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Hallucinogens: An Update

Editors:

Geraline C. Lin, Ph.D.
National Institute on Drug Abuse

Richard A. Glennon, Ph.D.
Virginia Commonwealth University

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Preface

Geraline C. Lin

Despite a general trend of declining substance abuse by high school seniors and college students in the United States from 1985 to 1991, the most recent (1992) National Institute on Drug Abuse (NIDA) National High School Senior Survey (currently known as Monitoring the Future) has found that annual prevalence of lysergic acid diethylamide (LSD) use has risen for a third consecutive year from 1989 to 1992 among college students and young adults aged 19 to 28. Moreover, from 1991 to 1992, an increase in LSD use by high school seniors comparable to the increase by college students and a trend of increasing annual prevalence of LSD use by 10th and 8th graders (although at a lower rate for the latter) were also observed.

Prompted by these observations and other independent sources indicating an increase in LSD use, the fact that the last comprehensive, indepth review of research in this area by NIDA was conducted well over 10 years ago, and the impressive advances made in and the tremendous research opportunities afforded by molecular biology and other neuroscience disciplines during the past decade, NIDA undertook the present technical review to examine current knowledge on hallucinogen research and to identify research priorities in this area.

The technical review meeting entitled "Hallucinogens: An Update" was held July 13 and 14, 1992, in Bethesda, MD. The objectives of the meeting were: (1) to update current knowledge on hallucinogen research; (2) to identify future preclinical and clinical research needs; (3) to discuss problems and possible solutions associated with hallucinogen research, especially relating to human studies; (4) to explore the potential therapeutic utility, if any, of classical hallucinogens; and (5) to address issues related to substance abuse such as how hallucinogen research can contribute, directly and indirectly, to drug abuse research and help prevent, ameliorate, and resolve problems associated with hallucinogen abuse.

The meeting covered qualitative and quantitative studies in both animals and humans on a wide range of classical hallucinogens, including investigational new drug (IND) clinical studies on N,N-dimethyl-tryptamine (DMT). Presentations addressed behavioral, drug discrimination (DD), and operant conditioning experiments performed

with whole animals as well as electrophysiological and neurochemical studies' exploring receptors, second messenger systems, and structure-function relationships of the 5-hydroxytryptamine, (5-HT_2) receptor at the molecular level. It might be noted, as an aside, that progress in serotonin research has been moving at a rapid pace. Since this technical review was held, there have been some changes in serotonin receptor nomenclature. The originally defined 5-HT_2 receptors mentioned in this monograph are now referred to as 5-HT_{2A} receptors, whereas 5-HT_{1C} receptors are now termed 5-HT_{2C} receptors. Both receptors, therefore, are considered as members of the same subfamily.

Applications of autoradiography, position emission tomography (PET) scanning, and other imaging techniques for identifying anatomic loci of action also were presented at the review. Other topics addressed structure-activity relationships (SAR) of ergolines, use of molecular graphic models of 5-HT_2 receptors for elucidating the action of hallucinogens (i.e., whether it be agonist, partial agonist, or antagonist), and identifying amino acid residues important in ligand binding. A discussion of the potential psychoheuristic value of hallucinogens also took place. Human studies of hallucinogens have recently resumed. A description of the effects of DMT in humans is provided in this monograph, and a Hallucinogens Rating Scale (or, more accurately, a DMT-like rating scale) is described (Strassman, this volume). Finally, the meeting concluded with a summary highlighting challenges and opportunities and identifying future research needs.

This monograph represents a state-of-the-art information resource concerning classical hallucinogens. It is hoped that this monograph will serve to stimulate further research in this area. Hallucinogen research, in addition to its relevance to hallucinogen abuse due to the unique actions of hallucinogens on human perception, cognition, and behavior, also affords an opportunity to unveil some fundamental brain processes through which these functions are organized and manifested. Therefore, an understanding of the mechanism of the action of hallucinogens not only would allow for opportunities to develop strategies and/or modalities for combating hallucinogen abuse but also would have profound consequences on individual and public health.

The monograph should be valuable to members of the scientific community who are involved in drug abuse research and neuroscience research in general; to those interested in the field of classical hallucinogens, including professionals in mental health, psychiatry,

public health, and education; and to Government agencies with regulatory responsibility, drug enforcement responsibility, or both.

AUTHOR

Geraline C. Lin, Ph.D.
Biomedical Branch
Division of Basic Research
National Institute on Drug Abuse
National Institutes of Health
Parklawn Building, Room 10A-19
5600 Fishers Lane
Rockville, MD 20857

Classical Hallucinogens: An Introductory Overview

Richard A. Glennon

INTRODUCTION

Classical hallucinogens may be broadly divided into two categories: indolylalkylamines and phenylalkylamines. The indolylalkylamines may be further divided into:

- simple tryptamines (e.g., N,N-dimethyltryptamine [DMT], 5-methoxy DMT, psilocin);
- α-methyltryptamines (e.g., a-MeT, 5-methoxy a-MeT);
- ergolines (e.g., (+)lysergic acid diethylamide [(+)LSD]); and
- ß-carbolines (e.g., harmala alkaloids).

Phenylalkylamines may be subdivided into:

- phenylethylamines (e.g., mescaline) and
- l phenylisopropylamines (e.g., 1-(2,5-dimethoxy-4X-phenyl)-2-aminopropanes where X = methyl, bromo, or iodo (i.e., DOM, DOB, and DOI, respectively).

For general reviews, see Nichols and Glennon (1984).

What constitutes a hallucinogenic agent? There have been various attempts to define the term "hallucinogenic," but none of the definitions seems to adequately, accurately, and completely describe the actions of these agents. Perhaps one of the better definitions-actually, a set of criteria-is that provided by Hollister (1968): (1) in proportion to other effects, changes in thought, perception, and mood should predominate; (2) intellectual or memory impairment should be minimal; (3) stupor, narcosis, or excessive stimulation should not be an integral effect; (4) autonomic nervous system side effects should be minimal; and (5) addictive craving should be absent.

It is recognized that not all classical hallucinogens necessarily produce identical effects. In fact, it has been said that a dose of a given agent may produce different effects in the same individual upon different occasions

of administration (Naranjo 1973), and that the human subject is as much a contributor to the final definition of a drug's action as is the drug itself (Shulgin and Shulgin 1991). Clearly, there exist some differences in effect. How can these differences be rationalized? There are several likely explanations: (1) effects may be dose-dependent (and additional examination of more doses of more agents in more subjects may reveal greater similarity than difference); (2) side effects may contribute substantially to the observed differences; (3) the agents may not constitute a mechanistically homogeneous group of compounds; and/or (4) the classical hallucinogens may act via similar but nonidentical mechanisms that share a common mechanistic component.

Because there is some evidence for similarity of effect, the last explanation (the common component hypothesis) provides a framework for mechanistic investigations. That is, the effects produced by hallucinogens may be likened to response patterns formed by certain neurohumoral "keys" played on a piano with the resulting chords being manifested as differently perceived behavioral effects (Glennon 1984). Identification of a common key may be important to further understanding of these agents. Agents lacking a common component are likely acting via a different mechanism; such agents may need to be categorized separately, and such categorization may influence future treatment modalities.

HALLUCINOGENIC AGENTS: METHODS OF INVESTIGATION

Hallucinogenic agents have been investigated using both human and nonhuman subjects. Obviously, only human subjects possess the faculties required to accurately assess and describe the subjective effects of these agents. However, relatively few hallucinogens have been examined in humans (however, see Shulgin and Shulgin 1991), and legal constraints discourage new clinical studies. Investigations involving nonhuman subjects are much more common, allow the examination of greater numbers of agents in large numbers of subjects, and are certainly less restrictive in terms of governmental regulation. However, there are obvious limitations to this approach, the most prominent being that it is not known if animals experience subjective effects identical to those experienced by humans. On the other hand, lacking the sophisticated behavioral repertoire of humans, animals may (?) be better able to focus on the common effects produced by these agents.

Animal studies tend to fall into two categories: investigative (i.e., observational) and interpretive. The former simply categorizes the effects of known hallucinogens in animal subjects (e.g., effect on electroencephalographic patterns, social behavior, and sleep cycles) in an attempt to catalog their pharmacological effects without further interpretation. The latter addresses possible mechanisms involved in the production of these effects. Mechanistic interpretation must necessarily be conservative, and identified mechanisms may or may not be related to the hallucinogenic activity of the agents under investigation.

Another type of investigation involving animals is the development of animal models to identify novel hallucinogens. Such studies begin with examination of known hallucinogens to determine what effects are common to a series of agents but absent upon administration of inactive agents. Once such an effect has been identified, the model ideally is challenged with other hallucinogens and nonhallucinogens and ultimately with novel agents. It never can be assured that novel agents identified in this manner will be hallucinogenic until they have been evaluated in human subjects. Nevertheless, robust and reliable animal models can be valuable for further mechanistic investigations by allowing experimentation not appropriate (or allowed) in humans. Here also, greater numbers and doses of agents can be evaluated in relatively large subject populations.

Thus, studies involving human and nonhuman subjects have their own peculiar limitations, advantages, and disadvantages. The ideal situation likely would be investigations involving both types of subjects.

MODELS OF HALLUCINOGENIC ACTIVITY

Animal Models

Over the years there have been numerous reports of animal models that might be useful for examining hallucinogenic agents (reviewed: Glennon 1992). Animal models are of two types: behavioral and nonbehavioral. The behavioral models are further divided into analog models and assay models (Stoff et al. 1978); others have referred to these models as "isomorphic models" and "parallel models," respectively (Jacobs and Trulson 1978). Analog models are correlational; that is, they rely on some drug-induced animal behavior for which there is an intrinsic similarity in human effect (e.g., exploratory behavior and stereotypy).

Assay models are inferential; that is, there need not be a relationship between the animal and human behavior so long as the test drugs produce a dose-related effect that parallels human hallucinogenic potency. It has been whimsically suggested that if hallucinogens elicited tail-biting behavior in rodents in a dose-dependent manner with a potency that parallels human hallucinogenic potency, then tail-biting could be a useful assay model of hallucinogenic activity (Stoff et al. 1978).

Nonbehavioral animal models may be of an analog or assay nature but simply rely on effects that are not necessarily behavioral (e.g., contraction of isolated muscle tissue in a muscle bath). Some common explicit or implicit animal models include (1) the serotonin syndrome; (2) ear-scratch reflex or scratch reflex stereotypy; (3) head-twitch response; (4) rabbit hyperthermia; (5) limb-flick behavior in cats or limb-jerk in monkeys; (6) startle reflex; (7) investigatory behavior; (8) disruption of fixed-ratio responding, the so-called hallucinogenic pause; and (9) drug discrimination (DD) using animals trained to standard hallucinogens (reviewed: Glennon 1992). Combinations of these and other assays have been employed as test batteries (Otis et al. 1978; Stoff et al. 1978) with the hope that a combination of tests might prove more reliable. To date, however, there is no foolproof animal model that allows reliable predictions of hallucinogenic activity. That is not to say that the use of animal models is not worthwhile; indeed, they have enhanced the understanding of hallucinogenic agents significantly, Unfortunately, each model has resulted in some false positives (i.e., has identified an agent known to be inactive in humans as being potentially hallucinogenic) and/or false negatives (i.e., has identified a known hallucinogen as being potentially inactive).

Nonanimal Models

Nonanimal techniques have been employed to investigate hallucinogenic agents and, in particular, the structure-activity relationships (SAR) of such agents. These may be classified as stochastic interaction models, conservative molecule models, and mechanistic models (Kier and Glennon 1978). Stochastic interaction models are simulations of drug-receptor interactions in the absence of any understanding of the receptor involved (i.e., the model features interactions between an active drug molecule and some hypothetical receptor feature). The conservative molecule approach is an investigation of the structural influence (e.g., physicochemical or quantum chemical properties) of active agents on hallucinogenic activity. This approach is amechanistic and is simply

an attempt to correlate hallucinogenic activity/potency with chemical structure. The mechanistic model is similar to the conservative molecule approach except that it allows development of quantitative relationships between properties of drugs and pharmacological activities with potential mechanistic relevance (e.g., the influence of lipophilicity on receptor affinity for a series of active agents).

Although all three of these models may be of some predictive or mechanistic value, each requires animal or human data for initial input and, as such, cannot be considered a substitute for animal models. One of the more exciting techniques explored recently is the modeling of drug-receptor interactions using graphics models of neurotransmitter receptors. Because the precise three-dimensional structures of neurotransmitter receptors are unknown at this time, different models, and indeed different hypothetical modes of drug-receptor interaction, are possible (reviewed: Westkaemper and Glennon 1991). Thus, these models will require buttressing and validation by empirical methods such as site-directed mutagenesis, ligand binding utilizing chimeric receptors, or both. Nevertheless, such investigations have propelled the study of hallucinogens to the submolecular level.

ENIGMATIC AGENTS

Certain agents are continually identified by various animal models as being "active," when in fact there are little or no supporting human data. These agents fall into three broad categories. First, there are agents known to lack hallucinogenic activity in humans when administered in a single dose. Amphetamine, an example of such an agent, is active in several animal models (e.g., rabbit hyperthermia). Second, there are agents that generally are regarded as lacking hallucinogenic properties and that may even be widely used therapeutically, but for which there are scattered accounts of hallucinogenic episodes in humans. Lisuride is typical of this type of enigmatic agent. Third, there are those agents for which human data are very limited. Quipazine, for example, is active in many, if not most, animal models. It is this last category of agents that is most troublesome. Until additional clinical studies are conducted, it can never be known with certainty if these types of agents truly are without hallucinogenic effects. Nevertheless, these agents should continue to be used in future studies with animals in order to challenge new models as well as to gain additional insight about the agents themselves.

THE DRUG DISCRIMINATION PARADIGM

The DD paradigm was listed above along with other animal models of hallucinogenic activity. In fact, it has never been claimed that the paradigm is a model of hallucinogenic activity; however, it has been quite successful in qualitatively and quantitatively identifying hallucinogenic agents. The author has used this method extensively. Because some of the results described below require an understanding of this method, a brief description will be provided here (see Glennon et al. 1991*a* for a review and additional detail).

In the DD paradigm, animals are trained to elicit a particular response when administered a specific dose of a hallucinogenic agent and to elicit a different response when administered vehicle. Thus, animals can be trained to discriminate a drug from nondrug condition by, for example, responding on one of two levers in a two-lever operant procedure. Once animals have been trained to discriminate a specific hallucinogen from saline, various pharmacological investigations can be conducted (e.g., determination of median effective dose [ED,] values, time of onset, and duration of action).

Of particular interest are tests of stimulus generalization and tests of stimulus antagonism. In the former, also referred to as challenge tests or substitution tests, doses of different agents are administered to animals trained to discriminate a specific hallucinogen from saline. Such studies allow the identification of other agents that produce stimulus effects similar to those of a common training drug. That is, the animals are in effect identifying novel agents that presumably are perceived to possess similar properties. The results of these studies also allow for interagent potency comparisons for those agents identified as being active.

Tests of stimulus antagonism are quite similar and are based on the presumption that administration of the appropriate neurotransmitter antagonist in combination with the training drug will result in nondrug (i.e., vehicle-appropriate) responding. Such studies are useful for the identification of potential antagonists or, given the appropriate neurotransmitter antagonist, may be useful in identifying mechanisms of action. The DD paradigm has proven to be quite effective for the investigation of hallucinogenic agents as well as other drugs of abuse, including amphetamine, cocaine, phencyclidine (PCP), opioids, barbiturates, and ethanol (Glennon et al. 1991*a).*

Examples of each major category of classical hallucinogens (with the exception of the ß-carbolines) have been used as a training drug in DD studies. Mescaline, (+)LSD, 5-methoxy-DMT (5-OMe-DMT), and DOM, representative of the four major subclasses of classical hallucinogens, have seen the most extensive application. Stimulus generalization occurs among all four of these agents regardless of which is used as the training drug. This is one reason why such agents have been classified under the common heading of classical hallucinogens and has some bearing on the above mentioned suggestion that classical hallucinogens (although perhaps capable of producing slightly different effects) seem able to produce a common effect. This hypothesis has been further tested using rats trained to discriminate DOM from vehicle. A large number of agents, including simple tryptamine hallucinogens (for example, see figure 1 for 5-OMe-DMT, 4-methoxy-DMT (4-OMe-DMT) and DMT), a-methyltryptamines (figure 2), ergolines (see figure 1 for (+)LSD), phenylethylamines, phenylisopropylamines (see figure 3 for some examples), and ß-carbolines (see figure 4 for harmaline and 6-methoxyharmalan) have now been examined. To date, there have been no reports of false negatives. Furthermore, structure-activity relationships (SAR) have been formulated, mechanistic studies have been conducted, and, for a series of agents for which human data are available, there is a significant correlation ($r > 0.9$) between discrimination-derived ED, values and human hallucinogenic potencies (reviewed: Glennon 1991).

MECHANISM OF ACTION OF CLASSICAL HALLUCINOGENS

The 5-HT$_2$ Hypothesis

Classical hallucinogens are structurally similar to several major neurotransmitter substances, including serotonin (5-HT), norepinephrine (NE), epinephrine, and dopamine (DA). Over the years, it has been variously proposed that each of these substances (and other neurotransmitters or putative neurotransmitters such as histamine and tryptamine) may be involved in the mechanism of action of hallucinogenic agents. Indeed, there is some evidence that certain hallucinogens, most notably (+)LSD, interact at each of these types of receptors. Historically, however, there is little support for involvement of most of these neurotransmitters in the common actions of the classical hallucinogens. In contrast, 5-HT has been implicated consistently in the actions of hallucinogens ever since its discovery.

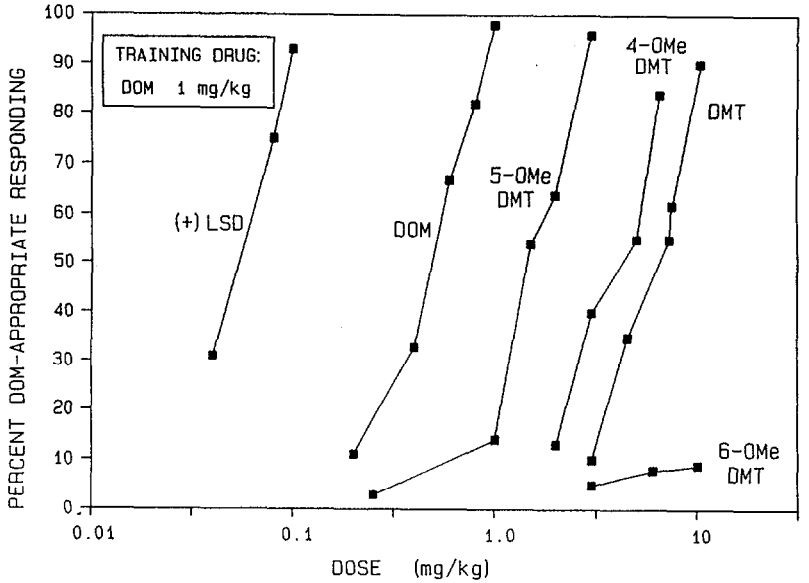

FIGURE 1. *DOM-stimulus generalization to examples of indolylalkylamines including (+)-LSD; N,N-dimethyltryptamine (DMT); 5-OMe-DMT; and 4-OMe-DMT as well as lack of DOM- stimulus generalization to 6-OMe-DMT. The dose-response curve for the training drug (i.e., DOM) is shown for the purpose of comparison.*

Controversy arose during the 1950s with the discovery of two distinct populations of peripheral 5-HT receptors (D receptors and M receptors). Do hallucinogens act at 5-HT receptors? If so, do they act as 5-HT agonists or antagonists? During the 1980s, identification of multiple populations of central 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_2, 5-HT_3, and 5-HT_4) only served to complicate the issue further. Most of the 5-HT_1 (and probably 5-HT_4) receptors belong to a G-protein coupled superfamily of receptors involving an adenylate cyclase second messenger system; 5-HT_2 and 5-HT_{1C} receptors (now referred to as 5-HT_{2A} and 5-HT_{2C} receptors, respectively) also belong to this family but are linked to a phosphoinositol (PI) second messenger system. 5-HT_3 receptors are distinct in being ligand-gated ion channel receptors.

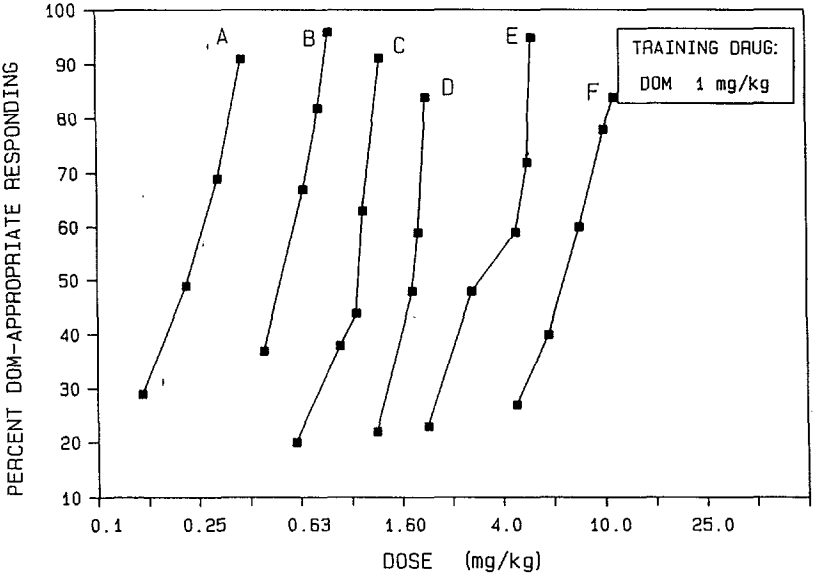

FIGURE 2. *DOM-stimulus generalization to (+)5-methoxy-α-methyltryptamine (5-OMe-α-MT; A), (±)5-OMe-α-MT (B), (-)5-OMe-α-MT (C), (+)α-MT (D), (±)α-MT (E), and racemic α-ethyltryptamine (F).*

The issue now becomes even more complicated: which population(s) of 5-HT receptors are involved in the actions of classical hallucinogens? On the basis that the discriminative stimulus effects of DOM and DOM-stimulus generalization to (+)LSD, 5-methoxy DMT, and mescaline could be potently antagonized by 5-HT_2 antagonists, it was proposed that the classical hallucinogens act as agonists at 5-HT_2 receptors (Glennon et al. 1983). To support this hypothesis, the binding of various hallucinogens at the different populations of 5-HT receptors was examined using radioligand binding techniques. Indolylakylamine hallucinogens are fairly nonselective and bind with high affinity at multiple populations of 5-HT receptors. In contrast, the phenylisopropylamine hallucinogens such as DOM, DOB, and DOI bind rather selectively at 5-HT_2 receptors. Furthermore, there is a significant correlation ($r > 0.9$) between 5-HT_2 receptor affinity and both discrimination-derived ED, values and human hallucinogenic potencies (Glennon 1990). For the first time, there was now evidence for the 5-HT_2 hypothesis of hallucinogenic activity.

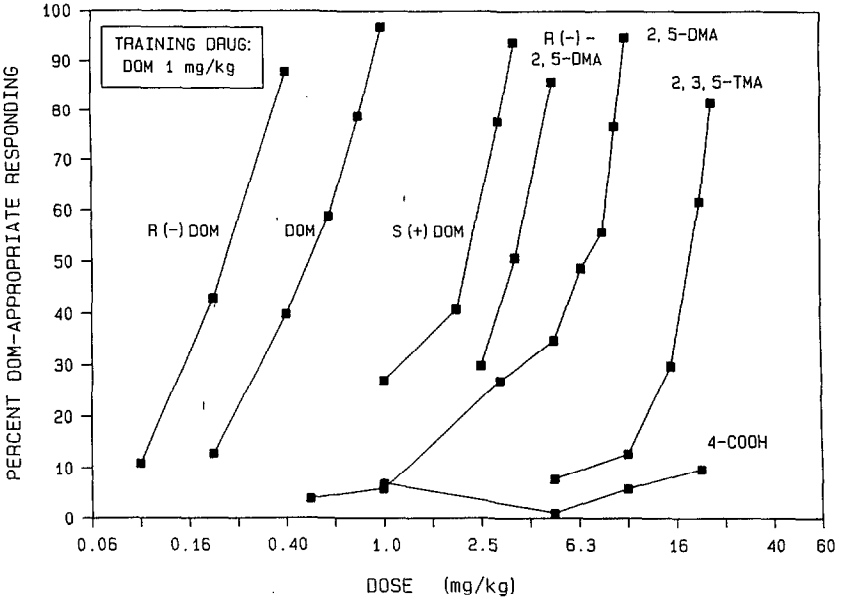

FIGURE 3. *DOM-stimulus generalization to R(-)DOM; (±)DOM; S(+)DOM; R(-)2,5-DMA; (±)2,5-DMA; and 2,3,5-TMA; and lack of stimulus generalization to 2,5-DMA 4-carboxylic acid ("4-COOH"), a metabolite of DOM*

DOB and its demethylated counterpart, α-desmethyl-DOB, produce similar yet distinguishable effects in humans. Consistent with the common component hypothesis, these agents produce similar stimulus effects in animals and bind with similar potencies at 5-HT_2 receptors; however, α-desmethyl-DOB binds in a less selective manner than DOB (Glennon et al. 1988). Thus, it could be the less selective nature of α-desmethyl-DOB that accounts for its distinguishability from DOB.

