nucleus

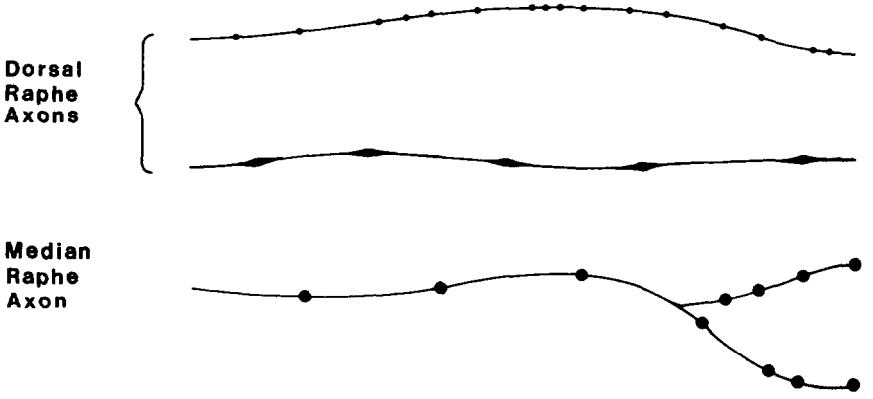

FIGURE 2. *A schematic representation showing the two classes of raphe-cortical axon terminals that were identified by anterograde axon transport*

NOTE: Axons that arise from cells in the DR nucleus are extremely fine with minute pleomorphic or fusiform varicosities. Axons from the MR nucleus have a beaded appereance characterized by large, spherical varicosities.

SOURCE: Adapted from Kosofsky and Molliver 1987. Copyright 1987, Alan R. Liss, Inc.

are of very fine caliber and typically have minute, pleomorphic varicosities that are often granular or fusiform in shape. The fine axon terminals are the most widespread and abundant type in cortex, while the beaded axons have a more restricted and characteristic distribution. Distinctive beaded 5-HT axons have been described in other areas of forebrain, e.g., in the entorhinal cortex (Kohler et al. 1980), in the olfactory bulb (McLean and

Shipley 1987), and in the hippocampus (Lidov et al. 1980; Zhou and Azmitia 1986). In addition, two corresponding morphologic axon types were found in the cat to form mutually distinct axon systems (Mulligan and Tork 1987; Mulligan and Tork 1988). Moreover, similar, morphologically distinct axon types have been described in neocortex and hippocampus in the macaque monkey (Wilson et al. 1989). Preliminary reports state that beaded 5-HT axons may form pericellular baskets around nonpyramidal neurons in cortex of the marmoset (Homung et al. 1987) and that similar 5-HT axons may terminate upon GABA-positive cells in the cat (Tork and Homung 1988).

SEROTONERGIC RECEPTORS IN CORTEX

Binding sites for 5-HT are present in high density throughout the brain, and these receptors have been the subject of recent reviews by Altar et al. 1986, Peroutka 1988, and Sanders-Bush 1988. One of the major discoveries in 5-HT pharmacology during the past decade has been the identification of multiple 5-HT binding sites originally classified by Peroutka and Snyder 1979 and 1981, and designated as 5-HT_1 and 5-HT_2 receptor types. Similar numbers of both types are found in cerebral cortex, yet each differs in its anatomic distribution and in the specific second messenger that is activated (Conn and Sanders-Bush 1987). Using a new ligand for detecting 5-HT_2 receptors (^{125}I-MIL), which was developed by Dr. P. Hartig and Blue and coworkers have compared the distribution of 5-HT axons in cortex with that of 5-HT_2 receptors. It was noted that the fine type of 5-HT axon terminals (DR origin) was closely associated with 5-HT_2 receptors, a relationship especially evident in rat somatosensory cortex where both terminals and receptors are extremely dense in the upper portion of layer V (Blue et al. 1986, Blue et al. 1988b). These results raise the possibility that 5-HT_2 receptors may be generally associated with fine axon terminals from the DR and that separate 5-HT projections may form multiple and distinct functional systems. The association of different classes of 5-HT axons with different receptors and second messenger systems is further evidence of the multiplicity and functional specificity of ascending 5-HT projections. The association of fine axon terminals with 5-HT_2 receptors is particularly relevant to the action of certain psychotropic drugs, which are postulated to act primarily at this receptor subtype (Glennon et al. 1984; Glennon and Lucki 1988; Heym et al. 1984).

NEUROTOXICITY OF AMPHETAMINE DERIVATIVES

While neurotoxic effects of amphetamines upon MA neurons had been reported in previous biochemical studies, a seminal paper from the University of Chicago has stimulated a new wave of interest in the neurotoxic effects of substituted amphetamines upon 5-HT projections. Large doses of the ring-substituted amphetamine derivative (±)3,4-methylenedioxyamphetamine (MDA) repeatedly administered to rats

by subcutaneous injection produced lasting reductions in biochemical markers for 5-HT in forebrain. Brain levels of 5-HT, levels of the metabolite 5-hydroxyindoleacetic acid (5-HIAA), and 5-HT uptake into synaptosomal suspensions were all substantially decreased 2 weeks after drug treatment (Ricaurte et al. 1985). For example, 5-HT levels in striatum and hippocampus were decreased more than 70 percent below control values. These findings were extended and confirmed for both MDA and its N-methyl analog 3,4-methylenedioxymethamphetamine (MDMA) by independent investigators at Chicago, the University of Utah, and elsewhere (Commins et al. 1987; Schmidt 1987; Stone et al. 1986; Stone et al. 1987a; Stone et al. 1987b). The latter study from Gibb's laboratory showed that the effects of MDA and MDMA were highly specific for 5-HT axons and that repeated doses produced greater than 90-percent decreases of tryptophan hydroxylase activity in cortex. These results were interpreted as indicating that these hallucinogenic amphetamine derivatives may cause initial release of 5-HT followed by lasting degeneration of 5-HT projections to forebrain; they thus appear to be similar to parachloroamphetamine (PCA) in their action (Schmidt 1987a).

IMMUNOCYTOCHEMICAL (ICC) STUDIES OF MDA AND MDMA TOXICITY

Based on the biochemical studies that psychotropic amphetamines act largely upon 5-HT neurotransmission and that prolonged exposure may be toxic to 5-HT neurons, it was of interest to examine the effects of MDA and MDMA upon the morphology of 5-HT neurons, in order to determine whether there may be evidence for structural damage to these cells or their processes, Consequently, an ICC study of the neurotoxic effects of MDA and MDMA was conducted in this laboratory by E. O'Hearn, in collaboration with Battaglia, De Souza, and Kuhar from the National Institute on Drug Abuse (NIDA) Addiction Research Center. In previous studies, evidence for axon degeneration was reported in the striatum following administration of MDA or MDMA (Ricaurte et al. 1985; Commins et al. 1987b) using the Fink-Heimer method, a silver stain for degenerating axons. However, because of low sensitivity for 5-HT axons, the silver-staining methods do not accurately depict the full extent or regional distribution of degenerating 5-HT axons, nor has any other conventional anatomic method proven satisfactory for thii purpose. Due to their limited sensitivity, the silver stains even fail to detect forebrain axon degeneration of MA projections following lesions of the medial forebrain bundle (MFB) (Moore and Heller 1967). At best, variants of the silver methods stain a small fraction of degenerating 5-HT axons, primarily in cingulate cortex, following raphe lesions (Hedreen 1973) or in hippocampus (Conrad et al. 1974). In order to characterize the cytotoxic effects of MDA and MDMA, it is important to determine whether there is morphologic evidence for degeneration of specific monoaminergic axons following drug administration. A central goal of this study was therefore to obtain

anatomic evidence that would establish whether or not 5-HT neurons degenerate following exposure to these drugs. Transmitter immunocytochemistry was employed for the visualization of 5-HT and catecholamine axons in order to determine whether there is structural evidence for degeneration, to identify the specific neuronal structures and neuronal compartments that are damaged by the neurotoxic drugs, and to determine the regional distribution of the effect 5-HT neurons, their axonal pathways, and axon terminals were visualized by 5-HT immunocytochemistry using an antibody to conjugated 5-HT, and cell bodies were examined in Nissl-stained sections. Catecholamine axons and cell bodies were visualized using an antibody to tyrosine hydroxylase (TH).

In initial ICC studies, animals were treated with MDA or MDMA using the protocol described by Ricaurte et al. (1985). Adult Sprague-Dawley rats (150 to 200 g) received subcutaneous injections of racemic MDA or MDMA every 12 hours for 4 days. Each dose was equivalent to 20 mg/kg of the free base. The rats were sacrificed by intracardiac aldehyde perfusion 2 weeks after the final dose. In order to study subacute effects for evidence of degeneration, additional rats received MDA every 12 hours for 2 days and were sacrificed 24 hours after the last injection. Additional experimental details are described elsewhere (O'Hearn et al. 1986; O'Hearn et al. 1988). A series of animals treated identically and in parallel were analyzed for changes in 5-HT levels and density of uptake sites using paroxetine binding (Yeh et al. 1986; Battaglia et al. 1987).

The biochemical and pharmacologic results were largely in agreement with previously reported effects of MDA and MDMA described above. The main neurochemical results of these studies (see also Battaglia and De Souza, this volume) confirm that, at 2 weeks after treatment, MDA and MDMA produced marked reductions in the content of both 5-HT and its metabolite 5-HIAA in most brain regions, with MDA causing a somewhat more potent effect. For example, in frontal cortex, 5-HT and 5-HIAA levels were reduced to 40 to 60 percent of control values; regional differences are evident in that smaller reductions of approximately 30 percent were found in the hypothalamus. The density of 5-HT uptake sites determined by paroxetine binding in homogenized tissue blocks showed highly significant reductions in cerebral cortex (60 to 70 percent), hippocampus (70 to 75 percent), and hypothalamus (40 to 50 percent) (Battaglia et al. 1987). No significant changes were found in markers for catecholamines. The above changes were closely matched by anatomic changes found in ICC preparations, described below (O'Hearn et al. 1988).

Neurotoxicity of MDA and MDMA

It was previously shown that immunocytochemistry with an antibody directed against 5-HT provides specific and highly sensitive visualization of 5-HT-containing cell bodies and nerve fibers throughout the central nervous

system (CNS) (Lidov et al. 1980, Lidov and Molliver 1982; Steinbusch 1981). The results of O'Hearn et al. (1988) showed that repeated doses of MDA or MDMA cause, at 2 weeks survival, profound loss of serotonergic axons throughout the forebrain, especially severe in neocortex, striatum, and thalamus (figure 3). Catecholamine innervation was unaffected, since no differences were seen between control and treated rats using TH immunocytochemistry. Both MDA and MDMA produce a similar pattern of denervation in cortex and other parts of the brain, but there is a smaller reduction in 5-HT axon density following MDMA than after MDA. Therefore, both drugs have similar effects, but MDA is more potent at the

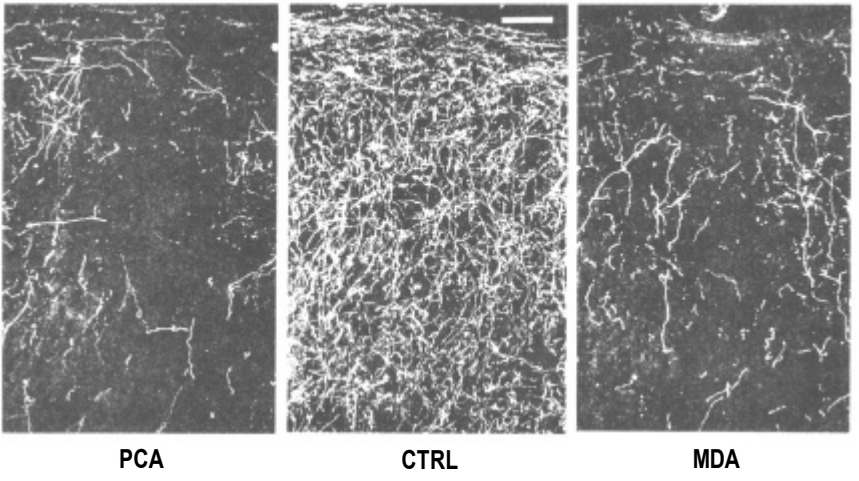

FIGURE 3. *Neurotoxic effects of psychotropic amphetamines upon 5-HT axon terminals in rat neocortex*

NOTE: Serotonin axons are visualized by 5-HT immunocytochemistry in parietal cortex. The central panel shows the normal pattern of 5-HT innervation in a control animal. In the right panel, there is a marked decrease in fine axon terminals 2 weeks following repeated systemic injections of MDA (20 mg/kg). A similar loss of fine axons is seen in the left panle 2 weeks following a single dose of PCA (10 mg/kg). Scale bar=100 μm. Darkfield photomicrograph. If examined with high magnification brightfield microscopy, the spread axons in both treated animals are all of the beaded type.

same dosage. The loss of 5-HT axons exhibits regional differences in neurotoxic effects, which are exemplified by partial sparing of 5-HT axons, particularly evident in hippocampus (figure 4), hypothalamus, basal

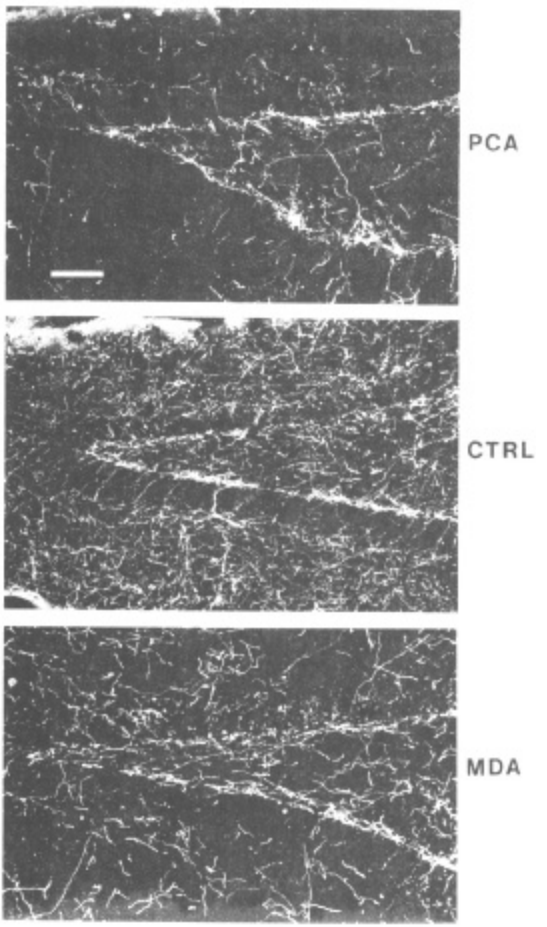

FIGURE 4. *Serotonergic innervation of the dentate gyrus in rat hippocampal formation*

NOTE: Serotonin axons visualized by 5-HT immunocytochemistry in darkfield microscopy. A high denisty of axons is seen in the control animal (central panel). Following multiple systemic dosesof MDA (bottom panel) or two doses of PCA (top panel), most fine axon terminals degenerate, as seen here at 2 weeks survival. However, there is consistent sparing of beaded axon terminals, especially marked along the inner surface of the dentate granule cell layer. Despite the loss of fine axons terminals, the 5-HT innervation in this area, as compared with neocortex, appears relatively spared following administration of neurotoxic amphetamine derivatives. Scale bar = 100 μm.

forebrain, and much of the brain stem, except for superior colliculus, which is markedly denervated. The forebrain denervation indicates a pronounced, but consistently selective, loss of 5-HT axons at 2 weeks after drug

treatment, which persists for many months. as found in later studies (Molliver et al., in press). The persistent loss of axon terminals reflects lasting denervation of target structures and parallels the reduction in 5-HT uptake sites. A study of the timecourse of regeneration and the origin of regenerating axons is currently in progress.

Axon Terminals Are Selectively Damaged

The fme morphologic detail afforded by the use of transmitter immunocytochemistry has made it feasible to identify the specific cytologic compartments that arc affected by these neurotoxic drugs. At the 2-week survival times that were analyzed, intact portions of the neurons are stained by 5-HT immunocytochemistry, while processes that have degenerated cannot be visualized. A consistent finding was that raphe cell bodies remain normal in density and ICC staining intensity, and that many smooth, straight, tangentially oriented 5-HT axons remain in deep layers of cortex, in subcortical white matter, and in basal forebrain and lateral hypothalamus. The disappearance of fine, highly arborized axons with sparing of the straight preterminal axons is evidence for selective vulnerability of serotonergic axon terminals. Intense 5-HT immunoreactivity seen in dilated axons of passage (especially in basal forebrain, in deep layers of frontal cortex, and in MFB) is presumably due to damming up of neurotransmitter and other axonal constituents in axon stumps secondary to ablation of the axon terminals. The accumulation of 5-HT and other contents in preterminal axons and cell bodies indicates that these cellular compartments remain functionally intact and that transmitter synthesis and anterograde axonal transport are not evidently impaired. The selective destruction of axon terminals is consistent with the large decrease in density of 5-HT uptake sites reported by Battaglia et al. (1987).

Raphe Cell Bodies Are Spared

In Nissl-stained sections, the cell bodies in the raphe nuclei are indistinguishable from those in control brains. The morphology of cell bodies and dendrites appears unremarkable, and the cells exhibit normal shape and size and show no evidence of increased staining nor any loss of cytoplasmic Nissl substance that would reflect chromatolysis. Moreover, Nissl-stained sections throughout other brain regions including cortex indicate no evidence of altered cellular morphology. Inclusion bodies in DR neurons of the monkey that are described elsewhere (Ricaurte et al. 1988) were not seen in raphe neurons in the rat. The lack of retrograde cytologic changes in raphe cell bodies is somewhat surprising considering the extensive loss of fine axon terminals. However, the sparing of cell bodies and of preterminal axons suggests that there may be substantial potential for recovery and regeneration of 5-HT projections. The failure to detect cytologic alterations in raphe cell bodies may reflect technical limitations in the experimental preparations. First, subtle cytologic changes in DR cell bodies would not

be easily detected because these cells normally have fine, dispersed Nissl substance and eccentric nuclei. Moreover, the use of frozen sections fixed for immunocytochemistry does not reveal cytologic features at the highest resolution, and subtle changes might not be visualized. Therefore, more sensitive cytochemical methods are needed to determine whether there may be subtle retrograde changes in the raphe neurons.

AXON DEGENERATION

One of the goals of this study was to obtain evidence that would establish whether or not serotonergic axons are damaged or degenerate following exposure to psychotropic drugs such as MDA or MDMA. At short survival times (24 hours after drug administration), while there is a marked decrease in the number of stained axons, cytopathologic changes are seen in some of the remaining immunoreactive processes. The most frequent abnormalities are markedly dilated axons with irregular diameter, giant varicosities, and fragmentation of axon segments. Giant, swollen varicosities were found in all cortical areas of treated rats but were never observed in controls. Their diameter was at least 4 times that of the largest axonal varicosities found in the normal brain. Moreover, the giant varicosities differ in their regional distribution from the normal, beaded class of 5-HT axons and appear to be newly formed structural abnormalities. Greatly swollen axonal stumps are especially prominent in the basal forebrain and ventral to the genu of the corpus callosum. At longer survival times, swollen axons are not found, and the persistent loss of fibers reflects lasting denervation. Several examples (figure 5) of swollen, fragmented axons are shown in figure 6 of the report by O'Hearn et al. 1988.