5-HT_2-Related Problems

Several problems have arisen regarding the 5-HT_2 hypothesis:

Do hallucinogens act as 5-HT_2 agonists or antagonists?
Are there subpopulations of 5-HT_2 receptors?
May some other population of 5-HT receptors (instead of 5-HT_2 be involved in the actions of hallucinogens?

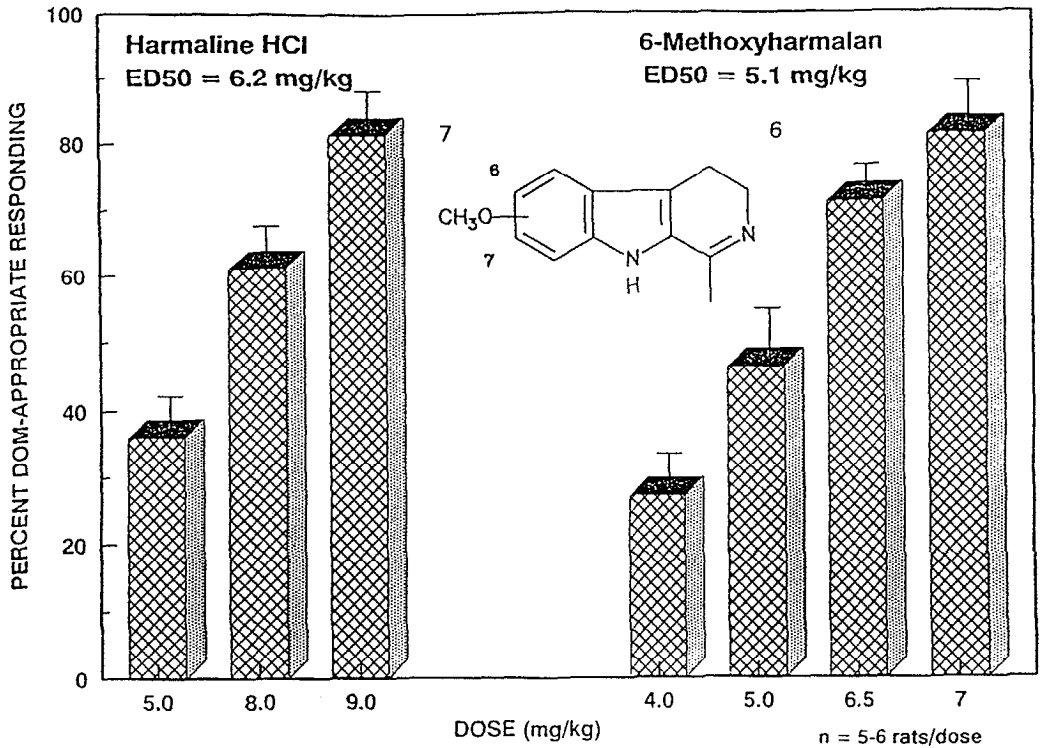

FIGURE 4. *DOM-stimulus generalization to two hallucinogenic ß-carbolines, harmaline and 6-methoxyharmalan, in studies using rats trained to discriminate 1 mg/kg of DOM from saline.*

On the basis of previous DD studies, it was originally suggested that classical hallucinogens act as 5-HT_2 agonists. However, Pierce and Peroutka (1988) recently challenged this concept and have suggested that hallucinogens, particularly (+)LSD, act as 5-HT_2 antagonists. This agonist-versus-antagonist controversy was reexamined, and it was concluded that hallucinogens are not 5-HT_2 antagonists; classical hallucinogens are agonists, or at least partial agonists, at 5-HT_2 receptors (Glennon 1990). Certain hallucinogens, including LSD, may possess a low intrinsic activity; thus, given in combination with a full agonist, such agents might occasionally appear to behave as antagonists in some pharmacological assays.

Lyon and colleagues (1987) have proposed that 5-HT_2 receptors exist in a low-affinity state and an agonist high-affinity state (i.e., the two-state hypothesis). [^{3}H]Ketanserin, a 5-HT_2 antagonist, labels both states of the receptors, whereas the agonist radioligands [^{3}H]DOB and [^{125}I]DOI apparently label the agonist high-affinity state. Pierce and Peroutka (1989) later conducted related investigations using [^{77}Br]DOB and proposed an alternative explanation: there exist two different populations (i.e., subpopulations) of 5-HT_2 receptors (the two-site hypothesis). The results of recent cloning studies favor the two-state concept in that a single 5-HT_2 receptor is expressed that behaves in a manner reminiscent of a two-state receptor population (reviewed: Weinshank et al. 1992). It might be noted, however, that the possibility of two different (overlapping) binding domains has not yet been excluded; that is, agonists and antagonists may bind in a slightly different manner at the same population (or state) of 5-HT_2 receptors.

Finally, there is the issue of involvement of other (or additional) populations of 5-HT receptors in the actions of hallucinogens. This is discussed below.

Involvement of 5-HT_{1C} Receptors

Shortly after the 5-HT_2 hypothesis was proposed (Glennon et al. 1983, 1984), Pazos and coworkers (1984) described their discovery of 5-HT_{1C} receptors. The binding of hallucinogens at these receptors was subsequently examined, and little difference between their 5-HT_2 and 5-HT_{1C} affinities was found (Titeler et al. 1988); indeed, later studies have shown less than a tenfold difference in receptor affinity for a large series of phenylalkylamine derivatives (Glennon et al. 1992). As with 5-HT_2 receptor affinities, 5-HT_{1C} affinities also are correlated both with

discrimination-derived ED, values and human hallucinogenic potencies. In addition, Burris and Sanders-Bush (1988) reported that DOM acts as a 5-HT_{1C} agonist. Furthermore the 5-HT_2 hypothesis was based, in part, on the finding that 5-HT_2 antagonists (such as ketanserin and pirenperone) antagonize the stimulus effects of hallucinogens. It is now recognized that these 5-HT_2 antagonists are described more accurately as 5-HT_2 and 5-HT_{1C} antagonists. Thus, the likelihood exists that 5-HT_2 and/or 5-HT_{1C} receptors are involved in the actions of hallucinogenic agents. It may be this interaction that constitutes the common "key" mentioned at the beginning of this chapter, and it may be this common interaction that allows animals to reliably discriminate classical hallucinogens from the vehicle.

It is quite difficult to ascribe a specific role for 5-HT_{1C} versus 5-HT_2 receptors in the mechanism of action of hallucinogens due to the lack of agents that display selectivity for one of these populations of receptors over the other. Nearly all agents that bind at 5-HT_2 receptors bind at 5-HT_{1C} receptors. However, there are a few agents that might offer some hope in resolving this problem. The DA 5-HT_{1A} antagonist spiperone binds with approximately 500-fold selectivity for 5-HT_2 versus 5-HT_{1C} receptors. An attempt was made to antagonize the stimulus effects of DOM using various doses of spiperone with the intention that it might be more difficult to antagonize the DOM stimulus if the stimulus was 5-HT_{1C} mediated. Unfortunately, the results of these studies were inconclusive due to the severe disruptive effects of low doses of spiperone in combination with DOM (Glennon 1991).

Another agent of interest is l-(3-trifluoromethylphenyl)piperazine (TFMPP). Although TFMPP binds at multiple populations of 5-HT receptors, evidence suggests that TFMPP is a 5-HT_{1C} agonist but a 5-HT_2 antagonist (or, at best, a 5-HT_2 partial agonist). Administration of TFMPP to animals trained to discriminate DOM from saline failed to result in stimulus generalization. In a parallel study, administration of DOM (or DOI) to animals trained to discriminate TFMPP from vehicle resulted in only partial generalization followed at slightly higher DOM (or DOI) doses by disruption of behavior. Thus, the results were again inconclusive. In a third series of studies, rats trained to discriminate 0.5 milligrams per kilogram (mg/kg) of TFMPP from the vehicle were administered doses of DOM in combination with 0.2 mg/kg of TFMPP (ED, dose = 0.17 mg/kg). The rationale for this investigation was that lower (i.e., nondisruptive) doses of DOM should potentiate the effect of the near-ED, dose of TFMPP if both agents act via a common

mechanism. The results (shown in figure 5) were somewhat surprising in that low doses of DOM, rather than potentiating the effect, actually antagonized the effect of TFMPP. For all practical purposes, the results of this study also were inconclusive; however, they suggest that DOM and TFMPP are likely producing their stimulus effects via different mechanisms.

There is one additional piece of information that perhaps has some bearing on the 5-HT_2 versus 5-HT_{1C} controversy. Several years ago, Glennon and Hauck (1985) reported that the DOM stimulus generalizes to lisuride. This was a rather unexpected finding. Subsequently, the author and coworkers reevaluated lisuride as a potential DOM antagonist and found that it attenuates the DOM stimulus by 50 percent at very low doses (i.e., at one-sixtieth of the dose that results in stimulus generalization). This led to speculation that lisuride may be acting as a partial agonist (Glennon 1991). Sanders-Bush has recently demonstrated (this volume) that, whereas lisuride is a pure 5-HT_{1C} antagonist, it behaves as a partial agonist at 5-HT_2 receptors. These results are consistent with the present DD studies. Thus, although hallucinogens unquestionably bind at both populations of receptors and whereas a mechanistic role for 5-HT_{1C} receptors cannot yet be eliminated, it would appear on the basis of all the above mentioned studies that the DOM stimulus involves primarily a 5-HT_2 mechanism.

Before leaving the topic of 5-HT_2 and 5-HT_{1C} receptors, it might be mentioned that certain of the animal models described earlier appear to involve actions mediated by these receptors. For example, the head-twitch response has been proposed to involve such a mechanism (Glennon 1992). In retrospect, some of these models may be less farfetched and more mechanistically relevant than once suspected.

Involvement of Other 5-HT Receptors

It was recently reported that there may be functional interactions between different populations of 5-HT receptors such that action at one may modulate activation of another (reviewed: Glennon et al. 1991*b*). Thus, interaction of an agonist at one population of 5-HT receptors may modulate the effect of the interaction of a second agonist at a different population of receptors. This could have far-reaching consequences. For example, what is the effect of a nonselective agonist that interacts at more than one population of receptors at the same time? What about a nonselective agent that is an agonist at one population and an antagonist

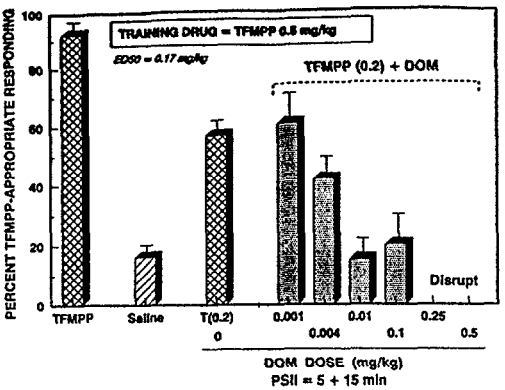

FIGURE 5. *The effect of DOM in combination with a near-ED_{50} dose of TFMPP in rats trained to discriminate TFMPP (0.5 mg/kg) from saline. TFMPP elicits > 90 percent TFMPP-appropriate responding (ED, = 0.17 mg/kg); 0.2 mg/kg of TFMPP [T(0.2)] elicits 57 percent TFMPP-appropriate responding. Administration of various doses of DOM 5 min prior to administration of 0.2 mg/kg of TFMPP results in attenuation of TFMPP-appropriate responding. Administration of 0.25 and 0.5 mg/kg of DOM in combination with 0.2 mg/kg of TFMPP resulted in disruption of behavior (i.e., no responding).*

at another? Experiments necessary to sort out these types of interactions could be rather labor intensive and their interpretation quite complicated. Worse yet are cases where such types of interactions are possible but unrecognized.

It was previously shown that the DOM stimulus does not generalize to the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), and that an 8-OH-DPAT stimulus does not generalize to DOM. Furthermore, DOM does not bind at 5-HT_{1A} receptors, nor does 8-OH-DPAT bind with significant affinity at 5-HT_2 receptors. More recently, it was demonstrated that very low doses of 8-OH-DPAT amplify the stimulus effects of DOM in DOM-trained rats. For example, animals given 0.05 mg/kg of 8-OH-DPAT in combination with the ED, dose of DOM behave as if they have received the training dose of DOM (i.e., stimulus generalization occurs upon administration of the ED, dose of DOM) (see inset, figure 6). Furthermore, pretreatment of animals with 0.05 mg/kg of 8-OH-DPAT results in a leftward shift of the DOM dose-response curve (figure 6). These results would seem to suggest that low doses of the 5-HT_{1A}-selective agonist influence the stimulus effects of DOM. Additional studies are required to further understand the details of this interaction.

Similar studies were conducted with 5-HT_3 agents. For example, very low doses of the 5-HT_3 antagonist zacopride attenuate the stimulus effects of DOM even though zacopride does not bind at 5-HT_2 receptors, and DOM does not bind at 5-HT_3 receptors. A dose of 0.001 mg/kg of zacopride in combination with the training dose of DOM results in about 30 percent DOM-appropriate responding. Higher doses appear to have less of an attenuating effect (figure 7). The 5-HT_3 (partial) agonist meta-chlorophenylbiguanide (mCPBG) also has an unusual effect on the DOM stimulus (figure 8). A dose of 0.5 mg/kg of mCPBG seems to attenuate the stimulus effects of 1 mg/kg of DOM; higher doses have less of an effect. However, administered alone, mCPBG seems to result in partial generalization. Doses higher than those shown resulted in disruption of behavior.

Parallel studies were conducted using rats trained to discriminate the structurally related agent N-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA) from saline. Zacopride and LY 278584, at doses of between 0.0003 and 0.001 mg/kg, decrease MDMA-appropriate responding to about 20 percent (figure 9). The effect of mCPBG (figure 10) is not unlike that seen with DOM. The results suggest a possible

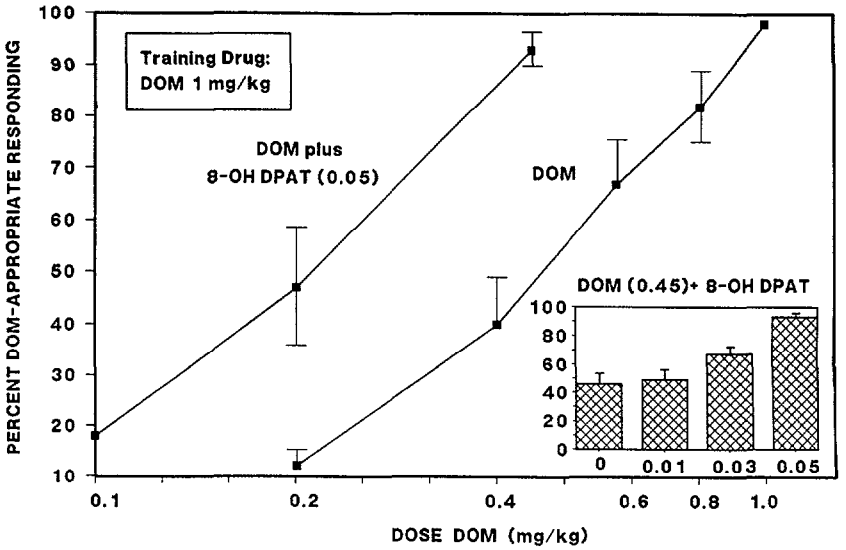

FIGURE 6. *Effect of the 5-HT$_{1A}$ agonist 8-OH-DPAT in combination with DOM in rats trained to discriminate DOM from saline. Dose-response curve for DOM (right) and for DOM in animals pretreated with 0.05 mg/kg of 8-OH-DPAT (left). Inset shows effect of different doses of 8-OH-DPAT administered in combination with the ED, dose (0.45 mg/kg) of DOM. Data previously reported (Glennon 1991).*

modulatory effect by 5-HT$_3$ receptors on the DOM and MDMA stimulus. It might be noted, for purpose of comparison, that zacopride had essentially no effect on amphetamine-appropriate responding in rats trained to discriminate (+)amphetamine from vehicle. These types of unexpected interactions open up entirely new lines of investigation regarding classical hallucinogens and may (?) hint at a possible role for 5-HT$_3$ antagonists in the treatment of drug abuse involving hallucinogens.

SAR AND STRUCTURALLY RELATED AGENTS

SAR have been formulated for hallucinogenic activity, DOM-stimulus generalization, and 5-HT$_2$/5-HT$_{1C}$ binding. The best investigated agents are the phenylalkylamines and, to a somewhat lesser extent, the simple tryptamines. ß-Carbolines and ergolines have received much less attention. Abuse of ß-carbolines does not seem to be a significant

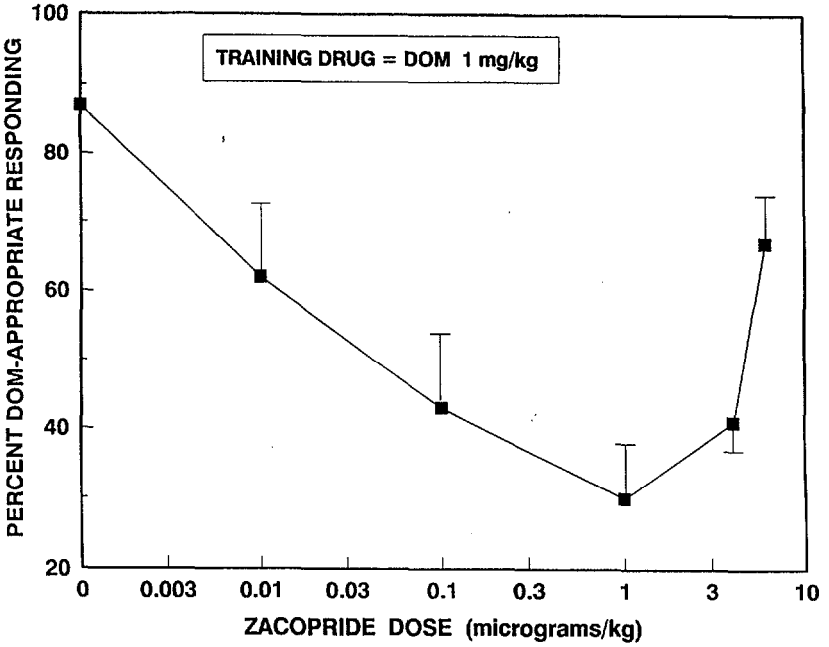

FIGURE 7. *Effect of the 5-HT_3 antagonist zacopride administered in combination with the training dose of DOM to rats trained to discriminate 1 mg/kg of DOM from saline.*

problem, and it is perhaps this reason that accounts for the lack of interest or urgency to study these agents. Ergolines, on the other hand, can offer, a significant synthetic challenge and relatively few agents are readily available. The SAR of classical hallucinogens has been reviewed (Nichols and Glennon 1984).

Many investigations of classical hallucinogens are limited to a small handful of standard agents (e.g., LSD, mescaline, DOM). Far fewer studies have examined some of the more novel or structurally distinct agents, or have examined series of agents. It would seem prudent to examine additional agents and structurally related analogs in order to define exactly what structural features contribute to activity. A classic example is the phenylisopropylamine amphetamine. The amphetamine structural backbone is contained in, for example, the hallucinogen DOM and the designer drug MDMA; and yet each of these three agents produces effects in animals and humans that are clearly distinguishable from one another (Shulgin and Shulgin 1991). As the structure of one of these agents is gradually modified to one of the others, at what point does

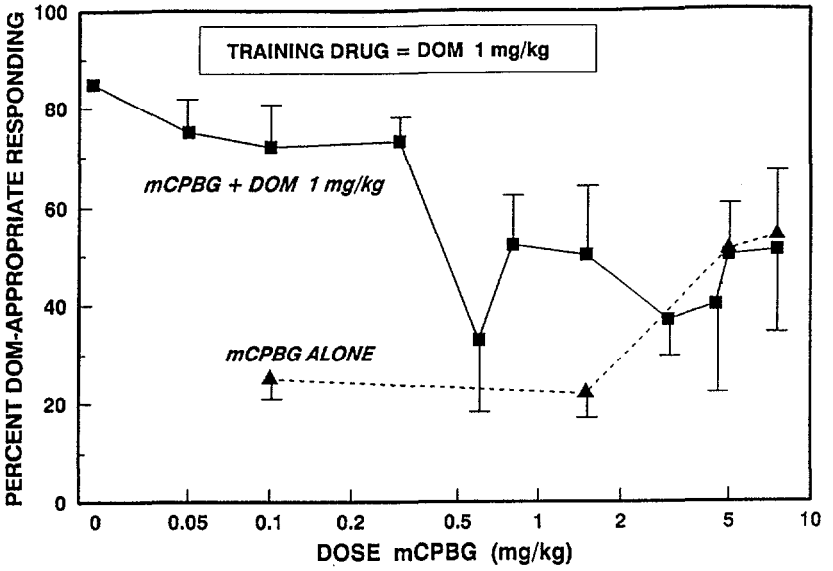

FIGURE 8. *Effect of the 5-HT$_3$ partial agonist meta-chlorophenylbiguanide (mCPBG), administered either alone (broken line) or in combination with the training dose of DOM (solid line) in rats trained to discriminate DOM (1 mg/kg) from vehicle.*

an amphetamine-like agent become, for example, a hallucinogenic agent? Is there some structure that possesses both properties?

This would seem to be the case. It has been demonstrated in tests of stimulus generalization that 1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDA) produces both amphetamine-like and DOM-like effects as well as MDMA-like stimulus effects. The amphetamine-like effect rests primarily with the S(+)isomer, whereas the R(-)isomer is the more DOM-like. Furthermore, it was recently demonstrated that rats can be trained to discriminate 1.25 mg/kg of S(+)MDA from 1.25 mg/kg of R(-)MDA using a three-lever operant paradigm (Glennon and Young, unpublished findings). The stimulus effects of the isomers of MDA are thus clearly distinguishable from one another.

Agents were also examined using rats trained to discriminate either DOM, (+)amphetamine, or MDA from vehicle. Some of these results are shown in figure 11. It can be seen that agents such as (+)LSD produce DOM-like effects, and cocaine produces (+)amphetamine-like effects, but

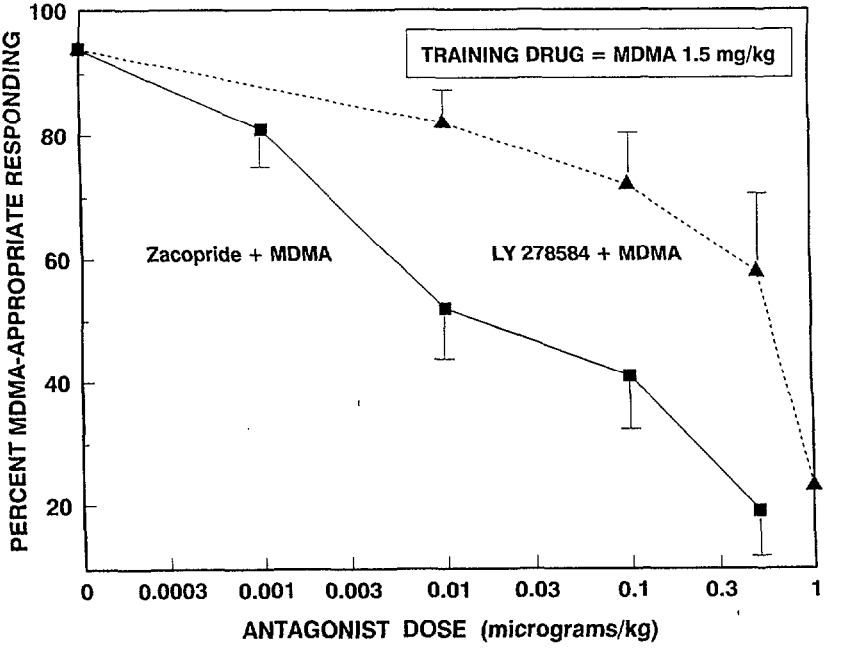

FIGURE 9. *Effect of the 5-HT_3 antagonists zacopride and LY 278584 administered in combination with the training dose of MDMA in rats trained to discriminate MDMA (1.5 mg/kg) from saline.*

both agents result in stimulus generalization in rats trained to discriminate racemic MDA from vehicle. Furthermore, agents such as 1-(3,4-dimethoxyphenyl)-2-aminopropane (3,4-DMA), which produces neither amphetamine-like nor DOM-like stimulus effects, produces MDA-like stimulus effects (figure 11). Clearly, minor structural changes have a profound influence on the stimulus effects of these agents.

Agents such as α-ethyltryptamine (ET), which is currently popular on the clandestine market, produce DOM-like effects (figure 2) but also result at least in partial generalization in rats trained to discriminate either (+)amphetamine or MDMA from vehicle (figures 12 and 13). Perhaps certain tryptamine derivatives will eventually be discovered to bridge several pharmacological categories in a manner similar to that of MDA, and the results of studies with ET (figures 12 and 13) certainly suggest that its individual optical isomers be examined.

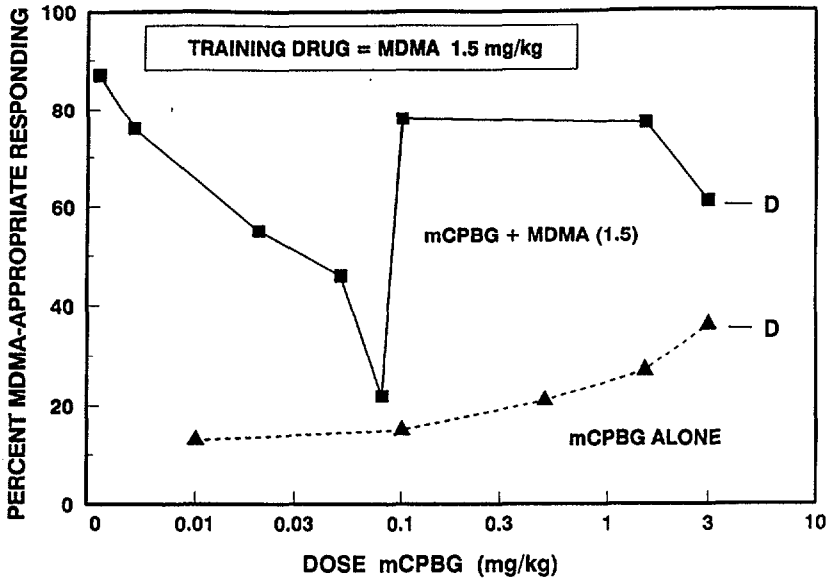

FIGURE 10. *Effect of the 5-HT_3 partial agonist mCPBG administered either alone (broken line) or in combination with the training dose of MDMA (solid line) in rats trained to discriminate MDMA (1.5 mg/kg) from saline.*

Thus, there is a need to continue examination of new structural analogs not only with the intent of formulating and challenging SARs, but also for the purpose of elucidating mechanisms of action and classifying what agents produce what effects.

FUTURE DIRECTIONS

The present technical review focused entirely on classical hallucinogens. Figure 14 shows the extensive nature of the investigations addressed at this meeting. With the above discussion as background, there are several problems that need to be addressed:

- An exacting definition is still required for the effects produced by hallucinogenic agents. Furthermore, addtional work needs to be done on what agents fall into this category on the basis of whether or not they produce a common effect.

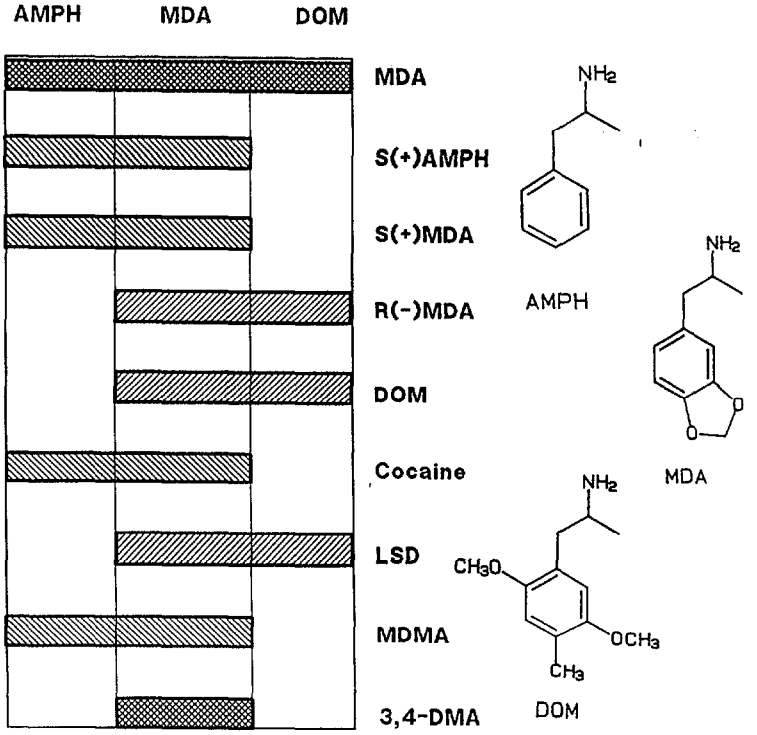

FIGURE 11. *Stimulus-generalization profiles of various agents in rats trained to discriminate either (+)amphetamine sulfate (AMPH), MDA HCl, or DOM HCl from saline. Once animals were trained to discriminate one of the training drugs (AMPH, MDA, or DOM), tests of stimulus generalization were conducted with the agents listed on the right. A darkened bar represents the group(s) of animals in which stimulus generalization (i.e., > 80 percent drug-appropriate responding) occurred. For example, both the AMPH stimulus and the MDA stimulus, but not the DOM stimulus, generalized to cocaine.*

- Additional clinical data are required to validate previously published animal data. Animal models are now realized to possess shortcomings, but there is a significant amount of animal data available that could assist the understanding of hallucinogens.

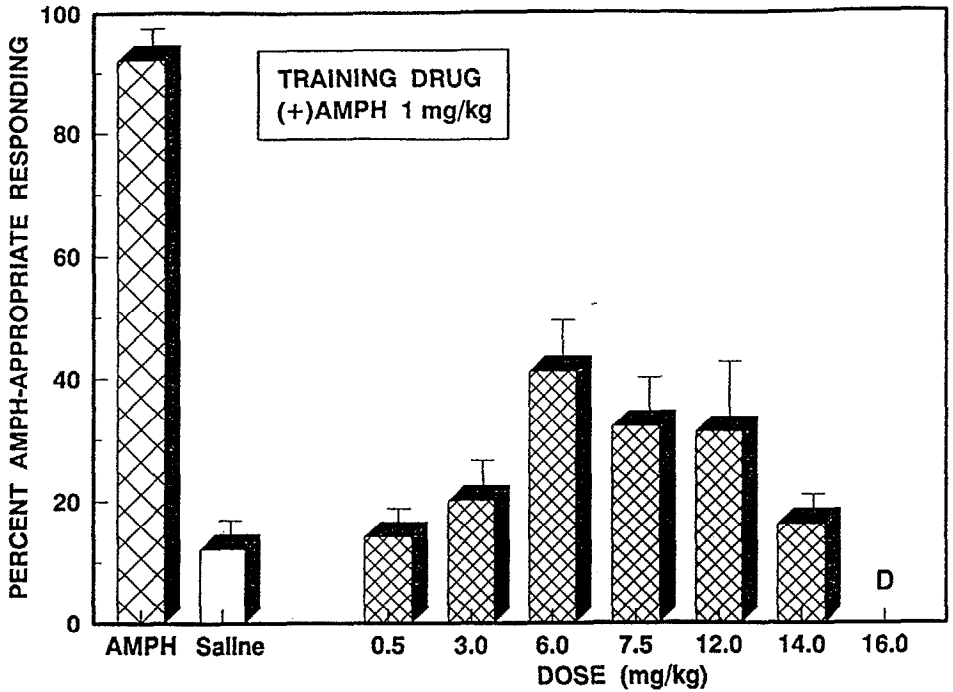

FIGURE 12. *Stimulus-generalization studies with racemic α-ethyltryptamine acetate in male Sprague-Dawley rats (n = four to five animals per dose) trained to discriminate (+)amphetamine sulfate from saline. At doses ≥ 7.5 mg/kg, the animals' response rates were depressed by about 50 percent; disruption of behavior occurred at 16 mg/kg.*

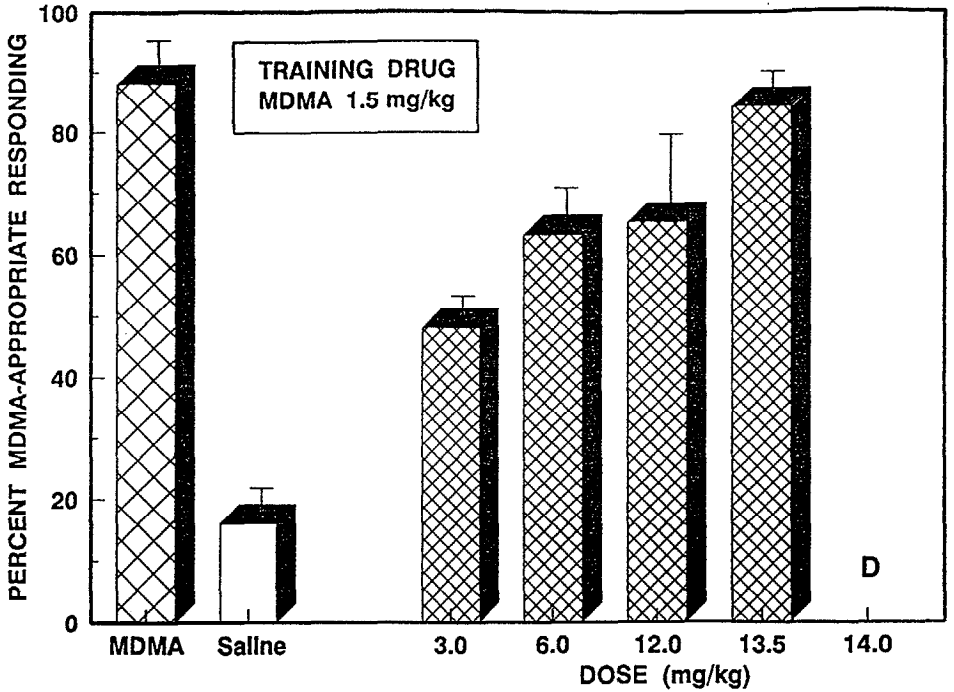

FIGURE 13. *Stimulus-generalization studies with racemic α--ethyltryptamine acetate in male Sprague-Dawley rats (n = three to four animals per dose) trained to discriminate MDMA hydrochloride from saline. At 13.5 mg/kg, only two of four animals responded; response rates were comparable to control response rates except where disruption of behavior occurred (i.e., at 14 mg/kg; n = 1/3).*

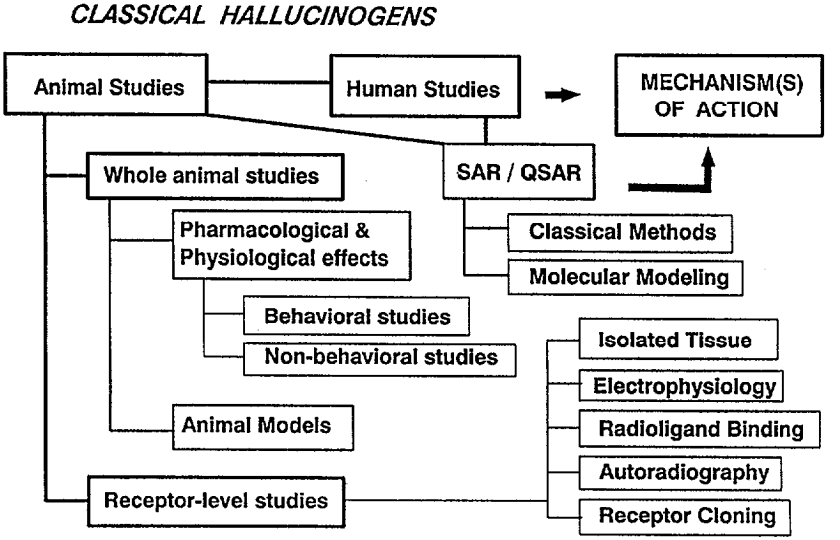

FIGURE 14. *An outline of various studies conducted with classical hallucinogens (described at the NIDA Technical Review on Advances in Data Analysis for Prevention Intervention Research).*

- Some SARs have been formulated, but more work needs to be done. Minor structural variation has a profound and intriguing effect on pharmacological activity. Minimal data are available on quantitative structure-activity relationships (QSAR).

- The mechanism of action of classical hallucinogens is not fully understood. Although 5-HT_2 and 5-HT_{1C} receptors have been implicated as playing a major role and are currently the primary mechanistic focus of many investigations, the role of other neurotransmitters requires examination.

- Functional interactions between receptor populations, with consequent modulation of agonist effects, may represent an entirely new method for treating drug abuse. Such functional interactions deserve further investigation.

- The locus of hallucinogen action in brain requires additional study. New scanning and autoradiographic techniques may aid in this regard. Second messenger systems, as well as differential regulation of receptor number versus second messenger systems, should be pursued.

- Is there a prototypic classical hallucinogen? Many investigations with hallucinogens involve the same small number of agents, in particular LSD. Should LSD be considered a prototype agent? Or is it possible that investigation of one or two agents in great depth may lead researchers astray by providing information that is unique to a specific agent, rather than information that may be more germane to classical hallucinogens as a group? The same may be said for animal models. Perhaps future studies should not rely solely on investigating the same small number of standard agents nor rely only on a few pharmacological test procedures.

NOTE

Since the original submission of this manuscript, the terms "5-HT_2 and 5-HT_{1C} receptors" have been replaced by "5-HT_{2A} and 5-HT_{2C} receptors," respectively.

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AUTHOR

Richard A. Glennon, Ph.D.
Professor of Medicinal Chemistry
Department of Medicinal Chemistry
School of Pharmacy, Box 980540
Medical College of Virginia
Virginia Commonwealth University
Richmond, VA 23298-0540

Are Hallucinogens Psychoheuristic?

Stephen Szára

"When I use a word," Humpty Dumpty said in rather a scornful tone, "it means just what I chose it to mean—neither more nor less."

"The question is," said Alice, "whether you *can* make words mean so many different things."

"The question is," said Humpty Dumpty, "which is to be master—that's all." (Carroll 1946, p. 229)

INTRODUCTION

One of the hallmarks of hallucinogenic drugs such as lysergic acid diethylamide (LSD), N,N-dimethyltryptamine (DMT), and mescaline is the extreme variability of the effects produced in human subjects that are not only dose dependent but also heavily influenced by the mental set, or expectation, of subjects and the environmental setting that surrounds them (Faillace and Szára 1968; Freedman 1968; Osmond 1957). This variability is also reflected in the names that have been suggested for hallucinogens in the past, for example, psychotomimetic, psycholytic, psychedelic, mysticomimetic, cultogenic, and entheogenic (Freedman 1968; Osmond 1957; Ruck et al. 1979; Szára 1961).

Is another name for hallucinogens really needed? This chapter argues that the names used in the past have largely lost their usefulness and may be even misleading, and that recent advances in the neurosciences and cognitive sciences have created opportunities for using hallucinogens as tools in attacking the supreme mystery: How does the brain work? In this quest, the author starts with a brief review of the past 35 to 40 years of use of these drugs in which several distinct trends, referred to as eras, can be distinguished. Although the eras are overlapping, some with clear beginnings and fading trails, others survive today to some extent in different contexts.

HALLUCINOGEN ERA

The term "hallucinogen" is widely used and understood in both professional and lay circles, in spite of the fact that hallucinations in the strict psychiatric sense of the word are a relatively rare effect of these drugs (Hollister 1962). What is probably the first reference to hallucinations as produced by peyote appears in Louis Lewin's book published in 1924 in German and later translated into English with the nearly identical title *Phantastica* (Lewin 1924, 1964). In this book by the noted German toxicologist, the term "hallucinatoria" appears as a synonym for phantastica to designate the class of drugs that can produce transitory visionary states "without any physical inconvenience for a certain time in persons of perfectly normal mentality who are partly or fully conscious of the action of the drug" (Lewin 1964, p. 92). Lewin lists peyotl (also spelled "peyote") *(Anhalonium lewinii),* Indian hemp *(Cannabis indica),* fly agaric *(Agaricus muscarius),* thornapple *(Datura stramonium),* and the South American yahe (also spelled "yage") *(Banisteria caapi)* as representatives of this class.

As Lewin explains: "Are not 'internal visions,' subjectively considered, real happenings which he who experiences such inward perceptions may regard as true? That is my own view." (Lewin 1964, p. 89) Today's psychiatry makes sharp distinction between illusions (internal visions) and hallucinations. Hallucination is defined as "sense perception to which there is no external stimulus" (Campbell 1989, p. 314). Illusion, on the other hand, is "erroneous perception, a false response, to a sense stimulation" (Campbell 1989, p. 354). The administration of a hallucinogenic drug can be regarded as an external stimulus to which a false response (geometric visual imagery) is made by the human organism. For this reason, "illusion" is a more appropriate term for this effect as long as the subject is aware of the reality of having taken a drug. "Hallucination" indicates a psychotic disturbance only when associated with impairment in reality testing (Kaplan and Sadock 1989).

The term "hallucinogen" was first used by Hoffer and colleagues (1954) and has remained popular ever since, in spite of numerous well-controlled clinical studies with drugs such as LSD, mescaline, DMT, psilocybin, 2,5-dimethyoxy-4-methylamphethmine, or methylenedioxy-amphetamine that found bona fide hallucinations, to which the subjects reacted as real, were a minor consequence of the drug (Cohen 1985; Fischman 1983; Freedman 1968; Hollister 1962).

The report by Hoffer and colleagues (1954) is considered by many as the start of a new era in psychiatric research, taking the suggestion seriously that these drugs reproduce, in normal subjects, some symptoms of schizophrenia or similar psychoses. The drugs, therefore, are psychotomimetic; the terms "psychosomimetic" and "psychotogenic" are also used in this sense. During the mid-1950s, chlorpromazine was made available to treat psychotic patients. Serotonin, norepinephrine (NE), and gamma aminobutyric acid (GABA) were found in synapses in the brain. Reports started to appear implicating the action of these drugs on synapses as the most likely mechanism of psychoactivity (for a review, see Cooper et al. 1974). Psychopharmacology as a discipline was born. There was much excitement, and expectations, among psychiatrists that their profession might finally become scientifically based, and that the knowledge gained would help to develop more effective treatment for their patients.

PSYCHOTHERAPEUTIC ERA

The psychotomimetic era for hallucinogens gradually gave way to a seemingly perverse movement that claimed that hallucinogens could actually help certain psychiatric patients and advocated a psychotherapeutic use of these drugs. The justification was provided by some of the unique and peculiar effects seen and/or experienced in certain situations, such as the loss of ego boundaries and regression to a more primitive, childlike functioning of the ego that seems to facilitate the recall of early childhood memories that have been forgotten or repressed. These effects are utilized by the psycholytic approach to the treatment of chronic alcoholism. It was rationalized that abolishing the distinction between subject and object (ego boundary) and conscious and unconscious self (regression) would cause a lessening of alienation from the world, a rediscovery of the self, and a learning of a new set of values; thus a new beginning could be achieved (Savage et al. 1962). Combining this approach with hypnosis gave rise to the *hypnodelic* strategy for the same purpose. The therapeutic results, however, only lasted for a few months at best, and longterm followup indicated relapse of drinking behavior to essentially pretreatment levels (Faillace 1966; Faillace et al. 1970; Levine and Ludwig 1965).

PSYCHEDELIC ERA

Another peculiar effect of these drugs is a dramatic change in perception: it appears to the person as if the eyes (the "doors of perception") have been cleansed and the person could see the world as new in all respects—"as Adam may have seen it on the day of creation" as Aldous Huxley (1954, p. 17) pointed out in his popular and influential book. This new reality is perceived and interpreted by some individuals as manifestation of the true nature of their mind; hence, the term "psychedelic" was suggested by Osmond (1957). This interpretation has been embraced not only by professional therapists but also by some segments of the public, and gave rise to the "Summer of Love" in San Francisco in 1967 with free distribution of LSD. This perception resulted in the formation of numerous cults, communes, and drug-oriented religious groups (Freedman 1968), permeated the lyrics and style of popular music (acid rock), and was viewed by some as one of the contributing sources of the occasional resurgence of popularity of illegal drug use (Cohen 1966, Szára 1968).

BEHAVIORISTIC ERA

In a review of the clinical use of psychotomimetic drugs, Faillace referred to a group of investigators as "behaviorists...for lack of a better term" (1966, p. 15). This group, he said, "is not principally interested in treatment but is trying objectively to determine the actions of these drugs. A great many investigators from many divergent disciplines are included in this group" (Faillace 1966, p. 16). He then cited four groups as examples.

1. Hoch and coworkers in New York explored the effects of LSD, mescaline, and other similar drugs on psychotic patients and concluded that these drugs aggravate schizophrenic symptoms. The group showed that the drugs brought forth the same type of psychodynamic material in their patients and there was nothing particularly specific for any of these drugs.

2. Isbell and coworkers at the Addiction Research Center, then in Lexington, KY, conducted a series of investigations on former narcotic addicts. They observed rapid development of tolerance to the effects of LSD and also showed the development of cross-tolerance between LSD and psilocybin. This group demonstrated the

feasibility of obtaining good dose-response relationships utilizing an array of physiological tests (e.g., pupil size, blood pressure). The group developed a standardized questionnaire, the Addiction Research Center Inventory (ARCI), that became widely accepted and is used for assessing the subjective effects of psychoactive drugs in a quantitative fashion.

3. Delay and coworkers in Paris carried out an intensive study of psilocybin and showed that the phosphoryl group does not contribute to its psychoactive effects because the dephosphorylated derivative, psilocin, has equal potency in humans. They observed an intensification of psychotic and psychoneurotic symptoms in 90 mental patients and 47 so-called normals, and suggested that psilocybin may offer diagnostic possibilities in difficult clinical cases.

4. Faillace referred to the work of the author and colleagues at Saint Elizabeth's Hospital in Washington, DC, as the last example of nontherapeutically oriented clinical research with hallucinogenic drugs. This work focused primarily on tryptamine derivatives such as DMT. In the course of investigation of the metabolism of these compounds that included the N,N-diethyl- and N,N-dipropyl-derivatives of tryptamine (DET and DPT, respectively), the conclusion was reached that 6-hydroxylation of the indole ring might be an important biological mechanism for the psychoactivity of these compounds. To provide further evidence, the Saint Elizabeth's Hospital laboratories synthesized a number of derivatives of these compounds that were blocked by substitution at the 6-position so as to prevent hydroxylation at this position (Kalir and Szára 1963). One of these, the 6-fluoro derivative of DET, was shown in clinical tests to produce autonomic symptoms and mood changes without the characteristic perceptual and thinking disturbances usually observed with psychotomimetic agents. Although there are some doubts whether 6-hydroxylation is responsible for psychoactive metabolites (Rosenberg et al. 1963), this fluorinated derivative of DET might be useful as an active placebo in clinical studies with hallucinogens (Faillace 1966).

ERA OF LEGAL LIMBO

All these studies were done before 1966, the year that was a turning point in research with these drugs. In response to public anxiety about drug

abuse, Congress passed the Drug Abuse Control Amendment (Public Law 89-74) that went into effect in May 1966. This amended law banned public use and sale of peyote, mescaline, LSD, DMT, and several other similar drugs. Pharmaceutical companies were forced to stop manufacturing LSD and turn over their supplies of the drug to the National Institute of Mental Health (NIMH).

That same month the author was invited to give a paper at the 122nd Annual Meeting of the American Psychiatric Association held in Atlantic City. The original stated, in part:

> This publicity pressure threatens serious scientific research not only with LSD but with the entire class of hallucinogenic drugs. We cannot put blame on the drugs; we can only put blame on the manner and the ways they are being used. It is my belief that it would be most unfortunate if we were to permit undue hysteria to destroy a valuable tool of science and evaporate an eventual hope for the many hopeless (Szára 1967, p. 1517).

Many other investigators voiced similar concerns (Cohen 1966; Dahlberg 1966; Freedman 1966; Klee 1966) before congressional committees and other appropriate forums (Szára and Hollister 1973), but the situation remains the same today. Clinical research with these drugs essentially stopped, with the exception of Strassman's work on DMT (Strassman, this volume) and some treatment-oriented work with LSD such as that on dying cancer patients (Yensen 1985). Some figures on human studies with LSD during the period of 1953-73 are shown in table 1.

THE PSYCHOHEURISTIC ERA

After more than 20 years of deliberate legal neglect and constraints, it is time, especially in view of the current focus on the "Decade of the Brain,"[1] to recognize and emphasize the potentially immense heuristic value of these drugs in helping to explore the neurobiological bases of some fundamental dimensions of psychic functions. With this in mind, the author suggests changing the point of view or attitude of professionals and of the public by calling these drugs by a name other than hallucinogens, psychotomimetics, or psychedelics. These names all suggest that, when these drugs are consumed, they will do something: produce hallucinations (rarely), mimic psychoses (questionably), or "manifest the mind" (whatever that means). In other words, these names

TABLE 1. *Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA) studies of LSD in human subjects, 1953-73*

Type of Projects	No. of projects	Estimated no. of subjects	Estimated funding (in millions)
Intramural projects			
Clinical Center	20	150	$0.5
Addiction Research Center	66	300	0.8
Extramural projects	30	1,300	2.7
Total ADAMHA	116	1,750	$4.0

NOTE: This table appeared in an internal ADAMHA document, "Report on ADAMHA Involvement in LSD Research," prepared by the staff about 1975, based on records available in the agency and on telephone interviews with extramural investigators and former staff members. It contains this statement: "At the present time, ADAMHA does not fund any research involving administration of LSD to humans. This is not a policy, but rather the result of accumulated findings in the field."

suggest that the drugs are in control. In contrast, the psychotherapeutic labels have a different focus and connotation: The drugs are used as tools to achieve some medically desirable effect (either alone or in combination with analytic therapy or hypnosis), that is, the physician is in control in producing some beneficial effects for the patient. It is for this reason (i.e., implying that it is not that drugs are in control, as it is usually assumed in a drug abuse context, but that the physicians and researchers are using them as tools) that the use of the term "psychoheuristic" is proposed.