Specific evidence for axon degeneration, especially for MA terminals, is difficult to establish defmitively. The criteria for degeneration applied in this chapter are based on previously documented changes in degenerating MA axons observed by histofluorescence (Baumgarten et al. 1972; Baumgarten et al. 1973; Bjorklund et al. 1973; Bjorkhmd and Lindvall 1979; Wiklund and Bjorklund 1980; Jonsson and Nwanze 1982). In this study, the direct visualization of greatly swollen and fragmented nerve fibers demonstrates that 5-HT axons are structurally damaged by exposure to MDA and MDMA. These changes are presented as positive evidence for acute degeneration of axon terminals. This conclusion is further supported by the damming up of transmitter in swollen preterminal fibers that appear after the destruction of axon terminals. The subsequent disappearance of these damaged fibers and persistent loss of fine axon terminals reflects lasting degeneration. A limitation of transmitter immunocytochemistry for studying neurotoxicity is that visualization of axons depends upon retention of the neurotransmitter. Since axons that are depleted of 5-HT cannot be detected by this method, the present results, while providing positive evidence for degeneration, are likely to underestimate the number of axons that are degenerating at any one time. While not currently available, the

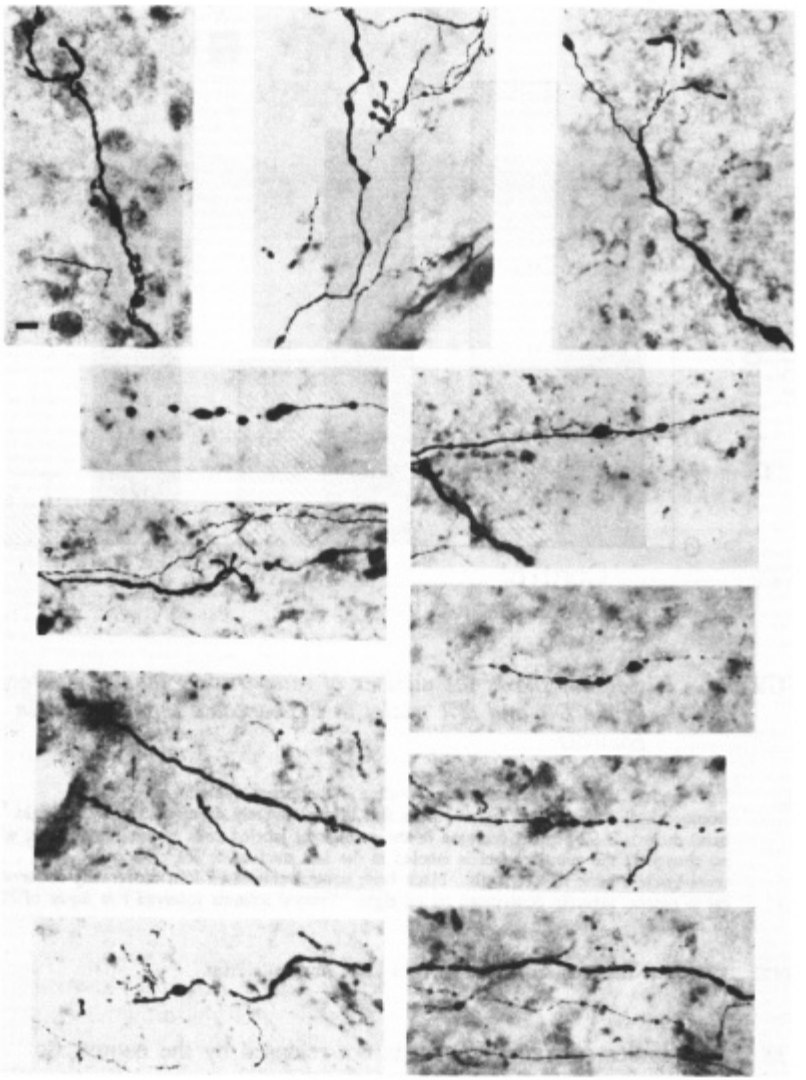

FIGURE 5. *Acute degeneration of 5-HT axons at 1-day survival following four doses of MDA*

NOTE: These axons exhibit cytopathologic changes, such as large, swollen varicosities, irregular thickening, and fragmentation of fibers. Dilatations of this type are severalfold larger than the largest 5-HT axon seen in control sections. These changes show evidence of structural degeneration in 5-HT axons following treatment with MDA. Scale bar = 10 μm.

SOURCE: O'Hearn et al. 1988, Copyright 1988, Oxford University Press.

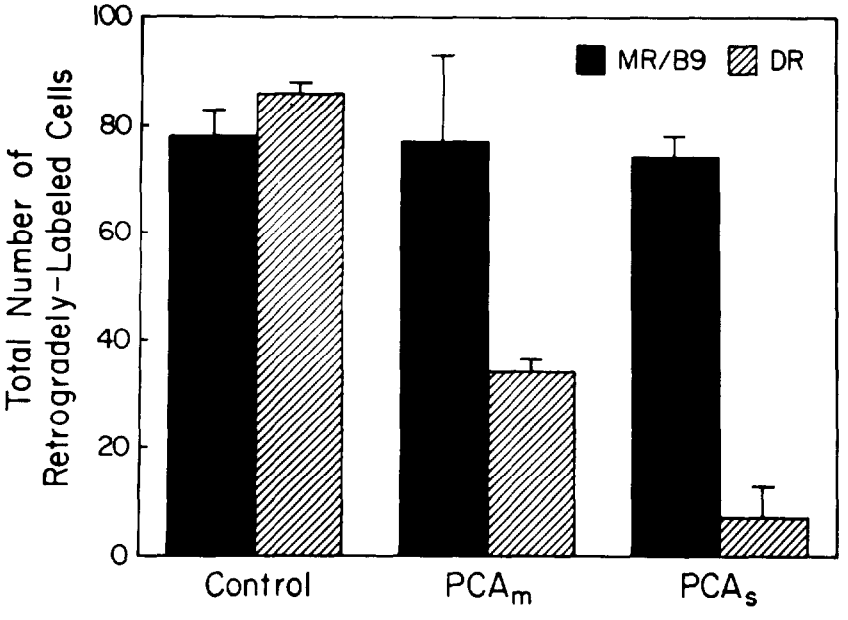

FIGURE 6. *Histogram shows the nunber of retrogradely labeled neurons in the DR and MR nuclei in PCA-treated animals and in controls*

NOTE: Retrogradely labeled cells were counted after a fluorescent dye was injected in frontoparietal cortex. This figue shows that in rats severely denervated by PCA (right bars) there is a 92-percent decrease in the number of labeled cells in the DR nucleus, with no change in the member of cells labeled in the MR nucleus or B9. DR cells: cross-hatched bars; MR/B9 cells: black bars; control animal on left; moderately denervated rat in center, severely denervated rat on right. Treated animals received two doses of PCA (6 mg/kg).

SOURCE: Mamounas and Molliver 1988, Copyright 1988, Academic Press.

use in future studies of a marker that is not released by the neurotoxic drugs is likely to provide evidence of more extensive terminal degeneration.

The occurrence of drug-induced structural damage and degeneration of 5-HT axons is further supported by the complete profile of effects produced by psychotropic drugs such as MDA, PCA, and fenfluramine. Structural evidence for axon damage is provided by the presence of enlarged varicosities and swollen fragmented axons in identified 5-HT-containing fibers at 1 to 2 days after MDA treatment. The formation of enormous, swollen axons is even more marked after treatment with a structurally related amphetamine derivative, fenfluramine (5.0 mg/kg) (Molliver and

Molliver 1988; Molliver and Molliver, in press). Additional structural evidence for axonal degeneration is summarized above and includes Fink-Heimer-positive axons (Ricaurte et al. 1985), persistent loss of fine axon terminals lasting many months, enlarged axon stumps with intense immunoreactivity for 5-HT, and the loss of retrograde axonal transport to DR cell bodies (described below). This structural evidence is accompanied by the loss of most biochemical markers for 5-HT axon terminals (as noted previously), including decreases in 5-HT levels, 5-HIAA, tryptophan hydroxylase activity, and 5-HT uptake sites. Despite this constellation of findings indicative of 5-HT axon degeneration, one may still speculate that (however unlikely) axon terminals may remain present yet lack any detectable properties.

DIFFERENTIAL VULNERABILITY OF 5-HT AXON TYPES

The two morphologic classes of 5-HT axons described earlier (Kosofsky and Molliver 1987) are differentially vulnerable to the neurotoxic effects of MDA, MDMA, and certain other neurotoxic amphetamine derivatives. The denervation caused by MDA and MDMA is subtotal, and some 5-HT axon terminals are consistently spared in most regions of cortex; them. is a characteristic regional pattern of axon sparing, as noted above. The analysis of ICC sections from MDA-treated rats using high-resolution brightfield microscopy reveals that there is a selective loss of fine axon terminals, which are almost completely ablated, nearly all of the spared 5-HT axon terminals in cortex and elsewhere are of the beaded type with large varicosities (O'Hearn et al. 1988; Mullen et al. 1987). Further analysis of additional treated and control material shows that the spared, beaded axons are identical in morphology and distribution to beaded axons that are found in control animals (Mamounas et al. 1988). The differential vulnerability of two axon types has been consistently confirmed in a series of additional studies. The effects of MDA and MDMA were compared with those of two other substituted amphetamines that were previously shown to cause similar decreases in biochemical markers for 5-HT, namely PCA and fenfluramine (Mamounas et al. 1988; Molliver and Molliver 1988; MolIiver and Molliver, in press). Both of these compounds produced a loss of 5-HT axon terminals that was indistinguishable from that produced by MDA or MDMA. In a comparative study of drug effects, PCA administered as two subcutaneous doses of 10 mg/kg produced a profound loss of 5-HT axon terminals throughout the rat forebrain, with a regional distribution identical to that described for MDA (Mullen et al. 1987; Mamounas et al. 1988; Mamounas et al., in preparation; Mamounas and Molliver 1988). As with MDA, treatment with PCA (or with fenfluramine) caused a preferential loss of fine 5-HT axon terminals, while terminals with large, spherical varicosities were unaffected by these drugs. The spared, beaded axons are identical in morphology to those found in control animals, and they have the same regional and laminar distribution. The beaded axons that are spared are consistently found in layers II to III of parietal and occipital

cortex, in the hippocampus where they are located in the subgranular zone of the dentate gyrus and in the stratum lacunosum of CA1, in layer III of lateral entorhinal cortex, in the olfactory glomeruli and in other regions including amygdala, lateral hypothalamus, and most of brain stem (Mullen et al. 1987; Mamounas et al. 1988; Mamounas et al., in preparation). Beaded, relatively coarse 5-HT axons that line the ependymal surface of the lateral ventricle, third ventricle, and aqueduct form a unique group of 5-HT axon terminals that are also consistently spared by all of the neurotoxic amphetamines that have been tested. A similar regional distribution of axon loss was obtained after giving the anorexic drug fenfluramine. Repeated doses of (±)fenfluramine at 12-hour intervals (n=4 to 8 doses) administered subcutaneously in doses of 5, 10, or 20 mg/kg produced a persistent loss of 5-HT axons at 2-week survival times with the identical anatomic distribution and morphologic features of spared axons seen with MDA and PCA (Molliver and Molliver 1988; Molliver and Molliver, in press). Using three doses at the 5-mg/kg level and shorter survival times (36 hours), 5-HT immunocytochemistry revealed a large number of enormously swollen, fragmented 5-HT axons with giant varicosities that are typically over 10 times the size of normal beaded axons. Thus, fenfluramine produces the same pattern of axon degeneration as that seen with MDA and PCA (Molliver and Molliver, in press). Selective neurotoxic effects of d-fenfluramine, similar to those found in the rat, have also been observed in cerebral cortex of the primate (Ricaurte et al., in press). These results indicate that MDA, MDMA, PCA, and fenfluramine, when administered in moderately large doses, have nearly identical neurotoxic effects upon 5-HT axons. Moreover, these studies distinguish two classes of 5-HT axons that differ in their morphology, regional distribution, and differential vulnerability to psychotropic drugs. In all cases, the fine axon terminals show consistent vulnerability to the effects of these compounds, while the beaded axons appear to be unaffected even at relatively large doses (e.g., 40 mg/kg of PCA) (Mamounas et al. 1988; Mamounas et al., in preparation).

While the results of the ICC studies summarized above indicate that two classes of 5-HT axons are differentially affected by particular neurotoxic amphetamines, analogous examples of selective vulnerability to degeneration also occur among dopamine (DA) and norepinephrine (NE) neurons in response to different drugs. Thus, differential vulnerability of specific subtypes of MA axons appears to be a common feature of these neuronal systems. For example, the DA neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), selectively damages nigro-striatal DA projections, while sparing most DA axons that arise from the ventral tegmental area (Langston et al. 1984; German et al. 1988). Moreover, in human cases of Parkinson's Disease, a selective loss of DA has been reported in the putamen, sparing DA projections to the caudate nucleus (Kish et al. 1988). In addition, recent studies of the neurotoxin, DSP-4, indicate that NE axons arising from the locus coeruleus are more susceptible to this compound than are those that arise from other NE cell groups (Lyons et al. 1989; Fritschy

and Grzanna 1989). The above results suggest that a general principle applying to alI types of monoaminergic neurons may be proposed for experimental verification: at least two classes of neurons utilize each MA transmitter, and these neuron subtypes are differentially vulnerable to neurotoxic or cytopathologic agents. The mechanisms that determine the differential vulnerability of particular MA cell types are currently unknown, but are of considerable importance for further understanding of the causes of neurotoxicity.

DIFFERENTIAL ORIGIN OF 5-HT AXONS

The differential vulnerability of fine and beaded 5-HT axons, combined with evidence from anterograde transport that fine and beaded fibers arise from the DR and MR nuclei. respectively, led to the proposal that axons from the DR nucleus are selectively vulnerable to the neurotoxic effects of psychotropic amphetamines, while the MR projection is resistant. The prior anterograde transport study (Kosofsky 1985; Kosofsky and Molliver 1987) sampled a relatively small number of neurons in the central portions of the DR and MR nuclei and suggested a predominantly differential origin of the two axon types. In order to determine directly whether the DR and MR projections are differentially sensitive to psychotropic amphetamines, L. Mamounas conducted a retrograde axonal transport study using fluorescent dyes in animals treated with PCA and in controls. PCA is a useful model experimental drug for the neurotoxic amphetamines, since, when administered in a single dose. it produces the same pattern of degeneration as MDA. Fluorescent dye was injected in the cortex and retrogradely labeled cell bodies were mapped; only those axons that survive PCA administration are able to take up the label and retrogradely transport it to cell bodies of origin. Thus, by comparing the number and locations of cortically projecting raphe neurons in control and treated animals, identification of the nuclei of origin of drug-sensitive vs. resistant axon terminals has been possible. The number of labeled neurons (figure 6) in the DR nucleus of PCA-treated animals was decreased by 77 to 90 percent; in contrast, the number of labeled neurons in the MR was unchanged (Mamounas and Molliver 1987; Mamounas and Molliver 1988). These results demonstrate that DR and MR projections are differentially vulnerable to PCA, and they confii that fine axon terminals, which are highly sensitive to the neurotoxic effects, arise from neurons in the DR. Moreover, the loss of the capability for axonal transport is additional evidence in support of axonal damage and degeneration. These results lead to the proposal that there are dual 5-HT projections to cortex that are anatomically and functionally distinct. These projections have different nuclei of origin, axon morphology, regional distributions, and pharmacologic properties. These findings lead to the further proposal that psychotropic amphetamines act preferentially upon serotonergic projections from the DR nucleus and that DR neurons may therefore be involved in the control of affective state and perceptual integration. While the selective vulnerability of DR axons is

likely to be of general validity, at the present time the direct demonstration by retrograde transport showing a loss of DR projections is based solely on studies of frontoparietal cortex after using the drug PCA. The consistent morphology of spared axons suggests that beaded axons in other regions of the forebrain, which are resistant to these drugs, also arise from the MR nucleus. However, further studies combining retrograde axonal transport from a variety of forebrain areas and treatment with MDA and/or MDMA would be desirable to establish the generality of these findings.

EFFECTS OF MDMA IN PRIMATES

Evidence that MA neurons in the primate brain are susceptible to the toxic effects of amphetamines was first reported following chronic methamphetamine treatment in rhesus monkeys (Seiden et al. 1975) before the toxicity was characterized in rodents. Most studies of amphetamine neurotoxicity have been conducted in rats; however, data from rodents do not always predict the mechanism of drug action or degree of toxicity in primates. In order to predict the potential neurotoxic effects of MDMA in humans, it is essential to analyze the drug effects in monkeys, since there is evidence that the metabolism of amphetamines in primates differs substantially from that in other species (Caldwell 1976). In order to determine the sensitivity of 5-HT neurons to MDMA, Ricaurte and colleagues administered various doses of this drug (2.5 to 5.0 mg/kg) to a series of squirrel monkeys using subcutaneous injections, repeated twice daily for 4 days. Determination of 5-HT levels by HPLC revealed that multiple doses of MDMA produced large depletions of 5-HT in many parts of the forebrain including neocortex, caudate nucleus, hippocampus, and hypothalamus (Ricaurte, this volume). At 2 weeks after the last dose, the neocortex was markedly depleted of 5-HT, with the lowest dose (2.5 mg/kg) producing a 44-percent depletion and the highest dose (5.0 mg/kg) producing a 90-percent depletion of 5-HT (Ricaurte et al, 1987; Ricaurte et al. 1988). Immunohistochemical preparations from treated monkeys revealed a marked reduction in the number of serotonergic axon terminals throughout cerebral cortex at 2 weeks survival, with the persistence of some structurally damaged intracortical axons that were abnormally swollen (Ricaurte et al. 1988). In addition, examination of cell bodies showed the presence of abnormal cytoplasmic inclusion bodies in the DR nucleus. These inclusions were periodic acid-Schiff (PAS)-positive and appeared to contain lipofuscin. Based on the evidence that MDMA is highly toxic to serotonergic neurons in primates, a detailed neuroanatomic analysis was conducted by M.A. Wilson to characterize the morphology and regional distribution of 5-HT axons that are affected by MDMA in the macaque monkey. Two weeks after treatment with MDMA (eight doses, 5 mg/kg, subcutaneous) the density of 5-HT immunoreactive axon terminals was strikingly reduced throughout the cerebral cortex (Wilson et al. 1987; Wilson et al. 1989). A characteristic regional distribution of serotonergic denervation was found in different cortical areas. For example, in somatosensory cortex, which is

densely innervated by 5-HT axons in control animals, few 5-HT axon terminals remain after MDMA treatment, except for beaded axons in layer I. In other regions, there was a significant denervation, yet a subgroup of 5-HT axons consistently survives, e.g., in visual cortex, hippocampus, dentate gyrus, and amygdala

As in the rat, the morphology of 5-HT axons in the normal primate is heterogeneous, with both fine and beaded axon terminals intermixed. The fine axon terminals are profoundly vulnerable to MDMA, as found in the rat, while nearly all of the surviving axons are of the beaded type with large spherical varicosities. The loss of 5-HT axons in monkeys is greater than that in rats that were given a fourfold higher dose of MDMA and, therefore, MDMA is far more neurotoxic in the primate than in the rat (Ricaurte et al. 1988; Wilson et al. 1989). While cell bodies and preterminal axons are stained, the morphologic changes in some 5-HT axons and the persistent loss of fine axon terminals provide evidence that MDMA produces axon terminal damage and degeneration in the primate cortex. Furthermore, the selective vulnerability of fine axon terminals and the sparing of beaded axons indicates that multiple classes of 5-HT axons can be distinguished in primates, as in rodents. Thus, the morphologic differences between 5-HT axons, their differential vulnerability to psychotropic drugs, and characteristic regional distributions suggests that--in primates--there may be two parallel, ascending serotonergic projections subserving different functions. The susceptibility of fine axons to MDMA supports the hypothesis that these axons are one of the sites of action of this drug and am involved in the control of affective state.

The effects of MDMA in the primate indicate that the anatomic organization and pharmacologic properties of ascending 5-HT projections in the primate are similar to those in the rodent These studies employing relatively large, repeated, subcutaneous doses of MDMA were not designed to analyze the toxicity of this drug in humans, but to obtain results indicating the potential toxicity, site of action, and biological effects of this drug on 5-HT neurons. It should be noted that the drug administration schedule may not be comparable to typical human use, that humans generally take MDMA via the oral route, and that the sensitivity of human and subhuman primates to the effects of MDMA may not be the same. However, in view of the extensive destruction of 5-HT axon terminals at doses that are approximately twice that commonly used for recreational purposes by humans, MDMA may have a relatively small margin of safety, and it would be prudent to consider this drug potentially hazardous for human use. Therefore, if human administration of MDMA-like compounds is considered clinically efficacious, further studies are needed to determine whether there may be a safe dose range or if there may be related compounds with less potential toxicity and similar beneficial effects. The studies reported here, and in other papers in this volume, describe several methodological approaches and well-characterized parameters to study the effects and

neurotoxicity of new psychoactive compounds. The effects of such drugs on pharmacologic and structural properties of 5-HT neurons in rodents are highly predictive of the action of these drugs in primates. However, it is clear from the above results that drug potency varies considerably among species and must be evaluated separately in primates. The results indicate that MDMA, combined with biochemical and immunohistochemical studies, provides a useful experimental tool to study the activity of such drugs and their neurotoxicity.

It would also be important to determine whether 5-HT axons are altered in clinical dementias, since a preliminary study shows that swollen 5-HT axons are associated with amyloid-containing plaques in aged monkeys (Kitt et al. 1989). Since the swollen 5-HT axon terminals in Alzheimer-like plaques are similar to degenerating axons seen after MDMA treatment, it is possible that endogeneous or environmental toxins derived from phenethylamines may play a role in the etiology of dementias. Since illicit recreational use of MDMA and related drugs may produce similar structural damage to 5-HT axons, it is plausible that the long-term effects of such damage might predispose susceptible individuals to degenerative disorders of the Alzheimer's type. Although this possibility is highly speculative, long-term prospective followup of MDMA users for subtle psychological changes in memory and cognitive processes are certainly warranted.

MECHANISM OF MDMA ACTION AND TOXICITY

The mechanisms by which MDMA and related drugs produce their pharmacologic actions and neurotoxic effects are not well understood, making it difficult to predict what structural or metabolic differences may account for the differential vulnerability of specific 5-HT axon types. However, new information from several laboratories has provided insight into the mechanisms of these drugs and indicates the importance of multidisciplinary approaches in this area of investigation. The commonality of both morphologic and biochemical effects of the methylenedioxy-substituted amphetamines with fenfluramine and PCA suggests that all these compounds may act via the same mechanism. Both *in vivo* and *in vitro* preparations have shown that this class of compounds acts by acutely releasing serotonin from 5-HT axon terminals in forebrain (Fuller et al. 1975a; Nichols et al. 1982; Johnson et al. 1986; Sanders-Bush and Martin 1982; Schmidt 1987a; Trulson and Jacobs 1976). Several studies have shown that the acute release of 5-HT is distinct from the long- term neurotoxic effects produced by PCA or MDMA and that separate mechanisms may be involved (Fuller et al. 1975b; Sanders-Bush et al. 1975; Schmidt 1987a); both effects depend on a carrier-mediated mechanism. In particular, the long-term degenerative effects can be prevented by administration of fluoxetine or citalopram, which block the 5-HT uptake carrier (Fuller et al. 1975b; Schmidt 1987a). The role of a carrier-mediated mechanism is further supported by high affinity of MDMA for the 5-HT uptake site

(Steele et al. 1987; Battaglia et al. 1988). In summary, previous pharmacologic studies indicate that MDMA and related psychotropic amphetamines have a multiphasic effect marked by an acute release of 5-HT, which may be reversible, followed by a chronic decrease in 5-HT markers probably due to axon degeneration.

Morphologic studies from this laboratory provide strong support for the multiphasic mechanism described above, and also indicate that fine axon terminals are selectively affected. In a series of acute *in vivo* experiments, a single intraperitoneal injection of PCA (10 mg/kg) or MDA (20 mg/kg) produced a dramatic reduction in the number of 5-HT immunoreactive axons in cortex and hippocampus of rats at survivals of 30 minutes to 4 hours following treatment (Berger et al. 1987). When administered by itself, the 5-HT uptake inhibitor fluoxetine had no effect on the staining or density of 5-HT axons after acute or repeated doses (Berger et al. 1987; Berger et al., in preparation); however, fluoxetine (10 mg/kg) coadministered with either MDA or PCA completely prevented the acute and chronic decrease in 5-HT immunoreactive axons. The results of these and further studies demonstrate that both MDA and PCA cause acute depeletion of 5-HT from fine axon terminals; however, the beaded axons stain intensely and appear unaffected (Berger et al. 1987; Mamounas et al. 1988). It is of interest that single or repeated doses of two other ring-substituted psychotropic amphetamines, DOM and 2,5-dimethoxy-4-ethylamphetamine (DOET), did not produce a reduction in 5-HT levels or the staining of 5-HT axons (Berger et al. 1987), consistent with the evidence that the latter drugs act at 5-HT_2 receptors (Glennon 1985). The contrasting effects of these several drugs led to the proposal that there are at least two classes of psychotropic amphetamines with different sites of action: one type exemplified by DOM or DOET acts postsynaptically at 5-HT_2 receptors, while the other type, such as PCA and MDA, acts presynaptically by a carrier-mediated mechanism to release 5-HT from axon terminals (Berger et al. 1987). Since PCA and MDA (but not DOM or DOET) cause degeneration of 5-HT axons, the ability of amphetamine derivatives to cause massive release of 5-HT appears related to the neurotoxicity of these compounds. The fact that fluoxetine prevents the neurotoxicity supports the idea that the neurotoxic amphetamines act at a presynaptic site located on 5-HT axon terminals and bind to the 5-HT uptake carrier. The selective releasing effect of psychotropic drugs such as MDA and PCA upon fine axon terminals is relevant to the finding noted above that these terminals appear selectively associated with 5-HT_2 receptors (Blue et al. 1988b). The release of 5-HT from this set of terminals may selectively activate 5-HT_2 receptors at postsynaptic sites that are linked to activation of phosphoinositide hydrolysis (Conn and Sanders-Bush 1987).

IS A NEUROTOXIC METABOLITE FORMED?

Based on observed differences between the *in vivo* and *in vitro* effects of amphetamine derivatives, several laboratories have suggested that the neurotoxic effects may depend upon the formation of an active drug metabolite (Sanders-Bush et al. 1972; Hotchkiss and Gibb 1980; Stone et al. 1987b); others have suggested that a metabolite might be formed from DA or 5-HT that is released in large quantities (Johnson et al. 1988; Commins et al. 1987a; Stone et al. 1988). To pursue this issue, this laboratory has employed several strategies in an effort to determine whether the parent amphetamine derivative is itself neurotoxic.

INTRACEREBRAL DRUG ADMINISTRATION

In view of the marked neurotoxicity of systemically administered MDA, E. O'Hearn administered MDA and/or MDMA directly into cerebral cortex by stereotaxic microinjection (6 μg in 0.5 μl). At both long and short survival times (3 days to 3 weeks) the 5-HT innervation density at the injection site could not be distinguished from that in normal animals or after saline injections (Molliver 1987; Molliver et al. 1986; O'Hearn et al., in preparation). These results suggested that large doses of MDA or MDMA administered directly into the brain are not neurotoxic and that the formation of a peripheral drug metabolite may be an essential step in inducing neurotoxicity. However, several caveats to this interpretation are raised, particularly that neurotoxic effects may require prolonged exposure to the drug and these lipophilic compounds are likely to diffuse rapidly from the injection site. To address the duration of exposure issue, U. Berger has done a series of experiments in which PCA was continuously infused directly into the cerebral cortex using an Alzet minipump at a rate of 10 μg per hour for 48 hours. Following a 2-week survival period, ICC preparations from the injection site revealed a small zone of local tissue damage due to the implanted cannula, similar in both drug-injected and saline control animals. However, despite continuous infusion of PCA (or MDMA) over 2 days, the serotonergic innervation in the surrounding tissue appeared normal, with no detectable loss of 5-HT immunoreactive staining. In contrast, a similar injection of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) produced a zone of total 5-HT denervation at least 2 to 3 mm in diameter. These chronic intracerebral microinjection experiments lend further support to the view that the parent compound is not itself neurotoxic (Berger 1989; Berger et al., in preparation; Molliver et al. 1986).

DRUG EFFECTS IN THE HIPPOCAMPAL SLICE PREPARATION

In order to circumvent the difficulties of maintaining known, constant drug concentrations in the brain *in vivo,* the hippocampal slice preparation was adapted to study the acute anatomic effects of psychotropic drugs. This method was implemented in conjunction with Drs. K. Stratton and

J. Baraban, who have experience in maintaining hippocampal slices under *in vitro* conditions for electrophysiologic recording. Several experimental paradigms have been tested with this method, showing that the hippocampal slice preparation combined with immunocytochemistry is a useful tool for studying *in vivo* and *in vitro* effects of psychotropic drugs. Freshly prepared hippocampal slices are incubated in oxygenated buffer, with or without drugs added, and are then immersion fixed and sectioned for ICC staining. The quality and sensitivity of axonal visualization in slices is equivalent to that in sections prepared from perfusion-fixed rats. In slices from control rats, a high density of morphologically intact 5-HT axons is seen in the hippocampus, with the same distribution as in conventional sections. In order to verify the 5-HT-depleting effects of *in vivo* treatment, hippocampal slices were prepared from rats that were given a single dose of PCA (10 mg/kg) subcutaneously, and the animals were sacrificed 3 hours later. A marked decrease of 5-HT immunoreactive axons was observed in slices that were fixed immediately after sacrifice of PCA-treated rats. Slices that were maintained *in vitro* in physiological saline showed progressive recovery of 5-HT axon staining over 0.5 to 2 hours. However, if the survival time of the animal after PCA treatment was extended for over 24 hours, then no recovery was seen, and the loss of 5-HT axons was irreversible. These results provide direct immunochemical and anatomic support for previous pharmacologic studies that showed a biphasic effect of PCA and related amphetamine derivatives, characterized by an early phase of 5-HT depletion that is potentially reversible during the first 24 hours (Fuller et al. 1975a; Sanders-Bush et al. 1975; Schmidt 1987a). During this acute phase, the fine axons are depleted of 5-HT but have not degenerated; the terminals retain the ability to synthesize and store 5-HT if the toxic compound dissociates, as observed in the slice incubation bath (Molliver et al. 1988). With longer *in vivo* survival times (4 to 6 days after PCA) the lack of subsequent recovery *in vitro* provides evidence for irreversible axon degeneration. The timecourse of this biphasic effect closely matches that reported by Fuller et al. (1975b) based on the use of 5-HT uptake blockers to displace PCA *in vivo*.

In order to test the cytotoxic potential of PCA alone, hippocampal slices from untreated control animals were incubated in buffer containing PCA, over a wide range of concentrations (typically 50 μM) for 2 to 3 hours. The incubation of slices directly in the parent compound (PCA) did not induce 5-HT depletion, and the 5-HT innervation in these slices was indistinguishable from that in control animals. Moreover, incubation of slices from PCA-treated animals in PCA-containing buffer did not prevent the recovery of 5-HT immunoreactive axons. The absence of 5-HT depletion after immersion of hippocampal slices in PCA strongly supports the proposition that PCA and related drugs are not directly neurotoxic. Thus, *in vivo* systemic administration of the drug appears necessary for the formation of a neurotoxic compound, such as a metabolite of the drug or of 5-HT, which is released.

THE PROTECTIVE EFFECT OF 5-HT DEPLETION

To determine whether the release of endogeneous 5-HT mediates the neurotoxic effects of PCA in the brain, several pharmacologic regimens were employed to deplete animals of 5-HT prior to treatment with PCA. In a series of studies conducted by U. Berger, rats were depleted of 5-HT by prior treatment with reserpine (2.5 mg/kg), the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) (250 mg/kg), or a combination of both drugs. These drugs initially produce depletion of 5-HT in brain and other tissues (over several days) followed by recovery to normal levels over 2 weeks; after that time, normal 5-HT ICC axon staining is obtained, and no evidence of axonal swelling or degeneration was observed. Animals depleted of 5-HT by different regimens were subsequently treated with PCA (10 mg/kg) and tested after 2 weeks for 5-HT neurotoxicity using both HPLC and immunocytochemistry. This study revealed a marked protective effect on 5-HT neurons after combined treatment with PCPA plus reserpine. After the extensive 5-HT depletion produced by combined treatment with both drugs, PCA produced only a small reduction in brain 5-HT levels, and nearly all 5-HT axons in forebrain were spared. Reserpine pretreatment alone, although producing substantially reduced brain MA levels, did not afford significant protection against the effects of PCA. This result indicates that depletion of 5-HT (and other biogenic amines) from vesicular storage sites in the brain does not provide significant protection against the neurotoxic effects of PCA, whereas more extensive depletion from brain, platelets, and intestine by inhibition of 5-HT synthesis does prevent the toxicity of PCA (Berger, in preparation). Since a primary pharmacological effect of PCA is the release of 5-HT from nerve terminals and platelets, these results suggest that the neurotoxicity of PCA is dependent upon the presence of a releasable pool of 5-HT and is not mediated directly by the drug or one of its metabolites. The site of the PCA-induced 5-HT release essential for neurotoxicity is not known, although depletion of vesicular 5-HT is not itself sufficient for protection. The data suggest that an extensive depletion of 5-HT pools is required to block the PCA-induced toxicity. These results lead to the suggestion that the 5-HT stores in platelets (or mast cells), the main 5-HT storage sites in the periphery, may play a central role in the neurotoxic mechanism (Berger et al. 1989). Therefore, it is postulated that a neurotoxic metabolite is formed from 5-HT released by the action of PCA on platelets or on other 5-HT storage sites. PCA is also a strong inhibitor of MA oxidase (Fuller 1966) and may therefore facilitate the formation of an unusual neurotoxic indolamine metabolite from peripherally released 5-HT. This metabolite, not yet identified, may enter the brain and cause selective destruction of 5-HT axons. This proposal is consistent with the findings from Seiden's laboratory that the neurotoxin 5,6-dihydroxytryptamine can be detected in the brain following PCA administration (Commins 1987a). Moreover, since reserpine depletes other biogenic amines, a role for catecholamines in the toxicity of amphetamine derivatives should be considered, as proposed

earlier (Johnson et al. 1988; Stone et al. 1988). While further investigation is needed to determine the origin and identity of the neurotoxic compound, the present studies indicate that exposure to the parent compound itself, e.g., PCA or MDA, is not sufficient to produce a lasting neurotoxic effect, nor does it produce acute 5-HT depletion.

CONCLUSION

The present studies demonstrate the value of combining morphologic with biochemical methods to study the neurotoxicity of psychotropic drugs upon central 5-HT neurons and to identify the specific neurons and neuronal compartments that are affected. There are two distinct serotonergic projections to forebrain that arise from the DR and MR nuclei, respectively, and have different patterns of termination in cortex, morphologically distinct axon terminals, and dissimilar pharmacologic properties. Substituted amphetamine derivatives PCA, MDA, MDMA, and fenfluramine have similar profiles of neurotoxicity in the brain and all act selectively upon the fine axon terminals that arise from the DR nucleus. Direct anatomic evidence for structural damage to 5-HT axon terminals has been obtained after treatment with MDA and with fenfluramine. These cytopathologic changes in axons combined with the pharmacological profile of effects, which include persistent decreases in 5-HT levels, turnover, synthesis, and uptake sites, provide convincing evidence that these psychotropic amphetamines can produce axon terminal degeneration. The exact mechanism of neurotoxicity has not yet been elucidated, nor has the specific neurotoxin been identified. Present evidence indicates that neither the parent compound alone nor a drug metabolite produces 5-HT depletion or degeneration. Preliminary evidence that depletion of central and peripheral 5-HT affords protection against the effects of RCA leads to the hypothesis that a metabolite of 5-HT released in the periphery, possibly from platelets, is essential for the expression of amphetamine-induced neurotoxicity. The selective toxic effect of these compounds upon one class of 5-HT axon terminals with sparing of other 5-HT axons and of raphe cell bodies provides a setting in which regenerative sprouting is likely to occur, a subject of ongoing investigation. The augmented neurotoxicity of MDMA in primates raises concern about the possible neurotoxic effects of this drug in humans. Further studies are needed to determine whether there may be a safe range of doses for human use of compounds in this class and whether clinically efficacious drugs similar to MDMA but without toxic effects can be designed. Current data also suggest that it may prove useful to explore experimentally the use of 5-HT uptake blockers such as citalopram, paroxetine, or fluoxetine to protect against the cytotoxic effects of drug overdose. The use of selective neurotoxic drugs in experimental studies should continue to enhance our understanding of the complex functional organization of 5-HT projections in the brain and the multiplicity of effects that have been ascribed to this neurotransmitter.

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AUTHORS

Mark E. Molliver, M.D.
Professor
Department of Neuroscience and Neurology

Laura A. Mamounas, Ph.D.
Fellow
Department of Neuroscience

Mary Ann Wilson, B.A.
Fellow
Biochemistry, Cellular, and Molecular Biology Program
Department of Neuroscience

Johns Hopkins University School of Medicine
725 North Wolfe Street
Baltimore, MD 21205

Studies of MDMA-Induced Neurotoxicity in Nonhuman Primates: A Basis for Evaluating Long-Term Effects in Humans

George A. Ricaurte

INTRODUCTION

Studies of (±)3,4-methylenedioxymethamphetamine (MDMA) neurotoxicity in nonhuman primates are potentially of great importance to both basic science and public health. Scientifically, such studies could shed light on the functional role of serotonin in the primate central nervous system (CNS). In this regard, it is pertinent to recall that it was not until the effects of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were explored in the monkey that its profound dopaminergic neurotoxic effects were noted (Chiueh et al. 1983) and then utilized to develop the first complete animal model of Parkinson's disease (Burns et al. 1983). Given this precedent, it seems not unreasonable to speculate that studies of MDMA in the primate could similarly enhance our understanding of seroto nergic function in higher animals. From a public health perspective, MDMA studies in monkeys are of value because they will help to define the risk that MDMA poses to humans. Additionally, these studies could help identify functional consequences of MDMA neurotoxicity in primates, and thus guide the clinical assessment of MDMA-exposed individuals.