"Heuristic" is derived from the Greek word "heuriskein," to invent or disover, and is defined in Webster's *New Twentieth Century Dictionary* (McKechnie 1983) as follows: "helping to discover or learn; specifically, designating a method of education or of computer programming in which

the pupil or machine proceeds along empirical lines, using rules of thumb, to find solutions or answers."

The Random House College Dictionary (Stein 1980) gives the following definitions for heuristic: (1) "Serving to indicate or point out; stimulating interest as a means of furthering investigation; (2) (of a teaching method) encouraging the student to discover for himself."

It is in the first, general sense of the dictionaries' definitions that the word psychoheuristic is meant to be used: "helping to discover" and "stimulating interest as a means of furthering investigation" into the mechanism(s) by which some of the unique psychological effects are produced by these drugs and, beyond that, to serve as keys to unlock the mysteries of the brain/mind relationship.

UNIQUE CHARACTERISTICS OF PSYCHOHEURISTIC AGENTS

What are the unique characteristics of the effects of these drugs that point to their potential as psychoheuristic agents? The vivid, mostly geometric visual illusions are one of the hallmarks of LSD, DMT, and other major psychedelics. These illusions are sometimes so intense that they are seen as superimposed on any outside surface, be it a plain white wall or people's faces. As pointed out earlier in this chapter, these visual patterns are seldom perceived as having real outside existence; so they are, strictly speaking, illusions rather than hallucinations. Nevertheless, they are sufficiently striking and sometimes spectacular, so that they have been of some interest to psychologists (Klüver 1967; Oster 1970; Siegel 1977), to physiologists (Evarts 1957; Purpura 1957), and even to mathematicians (Cowan 1988). Some other unique characteristics might be the alteration of time perception, synesthesia, dehabituation, the extreme individual variability of many of their actions, the religious or mysticomimetic properties, and the so-called cultogenic effects. The reader can probably name a number of others.

However, most of these effects are interpretations and/or secondary consequences of the drugs' disturbances of some fundamental physiological or psychological processes that underlie humans' capacity to attend, to be aware of, and to regulate their relationships with the physical and social environment. Thus, the author's recommendation is to use these drugs in a heuristic mode to explore the biological correlates

and perhaps the mechanism(s) of the fundamental process that is frequently referred to by the psychoanalytic term of disturbance of "ego boundaries" or "oceanic feeling." This aspect has been emphasized especially by psychiatrists, among others (Fischman 1983). Freedman, in his much-quoted landmark paper *On the Use and Abuse of LSD,* puts it this way:

> It is my impression that one basic dimension of behavior latently operative at *any* level of function and compellingly revealed in LSD states is "portentousness"—the capacity of the mind to see more than it can tell, to experience more than it can explicate, to believe in and be impressed with more than it can rationally justify, to experience boundlessness and "boundaryless" events, from the banal to the profound. (Freedman 1968, p. 331)

Grof, who has perhaps more clinical research experience than anyone else in the world with LSD and other hallucinogens such as DPT, has concluded that the major psychedelics do not produce specific pharmacologic states (i.e., toxic psychosis) but are unspecific amplifiers of mental processes (Grof 1980). In other words, rather than producing effects that are specific for the drug, they activate mostly unconscious mental processes from various deep levels. These mental processes are specific for the personality of the individual. The major focus of Grof's therapeutically oriented work was to interpret unconscious memories for the perinatal experience of pain and trauma as an example of what he called "temporal expansion of consciousness," and to deal with the so-called transpersonal experiences as a result of spatial expansion of consciousness. The common denominator, he said, "in this rich and ramified group of phenomena is the feeling of the individual that his consciousness expanded beyond the usual ego boundaries and limitations of time and space" (Grof 1980, p. 94).

BOUNDARIES IN THE MIND AND THE BRAIN

The subjective phenomenon of loss of ego boundaries is not restricted to psychedelic experiences. In the twilight states of falling asleep and waking, people go through such experiences every day, although not everyone is fully aware of them. LSD and similar drugs have the unique property of producing similar twilight states while a person is fully awake and aware of them. This characteristic makes these drugs specially suited

to exploring the full extent of these general phenomena, including their postulated biological bases.

The generality of these phenomena is underscored by a broad psychological theory of boundaries proposed recently by Hartmann, a well-known sleep researcher. He put forward this suggestion in his book *Boundaries in the Mind* and claims that these boundaries represent a major dimension of personality that had largely been neglected (Hartmann 1991). In the course of studies on people with nightmares, Hartmann was struck by the observation that such people have a group of common characteristics that could be described as open, unguarded, sensitive, fluid, artistic, and vulnerable. The description that seemed best to encompass all these people was that they had "thin boundaries" in many different senses. In contrast, among the control subjects, Hartmann found a significant group of people who could be characterized as having "thick boundaries" in the sense that they have a very solid, separate sense of self; they keep emotionally distant from most others; and they do not become overinvolved, sometimes appearing inflexible, even rigid. This distinction seems to hold in many areas of interpersonal relations in these extreme groups, but there were people who were in between.

The initial evaluation was based on Rorschach tests (popularly known as inkblot tests) of individuals participating in Hartmann's sleep electro-encephalogram (EEG) studies. Watson (1985) has found EEG correlates of this dimension: thin boundary individuals producing significantly larger numbers of phasic integrated potentials (PIP), also known as ponto-geniculo-occipital (PGO) spikes, on the boundaries of rapid eye movement (REM) and non-REM sleep stages.

Hartmann (1991) also has developed a 145-item questionnaire that could be used to quantify the thick and thin dimensions. The questionnaire covers 12 categories of psychological phenomena such as childhood experiences, interpersonal relations, habits, opinions, and sleep-wake and dream-recall patterns. Most people do not score in the extreme in each category but thick in some areas and thin in others. Among 300 subjects, there was some statistically significant correlation of this dimension to some of the scales of the Minnesota Multiphasic Personality Inventory (MMPI), but a closer analysis indicated that the boundary scale is definitely not measuring sickness or psychopathology.

The concept of boundaries, Hartmann (1991) claims, should be helpful in understanding and preventing the potential consequences of some

psychologically crippling problems and could be useful in counseling for marital problems and career choices. Hartmann did not point it out explicitly, but this concept may also be useful in rehabilitation of addicts, because thin-type personalities may be more vulnerable to peer pressure. Hartmann does mention that among the various psychoactive drugs, the psychedelic types are the only ones that produce a temporary thinning of inner boundaries. None of Hartmann's thick boundary subjects has reported ever taking LSD; only some of the mixed types did. However, one of his thin boundary subject said: "I can see why some people might like this sort of loosening or merging and the vivid images, but it's not for me. I'm too much like that anyway, without drugs." (Hartmann 1991, p. 239). One wonders whether this questionnaire would be useful in drug abuse prevention and counseling, but it would definitely be a good research tool in quantifying the boundary dimension in any clinical experimental study of this trait.

EXPLORING INNER BOUNDARIES

So much for the potential significance of the boundary concept. The question arises: How should one go about exploring the biology of this dimension of personality with the help of psychoheuristic drugs as tools?

Besides the tools that can be considered to be heuristic in the general sense, investigators need new concepts, new guidelines, and new models to serve as positive heuristics in the sense used by Lakatos, the noted philosopher of science, who focused on conceptual and strategic problems in the 1960s and 1970s in defining new research programs (Lakatos and Musgrave 1970). Lakatos' use of heuristics is in the second, specific dictionary sense: Defining some concrete rules that should guide the research. His striking example was from the historical development of quantum mechanics based on the positive heuristic model of the atom by Bohr. Bohr's model is now known to be only approximately true; nevertheless, it resulted in spectacular progress in research that resolved the structure of the atom. In the same spirit, one might use a version of the hierarchic models for the brain that may not be perfect but that might provide better conceptual guidance than the currently used information-processing paradigm.

The source for such a paradigm might be found in hierarchy theory as it is being developed primarily by evolutionary biologists and ecologists (Allen and Starr 1988; O'Neill et al. 1986; Salthe 1985) to come to grips

with the complexities of the thoroughly interconnected ecosystem. These researchers do not look at hierarchy in the sense of rigid organization or classification but as a dynamic control system that can be comprehended only as functioning on three levels simultaneously as a whole (also called a holon): the focal level that is the immediate object of scrutiny, the level above that serves as a boundary of constraints, and the level below that consists of the processes and semiautonomous entities under direct control of the focal level (figure 1) (Grene 1987; Salthe 1985). These scientists prefer to call these models holarchies rather than hierarchies, following Koestler (Koestler and Smythies 1969).

It is generally accepted that the brain is largely hierarchically organized. In the visual sensory system, for example, among the 305 pathways interconnecting 32 cortical visual areas, it is possible to identify 10 hierarchic levels of cortical processing of visual information (Van Essen et al. 1992). However, the application of information-processing concepts is limited to two levels at a time in identifying the anatomical areas where the pathways originate and terminate. The three-level control hierarchy concepts in Salthe's sense have not been applied to central control processes of the brain such as attention and memory or to sensations such as pleasure and pain.

Another discipline that could be tapped for conceptual guidance and for mathematical bookkeeping tools is Game Theory (Dyke 1988; Von Neumann and Morgenstein 1944). Concepts such as competition and cooperation have been widely used in enzymology, in receptor studies, and in neural network models, but Game Theory offers much more than these everyday concepts: mathematical rigor and heuristic techniques that could be applied to behavioral and brain processes for better payoffs (Maynard Smith 1984). Time and space do not allow for an elaboration on these suggestions at this time, but it may be important to stress a few points.

In terms of practical approaches to this level of complexity, researchers should take advantage of the availability of increasingly powerful (and relatively inexpensive) computers and test the concepts and hypotheses first in so-called neural network models. Such models could sharpen the conceptual frameworks and generate hypotheses that, in turn, could be tested on real brains and, to some extent, on real people (figure 2).

Among the other tools that have been refined to high sophistication in recent years is positron emission tomography (PET) scanning, which can

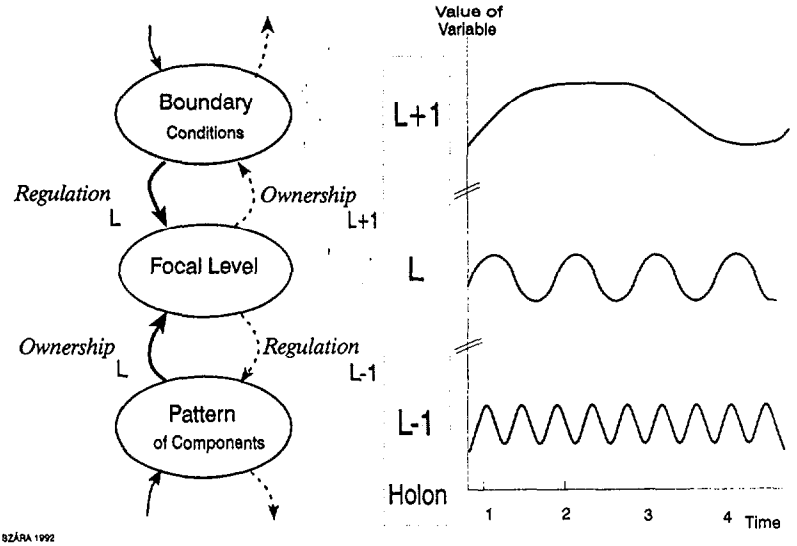

FIGURE 1. *Diagram sketching the principles and dynamic relationships among the components of the proposed game-holarchy paradigm for guiding research into subjective psychobiological phenomena such as boundaries.*

NOTE: In the center of figure 1, L+1, L, and L-1 designate three levels of a holon and refer to both the left and the right side of the diagram. On the left side, in line with L is the focal level of a currently active holon, above which and in line with L+1 are the boundary conditions (rules and regulations) for awareness and action. L-1 represents a pattern of components that is perceived as being under the control (ownership) of the focal level L. When a holon is engaged in a game with other holons, its choice of action is always constrained (regulated) by the rules of the boundary conditions (L+1). Cooperation and competition involve lower level components (residing in L-1) that can be lost or new components gained as payoffs. When the focal level shifts, say to level L-1, then the previous level L becomes the boundary condition that regulates the action of the new holon. Similarly, when the focus shifts to level L+1, the boundary conditions shift one level above, as suggested by the top arrows, and the previous level L becomes a pattern of components that is owned by the new focal level. The right side of the diagram symbolically represents the relative temporal dynamic relationships among the variables in the three levels of a holon.

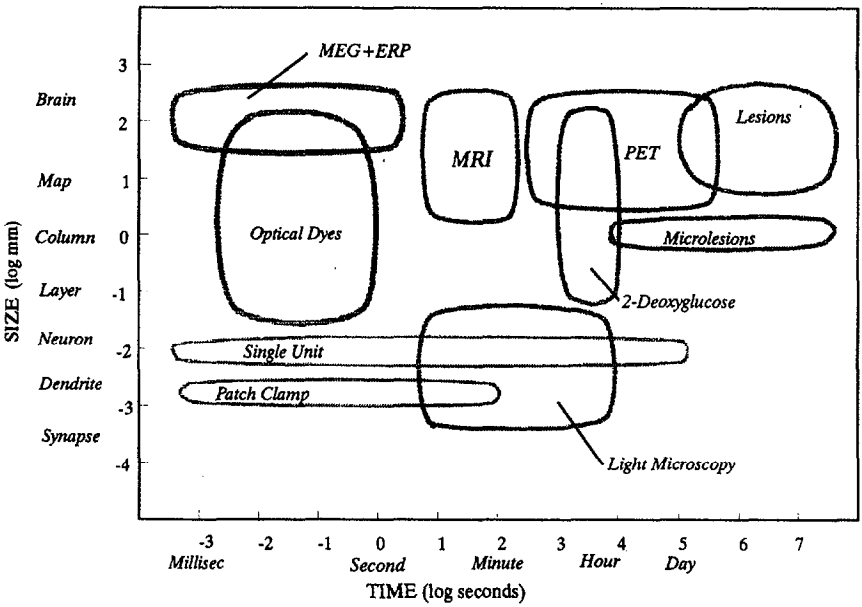

FIGURE 2. *Schematic illustration of the ranges of spatial and temporal resolution of various experimental techniques for studying the functions of the brain.*

KEY: MEG = magnetoencephalography, ERP = evoked response potentials, PET = positron emission tomography, MRI = magnetic resonance imaging.

SOURCE: Churchland, P.S., and Sejnowski, T.J. Perspectives on cognitive neuroscience. *Science* 242:741-745, 1988. Copyright ©1988 by the American Association for the Advancement of Science, Washington, DC.

be used for exploring spatial dimensions and localization of some biochemical (enzymes, receptors) or physiological (blood flow) processes relevant to hierarchic boundaries. Recently, a modification of magnetic resonance imaging (MRI), the so-called fast MRI, was shown to be capable of following regional blood flow in the brain that occurs within seconds after perceptual or cognitive stimulation, in contrast to the several minutes time scale needed for PET scan. A further advantage of MRI is its noninvasive nature; this technology uses intrinsic signals of venous blood oxygenation for imaging (Ogawa et al. 1992). Another technique, evoked response potentials (ERP), might also be useful to resolve temporal dimensions of the same boundaries. These are just a

few examples; many other methods could be profitably employed as required by the questions at hand (Churchland and Sejnowski 1988).

Last, but not least, there are some useful placebos that should sharpen any research design. Bromo-LSD could serve as a nonpsychoactive analog for LSD in a certain dosage range, and 6-fluoro DET can be used as an active placebo for the shorter acting psychoheuristic drug DET as demonstrated more than 25 years ago by Faillace and colleagues (1967).

SUMMARY

The author argues in this chapter for a reconsideration of the perception of hallucinogens as being only toxic, damaging, and therefore strictly condemnable for being abused. The author advocates that hallucinogens be viewed as powerful psychoheuristic tools that, in combination with other necessary conceptual (such as holarchic theory) and laboratory tools (such as PET scan or MRI), may help solve a major mystery of nature: the workings of human brains and minds.

NOTE

The National Institute on Drug Abuse is among the many Federal departments and agencies participating in programs and activities to observe the "Decade of the Brain" beginning January 1, 1990. Additional information about this effort can be found in the bill (H.J. Res. 174, March 8, 1989), which became Public Law 101-58 on July 25, 1989.

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AUTHOR

Stephen Szára, M.D., D.Sc.
Chief
Biomedical Research Branch (Ret.)
National Institute on Drug Abuse
10901 Jolly Way
Kensington, MD 20895

Lysergamides Revisited

Robert C. Pfaff, Xuemei Huang, Danuta Marona-Lewicka, Robert Oberlender, and David E. Nichols

INTRODUCTION

In discussions of hallucinogens in past years, most of the focus has been on phenethylamines and phenylisopropylamines, with a modest amount on tryptamines. A large gap always has been the lack of discussion of lysergamides. Lysergic acid diethylamide (LSD) is one' of the classic hallucinogenic agents, but substituted lysergamides always have seemed to be largely ignored. The lysergamides have been investigated on several historical occasions, but the late 1950s witnessed most of the recent work (Abrahamson 1959; Cerletti and Doepfner 1958; Gogerty and Dille 1957; Isbell et al. 1959). Within the past 8 years, there has been an attempt to fill in some obvious gaps that exist in the understanding of lysergamide-type hallucinogens.

The lysergamides are derived from the ergot fungus, well known throughout history as a source of various types of medications. Extracts of ergot have been recognized as legitimate pharmaceutical preparations since at least the Middle Ages. Lysergic acid is derived from hydrolysis of the ergot alkaloids. Although present-day methods of ergot production utilize submerged culture fermentation rather than cultivation on rye or other cereal grains, *Claviceps* is the ultimate alkaloid source.

It is also known that lysergamides have been employed as psychoactive preparations, with some of these uses stretching back to antiquity. For example, arguments have been made that the Greek mysteries at Eleusis were related to the ingestion of a preparation that contained lysergamides derived from an ergot fungus that infested the grass in that region. Another example is ololiuqui, a South American Indian and Mexican psychoactive preparation that was prepared from the seeds of certain morning glories, *Rivea corymbosa.* Ergine, or lysergic acid amide (figure 1), is the primary psychoactive component of morning glory seeds. In 1943, Hofmann discovered the unusual properties of LSD, the diethyl amide or lysergic acid.

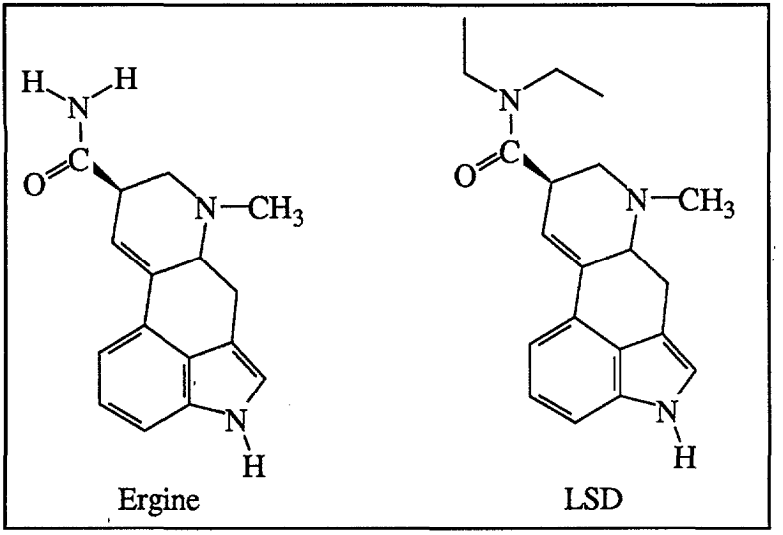

FIGURE 1. *Structural representations of ergine and LSD*

OVERVIEW OF THE ERGOLINES

All ergolines have a tetracyclic framework based on the aromatic two-ring indole nucleus. Lysergic acid has a carboxy function at the 8-position. Because of their structural complexity, lysergamides have several locations that can be modified for structure-activity studies of these hallucinogens. These are shown in figure 2.

Substitution at the N(1) position generally attenuates or abolishes hallucinogenic activity (Brimblecombe and Pinder 1975). Substitution at the C(2) position, particularly with a halogen such as bromine (e.g., BOL) or iodine, leads to compounds that not only are inactive as hallucinogens but also can antagonize the effect of a subsequently administered dose of LSD (Brimblecombe and Pinder 1975).

The stereochemistry is critical for the lysergic acid molecule. The *R* stereochemistries at both the C(5) and C(8) positions are essential. Inversion of either stereocenter abolishes hallucinogenic activity (Brimblecombe and Pinder 1975). C(5) inversion gives *l*-lysergic acid derivatives, as compared with the natural *d*-lysergic acid. Epimerization at the C(8) position gives the isolysergic acid or iso-LSD derivatives.

Reduction of the 9, 10-double bond also abolishes hallucinogenic activity (Brimblecombe and Pinder 1975). Ring substitution at the C(12) or

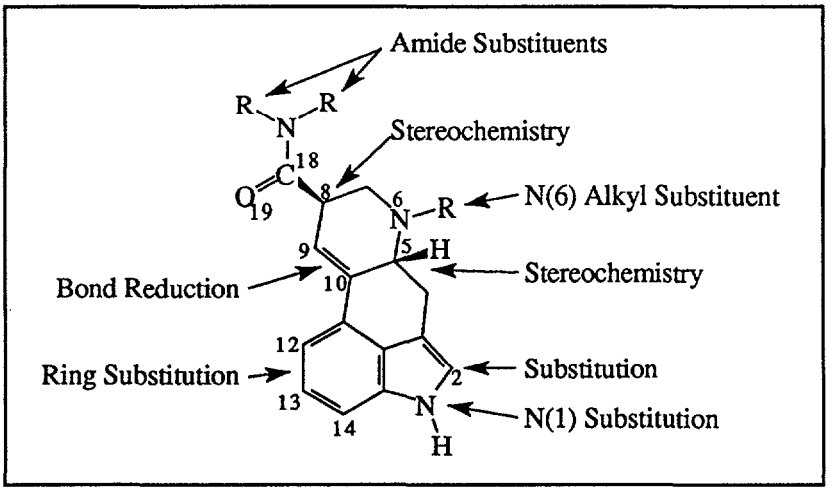

FIGURE 2. *Locations and types of structural modifications studied for lysergamides*

C(13) positions is fairly difficult. Because entire doctoral theses have been written about the total synthesis of lysergic acid, it is apparent that the synthesis of derivatives modified at the 12-, 13-, or 14-position would be quite a formidable task. Nevertheless, the 12-hydroxy compound was prepared years ago. The authors obtained a sample of this and performed drug discrimination (DD) studies in LSD-trained rats. It had unremarkable properties, with only about 20 percent of the potency of LSD (Pffaf et al., unpublished observations).

It also has been postulated that hydroxylation in vivo occurs at the 13-position. This occurrence would correspond to the 6-hydroxy-tryptamines that Szára (this volume) discusses. There is evidence in the literature that hydroxylation at this position confers high dopaminergic potency on ergolines, but it is not clear whether this could be related to the hallucinogenic properties of LSD.

These ring modifications are generally the only ones that have been studied, primarily because of the difficulty in carrying out chemistry on a complex molecule like an ergoline.

EFFECTS OF N(6) SUBSTITUTION

One major gap in the literature that the authors looked at some years ago was substitution on the basic N(6) nitrogen atom. Recently, the authors have examined substitutions on the amide nitrogen. Dopaminergic compounds had been studied for many years, and it was known that if the alkyl group on the N(6) nitrogen was extended from methyl to ethyl to propyl, the dopaminergic effects of the ergolines were maximal. About 10 years ago, the authors envisioned that a propyl group placed on the N(6) nitrogen of LSD might optimize its dopaminergic effects. Consequently, it might be possible to determine, in some way, the importance of that pharmacological component to the overall action of the drug. It was decided to make a series of N(6)-alkyl substituted compounds. At about the same time, Niwaguchi and colleagues (Nakahara and Niwaguchi 1971; Niwaguchi et al. 1976) also prepared a small series of N(6)-alkyl substituted lysergamides and examined them in smooth muscle preparations; they reported high activity. Therefore, the authors synthesized several N(6)-alkyl derivatives in the laboratory. Testing data for these are summarized in table 1 (Hoffman 1987).

TABLE 1. *Drug discrimination ED_{50} values and receptor affinities of N(6)-alkyl-nor-LSD derivatives*

Compound	DD* (µmol/kg)	K_I(nM)		
N(6)-alkyl	ED_{50}	[^{3}H]-5-HT	[^{3}H]-ketanserin	[^{125}I]-*R*-DOI
Allyl	0.013	15.5	8.1	3.4
Ethyl	0.020	3.8	5.1	5.1
Propyl	0.037	4.9	5.6	
Methyl (LSD)	0.046	5.9	5.2	5.1
CH_2-c-C_3H_5	0.067	10.9	7.7	
i-Propyl	0.10	21.4	14.1	
n-Butyl	0.36	45.7	5.2	
Propargyl	0.62	91.2	100.0	
2-Phenethyl	NS†			
H (nor-LSD)	NS†	30.2	158.0	

* Rats (n = 8) were trained to discriminate 0.08 mg/kg (+) LSD from vehicle.