This chapter will review some recently completed studies on the long-term effects of MDMA in nonhuman primates. The goals of these studies were to (1) determine if the neurotoxic effects of MDMA, which have been well documented in the rodent (see below), generalize to the primate; (2) compare the relative sensitivity of primates and rodents to the neurotoxic effects of MDMA; (3) ascertain if the toxic effects of MDMA in the monkey are restricted to nerve fibers (as they are in the rat). or if they involve cell bodies as well; (4) evaluate how closely toxic doses of MDMA in the monkey approximate those used by humans; and (5) examine whether 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) can be used to detect MDMA-induced serotonergic damage in the CNS of primates. Before presenting the results of these studies, previous results in the

rodent will be briefly summarized, so as to put findings in the primate in proper perspective.

PRIOR FINDINGS IN RODENTS

Bats given MDMA show prolonged reductions in the concentration of brain serotonin (Schmidt 1987; Commins et al. 1987; Stone et al. 1986; Battaglia et al. 1987; Mokler et al. 1987; Ricaurte et al. 1987), the number of serotonin uptake sites (Schmidt 1987; Battaglia et al. 1987; Commins et al. 1987), the level of 5-HIAA (Mokler et al. 1987; Stone et al. 1986), and the activity of tryptophan hydroxylase (TPH) (Stone et al. 1986). Correlative anatomical studies indicate that these neurochemical changes are due to damage of serotonergic axons (O'Hearn et al. 1988). Cell bodies in the brainstem of the rodent do not appear to be damaged by MDMA. Serotonin-containing perikarya in the raphe nuclei of rats have a normal cytological appearance and show no obvious reduction in number (Molliver 1987; O'Hearn et al. 1988). In guinea pigs, MDMA produces long-term neurochemical effects similar to those in rats (Commins et al. 1987). This is noteworthy because guinea pigs (like humans) metabolize amphetamine primarily by side-chain deamination, whereas rats do so mainly by ring hydroxylation (Caldwell et al. 1976). In contrast to guinea pigs and rats, mice do not develop long-term depletions of serotonin after MDMA, even after high doses (Stone et al. 1987). This provocative finding raises the important question of whether other animals might not also be resistant to MDMA's neurotoxic effects. In this regard, monkeys are of special interest because of their close phylogenetic relationship to humans and because they metabolize amphetamine in a manner similar to humans (Caldwell et al. 1976). For these reasons. as well as for those previously mentioned, studies were undertaken to evaluate the neurotoxic potential of MDMA in nonhuman primates.

OBSERVATIONS IN PRIMATES

Studies were performed in the squirrel monkey (Saimiri sciureus). This primate species was selected because of its size, availability, and prior use in neurotoxicity studies (Langston et al. 1984). Initial dose-response determinations were carried out using the following doses of MDMA: 2.50, 3.75, and 5.00 mg/kg. Each dose of MDMA was administered subcutaneously twice daily (at approximately 0800 and 1700 hours each day) for 4 consecutive days. This particular regimen of drug administration was employed because its prior extensive use in the rat (Commins et al. 1987; Battaglia et al. 1987; Ricaurte et al. 1987) would permit comparison of results in the monkey with those in the rat. Two weeks after drug treatment, the animals were killed, the brains were removed, dissected, and then analyzed for their regional content of serotonin, doparnine, and noradrenaline using the method of Kilpatrick et al. (1986), as previously described (Ricaurte et al. 1988a).

MDMA produced a dose-related depletion of serotonin without altering the concentration of either dopamine or norepinephrine in the monkey brain (table 1). Even the lowest dose of MDMA produced a substantial depletion

TABLE 1. *Selective dose-related depletion of serotonin in cerebral cortex of monkeys administered MDMA 2 weeks previously*

Treatment	5-HT	DA	NE
Saline	0.167 ± 0.015	10.4 ± 0.5	0.39 ± 0.03
MDMA - 2.50 mg/kg	0.093 ± 0.010* (-44%)	NT**	NT
MDMA - 3.75 mg/kg	0.037 ± 0.013* (-78%)	NT	NT
MDMA - 5.00 mg/kg	0.017 ± 0.003* (-90%)	9.7 ± 0.8 (ns)	0.41 ± 0.02 (ns)

*$p<0.05$, determined by individual comparison to control after one-way analysis of variance showed F value $p<0.05$

**NT=not tested because higher dose was without effect.

NOTE: Values in μg/g represent the mean ± SEM (n=3).

of serotonin in the cerebral cortex of the monkey (-44 percent). MDMA also reduced the concentration of cortical 5-HIAA (table 2). Reduced levels of serotonin and 5-HIAA were evident not only in the cerebral cortex, but also in the caudate nucleus (-86 percent), hippocampus (-77 percent), hypothalamus (-77 percent), thalamus (-84 percent), and putamen (-90 percent) (table 3). Anatomical studies were subsequently carried out in collaboration with Dr. Mark Molliver and Marianne Wilson of the Johns Hopkins University School of Medicine to determine if there was a structural basis for the serotonin and 5-HIAA depletions induced by MDMA. These morphological studies showed that there was a marked reduction of serotonin-immunoreactive axons in the monkey forebrain (figure 1). and that, at high power, some of the remaining axons appeared swollen and misshapen (Wilson et al. 1988). Coupled with the biochemical observations, these morphological findings suggest that MDMA produces neurochemical deficits by damaging serotonergic axons. Further, they demonstrate that the long-term effects of MDMA originally documented in the rodent generalize to the primate.

TABLE 2. *Decreased concentration of 5-HIAA in the monkey brain 2 weeks after MDMA (5 mg/kg)*

	N	Neocortex	Caudate	Hippocampus	Hypothalamus
Control	3	0.250 ± 0.010	0.232 ± 0.005	0.245 ± 0.006	0.897 ± 0.042
MDMA	3	0.040 ± 0.006*	0.056 ± 0.002	0.060 ± 0.001*	0.543 ± 0.084*

*p<0.05, two-tailed student's *t*-test.

NOTE: Values represent the mean ± standard error of the mean.

TABLE 3. *Regional concentrations of serotonin in the monkey brain 2 weeks after MDMA (5 mg/kg)*

Somatosensory Cortex	Caudate	Putamen	Hippocampus	Hypothalmus	Thalamus
		CONTROL (n=3)			
0.14 ± 0.01	0.21 ± 0.03	0.28 ± 0.02	0.13 ± 0.03	0.90 ± 0.06	0.73 ± 0.01
		MDMA (n=3)			
0.02 ± 0.01*	0.03 ± 0.01*	0.03 ± 0.01*	0.03 ± 0.01*	0.21 ± 0.01*	0.12 ± 0.01*

*p<0.05, two-tailed student's *t*-test.

NOTE: Values represent the mean ± standard error of the mean.

RELATIVE SENSITIVITY: PRIMATES VS. RODENTS

Next, the relative sensitivity of monkeys and rats to the serotonin-depleting effects of MDMA was evaluated. This was done by comparing dose-response data in these two experimental animals. In the monkey, a 2.5 mg/kg dose regimen of MDMA produced a 44 percent depletion of serotonin (figure 2). By contrast, in the rat, a 10 to 20 mg/kg dose regimen of MDMA was required to produce a comparable effect. Thus, monkeys are 4 to 8 times more sensitive than rats to the serotonin-depleting effects of MDMA. Inspection of the data in figure 2 also showed that the dose-effect curve of MDMA in the monkey is much steeper than in the rat. Consequently, small increments in dose cause large increases in serotonin depletion in the monkey but not in the rat. Clearly, this could have serious implications for humans experimenting with higher doses of MDMA, as it suggests that the margin of safety of MDMA in primates is narrow.

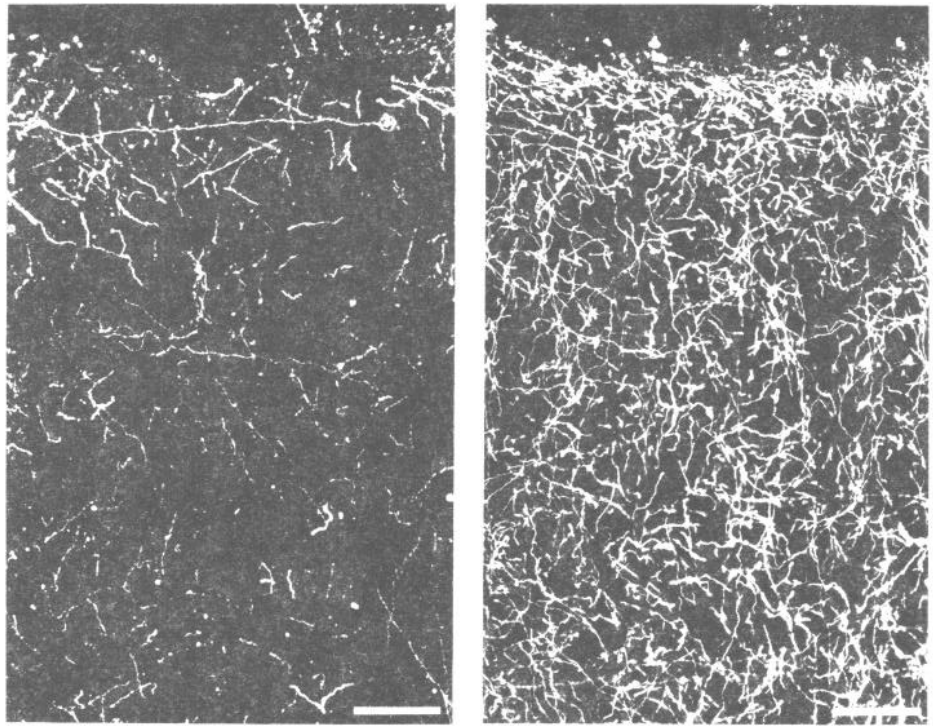

FIGURE 1. *Marked reduction of serotonin-immunoreactive axons in the somatosensory cortex of MDMA-treated monkey*

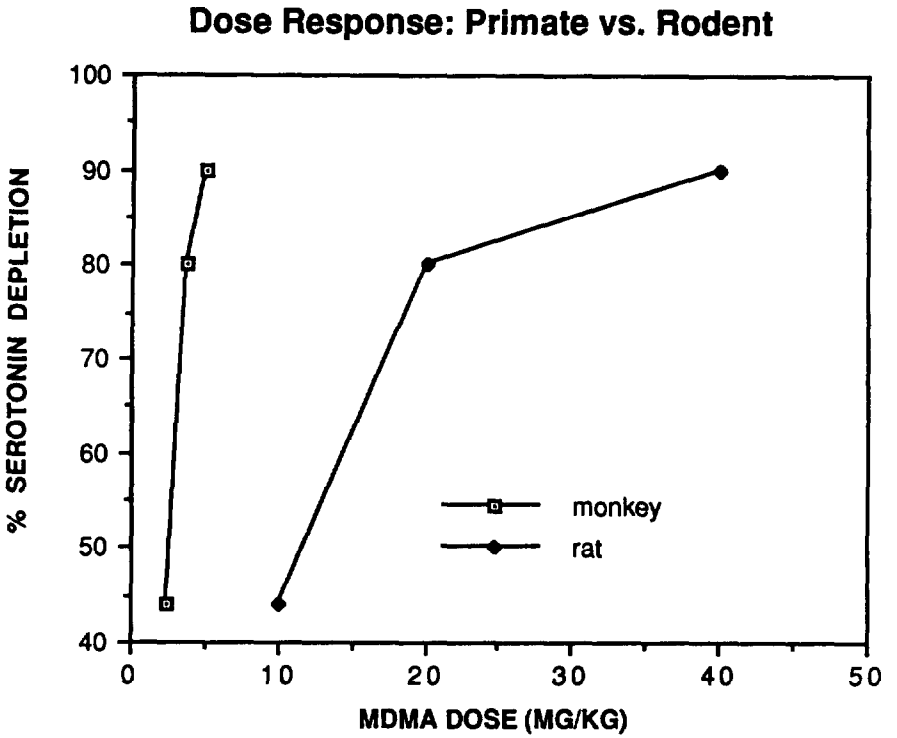

FIGURE 2. *Dose-response data in rats and monkeys administered MDMA 2 weeks previously*

INVOLVEMENT OF NERVE CELL BODIES

The severity of the axonal damage caused by MDMA in the forebrain of the monkey raised the question of whether cell bodies in this experimental animal might also be damaged by MDMA. As noted above, cell body damage does not occur in the rat (O'Hearn et al. 1988). However, it is to be recalled that in the case of MPTP, it was not until it was tested in the monkey that MPTP's toxic effects on cell bodies were appreciated (Chiueh et al. 1983; Langston et al. 1984). To determine if this was also the case for MDMA, the brainstem of monkeys treated with the high dose (5 mg/kg) regimen of the drug 2 weeks previously was examined histologically. MDMA-treated monkeys showed no obvious cell loss in either the dorsal or median raphe nuclei. However, there were clear cytopathologic changes in nerve cells of the dorsal (but not median) raphe nucleus. Specifically, nerve cells in the dorsal raphe nucleus appeared shrunken and contained brownish-red intracytoplasmic spherical inclusions, which were acid fast in Ziehl-Nielsen stain for lipofuscin, granular in LFB-PAS-stained sections, and

vividly PAS positive. While the significance of these inclusions remains unclear, their staining characteristics suggested the presence of an increased amount of lipofuscin. More recent studies investigating the fate of these inclusion-bearing nerve cells have suggested that they do not die, and that their survival is associated with partial recovery of serotonin in the monkey forebrain (Ricaurte et al. 1988c). Similar recovery of serotonin has been noted in MDMA-treated rats (Battaglia et al. 1988). It remains to be determined if recovery of serotonin is related to regeneration of serotonergic axons, and if the new axons innervate their original targets or form aberrant synaptic contacts.

RELEVANCE TO HUMANS

Clearly, much of the impetus for investigating the neurotoxic effects of MDMA in animals has come from concern that MDMA may produce similar toxic effects in humans. However, the extent to which findings in animals can be extrapolated to humans has been unclear, largely for three reasons: First, in most animal studies, MDMA has been administered subcutaneously or intraperitoneally (Schmidt 1987; Commins et al. 1987; Battaglia et al. 1987; Mokler et al. 1987), even though humans invariably take the drug orally (Seymour 1986). Second, most animal studies have used multiple doses of MDMA, and these have been given over relatively short periods of time (Commins et al. 1987; Battaglia et al. 1987; Mokler et al. 1987). By contrast, humans typically take single doses of MDMA, usually weeks apart (Seymour 1986). Third, doses of MDMA tested in animals have often far exceeded those used by humans.

In an effort to bridge the gap between studies of MDMA in animals and human MDMA use patterns, the following studies were performed. These studies tested the importance of dose, route, and schedule of drug administration as determinants of MDMA neurotoxicity. In the frost experiment, one group of monkeys received MDMA (5 mg/kg twice daily for 4 days) subcutaneously; another group received an identical dosage regimen of the drug orally. The animals were killed 2 weeks later, and regional brain serotonin concentrations were determined. Monkeys given oral MDMA showed depletions of serotonin that, depending on brain region, ranged from one-third to two-thirds of those found in monkeys given the drug subcutaneously (table 4). Recently, similar results have been obtained in rhesus monkeys (Kleven et al. 1989). Taken together, the results of these studies indicate that the oral route of administration does not afford significant protection against the long-term effects of MDMA on serotonin neurons.

A second study compared the effects of single versus multiple doses. One group of monkeys received a single 5 mg/kg dose of MDMA orally; another group received the same dose by the same route, but on a twice daily basis for 4 days. As before, the multiple dose regimen produced a

large depletion of serotonin in all forebrain regions examined (table 5). By contrast, the single dose produced a depletion of serotonin only in the thalamus and hypothalamus. In both brain regions, the depletion was smaller than that produced by the multiple dose regimen, but achieved statistical significance. The long-term effects of even single doses of MDMA further attest to the high sensitivity of the primate to the serotonin-depleting effects of MDMA. Further, they raise the question of whether humans might be similarly affected, particularly since they take doses that are only 2 to 3 times lower than the dose that produce an effect in the monkey (1.7 to 2.7 vs. 5.0 mg/kg).

STUDIES OF CSF 5-HIAA

Detecting a depletion of serotonin in the brain of a living human poses a major challenge. To date, only two methods have been attempted. The first involve measurement of 5-HIAA in the CSF (Garelis et al. 1974; Moir et al. 1970); the second calls for neuroendocrine challenge with various serotonergic agents (Cowen and Anderson 1976; Heninger et al. 1984). Unfortunately, both of these methods are indirect, and neither has been fully validated. Accordingly, it was necessary to test the usefulness of CSF 5-HIAA as a marker of MDMA neurotoxicity in the monkey before attempting to use it on humans.

To validate the CSF 5-HIAA method in the monkey, three monkeys were given MDMA at a dose that produces extensive serotonergic damage (5 mg/kg twice daily for 4 days, SC); three other age- and sex-matched animals were given saline and served as controls. Two weeks later, all of the animals were lightly anesthetized with ether, and 200 to 300 μL of CSF were removed by cervical puncture. Later that same day, all animals were killed for determination of regional CNS and CSF serotonin and 5-HIAA levels. These measurements showed that MDMA lowered the concentration of 5-HIAA in the CSF but not that of homovanillic acid (HVA) or 3-methoxy-4-hydroxyphenylene-glycol (MHPG) (table 6). The reduction of CSF 5-HIAA was associated with a marked depletion of serotonin in the CNS (table 7). The decrease in 5-HIAA in cervical CSF was smaller than the depletion of serotonin in the forebrain (59 percent vs. 90 percent), but greater than the depletion of serotonin in the cervical spinal cord (45 percent vs. 59 percent) (Ricaurte et al. 1988b). Hence, cervical CSF 5-HIAA underestimates serotonin depletion in the forebrain, but overestimates serotonin depletion in the cervical spinal cord. These results indicated that while 5-HIAA in CSF does not fully reflect the depletion of serotonin in the forebrain, it can serve as a partial indicator of serotonergic damage induced by MDMA in the forebrain of primates.

TABLE 4. *Effect of Oral vs subcutaneous MDMA on regional brain serotonin in the primate 2 weeks later*

	Somatosensory Cortex	Frontal Cortex	Caudate	Putamen	Hippocampus	Hypothalamus	Thalamus
Control (n=3)	0.14 ± 0.01	0.12 ± 0.03	0.21 ± 0.03	0.28 ± 0.02	0.13 ± 0.03	0.90 ± 0.06	0.73 ± 0.01
MDMA - SC (n=3)	0.02 ± 0.01* (-86%)	0.03 ± 0.01* (-75%)	0.03 ± 0.01* (-86%)	0.03 ± 0.01* (-90%)	0.03 ± 0.01* (-77%)	0.23 ± 0.01* (-75%)	0.12 ± 0.01* (-84%)
MDMA - PO (n=3)	0.06 ± 0.01** (-58%)	0.07 ± 0.01** (-42%)	0.15 ± 0.01** (-29%)	0.19 ± 0.01** (-33%)	0.07 ± 0.01** (-47%)	0.56 ± 0.03** (-38%)	0.28 ± 0.07** (-62%)

*p<0.05, determined by individual comparison to control after a simple one-way analysis of variance (ANOVA) showed F value p<0.05.