† No substitution occurred with these analogs.

KEY: DD = drug discrimination; nM = nanomolar; [^{125}I]-*R*-DOI = [^{125}I]-*R*-1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane

In the first column are DD data. Glennon (this volume) describes the DD paradigm, so it will be noted simply that LSD was used as the training drug at a dosage of 0.08 milligrams per kilogram (mg/kg) of the tartrate salt. The DD data are median effective dose (ED,) values for compounds that fully substituted. The ED, values are in micromolars (μmol)/kg, so direct potency comparisons can be made that take into account differences in molecular weight.

Displacement of tritiated serotonin in rat brain homogenate was investigated several years ago as a measure of affinity for serotonin receptors. This work was done prior to some of the differentiation of receptor subtypes; the data in column 2 probably closely represent inhibition constant (K_I) values at the 5-HT_{1B} receptor subtype (Pazos and Palacios 1985). Displacement of tritiated ketanserin from the serotonin 5-HT_2 receptor also has been investigated, and on a few compounds there exist data for displacement of [^{125}I]-*R*-DOI from the agonist state of the 5-HT_2 receptor.

The DD data indicate that the *n*-propyl is slightly more active than LSD, although not significantly so, but the ethyl and allyl compounds were significantly more potent than LSD. From the serotonin displacement data alone, there is no obvious basis for these results, although perhaps the ethyl and propyl compounds have slightly higher affinity. The cyclopropylmethyl compound is somewhat less active, but the isopropyl is even less so, indicating that branching adjacent to the nitrogen atom is not well tolerated. The data for the isopropyl derivative can be compared with those for the *n*-propyl derivative. The *n*-butyl compound drops off about tenfold in potency. There is a medicinal chemist's adage that says something like "ethyl, propyl, butyl, futile." However, it more properly should be stated as "ethyl, propyl, butyl is futile," because in most cases when *N*-alkylated amines are extended beyond propyl, a marked drop in activity is seen.

The allyl compound, which was the most potent, differs from the propargyl in that the allyl has two sp^2 carbon atoms and a double bond, whereas the propargyl has two *sp* carbon atoms and a triple bond. There is a dramatic difference in the activity of the allyl versus the propargyl. In general, a decrease in receptor affinity is seen with the less active compounds. For example, the propargyl has about one-twentieth the affinity of most of the more active analogs at ketanserin sites.

The 2-phenethyl derivative was included because, in the opiates, this substituent often gives high activity. This compound did not substitute in LSD-trained animals, so there was no followup with binding studies. NorLSD did not substitute either, although it has modest affinity for 5-HT_1 sites.

This was an area of the structure-activity relationships (SAR) that had been unfilled, and there is now some knowledge of the effect of the N(6) alkyl group on activity of lysergamide hallucinogens. If the animal data are used as a criterion, it is known that LSD is, in fact, not the most potent LSD-like agent; the ethyl and allyl compounds are more potent. Clinical data presented by Jacob and Shulgin (this volume) seem to corroborate this observation.

Figure 3 shows wire-frame stereo-pair representations of the energy-minimized structures of the N(6)-allyl, -ethyl, and -propargyl compounds viewed from the top, or ß, face. The authors attempted to determine whether there was any basis for the fact that the propargyl is not very active, whereas the allyl is potent. The N(6)-ethyl has a fair amount of flexibility; it tends to lie with the terminal methyl below the ring plane as shown in the figure. The allyl adopts a similar orientation but with the terminal CH_2 of the double bond projected further toward the back face of the molecule. The N(6)-propargyl also is projected in a similar orientation. Looking at these molecules from the edge (figure 4) shows that the CH_2 of the allyl group is projected toward the lower face.

The terminal alkyne of the propargyl is rigid and has a linear geometry; it may be that it is not flexible enough and is unable to get out of the way, preventing the molecule from interacting favorably with the receptor. Otherwise, there seems no obvious reason the allyl compound should be so potent whereas the propargyl has such low activity.

EFFECTS OF AMIDE SUBSTITUTION

The other major area now being examined is substitution on the amide function. Most lysergamides that have been subjected to clinical studies were reported in the mid- to late-1950s. Table 2 lists relative potency in humans for most of the amides that were studied. The amides were not studied systematically, and their characterization in clinical studies was rudimentary. The data for the compounds that were studied do not give any insight as to why the diethyl substitution of LSD should be so potent.

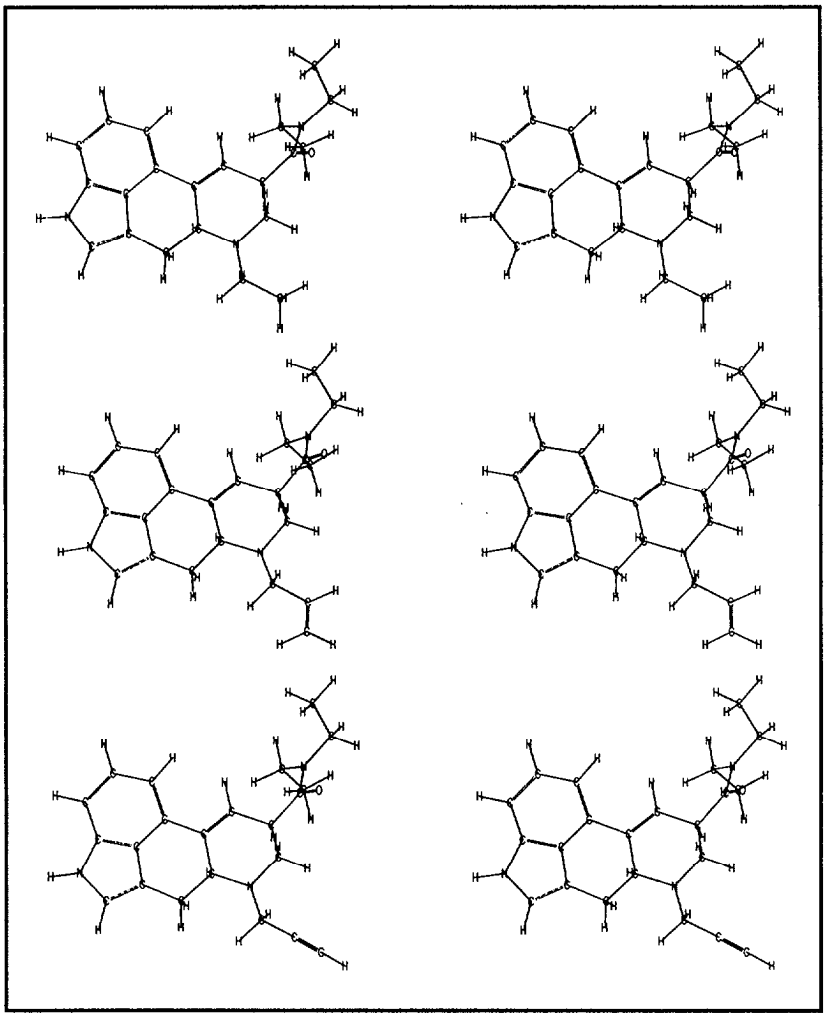

FIGURE 3. *Facial stereo views of the N(6)-alkyl LSD derivatives. Top: ethyl: center: allyl; bottom: propargyl*

It should be noted in table 2 that none of the compounds has more than about 30 percent of the activity of LSD. This is something that has always perplexed researchers in this field: What is it about the diethyl group that may be unique in this molecule? Even a substitution with the same number of carbon atoms, such as the *N*-methyl-*N*-propyl derivative, shows only about one-thirtieth the potency of LSD. The *N*-methyl-*N*-propyl presumably would have similar pharmacokinetics, with

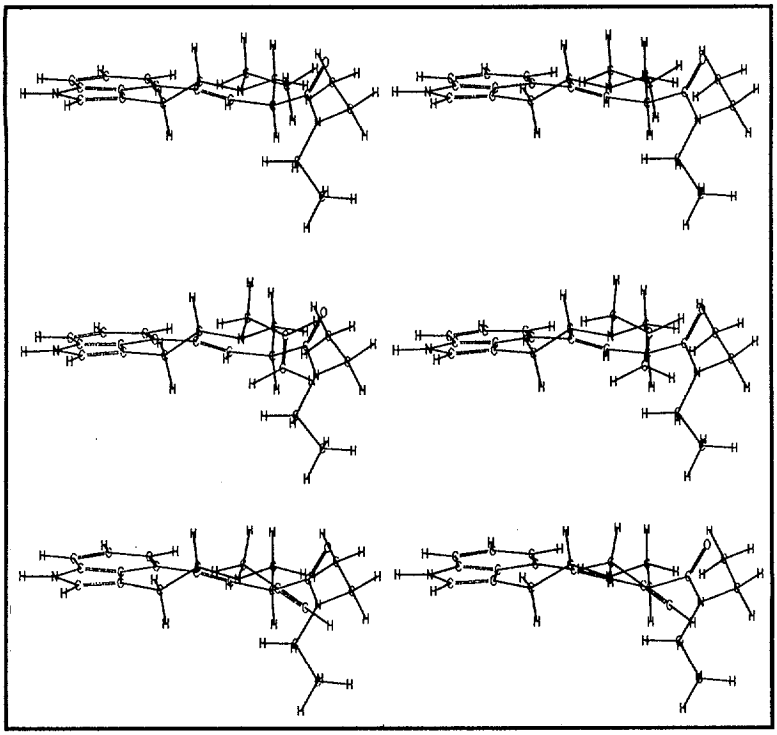

FIGURE 4. *Edge-on stereo views of the N(6)-alkyl LSD derivatives. Top: ethyl; center: allyl; bottom: propargyl*

comparable amounts of the drug expected to enter the brain, and yet this compound is only weakly active. Even the *N*-methyl-*N*-ethyl has low activity compared with LSD. With the *N,N*-diethyl (LSD) one sees optimum activity, but the *N*-ethyl-*N*-propyl is back to about one-third the potency of LSD, and *N,N*-dipropyl is down to one-tenth.

Compounds are being designed that might allow one to probe the dialkyl amide function and to try to understand what role it plays. If, in fact, the *N,N*-diethyl does have unique properties, why is this so? The authors' first attempt to address this problem was to "tie together" the two ethyl groups of LSD as a three-membered dimethylaziridine ring.

The target compounds are shown in figure 5. It was envisioned that the dimethylaziridines might resemble three possible conformations of the more flexible diethyl amide. There are three dimethylaziridines: a trans-

TABLE 2. *Relative human potency of lysergic acid amides**

R_1	R_2	Relative Potency
H	H (lysergamide, ergine)	3
H	CH_2CH_3	10
H	$CH(CH_2OH)CH_3$ (ergonovine)	3
CH_3	CH_3	10
CH_3	CH_2CH_3	3
CH_3	$CH_2CH_2CH_3$	3
CH_2CH_3	CH_2CH_3 (LSD)	100
CH_2CH_3	$CH_2CH_2CH_3$	32
$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	10
C_4H_8	(Cyclic pyrrolidide)	32
C_4H_8O	(Cyclic morpholide)	32

* Derivatives of ergine (figure 1) where the terminal amide -NH, has been replaced by $-NR_1R_2$

SOURCE: Data from Shulgin 1981

isomer with *R,R* stereochemistry, a transisomer with *S,S* stereochemistry, and a cis-meso compound in which both methyls are on the same side of the aziridine ring. These three molecules are isomeric probes that are similar in molecular weight to LSD. However, the methyls of what would be the corresponding ethyl groups in LSD are in different orientations. It was anticipated that evaluation of these compounds might offer insight into the role of the ethyl groups of LSD. Unfortunately, this was not to be. Acyl aziridines under acidic conditions are chemically labile. After condensation of the dimethylaziridines with lysergic acid—the amide protonates—the chloride then attacks the aziridine ring, which then opens to yield the chlorobutyl derivatives (Oberlender 1989) as shown in figure 6.

In the case of the trans-*R,R* enantiomer, chloride attack at either carbon 2 or 3 gives the same isomer, which has the 1*R*,2*S* configuration. Similarly, chloride attack at either of these positions in the trans-*S,S*. isomer gives the same diastereomer, the 1*S*,2*R* chlorobutyl. Depending on which center is attacked with the cis-meso compound, either the *R,R* or *S,S* diastereomer is obtained. When these four compounds (figure 7) were obtained, the authors carried out model reactions acylating the

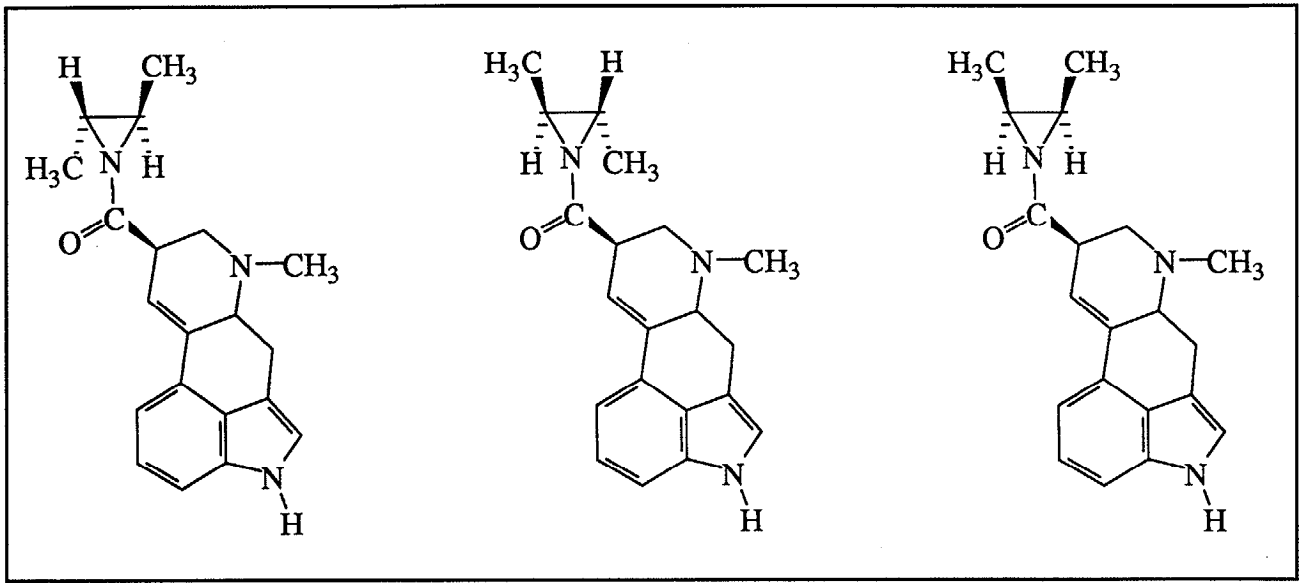

FIGURE 5. *The dimethylaziridinyl amides of lysergic acid. Left: R,R-dimethylaziridinyl; center: S,S-dimethylaziridinyl; right: cis-meso-dimethylaziridinyl*

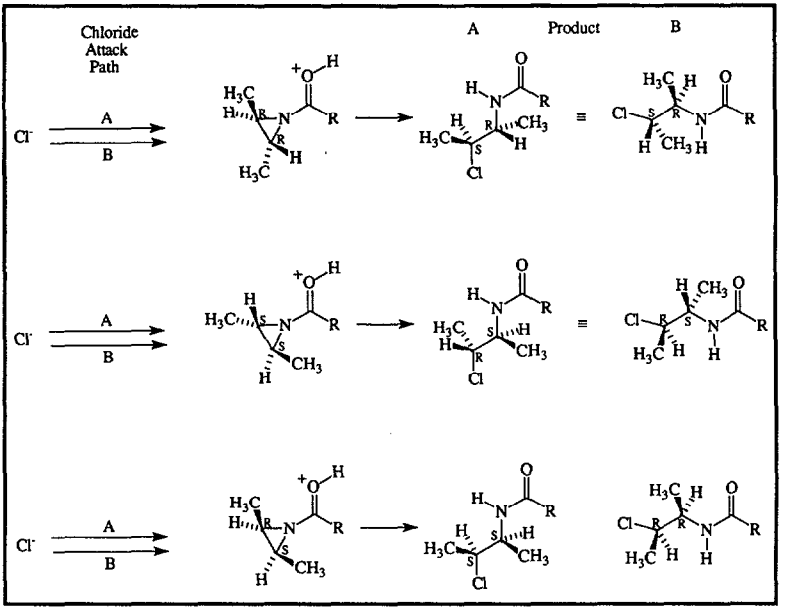

FIGURE 6. *Proposed mechanism of attack of chloride on the dimethylaziridinyl lysergamides*

dimethylaziridines with benzoyl chloride to prove that this occurred, and then chemically established that these were the products of the condensations between the dimethylaziridines and lysergic acid.

The four diastereomers were separated, purified, and tested in DD studies, and the data are summarized in table 3. It was found that one of the diastereomers was about 50 percent more potent than LSD. This was the first indication that something other than the diethyl amide might give high activity. Because this is a DD assay, it can give false positives, but it rarely, if ever, gives false negatives. So it seems probable that these compounds might have LSD-like activity. Yet, they are monoalkyl amides, not dialkyl amides. There is no precedent for a monoalkyl amide to have an activity approaching that of LSD. It should be noted that diastereomers 1 and 2 are obtained from racemic trans-dimethylaziridine, and diastereomers 3 and 4 are obtained from cis-dimethylaziridine. Diastereomers 1 and 3 are most potent, one derived from trans- and the other from cis-dimethylaziridine. Diastereomers 2 and 4 are much less potent. The pyrrolidide was included for comparison because it is known that in humans the pyrrolidide has about 10 to 15 percent of the potency

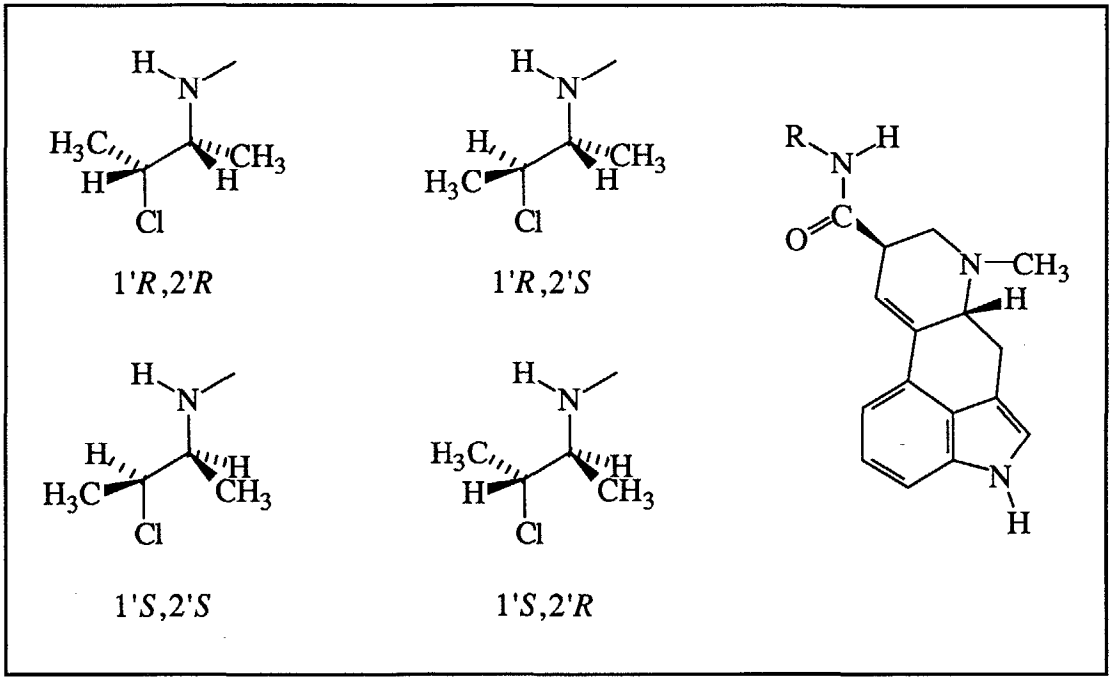

FIGURE 7. *The stereochemistries of the products of chloride attack on the dimethylaziridinyl lysergamides*

TABLE 3. *Potency of chlorobutyl and pyrrolidyl lysergamides in rats trained to discriminate 0.08 mg/kg of (+)LSD from vehicle*

Compound	ED_{50} (µmol/kg)	Relative Potency
Diastereomer 1	0.027	155
LSD	0.042	100
Diastereomer 3	0.156	27
Pyrrolidide	0.168	25
Diastereomer 4	0.387	11
Diastereomer 2	0.605	7

TABLE 4. *Radioligand-binding data for 2-butyl lysergamides*

Lysergic Acid Amide substituent	K_I (nM)		IC_{50} (nM)	
	5-HT_2 *	5-HT_{1A} †	D_1 ‡	D_2 §
N,*N*-diethyl (LSD)	6.31	5.05	60	13
R-2-butyl	2.63	2.01	44	15
S-2-butyl	7.76	4.61	70	37

KEY: Data are for the displacement of *[^{125}I]-*R*-DOI in rat frontal cortex homogenate, †[^{3}H]-8-OH-DPAT in rat frontal cortex homogenate (Oberlender et al. 1992), ‡[^{3}H]-SCH23390 in rat striatal homogenate, and §[^{3}H]-spiperone in rat striatal homogenate (unpublished results).

of LSD (Nichols et al. 1991). In DD trials, pyrrolidide shows considerably lower potency than LSD.

To simplify things, it was decided to prepare lysergamides from the enantiomers of 2-aminobutane, shown in figure 8. These were commercially available with either the *R* or *S* stereochemistry at the α-carbon (C-α).

The 2-aminobutane amide derivatives of lysergic acid were viewed as analogous to the chlorobutyl compounds but dechlorinated to remove the second stereocenter. Table 4 shows binding affinity data using

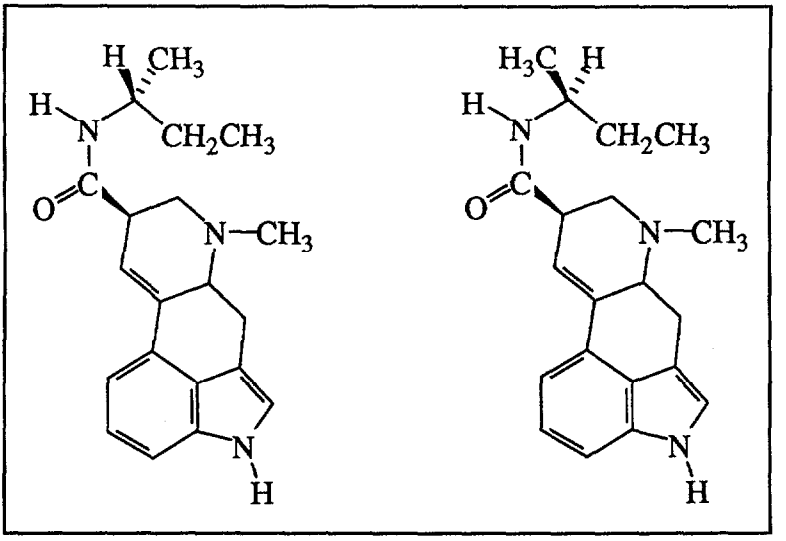

FIGURE 8. *The secbutyl lysergamides. Left: the diastereomer from the R-isomer; right: the diastereomer from the S-isomer*

[^{125}I]-*R*-DOI as a label for the 5-HT$_2$ receptor; [^{3}H]-8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) as a label for the 5-HT$_{1A}$ receptor; and [^{3}H]-SCH23390 and [^{3}H]-spiperone in rat caudate as labels for the dopamine (DA) type 1 (D$_1$) and type 2 (D$_2$) receptors, respectively. Some of the data were recently published (Oberlender et al. 1992).

As is already known, LSD has high affinity for the 5-HT$_2$ and the 5-HT$_{1A}$ receptors (Nichols et al. 1991). Interestingly, 60 nM is a respectable affinity for the D$_1$ receptor, and 13 nM is certainly a high affinity for the D$_2$ receptor. It should be noted that the dopaminergic component of LSD has not been examined in any detail. Therefore, it can be seen that LSD has high affinity at these four possible recognition sites, and it is known also to have high affinity for other sites. Note that of the two secbutyl diastereomers, with *R* and *S* stereochemistry in the side chain, the *R* isomer has significantly higher affinity than LSD at both serotonin recognition sites and comparable affinity at the D$_1$ and D$_2$ sites. This compound, at least from the receptor-binding profile, looks more potent than LSD. Even the diastereomer from the *S* isomer has affinities similar to LSD.

One interesting aspect that has not been studied is the significance of these high affinities for the D$_1$ receptor. This may be the first report of

the D_1 receptor affinity for LSD. Presently, there is great interest in the D_1 receptor, in part related to its functional interactions with D_2 receptors and in part because of the possibility that D_1 receptor activation is necessary for expressing the biological activity of compounds that have a D_2 dopaminergic effect. With affinities of this magnitude, the D_2 effect of LSD must be considerable, and it could be anticipated that the D_1 receptor also might play a role in the overall action of lysergamides. It never before has been recognized that these compounds have such high affinity for the D_1 receptor.