**p<0.05, determined by comparison to control and MDMA-SC after a simple ANOVA showed F value p<0.05.

TABLE 5. *Effect of single vs multiple doses of MDMA on regional brain serotonin in the primate 2 weeks later*

	Frontal Cortex	Hippocampus	Hypothalamus	Thalamus	Putamen	Caudate
Control (n=3)	0.12 ± 0.03	0.11 ± 0.01	0.85 ± 0.04	0.72 ± 0.02	0.28 ± 0.02	0.22 ± 0.02
MDMA Multiple (n=3)	0.07 ± 0.01*	0.07 ± 0.01*	0.56 ± 0.03*	0.28 ± 0.07*	0.19 ± 0.01*	0.15 ± 0.01*
DMA Single (n=3)	0.12 ± 0.02	0.13 ± 0.02	0.71 ± 0.03**	0.57 ± 0.07**	0.20 ± 0.02	0.22 ± 0.02

*p<0.05, determined by individual comparison to control after a simple one-way analysis of variance (ANOVA) showed F value p<0.05.

**p<0.05, determined by comparison to control and MDMA multiple after a simple ANOVA showed F value p<0.05.

TABLE 6. *Selective reduction in 5-HIAA in CSF of monkeys administered MDMA 2 weeks previously*

Treatment	N	5-HIAA	HVA	MHPG
Saline	3	101 ± 7	255±44	30 ± 4
MDMA	3	41 ± 7*	264 ± 20	40 ± 11

*p<0.05, two-tailed student's *t*-test.

NOTE: Values represent the mean ± SEM (expressed in µg/mg of tissue).

TABLE 7. *Selective reduction in serotonin in the caudate nucleus of monkeys administered MDMA 2 weeks previously*

Treatment	N	Serotonin	Dopamine	Norepinephrine
Saline	3	0.218 ± 0.023	11.6 ± 0.9	2.59 ± 0.18
MDMA	3	0.021 ± 0.005*	9.7 ± 0.2	2.84 ± 0.91

*p<0.005, two-tailed student's *t* -test.

NOTE: Values represent the mean ± SEM (expressed in µg/mg of tissue).

CSF 5-HIAA STUDIES IN HUMANS

In light of this, studies of CSF 5-HIAA have been initiated in a cohort of human volunteers with a history of extensive MDMA use. Most participants in the study are individuals who have recently learned of the neurotoxic properties of MDMA and have asked to be evaluated for possible serotonergic damage. To qualify for the study, subjects must (1) have used MDMA on at least 20 to 25 occasions, (2) be drug-free for at least 2 weeks prior to participating in the study, and (3) not have a history of neuropsychiatric illness thought to involve alterations in serotonin metabolism. To date, 34 individuals have participated in the study. The study is now in progress, and completion is anticipated by 1991. At this time, it would be premature to comment on the results.

NEUROENDOCRINE STUDIES

As noted earlier, the only other method presently available for detecting serotonergic dysfunction in living humans involves neuroendocrine challenge with serotonin-active drugs (Cowen and Anderson 1976). One such

neuroendocrine test is the L-tryptophan challenge test (Heninger et al. 1984). Briefly, this test calls for intravenous administration of L-tryptophan to human subjects with subsequent measurement of serum prolactin concentration. A rise in serum prolactin is taken as a measure of central serotonergic activity. Using the L-tryptophan challenge tests, serotonergic function was recently evaluated in nine MDMA subjects in collaboration with Drs. Price and Heninger of the Yale University School of Medicine. L-tryptophan induced a robust rise in serum prolactin in controls but not in MDMA subjects (figure 3). The peak change in serum prolactin concentration and the area under the prolactin response curve were diminished in MDMA subjects, but the difference was not statistically signifiit (Price et al. 1989). Additional studies are now in progress to assess the significance of these findings.

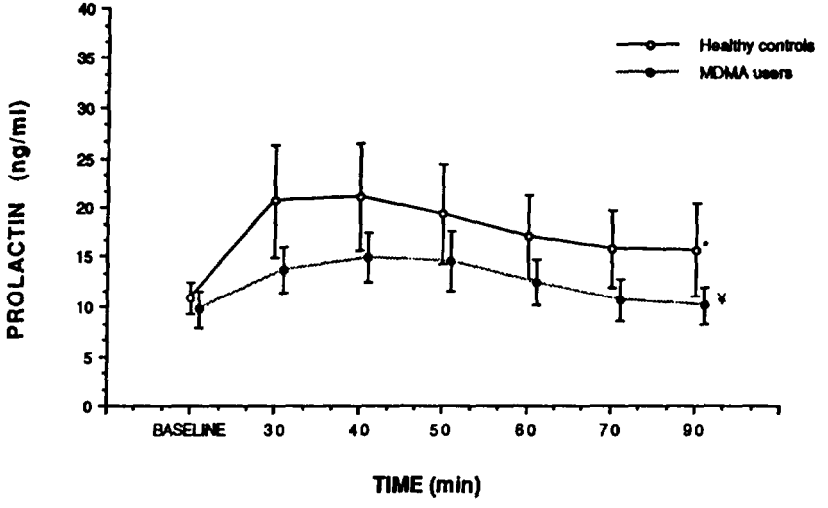

FIGURE 3. *Prolactin response to IV L-tryptophan in control and MDMA subjects (n=9)*

SUMMARY AND CONCLUSION

The results of the studies reviewed here show that the neurotoxic effects of MDMA generalize to the primate. Further, they indicate that monkeys are considerably more sensitive than rats to the serotonin-depleting effects of MDMA, and that the dose-response curve of MDMA in the monkey is much steeper than in the rat. Perhaps as a consequence of this, the toxic effects of MDMA in the monkey involve serotonergic nerve fibers as well as cell bodies, whereas in the rat, only nerve fibers are affected. The present studies also show that the toxic dose of MDMA in the monkey

(5 mg/kg) closely approaches the dose typically used by humans (1.7 to 2.7 mg/kg). This finding heightens concern that MDMA may be neurotoxic in humans, particularly since the steepness of the dose-response curve of MDMA in the primate suggests a narrow margin of safety. Finally, preclinical studies in monkeys have shown that CSF 5-HIAA can be used to detect MDMA-induced serotonergic damage in the primate CNS. Studies now underway in MDMA-exposed humans should help determine if MDMA exerts long-term toxic effects on serotonergic neurons in the human brain.

DISCUSSION

QUESTION: How long after the administration of the MDMA in your human subjects did you measure your parameter?

ANSWER: The request we made to the subjects was that they remain entirely drug-free for 2 weeks. We were trying to simulate the situation that we had in animals.

QUESTION: How long after the administration of the drug did you measure the 5-HIAA?

ANSWER: We did not administer MDMA. We were dealing with humans who had been previously exposed to MDMA. The request we made was that they not take the drug for at least 2 weeks. Some subjects had not taken the drug for over a year. Some had taken it as recently as 19 days.

QUESTION: Is there a decrease in serotonin metabolites, and does it show any relationship to age, cumulative dose, and so forth? Did you see anything there? You have that one person who had 42 grams. Was he any more or any less affected?

ANSWER: We are in the midst of analyzing the data. You will remember that the mean 5-HIAA level in control subjects is approximately 19 to 20. In that one individual, 5-HIAA level turned out to be 14 or 13. You might predict that he would have been the lowest one on the scale. I subsequently learned the importance of body height, and he happens to be a very short, stocky man. So we are now in the process of reanalyzing the data, trying to take in the appropriate variables into account. And, again, these arc studies that have to be replicated in our own hands and extended by others.

QUESTION: Do you see a progressive decline as a function of age, and if you have someone who has been damaged, is there any age relationship in this depletion?

ANSWER: No. Only one individual was 70 or 71 years old. By and large, the individuals are younger than 40. The other side of the coin, of

course, is does 5-HIAA really change with age in the control population? That was one of the variables that I listed. I would emphasize that the data for that are actually very weak. My guess is that there are no age-related changes in 5-HIAA, at least out to 50 to 60 years of age. But that is something we are going to have to contend with as well.

QUESTION: You showed CSF levels in monkeys after the 4-day treatment regimen, but you also showed neurochemical data after a single oral administration. Did you have the opportunity to look at the CSF levels after a single oral administration?

ANSWER: No. That study was actually completed before the CSF studies. We did not get CSF data on the animals that received the single dose because that study was done before the CSF studies were undertaken.

COMMENT: With regard to the CSF levels, I want to emphasize that the decreases that you get are probably a gross underestimate, as was pointed out, Another reason is that the ventricular plexus of serotonin fibers is completely unaffected, and that is probably a very large source. I do not know to what extent that contributes to CSF levels of S-HT and S-HIAA, but those fibers are in the CSF and bathing in it, and they appear to be quite active. So they must be biasing against your seeing an effect. The fact that you are getting such a sizable effect must mean that there is a very profound depletion in the forebrain.

QUESTION: Does anything suggest that people who have taken these drugs or MDMA for a period of time are subject to episodes of depressive disorders or affective disorders?

ANSWER: Frankly, at this point, we have only anecdotal evidence. And as Dr. Schuster mentioned yesterday, people's responses can be very misleading. I could cite three individuals who attribute some mood disturbances to their prior MDMA use, but one wonders how much their reports are based on what you want to hear.

One individual was prescient enough to realize that his depression coincided with loss of his job so he did not know if his depression was related to losing his job or to MDMA ingestion. I think these people are going to need to be looked at by people who know what they are doing in terms of analyzing depression, and that has not been done.

COMMENT: It is interesting in that letter that George Greer wrote to me informally, off the record, that he had seen 10 patients in psychotherapy who had been treated extensively with fenfluramine for dieting. And, after several weeks on fenfluramine, they became very depressed, and two of them committed suicide. So that is a very serious consideration.

RESPONSE: Another interesting consideration is that a number of the subjects have participated with the intent of helping the rest of the country see that this is not such a harmful drug. A number of the proponents of MDMA use the paucity of behavioral abnormalities in MDMA users to point to the fact that literally thousands of subjects have used the drug, and that they are not walking around like zombies; they do not appear to be harmed.

My answer always is that no one has yet done a detailed neurobehavioral study of these individuals and the deficit that they may have. It may be very subtle in nature, and I am not sure that we have the methods available to detect and quantify those deficits. The fact that these people are not walking in with overt behavioral disturbances as the people with MPTP did. I think, is related to the fact that, one, they may not have the kind of neurotoxicity we are suspecting, and two, if they do, the kind of functional consequences that you may get from serotonergic dysfunction may be much more subtle than the kind of functional consequences you get with dopamine dysfunction, where it is very easy to recognize the parkinsonian patient

QUESTION: Do you have any plans to study whether it would be possible to eliminate the large variation in dosage level and frequency and duration since the last dose by studying fenfluramine, where patients are receiving prescribed doses every day for finite periods of time? Perhaps one could set up a study where you sample CSF before and after the therapy so that you would avoid any concern about whether you had selected a group of patients who had low 5-HIAA levels.

ANSWER: Yes, that is one of the groups that we would hope to incorporate.

COMMENT: I am not an advocate of this view, but my colleague Efrain Azmitia from NYU has suggested that perhaps rather than seeing deficits, that pruning our serotonin projections now and then might be a very advantageous and beneficial thing to do.

RESPONSE: I am not going to try to follow up on that one.

QUESTION: We are used to seeing a lot of those big beaded axons which we, alter methamphetamine. have interpreted as damage. Do you think it is what is left over rather than big axons that are damaged?

ANSWER: Yes. We have very carefully evaluated that question and Dr. O'Hearn is a great skeptic who is forcing us to look at it very closely. We are finding that the large varicosity fibers that are left are identical in distribution, morphology, and density to those present in the normal fibers. The damaged fibers are of a completely different nature; they are 10 times

as big, fragmented, and very, very damaged. They can be easily distinguished.

QUESTION: So you get both?

ANSWER: Yes.

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AUTHOR

George A. Ricaurte, M.D., Ph.D.
Department of Neurology
John Hopkins University School of Medicine
Francis Scott Key Medical Center
4940 Eastern Avenue
Baltimore, MD 21224

Dose- and Time-Dependent Effects of Stimulants

Everett H. Ellinwood, Jr., and Tong H. Lee

INTRODUCTION

Considerable confusion abounds in discussion of stimulant-induced toxicity, pathology, psychopathology, and the mechanisms underlying these changes. Although there is laboratory and clinical evidence for histochemical and structural pathologies induced by chronic and high doses of stimulants, the specific relationship to behavioral pathology has not been clearly demonstrated. Clinically, the confusion originates in part from several sources including lack of clear distinctions (1) between phases of stimulant syndrome; (2) between the types of dosing, routes of administration, and differential pharmacokinetic parameters for different utilization styles; (3) between outcomes or other dependent variables; and (4) between proposed mechanisms mediating the outcome. In basic research, the confusion often results from description of a singular effect or even multiple effects of chronic stimulant treatment without clearly delimiting the time-frame, dosing schedule, or mutual exclusiveness of competing behavioral effects.

Our whole task from a clinical perspective includes (1) delineation of the patterns of behavioral pathology induced both during the active stimulant abuse phase and the phases of withdrawal; (2) description of the sequential profile of underlying structural and functional pathology at each of the clinical phases; and (3) an attempt to elucidate the relationship between (1) and (2). In addition, an understanding of the pharmacological and other parameters sufficient and necessary for inducing components of the stimulant syndrome is clearly needed and can be obtained only from basic laboratory studies.

In figure 1 we have outlined phases of stimulant abuse and withdrawal; this pattern does not depict the larger spectrum of patterns seen in stimulant abusers. Instead, we have emphasized what is called a high-dose transition pattern (Gawin and Ellinwood 1988), which leads to the greatest behavioral pathology. In focusing on the high-dose transitional form, the periodic dosing over months to years is deemphasized, as is considerable basic research literature dealing with once or twice-a-day dosing schedules.

Animal studies have shown that the periodic dosing regimen often leads to behavioral augmentation or "sensitization" in animals (Post 1981).

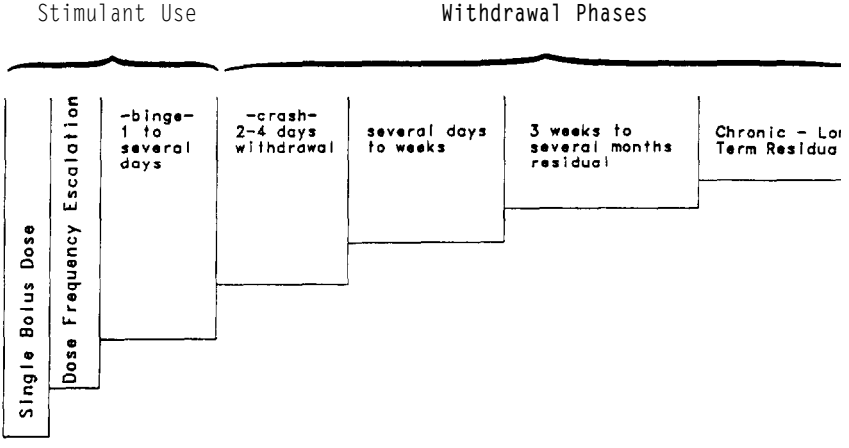

FIGURE 1. *Phases of stimulant abuse and withdrawal seen with high-transition pattern*

PHENOMENOLOGY OF STIMULANT ABUSE

High-Transition Pattern

Initial phases in high-transition pattern are similar to those in other abuse patterns. Typically, individuals are initially exposed to single doses of stimulants for therapeutic (e.g., weight reduction) or other purposes (Ellinwood 1973). Euphoria produced by single doses of stimulants before development of tolerance are proportional to plasma levels (Fischman et al. 1976; Javaid et al. 1978). Higher levels of euphoria are achieved with intravenous (IV) route secondary to rapid rise to a peak concentration (figure 2, A). During the "single bolus" phase, conditioning to euphoriant "rush" of stimulants is especially profound in individuals using a rapid route of administration (e.g., IV or smoking). The single bolus phase is followed by increasing doses and frequency ("dose frequency escalation" phase) mainly secondary to a development of tolerance to euphoriant effect of stimulants.

The high-dose transition is defined as a transition phase in which the individual suddenly increases the doses of stimulants or switches to smoking (e.g., cocaine "crack") or IV route of administration (Gawin and Ellinwood 1988). This change leads to a rapid escalation of plasma levels and intense euphoria (i.e.. rush) often with subsequent increase in dosing frequency. ln its most severe form, the high-dose pattern is characterized by binges of

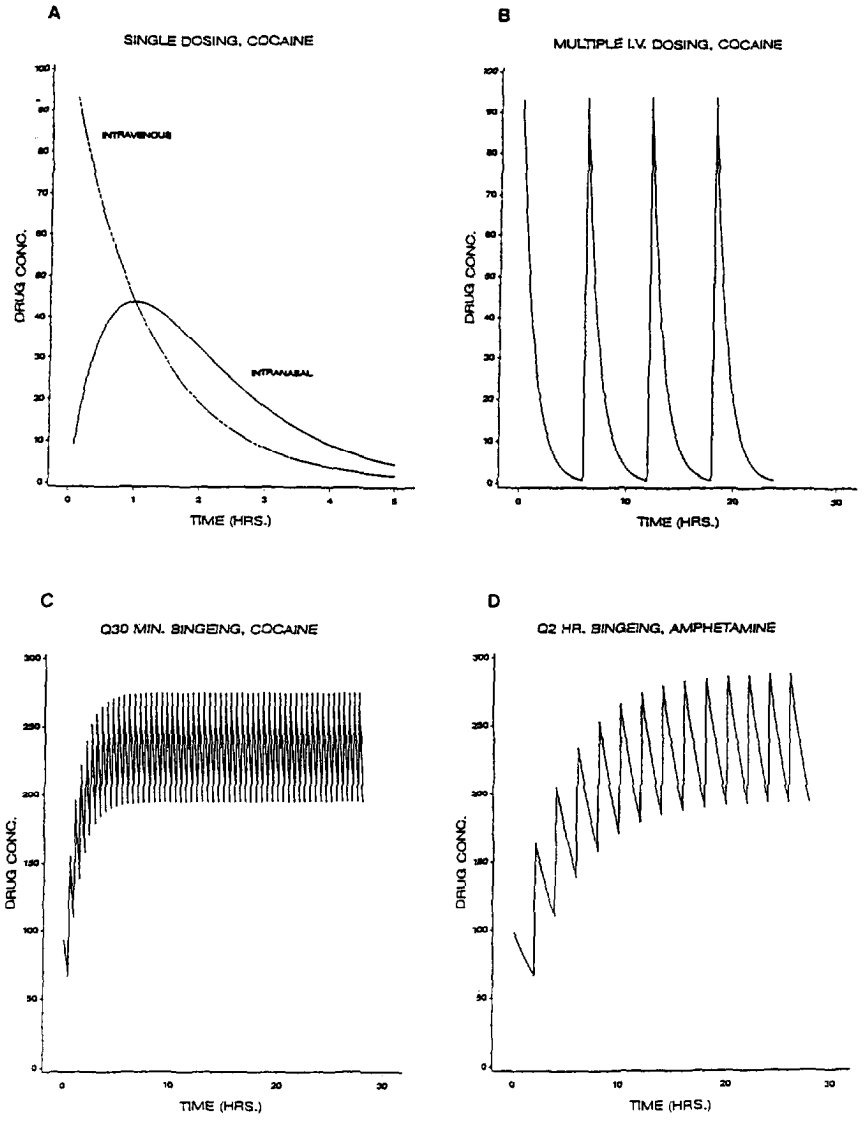

FIGURE 2. *Dose frequency escalation patterns, cocaine and amphetamine*

stimulant use, in which the individual repeatedly administers high doses of stimulant in an attempt to "chase" the euphoric state against the background of rapidly developing acute tolerance. Each bingeing episode can last from a few hours to days and is usually terminated by extreme physical

exhaustion and/or exhaustion of drug supply (Gawin and Ellinwood 1988). It is the high, sustained plasma levels that appear to lead to the greatest pathology in both stimulant abusers and laboratory animals (Ellinwood and Kilbey 1977). Figure 2, B through D, illustrates drug plasma levels during the high-dose transition and bingeing phase. A severe compulsive pattern of repeated cocaine administration is necessary to maintain sustained drug levels during cocaine binges, whereas, because of its longer plasma half-life, amphetamine bingeing is usually characterized by longer intervals between injections. During this compulsive phase, severely addicted individuals report stereotyped patterns of behavior and thiig with near exclusion of other concerns. Possible mechanisms responsible for different phases of high-transition pattern are summarized in figure 3 under abuse dependence. At present. there is only speculation on these mechanisms.

During the high-dose transition, the intense euphoria associated with the rush leads to profound conditioning of associated abuse behaviors; not only are the injection behaviors highly conditioned to the circumstances surrounding the injections, but the behaviors leading up to the procurement of the drug and the preparation prior to injection are also conditioned (Ellinwood 1973).

Aspects of Withdrawal

The clinical withdrawal period may be considered as a sequence of phases beginning with "crash" and ending with long-term withdrawal that can only be presented descriptively. Long-term residual effects may be noted weeks or months afterwards. Consideration for each different phase of withdrawal is critical in understanding and treating the evolving stages of withdrawal. The treatment consideration in the intermediate withdrawal phase is quite different from that in the long-term withdrawal phase (Gawin and Ellinwood 1988).

Crash, the initial phase of stimulant withdrawal, immediately follows a bingeing episode. Initially marked depressive dysphoria, anxiety, and agitation are noted, followed by craving for sleep over the next few hours (Ellinwood 1973; Gawin and Ellinwood 1988). Often the individual uses a wide variety of sedatives or anxiolytics, such as alcohol, to initiate a sustained hypersomnia. Prolonged sleep, often lasting 24 to 36 hours, is not unusual during this phase. Notably, addicts report minimal desire for the abused drug during this immediate phase of withdrawal (Gawin and Kleber 1986).

As the individual recovers from the crash phase, a period of anhedonia, dysphoria, and decreased mental and physical energy ensues (intermediate withdrawal phase). This phase can last from several days to weeks. Emerging from the mood and energy dysfunction, craving or high urge for the stimulant returns, frequently leading to recidivism (Ellinwood 1973).

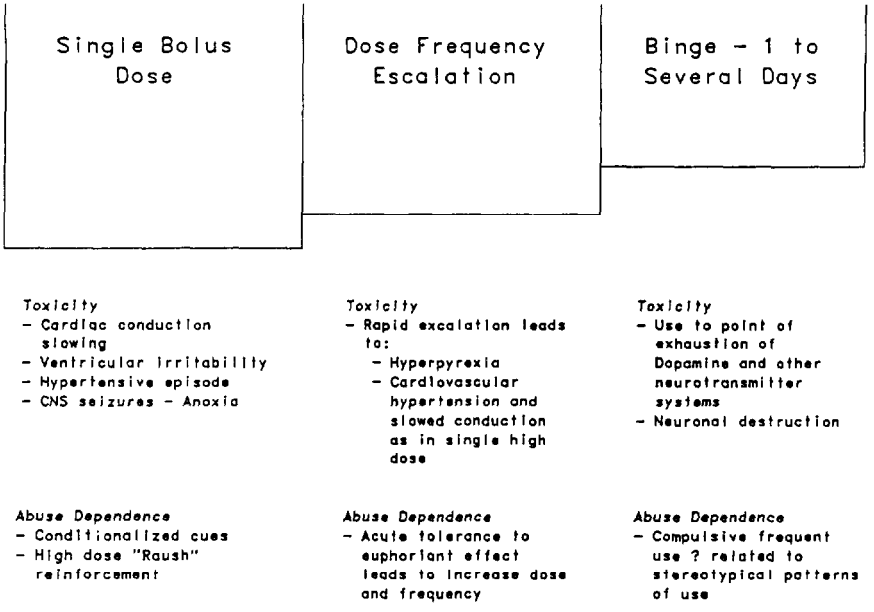

FIGURE 3. *Toxicity and development of dependence under single-dose, escalated, and binge conditions*

The stimulant urge-impulses are sensitive to environmental cues such as returning home and associated multiple situational stimuli (e.g., paraphernalia, friends, etc.). With continued drug availability, it is not unusual to observe repetitious cycles of bingeing with intervening crash and intermediate withdrawal phases over a period of months. It is noted that conditioned withdrawal responses are less pronounced than with opiates; yet, withdrawal appears to have a phase-specific relation to the reemergence of cue-sensitive responses that deserves further research.

With continued abstinence through the intermediate withdrawal phase, a more natural baseline affective state returns (long-term withdrawal phase). Although decreased in frequency and intensity, however, urges to return to stimulant use can recur after months to years of abstinence, again frequently triggered by environmental cues (Ellinwood 1973; Gawin and Kleber 1986). Moreover, the individual can exhibit a "grease slide" return to previously conditioned behavioral responses with a single "taste" of stimulants. Full expression of the drug-induced paranoid, stereotyped thinking pattern within minutes to hours of return to stimulant use is a well-documented long-term sequela of high-dose stimulant abuse (Ellinwood 1973; Bell 1973). This full expression of behavioral pathology may have originally taken weeks or months of chronic stimulant use to evolve. These examples highlight the latent propensity to recidivism even after long-term withdrawal from

stimulants. A question that arises from the residual behavioral pathologies is whether such changes are related to toxicity associated with stimulant use. Thus, careful consideration of the acute and chronic toxicity is warranted.

TOXICITIES ASSOCIATED WITH SINGLE DOSES OF STIMULANTS

Peripheral Toxicities

Several types of toxicities are responsible for stimulant-related morbidity and mortality (figure 3). Reported cardiovascular toxicities include acute myocardial infarction, "stunned" myocardium syndrome, arrhythmias, myocarditis, and ruptured aorta (Cregler and Mark 1986). Significantly, a history of underlying disease appears not to be a prerequisite. For example, acute myocardial infarction following administration of cocaine has been documented in patients without fixed or spastic coronary diseases or history of cardiac symptoms (Isner et al. 1986). Interestingly, cocaine appears to be more frequently associated with the above cardiac complications than are amphetamines. The exact reason for this preponderance of cocaine-associated toxicity is not clear; the significant local anesthetic effect of cocaine may contribute to its cardiac toxicity. Alternatively, because of its ultrashort half-life, cocaine may be more liable to overdosing with attempts to maintain effective plasma levels.

The relative contribution of different mechanisms to stimulant-induced cardiac toxicities is not known. Currently, sympathetic overstimulation is thought to mediate many of these effects (Cregler and Mark 1986). Increased oxygen demand secondary to increased heart rates and blood pressure has been hypothesized to lead to myocardial infarction (especially in patients with fixed coronary disease) and/or ventricular arrhythmias. In patients with no history of cardiac disease, cocaine is thought to induce acute ischemic complications via vasospasm of the coronaries (Ascher et al. 1988). In addition, Virmani et al. (1988) have reported a 20 percent incidence of myocarditis thought to be secondary to accumulated microvascular injuries.

One critical factor that has been neglected in considering mechanisms of cardiac fatalities is the timeframe for various types of toxicities. For example, a majority of cocaine-related fatalities and near fatalities reported from emergency rooms are attributed to one or more types of cardiac ischemic or hypertensive episodes (Isner et al. 1986). Thus, these studies may discount the cocaine-induced arrhythmias and conduction defects as important direct causes of fatalities. Yet, if coroner reports are used as data sources (Virmani et al. 1988; Wetli and Wright 1979; Mittleman and Wetli 1984), there are great numbers of deaths in which pulmonary effusion and lack of evidence for coronary occlusion, acute myocardial infarction, or

other events tend to discount the preponderance of coronary mechanisms as the key factor leading to death

The above discrepancy may be partly explained by studies by Kabas et al. (1988) demonstrating that, during the first 1 to 5 minutes of large IV cocaine dose, the His bundle-ventricular conduction time is markedly prolonged in dogs. These results indicate that an intense but transient conduction defect occurs almost immediately after escalation of plasma cocaine level. Local anesthetics impair cardiac conduction by interacting with the sodium ion channel (Starmer et al. 1984). A cardiac arrhythmia may develop rapidly secondary to combination of the conduction defect and cardiac irritability (due to massive cardiac stimulation by catecholamine potentiation). Furthermore, since the local anesthetic effect is potentiated by reduced extracellular pH (Moorman et al. 1986), acidosis due to increasing myocardial ischemia and/or seizure activity may potentiate the arrhythmogenic effect, ultimately leading to a fatal cardiac arrhythmia. Precipitous cardiac deaths, both with and without preceding seizure activities, have been documented following IV administration of cocaine (Wetli and Wright 1979). It should be mentioned that seizures are not necessary for the cardiac effect, and seizure threshold is above that necessary for cardiac conduction prolongation (Kabas et al. 1988). In conclusion, many cocaine-related sudden deaths coming directly to coroners' attention may be precipitated by a brief conduction defect leading to a terminal ventricular arrhythmia.

Central Toxicity

Although the incidence of cerebrovascular accidents from stimulant usage is low, case reports following acute intake of cocaine or amphetamines have appeared (Cregler and Mark 1986). Persons with subclinical cerebrovascular abnormalities such as arteriovenous malformation or cerebral aneurysm appear to be particularly susceptible. In addition to preexisting structural abnormalities, stimulants themselves, when abused chronically, may induce cerebral microarteriolar pathology predisposing individuals to stroke (Rumbaugh 1977). A sudden surge in blood pressure induced by the drug with the background of various types of vascular abnormalities is likely to mediate the cerebrovascular accidents. Intracranial hemorrhage should be included in differential diagnoses for patients complaining of headaches after stimulant use.

High doses of stimulants lead to progressive hyperthermia; death from a gradual overdose of stimulants (e.g., those occurring in "body packers") are often associated with hyperpyrexia, convulsions, and cardiovascular shock (Ellinwood 1973; Wetli and Wright 1979). Hyperpyrexia is more frequently noted with amphetamine, perhaps due to the longer half-life of this agent, Life-threatening hyperpyrexia usually ensues an hour or more following large doses of stimulants and is more prevalent in relatively naive

nontolerant users. In animals, amphetamines produce hyperthermia and death in a dose-dependent manner (Zalis et al. 1967). Stimulants increase body temperature by affecting both the central and peripheral temperature-regulating mechanisms, as well as by stimulating motor activity (Ellinwood 1973).

Not only do stimulants induce hyperthermia, but elevated ambient or body temperature itself may augment various effects of stimulants (Weihe 1973). For example, elevated environmental temperature has been associated with fatalities among amphetamine abusers taking their usual doses of the drug, and exercise potentiates the toxicity, as demonstrated by fatalities among athletes during the sixties when use of amphetamine to enhance performance was prevalent (Ellinwood 1973). The behavioral stereotypy induced by amphetamine is also potentiated by increased ambient temperature (Horita and Quock 1974), as is depletion of dopamine (DA) following chronic methamphetamine (METH) administration (Seiden and Ricaurte 1987). A recent study shows that acute hyperthermia may attenuate adaptive compensatory mechanisms of dopamine pathways (e.g., regulation of DA impulse-flow) in response to methylphenidate (Lee et al. 1988). This finding, then, suggests that the increased toxicity of stimulants under hyperthermic conditions may be due not only to the increased temperature per se but also to a direct impairment of the body's ability to compensate for stimulant toxicity.

Another modality of stimulant-induced toxicity is the induction of generalized seizures and associated anoxia (Ellinwood 1973; Jonsson et al. 1983). As noted above, the seizure during the hyperthermic condition is frequently associated with more gradual overdosing of stimulants, and, indeed, status epilepticus may ensue. The complication may also result from direct lowering of threshold by stimulants. For example, cocaine, via its local anesthetic properties, can alter amygdala electrical activity and produce seizures (Post et al. 1987); seizures due to local anesthetic effects, in contrast to hyperthermia-associated seizures, appear immediately after dosing. It should be mentioned that periods of nonfatal anoxia need to be considered in the accumulative neuropathology associated with chronic stimulant administration.

TOXICITIES ASSOCIATED WITH CHRONIC STIMULANT ADMINISTRATION

Peripheral Toxicities

During the escalation and bingeing phases of stimulant abuse, higher doses and frequency, as well as propensity for more rapid route of administration, may lead to increased susceptibility to various medical complications. On the other hand, development of either tolerance or sensitization to different stimulant effects is well known (Ellinwood 1973; Post 1981) and should be

properly assessed along with other variables already mentioned. For example, one recent report (Avakian and Manneh 1987) demonstrated that chronic cocaine pretreatment reduced susceptibility to epinephrine-induced arrhythmia in rabbits, suggesting chronic abusers may become tolerant to the arrhythmogenic effect of stimulants. It is not known whether this tolerance indeed develops in clinical settings and, if so, whether it shows time-, abuse pattern-, or dose-dependencies.

Central Toxicities

Effects in Laboratory Animals. As highlighted in other chapters, the central toxicities during and after repeated stimulant bingeing may be related to neuronal or terminal destruction and/or depletion of neurotransmitter in the brain. In monkeys and cats, the report by Duarte-Escalante and Ellinwood (1970) of neuronal chromatolysis associated with decreased catecholamine histofluorescence following chronic METH intoxication has been followed by extensive neurochemical demonstrations of damage to the monoamine pathways by chronic stimulants (Seiden and Ricaurte 1987). The most consistent changes have been observed in the DA systems with more variable effects on norepinephrine (NE) and serotonergic neurons.

Given current attempts in clinical neuroscience to relate monoamine changes to a variety of mental and movement disorders (including mood disorders and schizophrenia), reported changes in NE and serotonin levels following chronic stimulant administration deserve careful consideration, despite variabilities in findings. The earlier studies by Seiden et al. (1977) are interesting in that, in contrast to their later study using rats (Wagner et al. 1980), they demonstrated, in monkeys, 40 to 60 percent depletion of NE in the pons-medulla, midbrain, and frontal cortex regions, both shortly and 3 to 6 months after chronic METH treatment. Molliver et al. (this volume) also describe extensive loss of finely beaded serotonin terminal areas, yet no loss or even an increase in serotonin in the medial and posterior raphe. In an earlier study Duarte-Escalante and Ellinwood 1970) in cats and monkeys, we also found increase in serotonin histofluorescence in and around the medial raphe neurons. These findings are in sharp contrast to more frequently reported effects of METH on brain serotonin levels, i.e., a decrease (Seiden and Ricaurte 1987).

In addition to changes in monoamines, those in other modulators or transmitters may alter the functional responsiveness following chronic stimulant administration. For example, the marked increase in acetylcholinesterase noted in the mesencephalon and brain stem (especially in areas containing major catecholamine cell bodies) after chronic METH (Duarte-Escalante and Ellinwood 1970) takes an added significance in light of recent findings that this enzyme is, like DA, released from dendrites of DA cells in the substantia nigra (Greenfield 1984). Functionally, the released enzyme can inhibit DA cells firing in the compacta region. The effect of chronic

stimulants on other substances colocalized in DA neurons, such as cholecystokinin and cytochrome P450 reductase, has not been well studied. The latter enzyme has been proposed to participate in a possible endogenous formation of the neurotoxin 6-hydroxydopamine (Sasame et al. 1977); could the same enzyme be involved in stimulant-induced neurotoxicity via a similar mechanism as has been proposed by Seiden's group (Seiden and Ricaurte 1987)? Changes need to be assessed carefully in a wider spectrum of modulators, transmitters, and their possible functional consequences.

When determining effects of chronic stimulant administration, it is essential to distinguish specific drugs (e.g., d-amphetamine vs. METH), doses and regimens of administration, and differential sensitivities among species as well as the time at which measurements are made. For example, METH causes more serotonin depletion than does *d*-amphetamine (Seiden and Ricaurte 1987). Cocaine may not induce monoamine depletions (Kleven et al. 1988), although Hitori et al. (1989) have reported a selectively decreased binding to DA uptake sites in the prefrontal cortex. This issue awaits further evaluation. Evidence also indicates that monoamine damage induced by stimulants is more marked after continuous exposure (Lee and Ellinwood 1989) or higher doses of stimulants (Seiden and Ricaurte 1987), and this effect is perhaps more pronounced in higher animals such as the cat and monkey (Wagner et al. 1980; Owen et al. 1981; Trulson and Crisp 1985).

One of the most critical factors determining specific changes is the time of determination after chronic dosing. Yet this variable has not been carefully controlled in many basic and clinical studies. For example, too frequently in studies of neuronal damage (e.g., chromatolysis), deaths following chronic stimulant administration is the time variable neglected. Clear demonstration of the importance of time is provided by recent findings that the sensitivity of DA autoreceptors undergoes a rapid change (from sub- to supersensitivity) during the first week of withdrawal (Ellinwood and Lee 1983; Lee and Ellinwood 1989). The autoreceptor supersensitivity will be discussed further.

Effects In Humans. Neither postmortem nor functional cerebrospinal fluid (CSF) studies in humans provide firm evidence for similar, long-term damages or alterations to monoaminergic neurons in chronic stimulant abusers. In part, the lack of demonstrable neurochemical changes may well be due to the obvious preclusion of well-controlled prospective experimentation in humans, as well as to variability in critical variables (e.g., individual sensitivity or pattern of abuse) encountered in clinical research. Possible relationship of the various complications of stimulant abuse including hyperpyrexia, seizure, anoxia, and metabolic exhaustion to neuronal chromatolysis, terminal destruction. and monoamine and enzymatic depletion have not been systematically explored in human autopsy cases. It should be also noted that, under nonperturbed conditions, overt behavioral deficits are rare in

animals depleted of monoamines with chronic stimulants (Lee and Ellinwood 1989; Kokkinidis 1984). We need to evaluate carefully a possible relationship between the fatigue, neurasthenia, and mood dysfunction reported in the protracted stimulant withdrawal in humans and an underlying neurochemical or anatomical state.

MORE ISSUES IN CHRONIC STIMULANT RESEARCH

In chronic stimulant abusers, one observes interactions among direct long-term toxic consequences and various compensatory behavioral and physiological mechanisms; consequently, it is necessary to evaluate multiple effects over different phases of stimulant abuse, to sort out the contributions of each of these mechanisms. Lack of attention to the complex interaction has contributed to the confusion in stimulant research. Often, in basic research, a singular mechanism for effects of chronic stimulant treatment (e.g., those for stereotypy sensitization vs. tolerance) has been examined without consideration of other concomitant changes. For example, only a few investigators have attempted to sort out the conditioned effects in assessment of sensitization and tolerance (Post et al. 1987; Ellinwood et al. 1973). One goal of future research should be formulation of a clear concept of how the changes induced by chronic stimulants integrate over time and which mechanisms are "rate limiting" in induction of different functional changes.