Functional measures seem to parallel the serotonin receptor-binding data. Sanders-Bush (personal communication. 1992) has examined the ability of the 2-butyl lysergamides to stimulate phosphoinositide turnover in a cloned cell line transfected with 5-HT_2 receptor carrier deoxyribonucleic acid (cDNA). Both diastereomers were partial agonists, giving 75 to 80 percent of the response produced by 1 µM 5-HT. The diastereomer from the *R*-2-butyl enantiomer had approximately twice the potency of that from the *S* enantiomer. However, these diastereomers had only about 5 to 10 percent of the potency of LSD despite their comparable receptor affinity. Similarly, at the rat choroid plexus 5-HT_{1C} receptor, both diastereomers were partial agonists, with the diastereomer from the *R* enantiomer of 2-butylamine again having approximately twice the potency of that from the *S* enantiomer.

Mayaani (personal communication. 1992) also has examined these diastereomers for their ability to inhibit forskolin-stimulated adenylate cyclase in rat hippocampal homogenate. Both diastereomers were full agonists, comparable to 8-OH-DPAT. Again, the diastereomer from the *R* enantiomer of 2-butylamine was more potent than that from the *S* enantiomer.

Figures 9 and 10 illustrate edge-on and top views of the fully energy-minimized structures of LSD (top), the diastereomeric lysergamides of *R*-2-aminobutane (center), and *S*-2-aminobutane (bottom) as wire-frame stereo-pairs. Nothing is readily obvious from the structures, but it is possible to make an empirical observation that in the low-energy conformation of the diastereomer of the *R* enantiomer, the amide group "looks" a bit more like LSD. The amide of the diastereomer from the *S* enantiomer looks quite different in that all the bulk of the 2-butyl alkyl group is projected to the left of the molecule.

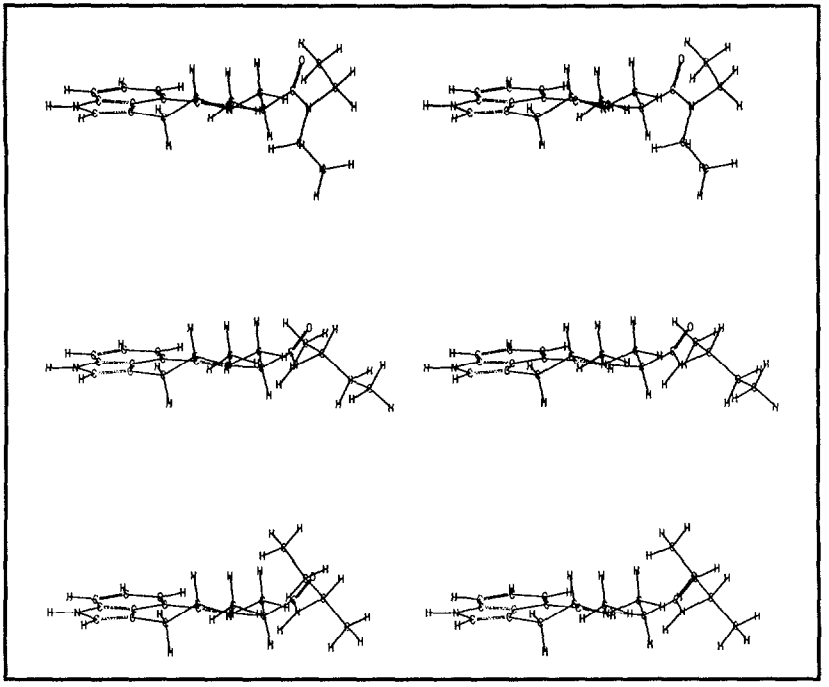

FIGURE 9. *Edge-on stereo views of LSD and the secbutyl lysergamides: Top: LSD: center: R-secbutyl lysergamide; bottom: S-secbutyl lysergamide*

These are flexible molecules, so no firm conclusions can be drawn. However, looking at the low-energy conformations, perhaps it can be said that the *R* isomer looks more like LSD than the *S* isomer. That begs the question and does not explain why LSD is active, but it is an interesting observation.

DISCUSSION

Much more work is needed on these amides. A series of compounds with a range of biological potencies is required to develop SARs. The authors now are trying to develop systematic series of amide-substituted compounds. Combining conformational analysis with biological activity data, some appreciation may be gained of the role of the amide in the lysergamides.

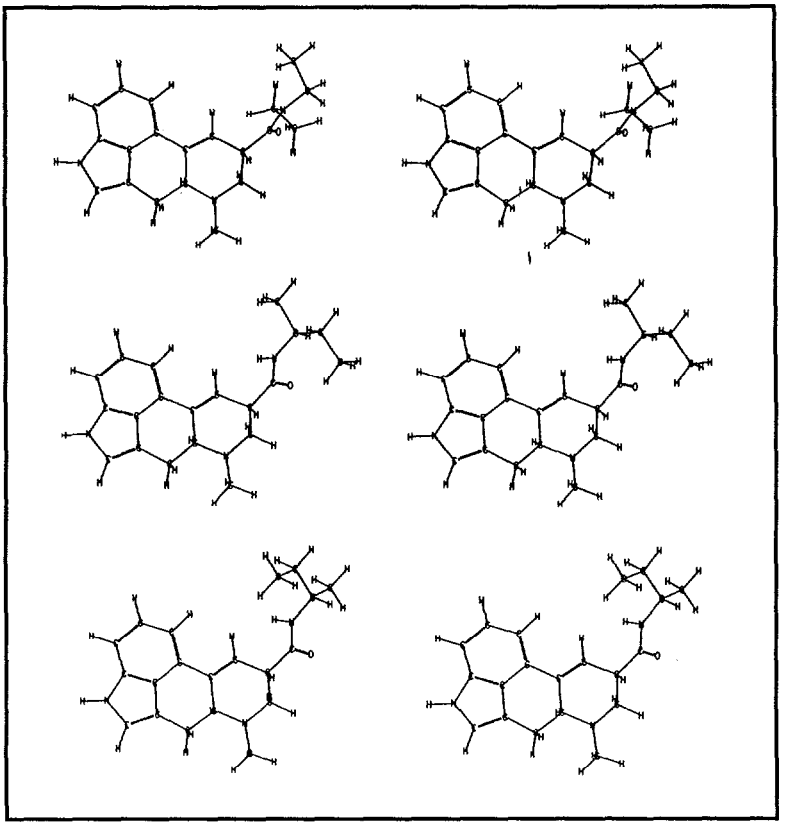

FIGURE 10. *Facial stereo views of LSD and the secbutyl lysergamides: Top: LSD; center: R-secbutyl lysergamide; bottom: S-secbutyl lysergamide*

Another compound to be studied is the methylisopropyl amide of lysergic acid (figure 11). This is an isomer of LSD where a methyl has been "removed" from one ethyl group and placed onto the α-position of the other. Receptor-binding data for that compound are compared with LSD in table 5. The affinity profile looks virtually identical to LSD for these four receptor types.

DD studies also have shown that this compound fully substitutes in animals trained to discriminate saline from LSD. Thus, it can be anticipated from the receptor-binding and behavioral data that this compound might be LSD-like.

The authors have been collaborating with Dr. Wilfred Dimpfel of the ProScience Private Research Institute in Germany, who employs a

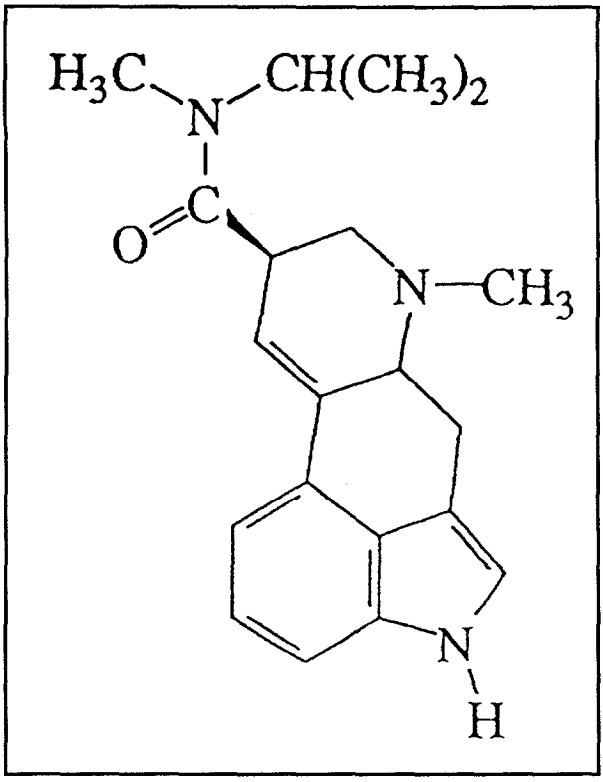

FIGURE 11. *The isopropyl amide of lysergic acid*

TABLE 5. *Radioligand-binding data for N-methyl-N-isopropyl lysergamide*

Lysergic Acid	K_I (nM)		IC_{50} (nM)	
Amide substituent	5-HT_2 *	5-HT_{1A} †	D_1 ‡	D_2 §
N,*N*-diethyl (LSD)	6.31	5.05	60	13
N-methyl-*N*-isopropyl	3.93	4.72	50	20

KEY: Data are for the displacement of *[^{125}I]-*R*-DOI in rat frontal cortex homogenate, [^{3}H]-8-OH-DPAT in rat frontal cortex homogenate (Oberlender et al. 1992), [^{3}H]-SCH23390 in rat striatal homogenate, §[^{3}H]-spiperone in rat striatal homogenate (unpublished results).

technique known as Tele-Stereo-EEG. Four bipolar stainless steel electrodes are chronically implanted into rat frontal cortex, caudate, hippocampus, and reticular formation. Field potentials in freely moving rats are recorded, processed by fast-Fourier transform, and filtered to generate dose- and time-dependent power spectra that give "EEG fingerprints" characteristic of the particular type of drug administered. LSD tartrate (0.01 to 0.05 mg/kg) has been compared by Dimpfel (personal communication 1992) with the *N*-methyl-*N*-isopropylamide tartrate (0.025 to 0.1 mg/kg) in this paradigm. In spite of the biochemical and DD data, the rat EEG studies give a surprising result. Although doses can be selected for the two drugs that have some similarities, the EEG fingerprint of the *N*-methyl-*N*-isopropylamide gives a best fit to the EEG fingerprint for dopamine D_1 agonists. This was totally unexpected and seems inexplicable. Perhaps when clinical trials are carried out with dopamine D_1 agonists, the result may be more understandable, but at the present time and in the absence of human data it is perplexing.

The series of isopropyl amides has recently been completed with the synthesis of the *N*-isopropyl, in addition to the *N*-methyl-*N*-isopropyl, *N*-ethyl-*N*-isopropyl, and the *N*,*N*-diisopropyl, as shown in figure 12.

With the exception of the *N*,*N*-diisopropylamide, all compounds completely substitute in the DD paradigm in rats trained to discriminate LSD from saline. In table 6, the receptor-binding data for displacement of [^{3}H]-ketanserin from rat cortical homogenate are shown. Although all *N*-isopropyl homologs have only 25 to 30 percent affinity of LSD for this site, it is interesting to note that the N-methyl-N-isopropyl compound discussed earlier has nearly equal affinity to LSD for the 5-HT_2 site labeled with the agonist ligand [^{125}I]-*R*-DOI. This illustrates the importance of determining the relative efficacy of these compounds rather than just receptor affinity.

The authors are continuing these studies with the *N*-alkyl-*N*-isopropylamides and also are expanding the studies with chiral alkyl groups in the amide function. Through a systematic approach utilizing modification of the amide alkyl combined with conformational and pharmacological analysis, the authors hope to identify correlations between activity and structure. Success in this endeavor might finally, after so many years, explain why LSD is such a potent compound and what actions at which monoamine receptors are the essential ones for expression of hallucinogenic effects.

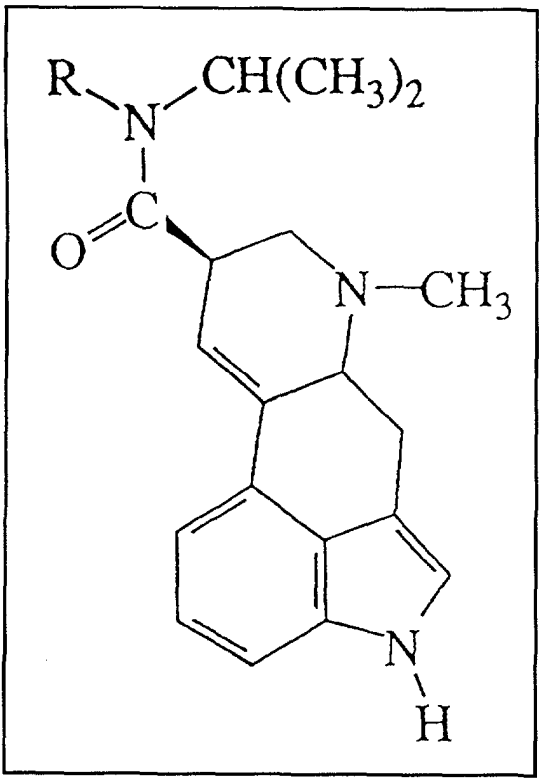

FIGURE 12. *The alkyl-isopropyl amides of lysergic acid*

TABLE 6. *Radioligand binding data for N-methyl-N-isopropyl lysergamides: [^{3}H]-ketanserin displacement (unpublished results)*

Compound	K_i (nM)	Hill Coefficient	N
LSD	4.8±0.5	0.94	3
N-isopropyl	26.2±2.2	0.89	4
N-methyl-*N*-isopropyl	28.1±4.5	0.89	4
N-ethyl-*N*-isopropyl	19.8±2.4	0.94	4
N,N-diisopropyl	20.2±2.4	0.90	3

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AUTHORS

Robert C. Pfaff, Ph.D.
Associate Professor of Chemistry
Department of Chemistry
St. Joseph's College
Rensselaer, IN 47978

Xuemei Huang
Graduate Assistant
Department of Pharmacology and Toxicology

Danuta Marona-Lewicka
Visiting Assistant Professor of Pharmacology
Department of Medicinal Chemistry and Pharmacognosy

David E. Nichols, Ph.D.
Professor of Medicinal Chemistry and Pharmacology
Departments of Medicinal Chemistry and Pharmacognosy and Pharmacology and Toxicology

Purdue University
West Lafayette, IN 47907

Robert Oberlender, Ph.D.
Assistant Professor of Medicinal Chemistry
Department of Pharmaceutics and Medicinal Chemistry
University of the Pacific
Stockton, CA 95211

Structure-Activity Relationships of the Classic Hallucinogens and Their Analogs

Peyton Jacob III and Alexander T. Shulgin

The path that leads to the appearance of a new psychotropic drug in the practice of medicine usually consists of four stages: discovery of activity, the development of animal behavioral models that can be correlated to this activity, the study of mechanisms of action and nature of toxicity, and the demonstration of effectiveness and benefit. The final studies of effectiveness for drugs intended for human use must be done in human subjects. This is the essence of the Phases 1 through 4 studies found in the Food and Drug Administration (FDA) regulations associated with the investigatory new drug (IND) application. With many drug families, the results of the animal model studies (steps 2 and 3) can allow prediction of new drug structures (step 1). However, with research in the hallucinogenic drugs (where the desired pharmacological activity can be demonstrated only in humans), the confirmation of activity must occur of necessity in humans. Therefore, it is of potential value for future research in this area to bring together in a single review the known human potencies of the classic hallucinogens and their analogs.

Two words in the title of this chapter must be defined: hallucinogen and classic. A hallucinogen is a drug that changes a person's state of awareness by modifying sensory inputs, loosening cognitive and creative restraints, and providing access to material normally hidden in memory or material of an unconscious nature. The changes thus gained are not masked by amnesia, although they will last only a finite period of time, and they are demonstrable only in humans. A generation ago these drugs were inaccurately called psychotomimetics, things that imitate psychosis. Today the term "hallucinogen" is allowed as a euphemism, although that term is also inaccurate because hallucinations are not part of the usual syndrome. In another generation, the synonym psychedelics may become acceptable in the medical and scientific literature.

Several chemical families that include drugs which have been clinically associated with the term "hallucinogen" have been excluded by design from this review, These substances have been the topics of other

conferences or monographs sponsored. by the National Institute on Drug Abuse (NIDA). Among these exclusions are marijuana, tetrahydrocannabinol (THC), ketamine, and related parasympatholytics such as the *Datura* alkaloids and the JB compounds, opiates, and agents related to 3,4-methylenedioxymethamphetamine (MDMA).

The term "classic" depends on the views of the person who defines it. A compound achieves a classic status when it has served as the focus of a considerable amount of research attention, With compounds such as mescaline, lysergic acid diethylamide (LSD), 2,5-dimethylthiophenethylamine (DOM), dimethyltryptamine (DMT), and psilocybin, the status as classic hallucinogens might be due to the extensive animal and clinical research that has appeared in the literature. But with other compounds such as thiomescaline, 2,5-dimethoxy-4-methylphenethylamine (2C-D), 2,5-dimethoxy-4-methylthiophenethylamine (2C-T), and 3,4,5-trimethoxyamphetamine (TMA), the bulk of the published literature has been focused at a structural and chemical level. The analogs of these nine prototypic classic hallucinogens are reviewed here, and nine tables list structurally related variants that have been explored in humans.

The oldest classic hallucinogen known to Western science is mescaline, the major alkaloid of the dumpling cactus peyote. Mescaline was isolated from the peyote cactus, the pharmacology was defined in 1896, and the structure of mescaline verified by synthesis in 1919. It is used as the potency standard against which all other phenethylamine bases have been compared. Table 1 shows the relative potency of mescaline along with the several alkyl homologs that have been studied with the oxygen atoms maintained in the vicinal 3,4,5-orientation.

A generalization is that there is an increase in potency with increasing the length of the alkyl group on the 4-position oxygen atom but not with such changes with the meta-oriented groups. The naming of these synthetic compounds has exploited the coincidence that mescaline carries the methoxy group at the 4-position and both words begin with the same syllable. The names "escaline," "proscaline," and "buscaline" follow easily as the groups become ethoxy, propoxy, and butoxy. The names for the diethoxy homologs (here and in table 2) incorporate the nomenclature prefix from being symmetrical (sym) or asymmetrical (asym) and having two (bis) ethoxy groups (bescaline).

TABLE 1. *Mescaline analogs*

Name	Code	Potency (mg)	Potency x mescaline
(4-Modified)			
Mescaline (4-methoxy)	M	200-400	1
Escaline (4-ethoxy)	E	40-60	6
Proscaline [4-(n)-propoxy]	P	30-60	7
Isoproscaline (4-isopropoxy)	IP	40-80	5
Buscaline [4-(n)-butoxy]	B	>150	<1
Cyclopropylmethyl-	CPM	60-80	5
Allyloxy-	AL	20-35	10
Methallyloxy-	MAL	40-65	6
Propynyloxy-	PROPYNYL	>80	<2
4-Desoxymescaline (4-methyl)	DESOXY	40-120	4
Phenescaline (4-phenethyloxy)	PE	>150	<1
(Other modified; substituent and location defined)			
Metaescaline (3,4-dimethoxy-5-ethoxy)	ME	200-350	1
Metaproscalme (3,4-dimethoxy-5-propoxy)	MP	>240	1
Asymbescaline (3,4-diethoxy-5-methoxy)	ASB	200-280	1
Symbescaline (3,5-diethoxy-4-methoxy)	SB	>240	<1
Trescaline (3,4,5-triethoxy)	TRIS	>240	<1
(Chain relocation)			
Isomescaline (2,3,4-trimethoxy)	IM	>400	<1
(Deuterium substitution)			
4-Trideuteromescaline	4-D	200-400	1
ß-Dideuteromescaline	ß-D	200-400	1

A comment is appropriate for the use of the symbols ">" and "<" in these tables. When a dosage weight is given as >250 milligrams (mg), the implicit statement is that no activity had been found at 250 mg. It is not known whether the compound is active at any dose, but, if it is, it will be at a dose greater than 250 mg. Thus, the potency relative to mescaline shown as < 1 means that if activity is found, it will be less than that of mescaline. There is no implication that the compound is or is not active.

There have been reports of mescaline analogs with a methoxy group removed. The analog 3,4-dimethoxyphenethylamine (DMPEA) has achieved some notoriety with the report of the observation of a pink spot in the thin-layer chromatographic analysis of the extracts of the urine of schizophrenic patients. The association of the spot with the diagnosis of schizophrenia has remained controversial, but the chemical identity has been shown to be DMPEA. Efforts to evoke some central nervous system (CNS) disturbance with this compound (orally, to > 1.5 grams (g); intravenously [IV], to > 10 mg) have produced no effects. Because of its close structural resemblance to the neurotransmitter dopamine (DA) (3,4-dihydroxyphenethylamine), this result has been disappointing. The 4-ethoxy homolog, 3-methoxy-4-ethoxyphenethylamine (2,3,4-trimethoxyphenethylamine) (MEPEA) has been assayed to 300 mg but has little if any activity.

An additional compound is isomescaline (2,3,4-trimethoxyphenethylamine). There is a fascinating report in the literature concerning its activity. It has been stated to be inactive in normal subjects but to promote a distinct intoxication in schizophrenic patients. If this effect were confirmed, it might play an interesting role as a marker or a biochemical probe of schizophrenia.

The two last compounds in table 1 are the only known deuterium analogs that have been explored in humans, and neither can be distinguished from mescaline. Other uniquely deuterated isotopomers that may be of interest are 3,5-(bis-trideuteromethoxy)-4-methoxyphenethylamine (3,5-D); 2,6-dideuteromescaline (2,6-D), and α,α-dideuteromescaline (α-D). The last compound, being deuterated at the most probable primary site for metabolic attack, might be of a different potency due to the kinetics of a-proton removal, and a study of the (R)-α-monodeuteroisotopomers [(R)α-D] and (S)-α-monodeuteroisotopomers [(S)α-D] might be informative. None of these latter compounds has as yet been studied.

The substitution of a sulfur atom for the 4-oxygen atom of mescaline yields the remarkably potent analog thiomescaline. Although this compound has not been widely studied in clinical trials, it has been the starting point of extensive synthetic studies that have further emphasized the importance of the 4-position of the aromatic ring of the phenethylamines (see table 2).

Homologation at the 4-position again increases or maintains potency until the chain reaches a length of three carbon atoms (4-thioproscaline), and then activity begins to disappear. None of the unsaturated (allylthio, methallylthio) or related electron-rich alkylthio counterparts (cyclopropylmethylthio) has been studied for comparison to the relatively potent oxygen counterparts (table 1). They should be reasonably simple to synthesize and might be exceptionally potent.

One additional degree of structural variation is introduced with the sulfur atom replacement for the oxygen. It may occupy either of two positions, para or meta, to the ethylamine side chain. The meta-sulfur positional isomers still emphasize the importance of the nature of the alkyl substituent on the para-heteroatom. The three possible thioanalogs of isomescaline were without activity.

The two remaining prototypes for structure-activity analysis are close relatives to well-known amphetamine counterparts. The well-studied drug DOM has a 2-carbon homolog, 4-methyl-2,5-dimethoxyphenethylamine (2C-D). The bromo counterpart 2,5-dimethoxy-4-bromoamphetamine (DOB) has a 2-carbon homolog, 2,5-dimethoxy-4-bromophenethylamine (2C-B). Both phenethylamines have engendered large families of analogs, and both amphetamines are presented in table 6.

The simplest 4-alkyl-substituted hallucinogenic compound is 2C-D. This base has been widely explored in the United States as a prototype for the exploration of new compound. In Germany 2C-D has been used as a psychotherapeutic agent in its own right, at larger dosages, usually under the code name of LE-25. The base without this para-alkyl group is 2,5-dimethoxyphenethylamine (2C-H), but if this group is homologated to an ethyl, the extraordinarily powerful and effective compound 2,5-dimethoxy-4-ethylphenethylamine (2C-E) is found. Potency continues to increase with further chain lengthening, but the positive nature of the observed psychopharmacological effects is lessened.

TABLE 2. *Thiomescaline analogs*

Name	Code	Potency (mg)	Potency x mescaline
(Sulfur para)			
4-Thiomescaline (3-Me-4-MeS-5-MeO)	4-TM	20-40	10
4-Thioescaline (3-MeO-4-EtS-5-MeO)	4-TE	20-30	10
4-Thioproscaline [3-MeO-4-(n)-PrS-5-MeO]	4-TP	20-25	10
4-Thiobuscaline [3-MeO-4-(n)BuS-5-MeO]	4-TB	60-120	4
4-Thioasymbescaline (3-EtO-4-EtS-5-MeO)	4-TASB	60-100	4
4-Thiosymbescaline (3-EtO-4-MeS-5-EtO)	4-TSB	>240	<1
4-Thiotrescaline (3-EtO-4-EtS-5-EtO)	4-T-Tris	>200	<1
(Sulfur meta)			
3-Thiomescaline (3-MeS-4-MeO-5-MeO)	3-TM	60-100	4
3-Thioescaline (3-MeS-4-MeO-5-MeO)	3-TE	60-80	5
3-Thiometaescaline (3-EtS-4-MeO-S-MeO)	3-TME	60-100	4
5-Thiometaescaline (3-EtO-4-MeO-5-MeS)	5-MTE	>200	<1
3-Thiosymbescaline (3-EtS-4-MeO-5-EtO)	3-TSB	>200	<1
3-Thioasymbescaline (3-EtS-4-EtO-5-MeO)	3-TASB	~160	<1
5-Thioasymbescalien (3-EtO-4-EtO-5-MeS)	5-TASB	~160	<1
3-Thiotrescaline (3-EtS-4-EtO-5-EtO)	3-T-Tris	>160	<1
(Chain relocation)			
2-Thioisomescaline (2-MeS-3-MeO-4-MeO)	2-TIM	>240	<1
3-Thioisomescaline (2-MeO-3-MeS-4-MeO)	3-TIM	>240	<1
4-Thioisomescaline (2-MeO-3-MeO-4-MeS)	4-TIM	>240	<1

TABLE 3. *2C-D analogs*

Name	Code	Potency (mg)	Potency x mescaline
(4-Alkyl groups)			
4-Proteo-2,5-DMPEA	2C-H	?	
4-Methyl-2,5DMPEA	2C-D(LE-25)[a]	20-60	8
4-Ethyl-2,5-DMPEA	2C-E	10-15	24
4-(n)-Propyl-2,5-DMPEA	2C-P	6-10	40
(3,4-Dialkyl groups)			
Dimethyl-2,5-DMPEA	2C-G	20-35	10
Trimethylene-2,5-DMPEA	2C-G-3	16-25	14
Tetramethylene-2,5-DMPEA	2C-G-4	?	
Norbornyl-2,5-DMPEA	2C-G-5	10-16	24
Naphthyl[b]	2C-G-N	20-40	10
(Other groups)			
1-Fluoro-2,5-DMPEA	2C-F	>250	<1
4-Chloro-2,5-DMPEA	2C-C	20-40	10
4-Bromo-2,5-DMPEA	2C-B	12-24	16
4-Iodo-2,5-DMPEA	2C-I	14-22	16
4-Nitro-2,5-DMPEA	2C-N	100-150	2
4-Isopropoxy-2,5-DMPEA	2C-O-4	>60	?
4-Methylthio-2,5-DMPEA	2C-T[c]	60-100	4
4-Methylseleno-2,5-DMPEA	2C-SE	ca. 100	ca. 3

[a]Higher levels have been used in psychotherapeutic research.
[b]1,4-Dimethoxy-2-(2-aminoethyl)naphthalene
[c]Extensively studied via homologation, see separate table.

As discussed with the 3-carbon amphetamine analogs below, it had been observed that the addition of a second alkyl group at the ring 3-position led to compounds that were of reduced potency but still maintained hallucinogenic activity (table 6). The 2-carbon counterparts are shown in table 3. They form an unusual group with unique properties. Of all the

phenethylamine/amphetamine pairs explored so far, the phenethylamine is of a lower potency than the amphetamine homolog, and the potency of each increases yet further with the increasing of the molecular weight of the 4-alkyl group. However, with these 3,4-dialkyl analogs, as the two alkyl groups become increasingly large and complex, not only do the phenethylamines become more potent than the amphetamine homologs, but the absolute potency also tends to increase with increased mass and bulk of these alkyl groups. The most potent compound yet found in this structural family is the illustrated norbornyl material 3,6-dimethoxy-4-(2-aminoethyl)-benzobornane (2C-G-5), with a total of 5 aliphatic carbons arranged between the 3- and 4-positions.

Many directions can be pursued here, both synthetically and pharmacologically. So far, all the compounds that have been prepared-those that have been evaluated psychopharmacologically and those whose evaluation has not yet been completed-are symmetrically substituted about the 3,4-axis. The use of a Diels-Alder reaction with benzoquinone as the dieneophyle ensures an almost unlimited degree of variation in the 3,4-dialkyl-2,5-dimethoxyphenethylamines. With the increase in mass suggesting greater potency, there may be some remarkable compounds here. Two further avenues of promising exploration are obvious. An asymmetric substitution is possible in which the 3- and 4-position groups are different from one another. Also, one must investigate the optical isomers of the racemates produced (as with 2C-G-5).

Two additional centrally active structural variations of the prototype 2C-D have been observed and explored to a small degree. The p-oxygenated analogs are known as the ß-methoxy-ß-arylethylamine (BOX) series, and the materials with ethoxy groups in place of either of the methoxy groups are called the tweetios. Neither family is entered in table 3, but both are logical extensions of it.

The BOX compounds are ß-oxy analogs of phenethylamines, masked as the methyl ether. The X then is the initial or identifier of the 2C analog that has been oxygenated. This manipulation introduces an oxygen heteroatom at a position identical to that found in the neurotransmitters norepinephrine (NE) and epinephrine. But it also introduces a new chiral center, and in the corresponding amphetamine derivatives a threo-erythro system of diastereoisomers that resembles that of ephedrine and pseudoephedrine would be produced. The ß-methoxy analogs of 2C-ß, ß,2,5-trimethoxy-4-bromophenethylamine (BOB), and 2C-D (ß,2,5-trimethoxy-4-methylphenethylamine [BOD]) are a little more potent than

their oxygen-free counterparts but not as interesting subjectively. There has been no research done on the pure enantiomers.

The two tweetio analogs (2-ethoxy and 5-ethoxy) of both 2C-D and 2C-B have been explored and have dramatically reduced activity. The 5-tweetio (5-ethoxy) compounds are of twofold lessened potency, and the 2-tweetio (2-ethoxy) materials are down by another factor of five. The bis-etios (2,5-diethoxy homologs of 2C-D and 2C-B) are not known to be active at all.

Near the bottom of table 3 is a sulfur compound, 2C-T. Although only modest in activity, its homologs show a wide and varied psychopharmacology and constitute yet another family of hallucinogenics. These 2C-T analogs are listed and compared in table 4.

The optimum alkyl substitution is two to three carbons, with 2C-T-2, 2C-T-4, and 2C-T-7 (the S-ethyl, S-isopropyl, and S-propyl) being both potent and LSD-like. The placement of a methoxy on the S-ethyl group of 2C-T-2 yields the active methoxyethylthio derivative 2C-T-13, and the replacement of this methoxy group with a fluorine gives the potent ß-fluoroethylthio-2,5-dimethoxyphenethylamine (2C-T-21). This structure is interesting because it is the first hallucinogenic drug with six separate elements in its formula (C, H, N, O, S, and F) and is potentially valuable as a vehicle for ^{18}F studies of brain kinetics with positron emission tomography (PET). In this case the fluorine atom is intrinsic to the expressed central activity.

There are two classic amphetamine hallucinogens that have provided the starting point for extensive structure-activity investigations. The first, based on the well-known 3-carbon homolog of mescaline 3,4,5-trimethoxyamphetamine (TMA), is shown in table 5. Here all compounds are characterized by the presence of an oxygen atom on the 4-position of the benzene ring, where the l-position is always defined as the point of attachment of the aminoalkyl side chain. All six possible positional isomers of TMA have been prepared and compared. Two isomers that stand out from the others are the 2,4,5- and the 2,4,6-isomers, TMA-2 and TMA-6. Both are active in the 20 to 50 mg range orally. The first of these has been broadly modified, with the most productive area of change being the nature of the alkoxy group in the 4-position to give 2,5-dimethoxy-4-ethoxyamphetamine (MEM) or the cycling of it into the 5-membered dioxole ring to give 2-methoxy-4,5-methylenedioxyamphetamine (MMDA-2). This latter methylenedioxy base also has been

TABLE 4. *2C-T analogs*

Name	Code	Potency (mg)	Potency x mescaline
(4-Alkylthio-2,5-DMPEA)			
Methyl-	2C-T	60-100	4
Ethyl-	2C-T-2	12-25	16
Propyl-	2C-T-7	10-30	15
Isopropyl-	2C-T-4	8-20	20
Sec-butyl-	2C-T-17	60-100	4
Tert-butyl-	2C-T-9	60-100	4
Cyclopropyl-	2C-T-15	>30	?
Cyclopropylmethyl-	2C-T-8	30-50	8
(4-Heteroalkylthio-2,5-DMPEA)			
2-Methoxyethyl-	2C-T-24	25-40	10
2-Fluoroethyl-	2C-T-21	8-12	30

subjected to positional isomerization. Dropping of the methoxyl group from MMDA-2 (or MMDA) provides one of the few known phenethylamine hallucinogens with only two ring substituents. This base, 3,4-methylenedioxyamphetamine (MDA), is also remarkable because the N-methyl homolog 3,4 (MDMA) has biological activity, although the nature of its action places it outside of this review. No other phenethylamine hallucinogen retains central activity on N-methylation. The mono-substituted analog 4-methoxyamphetamine (4-MA) is an active compound, but it is largely a cardiovascular stimulant.

A similar group of compounds is known that has the 4-alkoxy group replaced with something without an oxygen atom. These are gathered in table 6. Among the more potent of these are the halogen-containing analogs. DOM, DOB, and especially 1(2,5-dimethoxy-4-[^{125}I] iodo-phenyl)-2-aminopropane (DOI), have recently received much research attention as ligands in the study of serotonin receptors.

Two families related to DOM are mentioned here but are not included in table 6. A few 4-alkylthio analogs called the Aleph compounds are

TABLE 5. *TMA analogs*

Name	Code	Potency (mg)	Potency x mescaline
(Alkoxyamphetamine)			
4-Methoxy	4-MA	50-80	5
2,4-Dimethoxy	2,4-DMA	>60	?
2,5-Dimethoxy	2,5-DMA	80-160	2.5
3,4-Dimethoxy	3,4-DMA	in the 100s	<1
3,4,5-Trimethoxy	TMA	100-250	1.7
2,4,5-Trimethoxy	TMA-2	20-40	10
2,5-Dimethoxy-4-Ethoxy	MEM	20-50	10
2,5-Dimethoxy-4-Propoxy	MPM	>30	?
2,3,4-Trimethoxy	TMA-3	>100	?
2,3,5-Trimethoxy	TMA-4	>80	?
2,3,6-Trimethoxy	TMA-5	ca. 30	ca. 10
2,4,6-Trimethoxy	TMA-6	25-50	8
2,3,4,5-Tetramethoxy	TA	>50	?
(Methylenedioxyamphetamine)			
3,4-Methylenedioxy	MDA[a]	80-160	2.5
3-Methoxy-4,5-Methylenedioxy	MMDA	100-250	1.7
2-Methoxy-4,5-Methylenedioxy	MMDA-2	25-50	8
2-Methoxy-3,4-Methylenedioxy	MMDA-3a	20-80	6
4-Methoxy-2,3-Methylenedioxy	MMDA-3b	>80	?

[a]The N-methyl homolog of MDA (MDMA) is not appropriate to this review of hallucinogens.

known, These correspond exactly to the 2C-T bases listed in table 4. Aleph, Aleph 2, Aleph 4, and Aleph 7 are the 4-methylthio-, 4-ethylthio-, 4-isopropylthio-, and 4-propylthio-2,5-dimethoxyamphetamine isomers, respectively. They are consistently more potent than their 2-carbon phenethylamine counterparts.

TABLE 6. *DOM analogs*

Name	Code	Potency (mg)	Potency x mescaline
(4-Alkyl-2,5dimethoxyamphetamine)			
Methyl	DOM (STP)	3-10	50
Ethyl	DOET	2.0-6.0	80
Propyl	DOPR	2.5-5.0	80
Butyl	DOBU	>3	?
Iso-Butyl	DOIB	>10	?
Set-Butyl	DOSB	>25	?
Tert-Butyl	DOTB	>10	?
(4-Substituted-2,5dimethoxyamphetamine)			
Chloro	DOC	1.5-3.0	150
Bromo	DOB	1.0-3.0	150
Iodo	DOI	1.5-3.0	150
Nitro	DON	3.0-4.5	80
2-Fluoroethyl	DOEF	2.0-3.5	100
(3,4-Disubstituted-2,5-dimethoxyamphetamine)			
Dimethyl	G	20-32	10
Trimethylene	G-3	12-18	20
Norbornyl	G-5	14-20	18

The second group has a 2,4,6-substitution pattern. The majority of the compounds listed in the last few tables has carried the 3,4,5- or the 2,4,5-substitution pattern. The similarity of potency between TMA-2 and TMA-6 (the latter with the 2,4,6 substitution pattern, see table 5) has opened up a new family of hallucinogenic amphetamines, one of the authors' current areas of research. With this group also, the 4-position appears to dictate the potency and nature of response. It seems that each of the 2,4,5-substituted materials may have an active 2,4,6-counterpart. The isomer that corresponds to DOM (2,6-dimethoxy-4-methylamphetamine [pseudo-DOM]) is active at 15 to 25 mg orally. Synthetic procedures are now in hand to prepare the pseudo analogs of the 2C-T family with various alkylthio groups at the 4-position.

The first six tables have been devoted to the phenethylamine hallucinogens; the remaining three list the second "kingdom" of pharmacologically related compounds, the tryptamine hallucinogens. Table 7 lists the known active tryptamines other than the psilocybe group. The N,N-dialkyltryptamines are the oldest and most thoroughly studied. Those with low molecular weight groups, DMT and N,N-diethyltryptamine [DET], are presumably inactivated through metabolic deamination and hence must be administered parenterally or with some amine oxidase inhibitor. The presence of groups with increased bulk, such as isopropyl groups, on the nitrogen atom allows these compounds to be active orally.

The indole ring can carry a single oxygen substituent in the aromatic ring, and activity can be retained. The 4-substituted indoles are discussed below. The 5-hydroxylation of DMT (the substitution position of the neurotransmitter serotonin, 5-hydroxytryptamine [5-HT]) yields bufotenine, which is probably not a hallucinogen. Converting this to its methyl ether yields a group of N,N-dialkyl tryptamines whose parenteral/oral availabilities closely parallel the DMT counterparts, except that there is generally an appreciable increase in potency. The masking of the two vulnerable locales of serotonin, the O-methylation to allow entry into the CNS and the α-methylation to avert enzymatic deamination, provide α,, O-dimethylserotonin. This is an orally active hallucinogen of uniquely high potency. Any other substitution on the indole ring (6-, 7-, or multisubstitution) gives inactive compounds. Almost nothing is known about the oral versus parenteral requirements, potency, or nature of action of tryptamines (5-proteo and 5-methoxy) with mixed alkyl groups on the basic nitrogen atom. This is the second area of the authors' current research.

TABLE 7. *DMT analogs*

Name		Code	Potency (mg)	Potency x DMT
(N,N-dialkyltryptamine)				
H		Tryptamine	>100	<1
Dimethyl		DMT	60-100 (4-30 IV)	1
Diethyl		DET	60-150	1
Dipropyl		DPT	20-100 (100s po)	1
Methylisopropyl		MIPT	10-25	4
Diisopropyl		DIPT	40-100	2
Diallyl			80	1
(Ar-substituted — alkyltryptamine)				
H	α-Methyl	IT-290	20-40	3
4-Me	α-Methyl		>60	<1
4-OH	N,N-Dimethyl	(psilocin, see separate table)		
S-OH	N,N-Dimethyl	(bufotenine, not CNS active?)		
5-OCH_3	N,N-Dimethyl	5-MeO-DMT	6-10	10
5-OCH_3	N,N-Disopropyl	5-MeO-DIPT	8-12	7
5-OCH_3	N-Methyl-n-isopropyl	5-MeO-MIPT	4-6	15
5-OCH_3	a-Methyl	α,,O-DMS	3-5	20
5,6-OCH_3	N-Methyl-n-isopropyl	5,6-OMe-MIPT	>60	<1
5,6-OCH_2O	N-Methyl-n-isopropyl		>60	<1
6-OH	N,N-Dimethyl	6-OH-DMT	>80	<1
6-F	N,N-Diethyl	6-F-DET	>80	<1
6-OCH_3	N-Methyl-N-Isopropyl	6-MeO-DIPT	>50	<1
7-OCH_3	N-Methyl-N-Isopropyl	7-MeO-DIPT	>70	<1

IV = intravenous

TABLE 8. *Psilocybin analogs*

Name	Code	Potency (mg)	Potency x mescaline
(4-Oxy,N,N-dialkyltryptamine)			
Dimethyl (phosphate ester)	CY-39 (PSOP)	10-15	6
Dimethyl (free OH)	CX-59 (PSOH)	7-10	8
Methylpropyl (free OH)	4-OH-MPT	10-15	6
Methylisopropyl (methyl ether)	4-MeO-MIPT	20-30	3
Diethyl (phosphate ester)	CEY-19	20-30	3
Diethyl (free OH)	CZ-74	15-20	4
Diisopropyl (free OH)	4-OH-DIPT	15-20	4

An additional family of compounds should be mentioned here, the ß-carbolines. Their use has been well documented in the ethno-pharmacological literature as enzyme inhibitors that allow the tryptamines normally only active parenterally to be orally active, presumably by inhibiting first-pass metabolism in the liver. Also, they can be generated by the cyclization of 6-methoxytryptamine with a 2-carbon unit such as acetaldehyde. In nature, they usually are found in one of three degrees of hydrogenation: harmine, harmaline, and tetrahydroharmine. The isomers more closely related to serotonin are similarly formed, synthetically, from 5-methoxytryptamine. Only harmaline, one of the principal components of Ayahuasca, has a reputation for being intrinsically an active hallucinogen. The aromatic analog, harmine, has little if any psychotropic activity. No reports have been published concerning the three cyclization products of 5-methoxytryptamine.

The family of tryptamines with an oxygen function at the 4-position is based on the active alkaloids of the mushroom genus *Psilocybe* (table 8). The phosphate ester alkaloid is psilocybin, and the free phenolic counterpart is the less stable compound psilocin. The dephosphorylation of psilocybin to psilocin appears to occur in the body, as both are molecularly equipotent. The N,N-diethyl homologs have been synthesized and explored in connection with psychotherapy. They appear to be a little less potent than the methyl counterparts. Another study has looked at other groupings on the nitrogen atom, and all materials

investigated are potent and orally active. Both the monomethylated and nonmethylated homologs, baeocystin and norbaeocystin, occur as congeners of psilocin in some species of the mushrooms. They have not been assayed in humans.

The last and by far most potent family of the tryptamine hallucinogens is found in the ergolines related to LSD. These are listed in table 9. Classically, the diethylamide has been considered the most potent of all and the prototype for comparison. The earliest work done in this area usually gave human potencies as an explicit fraction of the potency of LSD itself, and some of these values have been derived from a single dosage administration. The microgram (μg) ranges offered have been obtained by back calculations from these fractions. The N-1 acetyl derivative is equipotent, probably due to an easy loss of the acetyl group by hydrolysis in the body. All variations studied on the amide nitrogen have led to compounds of diminished activity.

However, variations of the N-6 substitution have maintained the potency of LSD and in some cases enhanced it. No studies have been made of the more potent N-6 homologs with amide nitrogen substituents other than the diethyl group found in LSD.

CONCLUSION

This chapter presents a brief picture of the present state of knowledge of the analogs of the classic hallucinogens. Relating these to the logical neurotransmitters, no structural theme is apparent that would allow a working theory of their mode of action. Many attractive research directions are obvious from the omissions in these tables. Two have been mentioned as being in progress. If the 2,4,6-substitution pattern proves to provide consistently active compounds, hypotheses suggesting some involvement of a hydroquinone intermediate explaining the activity of the 2,4,5-substituted compounds will have to be reconsidered. Analysis of the geometry surrounding the basic nitrogen in the DMT homologs might accurately define the geometry requirements of the active site involving that location. These studies are also in progress.

Other provocative questions remain. Might the remarkable activity of the N-methyl N-isopropyl substitution patterns of the tryptamines apply to the phenethylamines? Why are the active phenethylamines so active

TABLE 9. *LSD analogs*

Name	Code	Potency (mg)	Potency x DMT
(Amide variations)			
Diethyl	LSD-25	50-200	1
(1-AC, 2-H)	ALD-52	100-200	1
(1-Me, 2-H)	MLD-41	200-300	0.3[a]
(1-H, 2-Br)	BOL-148	>1,000	<0.1
(l-Me, 2-Br)	MBL-61	>10,000	<0.01
Ethyl	LAE-32	500-1,400	0.1[a]
(1-AC, 2-H)	ALA-10	1,200	0.1
(l-Me, 2-H)	MLA-74	2,000	0.05[a]
Methyl		ca. 500	ca. 0.2[a]
Dimethyl	DAM-57	500-1,200	0.1
Methylpropyl	LMP	>100	<1
1-Hydroxy-2-Propyl	Ergonovine	10,000	0.01[a]
1-Hydroxy-2-Butyl	Methylergonovine	2,000	0.05[a]
(l-Me, 2-H)	UML-491 (Sansert)	4,000-8,000	0.02
-$(CH_2)_5$-(pyrrolidinyl)	LPD-824	800	0.1[a]
(l-Me, 2-H)	MPD-75	>1,600	<0.05
-$CH_2CH_2OCH_2CH_2$-(morpholinyl)	LSM-775	300-600	0.3
(N-6 variations)			
H	Nor-LSD	>500	<0.3
Methyl	LSD	50-200	1
Ethyl	EHLAD	40-80	2
Propyl	PROLAD	80-175	
Allyl	ALLYLAD	50-150	1
Butyl	BULAD	>400	<0.3
Phenethyl	PHENETHYLAD	>350	<0.3

"Relative potency is based on intensity of described effects, In some cases only a single dosage level was employed.

whereas common wisdom would predict that they should be deaminated and thus inactive? Also, with these compounds and their amphetamine homologs, why is it only the 4-position that allows such extensive structural manipulation without much attenuation in activity? In the tryptamine world, might there not be a host of active compounds to be found pursuing the amphetamine-like structure of α,O-DMS, and perhaps further homologs with one or two alkyl groups on the basic nitrogen?

The answers to these and other related questions might provide valuable information to help explain the remarkable activity of this class of psychotropic agents.

NOTE

Some of the data presented here have not been published previously. A comprehensive bibliography documenting the known data reviewed in this article would contain hundreds of citations. For leading reviews that provide these references, the following should be consulted.

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AUTHORS

Peyton Jacob III, Ph.D.
Research Chemist
3787 Highland Road
Lafayette, CA 94549

Alexander T. Shulgin, Ph.D.
1483 Shulgin Road
Lafayette, CA 94549

Human Hallucinogenic Drug Research: Regulatory, Clinical, and Scientific Issues

Rick J. Strassman

INTRODUCTION

It is likely that no other group of drugs has been subject to such intensive clinical study and to then nearly complete neglect in such a short a period of time as the hallucinogens. Mescaline, the most well-known psychoactive alkaloid of the peyote cactus, was studied in a limited fashion (Kluver 1928) before the discovery of lysergic acid diethylamide (LSD). However, Sandoz Laboratories' description of the microgram potency of LSD to induce psychotomimetic effects (Stoll 1947) generated tremendous excitement in psychiatry and may be seen as an equally important precursor to the modern era of biological psychiatry, as was the contemporaneous discovery of chlorpromazine's antipsychotic properties.

Thousands of subjects, both normal volunteers and psychiatric patients, received LSD and similar drugs in research in the United States and Europe. Szára (this volume) has summarized the research done and monetary effort expended toward understanding LSD's effects during the early 1950s through the early 1970s. Important insights into human cognitive, emotional, electroencephalographic, and perceptual functions in normal individuals and in persons with a broad range of psychiatric disorders were detailed, based on early psychopharmacologic investigations of LSD's effects (Hoffer and Osmond 1967). Drug-assisted psychotherapy, although not clearly proven efficacious before research with these drugs was stopped, had shown some promising results (Pahnke et al. 1970). Neither optimal patient selection nor psychotherapeutic approaches had been decided upon, nor had a firm theoretical basis for this type of work been developed (Grinspoon and Bakalar 1979).

There are several recently published popular histories of the psychedelic era: its beginnings, flourishing, and eventual decline within the scientific, legislative, and media communities (Lee and Shlain 1985; Stevens 1987). Unsupervised use of these drugs by millions of young adults and the

domestic instability caused by the Vietnam War combined to make use and abuse of hallucinogens a significant public health concern. In order to restrict their use by the public, hallucinogens were placed into Schedule I of the Controlled Substances Act of 1970. Drugs in this schedule have no known medical use, cannot be used safely even under medical supervision, and are highly abusable. However, the regulatory process involved in working with Schedule I compounds discouraged all but a few investigators from human research with these compounds. Recently, however, mescaline's psychological and electroencephalographic effects have been studied by a German group (Oepen et al. 1989). In addition, a group of Swiss clinical psychiatrists has been administering 3,4-methylenedioxymethamphetamine (MDMA) and LSD to psychiatric patients since 1985, but no data have been forthcoming from purely clinical use (Widmer 1992).

The confusing and often contradictory nature of the approaches taken in understanding and studying these compounds is reflected in the many names they have received. These names include but are not limited to: "hallucinogenic," "psychedelic," "psychotomimetic," "mysticomimetic," "entheogenic" (generating the divine), "psychodysleptic," "illusogenic," "phanerothyme" ("soul-feeling"), "psycholytic," "phantasticant," and "psychotogenic" (Stafford 1982). "Hallucinogenic," probably the most widely used term in the medical literature, is used in this chapter. However, it should be remembered that frank hallucinations with these drugs are rare, and hallucinogen is as much a term of convenience as of accuracy (Shulgin, this volume).