In addition to interaction among different mechanisms, we need to consider that there is a competitive economy of behaviors in the animal's repertoire, as these behaviors undergo time-dependent changes during chronic administration. If a single behavior, such as stimulant stereotypy, comes to the foreground, then other behaviors, such as locomotion or grooming, have to recede into the background, thus leading to constriction of behavioral repertoire. The response competition of species-specific behaviors (Ellinwood and Kilbey 1979) is rarely considered, but it may be a major contributor to the simultaneous appearance of tolerance and sensitization reported in many of the basic laboratory studies.

This constriction of behavioral repertoire occurs in the clinical setting. Examples include not only the compulsive profile of drug-seeking behaviors (with exclusion of other types of behaviors) but also compulsive ritualistic (1) "paranoid" thinking patterns, (2) sexual behavior, and (3) cleaning, sorting, collecting, and grooming behaviors. These are the same behaviors that rapidly reemerge shortly after readministration of drug following a long period of abstinence. Unfortunately, we have no clear perspective on whether or how central toxicity is involved in the initiation, maintenance, or reemergence of these psychopathologic changes.

Although the sequential periods of withdrawal from chronic stimulants are an integral part of an abuse pattern, detailed studies are lacking. In this respect, we have recently demonstrated that DA autoreceptor sensitivity

undergoes timedependent changes during withdrawal. Thus, 7-day infusion of amphetamine induces marked subsensitivity of both terminal and somadendritic DA autoreceptors immediately following the 7-day infusion; more important, these receptors become supersensitive over the next 7 days (Ellinwood and Lee 1983; Lee and Ellinwood 1989). This supersensitivity is manifested by enhanced effects of apomorphine in inhibiting cell firing and/or DA synthesis in the nigrostriatal and mesolimbic DA pathways. We have questioned whether the increased autoregulation may in part underlie the characteristic lethargy and loss of mental energy observed in human stimulant abusers during the intermediate withdrawal phase (Gawin and Ellinwood 1988). Enhanced autoregulation may lead to a decreased ability to "turn on" the DA transmission-regulating behavioral arousal systems. These functional changes due to changes in autoreceptor sensitivity or other variables could prove to be an important factor in pathogenesis and rational treatment of chronic stimulant syndrome.

CONCLUSION

Time is an important variable in the study of the neuropathological and psychopathological changes noted in chronic stimulant syndromes. It is important in (1) frequency, timing, and chronicity of dosing, (2) the evolution of neuropathology and behavioral changes over time; and (3) evaluation of reversible and residual stages of withdrawal. Careful delineation of the changes at each stage of the ontogeny and withdrawal of the stimulant syndrome is warranted. As is summarized in other chapters, there are many residual pathological changes following chronic amphetamine stimulant dosing. The relation of neuropathology to psychopathology in the stimulant abuse syndrome and withdrawal is tantalizing, yet essentially unknown. This lack of understanding of the relationship certainly applies to functional changes such as autoreceptor alterations. Whether and how the chronic waxing and waning atypical depression seen after withdrawal is related to the stimulant-induced central toxicities demonstrated in laboratory studies need to be determined. Is it related to the neuronal destruction and/or monoamine depletion in the brain, is a chronic functional state (e.g., DA autoreceptor supersensitivity) sufficient to facilitate this behavioral state, or is terminal depletion and some other change a necessary covariable? More important, can we develop a rational approach that allows the clinician to manipulate the mechanisms to prevent relapse? The marked variability of therapeutic agents tried for the stimulant withdrawal period (e.g., tricyclic antidepressants, monoamine oxidase inhibitors, DA agonists, and uptake inhibitors) attests to our lack of understanding of the rate-limiting mechanisms involved. Understanding of the relationship between the neuropathological and functional changes noted with the stimulant of these syndromes may lead to a more fundamental understanding of the development of psychopathology in the psychoses and addictions in general.

DISCUSSION

QUESTION: What is your view of the role of the supersensitive autoreceptor after 7 days? Can you precipitate or replicate a psychosis?

ANSWER: No, I am not relating it to psychosis.

COMMENT/QUESTlON: I was not relating it to psychosis either. I am trying to put it in a functional context. Have you speculated about the role of the supersensitive autoreceptor at that point? You could speculate early on that the subsensitivity autoreceptor favors the potentiation of the behavioral effect. But what might happen when it becomes supersensitive?

RESPONSE: We have primarily related it to the withdrawal phase of fatigue and lethargy. We have a system that is set to turn itself off as rapidly as possible. What are the treatments that reverse autoreceptor supersensitivity? Thinking ideologically, to be hit with this huge dose of amphetamine over and over again means doing whatever must be done for the brain to turn off that response. If you take out the more sensitive regulation, because these receptors are now supersensitive, you immediately turn off the impulse coupled with the release of dopamine. If you give even a small dose of amphetamine, you now have, if you are looking at this neuron in isolation, a terminal that is not being regulated by impulse-coupled mechanisms. I don't know how important that is.

COMMENT: I would favor the view that lethargy and fatigue of post-amphetamine withdrawal during the withdrawal phase would be consistent with the shutting off of the dopamine neuron. Still, it is hard to imagine how that would be. First, the amphetamine-induced release is not regulated by the autoreceptor. And, as you say, if it would be impulse related, however weak. it would be regulated. But we do know that after a period of amphetamine intoxication, an individual is supersensitive behaviorally.

QUESTION: Are you talking about augmentation?

ANSWER: Yes. I am talking about the influence of a subsequent dose on an individual who has had a repetitive binge of amphetamine. At that time, he or she is withdrawn. Then he or she comes back and you can give a relatively low dose that will reinstitute the endstage symptoms, as was being discussed earlier.

COMMENT: That particular phenomenon, maximum sensitization of augmentation, is best elicited by single daily doses. We were attempting to mimic the high-dose continuous binge phenomenon where you sustain plasma levels (in this case, for 7 days) with an Alzet pump. You don't see it going the same augmentation route in that regime. In these Alzet pump animals, at the end of 7 days, even though they are getting about a

5 mg/kg/day dose, there is very little stereotypy. There is massive tolerance.

I am not relating primarily the amphetamine response or the subsequent amphetamine response to this autoreceptor phenomenon. I have tried to say that, in the beginning, when you give a substantial dose of amphetamine, the autoreceptors are out of the picture. I don't think they play a part. If you give a substantial enough dose, it wipes out the autoreceptor response. I don't think that we are dealing with that part of the phenomenon. I hope we are dealing with the beginning model for this loss of mental energy, the incapacity of the normal responses during the intermediate withdrawal phase.

QUESTION: Have you looked at the behavioral consequence of the low dose of apolmorphine in these particular animals?

ANSWER: Yes. In very low doses it turns off the animals. We are talking about 50, 75 μg, IV. So they are more sensitive to turning down locomotion, which would fit in with the hypothesis that they would turn themselves off before they turn on.

There is no way to explain sensitization tolerance using autoreceptors.

QUESTION: Could these changes in the autoreceptors **account** for the cravings for cocaine or amphetamine? If you are shutting down dopamine activity, that may lead to the desire to return to cocaine.

ANSWER: Yes. We think that it is a neat hypothesis. In the absence of natural reinforcement forces, craving for cocaine becomes more intense. I think one of the things that would be important is to figure out some way of testing it.

QUESTION: A possibility that comes to mind is from reading Dr. Larry Stein's work. His theory of a reward system suggests that the cerebral cortex has basically inhibitory behavioral characteristics. And that the reward system, when it is activated, inhibits the cerebral cortex so that there is an inhibition of an inhibitory mechanism, thus releasing behavior. If that is a valid concept, could that have anything to say about the consequence of this supersensitization having behaviorally inhibitory effects?

ANSWER: I think at this point that even Larry Stein would agree that the norepinephrine is probably not the major mediator of the reward systems. I think that we have enough evidence to indicate that is not the case.

QUESTION: If the reward system is not being activated, for whatever reason, is dopamine considered to be more of a neurotransmitter of the reward system?

ANSWER: I think there is fairly good evidence that dopamine is substantially involved.

QUESTION: If anything was preventing a reward system from being operative, you would, perhaps, tend to see this inhibitory effect behaviorally. With supersensitization, do you have this kind of a consequence to beat the reward system not being activated?

ANSWER: Well, that certainly would be one of the things we would like to find some way of testing specifically.

COMMENT: The main point that I wanted to make is that it is very important to attempt to develop models where one is looking at least at some sort of *in vivo* integrated preparation. We look at serotonin depletion. We look at dopamine depletion. We have a variety of different mechanisms. Again we really do not know what, at this point in time, the serotonin depletion is doing. I think I know what it means if you deplete dopamine beyond a certain level. But even there, it is difficult to put an exact degree of impairment on the levels of dopamine depletion that we see in most of these models.

I would really like to see development of models that are explant or *in vivo* models, where we can see the animal in a more integrated role and look at the corresponding *in vitro* events.

I don't think we know what the rate-limiting mechanisms are for most of the behaviors that we think we are concerned with.

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AUTHORS

Everett H. Ellinwood, Jr., M.D.
Tong H. Lee, M.D., Ph.D.

Behavioral Neuropharmacology Section
Department of Psychiatry
Duke University Medical Center
P.O. Box 3870
Durham, NC 27710

Recommendations for Future Research on Amphetamines and Related Designer Drugs

Ray W. Fuller

INTRODUCTION

This volume has focused on several amphetamine analogs in addition to amphetamine and methamphetamine, especially 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA). Among the pharmacologic actions of these drugs, their behavioral effects in humans and in laboratory animals have been discussed, with some attention to electrophysiologic and electroencephalographic effects. Other functional effects, such as effects on neuroendocrine regulation, sleep, thermoregulation. and appetite and body weight have not been discussed. Consideration of toxic effects mainly focused on neurotoxic actions that the drugs can have on specific brain monoaminergic neurons. In relation to this action, two other amphetamine analogs, *p*-chloroamphetamine and fenfluramine, have been compared because of their similar neurotoxic actions in rats.

SOCIAL IMPLICATIONS

General concerns about abuse of amphetaminerelated drugs are similar to concerns about other illicit or addictive drugs. Dr. G. Nahas wrote an editorial for the *Wall Street Journal* arguing that "a strongly expressed sentiment of societal disapproval" of illicit drugs is necessary for prohibitive measures to be effective *(Wall Street Journal,* July 11, 1988, p. 16). He cited examples from history to support his contention that when illicit addictive drugs are socially accepted and easily available, they have a very damaging effect on individuals and on a society. His examples included the use of cannabis in the Islamic-dominated world several centuries ago, the chewing of coca leaf in Peru, the use of opium in China at the beginning of this century, the epidemic of amphetamine abuse in Japan in the 1950s, and others. In some cases, there was widespread social acceptance. Although there is acceptance of MDMA and amphetamines in only limited segments of our society, Nahas argues there is not forceful enough disapproval of illicit drugs in general in our society today.

The behavioral and dependence-producing effects of some of these drugs can be damaging to individuals, but neurotoxic damage to particular brain neurons can result when these drugs are given to animals, including nonhuman primates. There continue to be inadequate data about whether such damage occurs in humans.

Patterns of Abuse of Amphetamine Analogs

The need for more accurate and precise information about human use of MDMA and MDA was aptly stated by Dr. Gawin (this volume) and others. There is a general perception that these drugs are widely used, especially on college campuses, but there are relatively few hard data on the geographic distribution of use, on the pattern(s) and frequency of use, the doses used, and so on. For many reasons, such information is needed.

Behavioral Effects of Amphetamines: How Useful, What Mechanisms?

The behavioral effects of amphetamine, methamphetamine, MDMA, MDA, *p*-chloroamphetamine, and fenfluramine are not identical. Except for the last drug, all can cause some degree of behavioral stimulation, but exact behavioral effects differ markedly. More complete definition of their behavioral differences is a prerequisite to a better understanding of the mechanism(s) of these drugs.

Apparently there are psychiatrist and nonpsychiatrist clinicians whose experience convinces them that MDMA can have therapeutic uses, mainly as an adjunct to psychotherapy. Despite these convictions, there appear to be no published data to support these claims. There is an urgent need for objective data from well-controlled, blinded clinical studies, if these claims of therapeutic usefulness are to be taken seriously. If a bona fide use is evident, then it may be possible to produce other drugs with the same desirable action, lacking the toxicity inherent in MDMA.

NEUROCHEMICAL MECHANISMS

Aside from the therapeutic usefulness of MDMA, there is scientific importance to elucidating further the mechanism(s) involved in the seemingly unique behavioral effects of MDMA and MDA. Apparently, a major action of these drugs is the release of serotonin and dopamine from brain neurons, leading to enhanced serotonergic and dopaminergic input to those neuronal systems with which they make synaptic contact. In addition, MDMA has been shown to interact *in vitro* with sites including $5HT_2$, $5HT_{1A}$, and α_2 adrenergic receptors, among others (Battaglia, this volume). Do those interactions occur *in vivo,* and does MDMA interact as an agonist or as an antagonist at these sites? If the interactions occur *in vivo,* how do they contribute to the profile of behavioral effects of MDMA? These questions can and should be approached experimentally. Further unraveling

of the effects of MDMA and MDA on serotonergic and dopaminergic function is also needed. Serotonin neurons and dopamine neurons are known to interact in many brain regions (Bosler et al. 1984; Benkirane et al. 1987; Herve et al. 1987), so the release of dopamine may influence serotonergic function, just as the release of serotonin may influence dopaminergic function.

Neurotoxicity of Amphetamines

The recognition that amphetamines can be neurotoxic in brain can be traced back to *p*-chloroamphetamine studies. In the middle 1960s, *p*-chloroamphetamine and *p*-chlorometbamphetamine were found to cause selective depletion of brain serotonin (Pletscher et al. 1963; Fuller et al. 1965). The long duration of this depletion was not appreciated until later (Sanders-Bush et al. 1972). and it was subsequently established that the loss of serotonin was accompanied by changes in other parameters specifically associated with serotonin neurons, e.g., a loss in tryptophan hydroxylase, a loss in serotonin uptake capacity, and a reduction in serotonin turnover, as well as by histologic evidence of neurotoxicity (Puller and Snoddy 1974; Harvey et al. 1975; Sekerke et al. 1975; Massari et al, 1978). Fenfluramine was recognized to have similar effects on brain serotonin neuron parameters (Harvey and McMaster 1975; Clineschmidt et al. 1978), although there has been controversy about histologic changes (Sotelo and Zamora 1978).

During the 1970s, evidence accumulated that amphetamine and methamphetamine could also be neurotoxic (Ellison et al. 1978; Hotchkiss and Gibb 1980; Wagner et al. 1980). The effects of amphetamine seem mostly limited to dopamine neurons, whereas methamphetamine affects dopamine and serotonin neurons (Warren et al. 1984). Most recently, MDMA and MDA have been shown to produce neurotoxicity toward brain serotonin neurons much like that of the halogenated amphetamines (Ricaurte et al. 1985; Stone et al. 1986).

Role of Uptake Carriers

The neurotoxic effects of all these compounds are antagonized by inhibitors of monoamine uptake (table 1), implicating the membrane uptake carrier on serotonin and dopamine neurons in the mechanism of neurotoxicity. In this regard, these amphetamines are like a drug somewhat related in structure, namely 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a Parkinsonism-causing neurotoxic drug that has been studied intensely since 1983 (Langston and Irwin 1986). In the case of MPTP, the mechanism by which inhibitors of the dopamine uptake carrier block the neurotoxicity toward dopamine neurons (mainly nigrostriatal dopamine neurons) seems clear. A metabolite of MPTP, l-methyl-4-phenylpyridinium (MPP+), has been shown to be a substrate for the dopamine uptake carrier (Javitch et al. 1985). Thus accumulation of MPP+, formed metabolically from

MPTP, into dopamine neurons seems to be essential, and blockade of that accumulation prevents the neurotoxicity. MPP+ also can be transported into norepinephrine neurons (Javitch et al. 1985), leading to neurotoxicity toward cortical norepinephrine neurons, an effect blocked by inhibitors of the norepinephrine uptake carrier (Sundstrom and Jonsson 1985).

TABLE 1. *Characteristics of monoaminergic neurotoxicity induced by amphetamine and related compounds in laboratory animals*

Drug	Membrane Uptake Carrier Involved in Neurotoxicity	Drug Metabolite Involved in Neurotoxicity
Amphetamine	Yes	?
Methamphetamine	Yes	?
MDMA, MDA	Yes	?
p-Chloroamphetamine	Yes	?
Fenfluramine	Yes	?
MPTP	Yes	Yes

The mechanism by which uptake inhibitors block the neurotoxic effects of amphetamine, methamphetamine, MDMA, MDA, *p*-chloroamphetamine, and fenfluramine is not so clear. A simple explanation (analogous to that with MPTP) might be that these drugs are accumulated into serotonin or dopamine neurons via the membrane uptake carrier, and that uptake inhibitors prevent the neurotoxicity by preventing that accumulation. However, none of these compounds have been shown to be a substrate for dopamine or serotonin uptake carriers. Their lipophilicity leads one to believe they would enter neurons readily without requiring active transport. There may be, however, both entry and accumulation of these drugs. It is conceivable that the amphetamines do enter brain monoaminergic neurons and other cells by passive diffusion. They may in addition be accumulated by brain monoaminergic neurons, if amphetamines are substrates for the membrane uptake carriers. For example, *p*-chloroamphetamine may enter all cells, but may be selectively concentrated in serotonin neurons, due to its accumulation via the membrane uptake carrier. That concentration may be required for the short-term and long-term depletion of brain serotonin, so that inhibition of the uptake carrier blocks the depletion. No direct evidence to support this possibility is available. Uptake of radioactive *p*-chloroamphet-amine by the serotonin uptake carrier has not been shown *in vitro,* although *p*-chloroamphetamine does have high affinity for that carrier (Wong et al. 1973).

There has been some evidence that *p*-chloroamphetamine is preferentially localized in synaptasomal fractions of brain homogenates (Wong et al. 1972), and recently Ask and Ross (1987) have published evidence

consistent with an accumulation of pchloroamphetamine in serotonergic synaptosomes *in vitro*. They evaluated the ability of reversible inhibitors of monoamine oxidase to be accumulated in serotonin nerve endings by the membrane uptake carrier by comparing two conditions of serotonin deamination: first, when the radioactive serotonin was being deaminated, mainly inside serotonergic synaptosomes, after it was accumulated via the uptake carrier; and second, when serotonin was being deaminated by other synaptosomes, because its transport via the serotonin uptake carrier was blocked. In this way, they evaluated the ability of certain reversible inhibitors of monoamine oxidase to be themselves concentrated in serotonergic synaptosomes due to their transport via the membrane uptake carrier. Study of *p*-chloroamphetamine, which is a reversible inhibitor of monoamine oxidase (Fuller 1978), did indicate accumulation within serotonergic synaptosomes.

Further investigation of the possibility that inhibitors of the serotonin uptake carrier protect against serotonin depletion by *p*-chloroamphetamine, fenfluramine, MDMA, MDA, and methamphetamine because they prevent the accumulation of those drugs within serotonin nerve terminals is warranted, but at present compelling evidence for this mechanism does not exist.

Possible Role of Dopamine Release

A second possible mechanism, supported by some existing data on methamphetamine and MDMA, is that these drugs release dopamine, which is then taken up into serotonin neurons via the membrane uptake carrier, leading to neurotoxic effects on the serotonin neurons. Inhibitors of dopamine synthesis or of the dopamine uptake carrier, e.g., α-methyltyrosine and GBR 12909, have been reported to prevent the depletion of serotonin by methamphetamine and by MDMA (Schmidt et al. 1985; Gibb et al., this volume). MDMA does release dopamine both *in vitro* and *in vivo* (Yamamoto and Spanos 1988). Dopamine can be transported into serotonergic synaptosomes (Schmidt and Lovenberg 1985). Further investigation is needed, especially to see if the involvement of dopamine is a general phenomenon in the neurotoxic effects of amphetamines. We have found that potent inhibitors of dopamine uptake, including mazindol and nomifensin, block depletion of striatal dopamine by MPTP in mice, but do not block depletion of brain serotonin by *p*-chloroamphetamine in mice.

Possible Role of an Active Metabolite of the Drug in the Neurotoxicity of Amphetamine Analogs

The possibility that an active metabolite is involved in the neurotoxic effects of amphetamine analogs receives limited discussion in this chapter and has been considered previously, especially with *p*-chloroamphetamine (Miller et al. 1986.) Partly because the chemical structures of these amphetamines do not suggest ways in which they would be toxic to neurons, the

possibility that conversion to a more reactive metabolite accounts for the neurotoxicity has been attractive. The study of numerous analogs of *p*-chloroamphetamine and other neurotoxic amphetamines has not yielded a strong candidate for such a neurotoxic metabolite. Most potential metabolites of p-chloroamphetamine caused less depletion of serotonin (Fuller 1978). Although N-hydroxy-*p*-chloroamphetamine did deplete serotonin, it was metabolized rapidly and almost quantitatively to *p*-chloroamphetamine (Fuller et al. 1974). Inhibitors and inducers of drug metabolism have generally failed to influence neurotoxicity of amphetamines. MDA is metabolixed to a-methyldopamine (Marquardt et al. 1978; Midha et al. 1978), a metabolite that should be considered as a possible mediator of neurotoxicity, especially in view of the properties of dopamine discussed below.

Involvement of a Metabolite of the Neurotransmitter in the Neurotoxicity of Amphetamines

Also a possibility is that a product formed from one of the neurotransmitters affected mediates the neurotoxic effects of amphetamines. This possibility was suggested by Seiden and Vosmer (1984), who reported the presence of 6-hydroxydopamine in the rat caudate nucleus after a single injection of a high, neurotoxic dose of methamphetamine. They suggested that 6-hydroxydopamine was formed from endogenous dopamine released by methamphetamine and that the 6-hydroxydopamine was responsible for the neurotoxicity to dopaminergic terminals. Other investigators have not found 6-hydroxydopamine to be present in rat striatum after amphetamine or methamphetamine administration (Rollema et al. 1986).

Commins et al. (1987) have also reported the formation of 5,6-dihydroxytryptamine in rat hippocampus after a single, high doses of methamphetamine. They suggested that the formation of 5,6-dihydroxytryptamine, a known neurotoxic substance, may mediate the neurotoxic effects of methamphetamine toward serotonergic nerve terminals.

Molliver (this volume) made the provocative suggestion that a metabolite of serotonin released from blood platelets by *p*-chloroamphetamine may mediate the neurotoxic effects of *p*-chloroamphetamine on cortical serotonergic neurons in the rat. Such a possibility would be compatible with the observations of Molliver and his colleagues (this volume) that *p*-chloroamphetamine is not effective when pumped directly into the brain or when added to brain slices *in vitro.* Their demonstration that a combination of *p*-chlorophenylalanine and reserpine prevented the neurotoxic effects of *p*-chloroamphetamine led them to suggest that platelet serotonin stores were involved in the neurotoxic mechanism (Berger et al., submitted for publication). This interesting idea deserves testing in various ways. Since it would not cross the blood-brain barrier, 5,6-dihydroxytryptamine would not

seem to be a candidate for their hypothesized metabolite, unless the integrity of that barrier had been lost due to the drug treatment.

Role of Dopamine Involvement in the Neurotoxicity of Amphetamines

Some data suggest that dopamine itself is involved in certain of the neurotoxic effects. It is worth asking if dopamine might account for the neurotoxicity of all the amphetamine analogs toward both dopaminergic and serotonergic neurons. At least three ways in which dopamine might lead to cytotoxicity have been suggested, First, dopamine might be converted to 6-hydroxydopamine, a known neurotoxin, as discussed above. Second, dopamine metabolism by monoamine oxidase is known to produce hydrogen peroxide, and excess hydrogen peroxide formation from this source might, under some conditions, have deleterious effects on the cell (Cohen and Mytilineou 1985). Third, dopamine itself is known to undergo auto-oxidation analogous to, but slower than, that of 6-hydroxydopamine (Graham et al. 1978; Graham 1984). Persistently increased intraneuronal but extragranular concentrations of dopamine due to amphetamine-induced release of granular stores of dopamine and protection against dopamine oxidation by monoamine oxidase type A have been suggested as possibly mediating the neurotoxic effects of amphetamine (Fuller and Hemrick-Luecke 1982). Uptake of dopamine into serotonergic terminals (Schmidt and Lovenberg 1985) might lead to destruction of serotonergic terminals after treatment with drugs like methamphetamine and MDMA. It is not clear why such effects should be less with amphetamine than with methamphetamine, yet amphetamine seems to affect dopaminergic neurons primarily, whereas methamphetamine is neurotoxic toward serotonin neurons as well as dopamine neurons (Hotchkiss and Gibb 1980; Ricaurte et al. 1984). Investigation of the possible involvement of dopamine in the different neurotoxic process is needed.

IMPLICATIONS OF NEUROTOXICITY

Functional Deficits Resulting from Amphetamine Neurotoxicity

Rats that have lost dopamine and/or serotonin terminals following treatment with amphetamine, methamphetamine, MDMA, MDA, *p*-chloroamphetamine, or fenfluramine show little in the way of overt changes in appearance or behavior. Dr. Ricaurte (this volume) emphasized the need for more studies in primates, since MPTP-treated mice also show little in the way of observable functional changes, whereas MPTP-treated monkeys show marked neurologic deficits. It may be necessary to do more detailed analysis of specific behaviors and other functional outputs that are influenced by dopamine and/or serotonin neurons, to detect functional deficits induced by some neurotoxic drugs. For instance, specific behaviors such as appetite-controlled behavior (Leibowitz and Shor-Posner 1986), muricidal behavior (Katz 1980), and sexual behavior (Tucker and File 1983) elicited by drugs

or environmental conditions ate known to be influenced by serotonergic input. Careful analysis of these behaviors in rats that have received neurotoxic doses of *p*-chloroamphetamine, MDMA, MDA, fenfluramine, or methamphetamine may reveal functional deficits. Electroencephalographic patterns, nociception, sleep, thermoregulation, and endocrine regulation are other brain-controlled functions that are influenced by serotonergic pathways. Careful studies of these functions, especially measuring responses elicited by serotonergic drugs or by environmental stimuli whose actions are mediated by serotonergic systems (insofar as that is known) may reveal functional deficits associated with loss of serotonergic terminals. For example, we have found that the acute increase of serum corticosterone in rats given *p*-chloroamphetamine, an increase that appears to be mediated by release of serotonin from central neurons making input to cells that release corticotropin-releasing factor in the hypothalamus, is blunted in rats pretreated with a neurotoxic dose of *p*-chloroamphetamine. There are few examples of studies of this sort, in which a functional correlate of the loss in serotonin content has been sought in rats that have received neurotoxic doses of any of the amphetamine analogs in question. It seems important for such studies to be done for several masons, including the goal of learning more about physiologic functions of the serotonin and dopamine pathways that are affected, and to suggest ways in which possible neurotoxicity in humans might be investigated.

Does Neurotoxicity Occur in Humans

All the neurotoxic drugs discussed have been taken by human subjects. Amphetamine and methamphetamine have a long history of therapeutic use along with illicit misuse. To a limited extent, *p*-chloroamphetamine has been used in humans as an investigational drug (Van Praag et al. 1971). MDMA and MDA have no approved medical uses, but they appear to be rather widely abused drugs at present. Fenfluramine continues to be marketed as an appetite suppresant A key question, to which there is no current answer, is whether any of these drugs produce, in humans, neurotoxic effects on dopamine and/or serotonin neurons in brain analogous to those produced in rodents and in nonhuman primates (table 2). It is remarkable that no data exist on this issue, given that the neurotoxic effects of some of these drugs in animals have been know for more than a decade.

There are several ways in which possible neurotoxic effects might be studied. First, measurement of cerebrospinal fluid concentrations of dopamine or serotonin metabolites would be a straightforward way of assessing neurotoxicity. There are pitfalls in this approach (as outlined by Dr. Ricaurte (this volume), such as the facts that lumbar cerebrospinal fluid might reflect spinal cord neurochemistry more than it reflected brain neurochemistry, and drugs like *p*-chloroamphetamine affect serotonin neurons in spinal cord less than they do those in brain (Sanders-Bush

et al. 1975). Nonetheless, fenfluramine has been shown to produce marked decreases in 5-hydroxyindoleacetic acid concentration in the cerebrospinal fluid during treatment (Shoulson and Chase 1975), and it would be important to know if those concentrations return to control levels when fenfluramine is discontinued.

TABLE 2. *Nature of neurotoxic damage to brain monoaminergic neurons*

Drug	Brain Monoaminergic Neurons Showing Neurotoxic Damage	Neurotoxicity Occurring in Humans
Amphetamine	Dopamine	?
Methamphetamine	Dopamine, Serotonin	?
MDMA, MDA	Serotonin	?
p-Chloroamphetamine	Serotonin	?
Fenfluramine	Serotonin	?
MPTP	Dopamine, Norepinephrine	Yes

A second approach might be to measure dopamine and serotonin along with their metabolites and other specific neuronal constituents such as tyrosine hydroxylase and tryptophan hydroxylase or uptake carrier sites in brain tissue obtained at autopsy. Accumulating data in this way might be a slow and tedious process, and drug dosing history might be uncertain and variable; nonetheless, the approach deserves consideration.

A third approach would be to measure some indicator of functional output of dopamine and/or serotonin neurons. As mentioned previously, studies in laboratory animals can be invaluable in defining parameters that change in correlation with directly measurable neurotoxic effects in the brain. Changes in serum hormones elicited by a drug whose effects are mediated by dopamine or serotonin neurons are especially attractive possibilities, since these changes are already being used as a means of assessing the functional state of brain serotonergic pathways (Siever et al. 1984). In this regard, it is intriguing that Coccaro et al. (1987) have observed recently a blunted elevation in serum prolactin concentration elicited by fenfluramine in psychiatric patients who had received a previous dose of fenfluramine within a 12-day period. While there may be numerous possible explanations, one could be that the first dose of fenfluramine had damaged or destroyed a traction of the serotonin neurons from which release of serotonin is the mechanism of prolactin elevation by fenfluramine.

A fourth approach to evaluating the intactness of dopamine and/or serotonin neurons in human subjects who have taken one of the amphetamine analogs might be to use a probe for labeling a constituent of those neurons in position emission tomography scanning studies. A label for the serotonin or dopamine uptake carrier, or a label for tryptophan hydroxylase or tyrosine

hydroxylase, would be an ideal agent for use in studies of this sort. Such methods are not currently available, but the possibilities for development of methods like this seem excellent.

DISCUSSION

COMMENT: I would like to know why you thought the amphetamine model of dopamine neurotoxicity might be more suitable or more revealing for the study of Parkinson's disease than the MPTP model.

RESPONSE: We do not understand all there is to know about the mechanisms of MPTP neurotoxicity. but it seems to involve MPP+, which is potentially cytotoxic to all cells but that attains toxic concentrations after MPTP administration only in cells that concentrate MPP+. Dopamine apparently is not involved in the neurotoxic effects of MPTP. I am attracted to the idea that dopamine itself may be involved in the etiology of Parkinson's disease, that dopamine neurons may be at risk because of the nature of their neurotransmitter.

If there is anything to that line of thought then I am suggesting that the exact mechanisms involved in MPTP may not be like the mechanisms that are involved in the &generation of those dopamine neurons in Parkinson's disease. And that something like amphetamine neurotoxicity might have closer parallels to the degeneration in Parkinson's disease. That presupposes the way the story is going to end and obviously I don't know that any more than anybody else does.

I have argued in the past that looking further at amphetamine toxicity in terms of understanding the mechanism by which those neurons die, might be more revealing. That is not to belittle the importance of MPTP as a model of Parkinson's disease. Certainly in terms of effects in the MPTP-treated monkeys, these animals are of unquestioned value. But in terms of the mechanism by which the neurons die, that was the point that I was questioning, whether the MPTP model would mimic as well as the amphetamine model.

QUESTION: You mentioned the N-hydroxy parachloroamphetamine. Is it less or more toxic than PCA?

ANSWER: The same. And the reason is that it is converted very rapidly to PCA itself almost quantitatively.

QUESTION: If the oxidation of dopamine is proving to be toxic, are there any natural endogenous substances or nutrients that can help prevent that? Is it possible that perhaps ascorbic acid would keep the dopamine from being metabolized or oxidized?

ANSWER: I think that is an interesting possibility, since cells presumably have some kind of cytoprotective mechanism. It is possible that in patients predisposed to Parkinson's disease there is some breakdown of the patients' protective mechanisms in those neurons. That might be a reason why they develop the disease. Fortunately, all of us don't. I think we simply don't know. We should consider all possibiiities.

COMMENT: I would like to follow up with the point that Dr. Rebec, from the University of Indiana, made here at the NIH in January. The topic of his talk was ascorbic acid and dopamine in schizophrenia. In experimenting with amphetamine, he was finding with individual neuron investigation that amphetamine, in both low and high doses, was inhibitory in some neurons. In other neurons it was inhibitory in low doses, and in high doses it became excitatory. But in this investigation he claimed that he was finding some substance in the brain that was counteracting the effect of the amphetamine and, by analysis, he said it was determined to be ascorbic acid.

Now the use of molecular psychiatry of ascorbic acid in schizophrenia by Linus Pauling and others, where there seems to be some relationship to dopamine neurons, and fmding that dopamine-dopaminergic neurons or receptors are present in twice the normal amount, makes this an intriguing area of investigation.

Dr. Rebec also said that the brains on post mortem studies of schizophrenics tended to be mushy and to have very low levels of ascorbic acid in their constituent tissue.

RESPONSE [FROM AUDIENCE]: We tried an opposite strategy where we made guinea pigs scorbutic. We deprived them of their scorbic acid contents, then exposed them to amphetamine. In those studies we found that the ascorbutic animals were protected from some of the neurotoxicities of the amphetamines. It is a very complex issue. It is not just a matter of adding vitamin C or ascorbic acid and getting protection; it can work as a double-edged sword. It can work for or against you.

QUESTION: Has anyone given an uptake blocker in the chronic state? You showed that in the acute stage that you could get some reversal effects. Has anyone administered an uptake blocker after chronic use of amphetamines?

ANSWER: Even after one dose of *p*-chloroamphetamine, the depletion of brain serotonin cannot be reversed at later times. You lose the reversibility after several hours.

QUESTION: Totally lose it?

ANSWER: Yes. So I feel very sure that in the chronic state there will come a time when this is not reversible.

QUESTION: We have all these theories about serotonin being involved in depression. Do you have any explanation for why depression is not seen in humans if serotonin neurons have been damaged by these drugs?

ANSWER: I think that clearly it is possible that there is no neurotoxicity in humans. I think all of us would like for this to be the case. And maybe it is the case. We have talked about this a lot with fenfluramine, and we have done studies with parachloroamphetamine in which we have given it orally to rats at relatively low doses, but still anorectic doses, over 90 days. We have seen depletion of serotonin, but that was fully reversible depletion. It came back when the drug was stopped. I think there may be no neurotoxicity at the oral doses used in humans. I think that would be great if that is the case. That would explain why there is no depression or other kinds of symptoms. But I don't feel comfortable about relying on the lack of reporting of depression as real evidence that there is no neurotoxicity. We simply need to have better data on that.

COMMENT: I think another matter to take into account is that, at least from the experience of dopamine systems, in order to get overt behavioral dysfunction you really need a pretty whopping lesion. In the primate, to get the kind of Parkinsonism that people talk about in animal models, that animal model actually turns out to be very difficult to produce in chronic Parkinsonism. The problem is developing an animal that has 90 to 95 percent depletion of dopamine on a chronic basis. As you know, it is a very narrow window, and it is very difficult to produce that kind of animal preparation. So I think you have to consider the possibility that lack of symptoms after serotonergic lesions could, perhaps, be related to the fact that we are dealing with preparations where there is a 50, 60, 70 percent depletion where we don't have enough of a lesion to produce an overt behavioral disturbance.

RESPONSE: Perhaps I can bridge the dispute by suggesting it is probably going to vary among neuronal systems. There may be systems in which you must have a lot of depletion to see a functional change, and there may be others where it doesn't take very much.

COMMENT: I would dispute it within the dopamine system itself. And I would dispute it about Parkinson's disease. I think that if you did a proper neuropsychological exam that you would pick up even smaller depletion effects. I think if you are looking for an overt complete terminal Parkinson situation, yes, you need a 99 percent depletion. But part of the problem is that the Parkinson situation involves not only the nigrostriatal system but also the mesolimbic dopamine system. There are plenty of studies in rats, and it is very easy to produce a Parkinsonian rat with a very discrete

injection of 6-hydroxydopamine in the right place. It doesn't require a lot of work. I can take 2 micrograms of 6-hydroxydopamine and put it in exactly the right location in the ventral tegmental area, and I can produce a Parkinsonian rat that will die,

So I think you can debate that issue about 99 or 95 percent depletion. I think that if you probe those animals with the proper pharmacological agents and proper environmental situation, you will pick up deficits. I think the lack of knowledge about what the serotonin systems do is the basis of the problem here. We don't know what the behavioral consequences of the serotonin depletion are.

How are we going to probe a person's gestalt? I think that was brought out earlier. If we had proper probes we might see the effects. If we have proper probes for exaggerating serotonergic function or proper probes for exaggerating deficits associated with serotonergic function we would easily pick up things. Whether that is important or not, you know it might be good to trim our serotonin neurons slightly. Maybe we would be better off. Maybe we would all be somewhat anxiolysed. That is another question. But I think we have the tools in behavioral pharmacology to conduct tests in rats that will be sensitive to serotonin depletion. And I assume that those can be extrapolated to primates.

COMMENT: One of the problems that we have not addressed is the issue of potential recovery and regeneration.

One of the striking aspects of this toxicity of compounds is selective destruction terminals and the cell bodies that are left intact. Dr. De Souza has recently reported some biochemical evidence for recovery of serotonin. We have now found anatomic evidence for reinnervation of depleted areas by serotonin neurons. But it is going to be a while before we figure out whether their reinnervation is appropriate or perhaps aberrant. Do they end up with complete recovery, do they end up with a better system than they started with or one that malfunctions? I think that is an important area for future study.

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AUTHOR

Ray W. Fuller, Ph.D.
Research Advisor
Lilly Research Labortories
Eli Lilly and Company
Lilly Corporate Center
Indianapolis, IN 46285

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