A tremendous amount of publicity and scientific discourse took place regarding the adverse effects of hallucinogenic drugs. As early as 1960, Cohen had published data on nearly 5,000 individuals exposed to LSD either as research subjects or in drug-assisted psychotherapy. These cases were obtained from his and others' research caseloads. He concluded that the incidence of psychotic reactions lasting longer than 48 hours was 0.08 percent in controls and 0.18 percent in patients; the incidence of suicide attempts was 0 percent in controls and 0.12 percent in patients (Cohen 1960). Thousands of cases subsequently were reported of individuals presenting to emergency rooms and psychiatric clinics for treatment of adverse reactions. These included acute panic reactions, the bad trip, and "flashbacks," or spontaneous eruptions into awareness of one or more features of a previous drug experience.

These reports have been reviewed carefully, and it was concluded that nearly all of these contained serious methodological flaws (Strassman 1984). These unavoidable limitations to these descriptions of adverse effects included lack of knowledge of the actual identity or dose of drug, confounding combinations of other drugs or alcohol, sparse premorbid data on the subjects' psychiatric history, and selection bias. Furthermore, it appeared that in carefully screened, prepared, supervised, and followed-up subjects, both experimental and psychotherapeutic, the incidence of prolonged or delayed adverse reactions was extremely low (Strassman 1984).

The necessary interfacing between human psychopharmacology and basic neuropharmacology has proceeded for the psychotherapeutic drugs such as minor tranquilizers, antidepressants, neuroleptics, and lithium. These parallel courses of investigation have provided clinicians with new families of drugs based upon data generated in lower animal models. Examples include the 5-hydroxytryptamine ($5\text{-}HT_{1A}$) partial agonist and anxiolytic agent buspirone and the selective serotonin reuptake inhibitors fluoxetine and sertraline. Such interfacing work, however, has not been possible with the hallucinogens because of the difficulty associated with human research with them.

In spite of restrictions on human use of hallucinogens, basic research has proceeded at a rapid rate. For example, the original work describing the $5\text{-}HT_1$ and $5\text{-}HT_2$ subtypes used LSD (Peroutka and Snyder 1979); the later discovery of the $5\text{-}HT_{1C}$ subtype was made with LSD (Yagaloff and Hartig 1985); and phenethylamine hallucinogens are now the standard radioligands for localization of $5\text{-}HT_2/5\text{-}HT_{1C}$ subtypes in mammalian brain (Appel et al. 1990).

Several converging factors now provide a suitable backdrop for the resumption of human research with hallucinogens (Freedman 1984). The aforementioned advances in serotonin neuropharmacology have generated one of the major thrusts in current neuroscience research; that is, the role of serotonin in normal and aberrant brain function. Much human psychopharmacologic data are being generated on the effects of 5-HT agonists and antagonists, including L-tryptophan, L-5-hydroxy-tryptophan, fenfluramine, chlorimipramine, buspirone, b-chloro-(1-piperazinyl)pyrazine (MK-212), meta-chlorophenylpiperazine (MCPP), ritanserin, metergoline, methysergide, and cyproheptadine (e.g., Murphy et al. 1991). No 5-HT active compounds generate more profound or unusual effects on mental function than the hallucinogens,

and a firmer theoretical foundation is now available to begin assessing the relative contributions of specific 5-HT subtypes in their mechanism of action. In addition, the use of hallucinogenic drugs by young adults is growing, unlike the use of other illicit psychoactive drugs (NIDA 1991). Therefore, public health issues are addressed by resumption of careful study of the hallucinogens with the possibility of developing safe, effective, and rapid antidotes to drug-induced acute adverse reactions. Finally, the approaches taken by proponents of the psychedelic revolution may have faded sufficiently into the background of the American psyche to allow the resumption of systematic, hypotheses-based research into the effects and mechanisms of action, and perhaps therapeutic utility, of these most interesting drugs.

EPIDEMIOLOGY AND PUBLIC HEALTH SIGNIFICANCE

Hallucinogen use in this country had remained relatively constant over the last 20 years but now appears to be increasing. The 1990 high school drug use survey (Johnson et al. 1991*a)* found that approximately 10 percent of high school seniors polled had used hallucinogens at least once, an increase of 0.4 percent since 1989. These lifetime prevalence rates are about the same as those for cocaine, seven to eight times higher than for heroin, almost twice as high as for sedatives, and three times higher than for steroids. LSD ranks first in the categories "most intense" and "longest" high among respondents. The comparable population estimates for young adults and college students indicate a 0.8 percent rise in lifetime prevalence rates for LSD use (0.6 percent increase in annual prevalence rates) in 1990 compared to 1989; females' use had been converging on males, but "in 1990, an important increase in LSD use among males widened the difference again" (Johnson et al. 1991*b,* p. 92). Lifetime prevalence rates in young adults for LSD are three times those for phencyclidine (PCP) and more than half those for cocaine. The 1990 National Household Survey of Drug Abuse (NIDA 1991) reports that lifetime prevalence of hallucinogen use is 7.6 percent, compared with 11.3 percent for cocaine. The number of individuals in this country, therefore, who have used an hallucinogen at least once is estimated at between 13 and 17 million. Whites use hallucinogens more (8.7 percent lifetime prevalence) than either African Americans (3.0 percent) or Hispanics (5.2 percent), with a consistent male-to-female ratio of 2-3:1 across all ethnic groups.

The compound used in the study described below, N,N-dimethyltryptamine (DMT), is used by a seemingly small proportion of hallucinogen abusers. However, DMT is a prototypical tryptamine hallucinogen. Variations on its structure have generated other short-duration parenterally active drugs such as N,N-diethyltryptamine (Szára et al. 1966) and N,N-dipropyltryptamine (Soskin et al. 1973). Furthermore, designer drugs developed to circumvent existing scheduling laws and/or produce specific qualitative effects derive from this compound (Repke et al. 1985). Other recent developments also are important. Ayahuasca, an Amazonian plant extract (McKenna et al. 1984*a*), has become popular in some circles on the coasts, being imported from Peru and Brazil. It contains DMT, found in one of the plants *(Psychotria viridis),* and monoamine oxidase (MAO) inhibiting ß-carboline harmala alkaloids in another plant *(Banisteriopsis caapi),* allowing the DMT to become orally active (McKenna et al. 19848).

Space limitations prevent a detailed discussion of the relationship between hallucinogen use and other psychiatric disorders. However, the greater incidence of use of hallucinogens by individuals with schizophrenia than by a normal population (Mueser et al. 1990), the possible role of hallucinogens in the development of borderline psychopathology (Dulit et al. 1990), the higher incidence of hallucinogen use in nondrinkers who are first- and second-degree relatives of alcoholics compared to controls (Schuckit and Sweeney 1987), and the role of hallucinogens in precipitating schizophrenic and other psychoses (Vardy and Kay 1983) all bespeak the importance these drugs have for issues of public psychiatric health.

REGULATORY ISSUES

A detailed account of the process by which an investigational new drug (IND) permit was acquired for DMT has been published (Strassman 1991). This chapter summarizes the salient points.

There were several reasons DMT, rather than longer-acting, more popular hallucinogens, was chosen for a research proposal. First, it is a very short-acting hallucinogen, with peak effects obtained within 2 to 5 minutes after intramuscular (IM) injection (Sai-Halasz et al. 1958). This attribute is an advantage in the potentially anxiety-provoking environment provided by a modem clinical research center, where a renewal of human studies with hallucinogens must take place. That is, no

matter how troubled a subject's experience on DMT might be, it would be short-lived and relatively manageable.

Second, DMT is an endogenous hallucinogen whose existence in the human nervous system has never been adequately explicated (Gillin et al. 1976). Thus, an investigation into the mechanisms of action and effects of DMT might shed some light on endogenous hallucinatory states. Along this line of reasoning, antidotes to DMT might show efficacy as treatments of disorders with hallucinations such as schizophrenia. The importance of the 5-HT_2 receptor subtype in the mechanism of action of the novel antipsychotic drugs clozapine (Meltzer and Nash 1991) and ritanserin (Gelders et al. 1986) bespeaks the relevance of this approach. Finally, DMT abuse is not a widespread phenomenon. Thus, beginning human hallucinogen research with DMT would not cause the intense public scrutiny that might result from knowledge that LSD once again was being used in humans.

In order to use a drug in humans for either nonindicated purposes or a purpose with no currently established use, it is necessary to apply to the Food and Drug Administration (FDA) for an IND permit. However, for a Schedule I hallucinogen, none of which has been used in humans for many years, there is the additional problem of locating an acceptable source of the compound. A Drug Master File for the compound must be established for the FDA to determine basic safety and manufacturing data concerning any drug for human use. This file contains manufacturing and quality control data for the drug to which FDA may refer. FDA also is charged with concurrently establishing whether the proposed course of study will yield worthwhile data.

The proposed study was to establish normative dose-response data for DMT in a group of experienced hallucinogen users in a double-blind, randomized, placebo-controlled study design.

Experienced hallucinogen users were chosen for three reasons: experienced subjects would be less likely to panic than naive subjects in response to the sudden onset and intensely hallucinogenic effects of a short-acting drug such as DMT; experienced subjects would be more capable of carefully describing the effects of DMT than would subjects with no experience with these compounds; and liability for the subsequent development of substance abuse and/or other psychiatric pathology would be less sustainable in subjects with a prior history of use of these drugs.

The literature on the biological and psychological effects on humans and animals of hallucinogens in general and DMT in particular was reviewed, primarily from the perspective of recent advances in 5-HT neuropharmacology and psychopharmacology. Several hypotheses were then generated regarding what variables would be affected by DMT, what the interpretation of these effects would be, and subsequent experiments that would help prove or disprove these interpretations. The variables of interest were easily observable and verifiable. They included several neuroendocrine functions, cardiovascular effects, autonomic effects (pupil diameter and core temperature), and rating scale scores.

The first step in the application process for use of DMT was to submit a protocol to the Human Research Review Committee of the University of New Mexico School of Medicine's Institutional Review Board (IRB). The IRB is responsible for the protection of human subjects involved in any clinical research. Although the IRB is concerned primarily with risk-to-benefit ratios, it also may offer suggestions regarding the scientific quality of the proposed study. This latter function usually is subsumed by the Scientific Advisory Committee of the General Clinical Research Center (GCRC) of the University of New Mexico Medical School, where the actual study was to take place.

The IRB places great emphasis on the nature of the informed consent document and, at first, was inclined to have this document state that DMT "had no known medical use," one of the criteria for placement into Schedule I. However, the suggestion was made that, if FDA approval were subsequently obtained, this would not be the case, as DMT would have been approved as a medically useful compound (i.e., as a probe of human serotonergic psychopharmacology and neuroendocrinology). The IRB accepted this reasoning and did not insist on including this phrase, which would have unnecessarily alarmed the subjects who were already approaching the study with some trepidation. The IRB also wanted a statement in the informed consent document regarding what effects subjects might experience while under the influence of DMT.

Interviews had previously been conducted with 19 experienced DMT free-base smokers in order to draft a new rating scale for this study (see below). Thus, a relatively balanced account of the subjective effects of DMT could be provided without going into too much detail. The subjects interviewed spoke most frequently of positively charged effects, so these predominated in the informed consent. However, panic, fear, confusion, and other unpleasant experiences also were listed as possible

outcomes. Prolonged (> 24 hour) reactions to hallucinogenic drugs do occur in carefully controlled settings, although such reactions never have been reported for DMT. However, their overall incidence is quite low. The informed consent document, then, included a statement concerning the fact that subjects might experience prolonged adverse responses to the drug and that psychiatric hospitalization was available.

Finally, the IRB was told that local approval was necessary before applying for Federal permission to begin this study. Once Federal permission was acquired, the principal investigator (PI) would notify the IRB and begin, and not before.

The issues of confidentiality and anonymity also were of concern, since admitting to the use of Schedule I drugs is a crime, and a relatively high-functioning group was expected to volunteer for the study based on the socioeconomic status of the initial interviewees. Meetings with the university hospital counsel, directors of medical records and hospital admissions, GCRC head nurse, and administrative assistants led to a coding scheme by which subjects' confidentiality and anonymity were maintained, with only the PI having the key to the code.

The next step was presenting the protocol to the GCRC Scientific Advisory Committee for a thorough scientific critique. Issues of informed consent and ethical review were not taken up, except in a general way, as these concerns were the purview of the IRB.

After approval from both university committees charged with scientific and ethical issues, State Pharmacy Board approval was necessary to obtain and use a Schedule I drug. The protocol and the appropriate approvals had previously been submitted to the Drug Enforcement Administration (DEA) in Washington, DC, but approval had not yet been received. However, the State Pharmacy Board approved the protocol after it was explained that Federal DEA approval could not be received until State approval was obtained and that the project could not begin until all the requisite Federal permits were in hand.

The Federal approval process required simultaneous interaction with the FDA and the DEA. The applications for a Schedule I permit for DMT were sent to DEA, and the IND application was sent to FDA at the same time. Nearly 2 years were required to keep both of these processes on track until they were finally approved.

The application process to the DEA was complicated by requests for DMT for two separate reasons, possibly from two different sources, and of two different grades. DMT was required for the development of a laboratory assay for DMT in human blood and could be purchased from one of several chemical supply houses. However, laboratory grade DMT could not be used for human studies, even if it were purified to FDA standards. Thus, an additional source of pharmaceutical quality DMT was necessary for human administration. DEA was reluctant to approve the Schedule I application to ***possess*** laboratory grade DMT until the IND was received from the FDA. FDA could not decide whether to approve the lND request until pharmaceutical quality DMT was on hand and its appropriateness for human use could be confirmed. FDA finally wrote a brief letter to DEA saying that the science of the study was reasonable and that human studies of DMT would not begin until the FDA had deemed it safe to begin, but that DEA could approve the Schedule I permit to *obtain* laboratory grade DMT (so assay development could begin) and pharmaceutical grade DMT (to which the appropriate quality control procedures could be applied).

The next major effort involved finding a source of DMT approved by the FDA. The FDA required a pedigree of the DMT if it were to be administered parenterally, the only way DMT alone is active. The pedigree is a description of all precursors, intermediates, and impurities in these compounds, en route to the final synthesis of the DMT. A detailed chemical analysis of the final product also is required. Additionally, the manufacturer must provide FDA with details concerning the manufacturing and cleanup procedures to assure FDA that "good manufacturing procedures (GMP)" were followed. The National Institute on Drug Abuse (NIDA) had some DMT "on the shelf," as did Sigma Chemical (from which the laboratory grade DMT was purchased). Either sample could be purified to > 99 percent purity, but FDA would not accept DMT without a pedigree. Neither NIDA nor Sigma had the pedigree information. Several other possible sources also proved to be either legally or economically unfeasible.

Finally, the DEA determined that regulations allow the synthesis of small batches of Schedule I drugs by previously licensed research laboratories if the drug was for research rather than fiduciary purposes. The "coincidental activities" clause frees synthesizers of small batches of scheduled drugs from the much more rigorous and costly security arrangements required for manufacturers with primarily commercial interests. A colleague with a Schedule I permit for DMT then agreed to

make a small batch of DMT for this study and to provide the requisite documentation, a collaboration with which FDA was satisfied in principle.

The DMT was formulated for intravenous (IV) administration by the inpatient pharmacy of the University of New Mexico Hospital. The DMT then had to be put through the quality control steps required by FDA. Since pharmaceutical companies work with much larger lots of drugs than this small batch, it took some creative problem solving to determine the number of vials of injectable DMT that needed to be tested for pyrogenicity, sterility, and content uniformity. The last issue concerns both the concentration of representative samples of the product being within 5 to 10 percent of the hypothetical value (in this case 40 milligrams per milliliter [mg/ml]) and the maintenance of this concentration over time.

Satisfied with the quality of the DMT for human use and the scientific rigor of the study, FDA gave approval to begin. DEA was notified at this time. Amendments to this protocol, if not substantive, were discussed and approved by telephone. More substantial amendments, such as combining DMT with another drug, required both written and telephone consultation as well as formally requesting the manufacturer of the additional drug to provide written authorization for FDA to access *its* Drug Master File and IND information. DEA was sent copies of all changes to the original DMT protocol.

CLINICAL ISSUES

Recruitment, Screening, and Orientation of Subjects

Experienced hallucinogen users were recruited for this study by word of mouth, except for the last subject who responded to an announcement sent through the psychology department graduate student training office of a local university. Although this particular issue has not yet arisen, it was decided to take no more than two subjects from any particular department or division to prevent the development of cliques, an unfortunate occurrence in previous human hallucinogen studies using graduate students. Subjects initially called the PI to discuss the protocol. They were asked about current medication use or drug abuse and whether they had experience with hallucinogens. Those who were taking medication chronically, had an intercurrent medical illness, or were

currently abusing cocaine or alcohol were not accepted for further screening. Occasional marijuana use was accepted. The subjects were then interviewed in person to screen more carefully for current medical and psychiatric conditions and to assess the level of experience with hallucinogenic drugs. This interview assessed adaptive and maladaptive responses to dysphoric experiences with these drugs. That is, the investigators were interested in knowing how subjects managed bad trips and whether they repressed and denied, or acknowledged, the relevance of these experiences to their personal lives. Examples of this type of adaptive response to an acute adverse reaction are being more careful with whom the person takes hallucinogens and deciding not to take these drugs in a busy metropolitan setting.

Subjects would have been withdrawn if they currently suffered from a severe Axis I disorder (e.g., schizophrenia, major affective disorder, substance abuse, or anxiety disorder) or had a history of psychosis not due to either drugs or fever. One subject with an adjustment disorder related to divorce proceedings was included, as he appeared to be managing these stresses adaptively. A past history of major depression or substance abuse was not necessarily cause for exclusion from the study if subjects were in good remission for at least 1 year, understood the nature of their episode(s), and were not in life circumstances that could precipitate a recurrence during the study.

Subjects also were required to have relatively good object relations, as determined by stability of work, school, and interpersonal functions. In this study, the subject with the most chaotic life circumstances was the person who developed an intercurrent depression midway through the protocol and had to be withdrawn from further participation. Such individuals are not believed suitable for this type of project until their personal and/or work lives are more stable.

Subjects were interested in why this work was being done, the expected results, and the hypotheses. Honesty and tact are very important when orienting and preparing subjects for hallucinogenic drug studies. Such subjects, during the intoxication, may be exquisitely sensitive to interpersonal nuances, attempts to obscure or lie, and personal discomfort with particular issues. Any approach with these subjects that hints of deviousness, dishonesty, manipulation, or callousness is multiplied several times over when the actual drug intoxication is underway. Thus, starting off on the right foot is not only desirable but will prevent paranoid, panic, and dysphoric reactions later during the actual study.

At that time, the nature of the study was described, and subjects received a copy of the informed consent to review. The subjects were asked to think over their participation and were told to call the PI with their decision or any further questions. Prospective subjects were instructed to discuss their participation with their spouse or significant other. The handful of subjects with fearful and reluctant spouses needed more time to decide to participate; some required a meeting between the spouse and study staff to prevent study participation from generating unnecessary friction within a couple. Such friction could not help but carry over into the drug sessions and affect the quality of drug effects.

The informed consent document stated that the primary effects of DMT were psychological, although high doses can cause a transient moderately elevated blood pressure. Auditory and/or visual hallucinations might occur, and other unusual effects were likely. Sense of time might be altered; very powerful emotions, both pleasurable or unpleasant, might be experienced, including opposite feelings or thoughts at the same time. Extreme sensitivity to the environment and people, including frank paranoia, could occur; on the other hand, subjects might not notice anything at all in the environment. Separation of mental processes from all physical sensations could occur. Extreme changes of mood are not uncommon. The incidence of prolonged (> 24 hr) adverse reactions to hallucinogenic drugs in well-screened, prepared, and followed-up subjects was described as quite low. If prolonged adverse reactions to DMT did develop, 24-hour emergency psychiatric consultation was available. Psychiatric hospitalization could also be arranged.

Only 3 of the original 12 subjects had previous experience with DMT, and all were extremely interested in knowing as much about it as possible. Each subject received a copy of a chapter on the history, botany, chemistry, and effects of short-acting tryptamines written for an educated lay audience (Stafford 1982, pp. 308-331).

Prospective subjects then received a structured clinical interview (SCID) of the *Diagnostic and Statistical Manual of Mental Disorders III-Revised* (DSM-III-R), Outpatient Version (Spitzer et al. 1987), by a trained psychiatric research nurse. First-degree relatives' psychiatric histories were obtained during the SCID. Diagnoses were confirmed by discussion with two research psychiatrists. A medical history, a physical examination, and laboratory screening tests then were performed by the PI. Laboratory tests included a complete blood count with differential, 24-item chemistry panel, thyroid functions (including thyroid stimulating

hormone), routine urinalysis, and electrocardiogram (EKG). Subjects were withdrawn who demonstrated any significant abnormalities in this screening, including thyroid disorder, high blood pressure (diastolic over 90 mm Hg), heart disease/abnormal EKG, hepatitis, peptic ulcer disease, diabetes, or other serious medical problems.

The PI administered the medical work-up personally to establish a hands-on relationship with subjects that would facilitate their ability to more comfortably regress under the effects of DMT under supervision in a clinical research setting, and because the personal relationship might prove helpful. This was also the first opportunity for subjects to meet the research nurse who was to assist in the drug sessions. She introduced herself, drew the requisite screening blood samples, performed the EKG, and gave subjects a brief tour of the inpatient unit. This allowed subjects to initiate a relationship with the nurse and become accustomed to her manner of performing potentially painful, uncomfortable, and embarrassing procedures (even more so in the midst of the DMT intoxication). Subsequently, the PI and nurse discussed initial impressions of the subjects.

Nature of the Research Team

The importance of one clinical nurse being involved with subjects from start to finish of the protocol cannot be overemphasized. The nurse's presence is important to the development of subjects' trust and ability to attend completely to the effects of the drug, just as the subjects' trust and confidence in the PI as the only one ever to administer the drug is important. On the rare occasion when the nurse was unavailable for inpatient studies, things never went as smoothly, subjects were not as relaxed, and verbal and nonverbal signals between the substituting nurse and PI were not as clear and unimpeded as with the regular nurse. All of these issues are significant when subjects are in an extremely regressed, suggestible, and, at times, disoriented state.

In addition, the nurse preferably should be of the opposite gender to that of the PI. The regressed state induced by hallucinogens greatly enhances the subjects' tendency to project and overly identify with the experimental team. The presence of two authority figures of opposite genders may help provide a soothing transference template for subjects who may be experiencing helpless infantile feelings.

The PI for human hallucinogen studies should be a psychiatrist with experience in the interviewing, diagnosis, and treatment of psychotic (drug-induced or otherwise) states. The PI should have extensive psychotherapeutic expertise, as the transference and countertransference issues involved in dealing with childlike and regressed conditions requires great tact and sensitivity. If at all possible, the PI should also have had a successful course of psychotherapy/psychoanalysis in order to learn about his/her interpersonal style, nuances, idiosyncracies, and their effect on others.

DMT Sessions and Their Supervision

Before entering into the double-blind, placebo controlled, randomized arm of the study, all subjects received nonblind administrations of 0.04 and 0.4 milligrams per kilogram (mg/kg) IV DMT, usually on consecutive days. The DMT solution (40 mg/ml) was drawn into a sterile tuberculin syringe at a dose range of 0.04 to 0.4 mg/kg, and diluted with sterile saline to 1.0 ml before administration. Tolerance was not a concern, as previous work demonstrated no tolerance to twice daily IM administration of fully hallucinogenic doses of DMT for 5 consecutive days. The drug was administered IV rather than IM as had been reported previously (Szára 1957) because of the results of the initial dose-finding studies using a subject with experience smoking DMT free base, the usual form and route of administration by recreational users (Stafford 1982). The onset and intensity of effect of 1.0 mg/kg IM DMT fumarate were described by this subject as significantly less intense and hallucinogenic than a previous experience with the smoked drug. Further dose-finding studies with this subject and another experienced DMT smoker established that 0.4 mg/kg IV DMT fumarate produced a rush and hallucinogenic effects comparable to or slightly greater than those seen with a full dose of smoked DMT free base.

Subjects were admitted to a dimly lit (about 100 lux) room by 9:00 a.m. after having fasted since midnight. A butterfly style small gauge metal needle was inserted into a forearm vein and kept patent with heparinized saline. Subjects were allowed to relax for 30 minutes before the drug was given. DMT was infused over 30 seconds and flushed with 5 ml of sterile saline over the next 15 seconds. Blood pressure and heart rate were taken with an automatic cuff several times before and frequently during the 30 minutes following drug administration. IV diphenhydramine and diazepam and sublingual nitroglycerin were available for severe allergic, emotional, and hypertensive reactions, respectively. The

research team (a nurse and psychiatrist) sat quietly on either side of the subject, attentive to verbal and nonverbal cues, but did not offer any direction or advice unless absolutely necessary during the first several minutes, the time of peak effects of the drug. Verbal and emotional support were provided as necessary, but drug sessions were not exploratory or therapeutic in intent nor nature. The experienced nature of the subjects, their relatively stable object relations, and the consistent emphasis on objective data collection was helpful in focusing subjects' expectations toward these goals. As drug effects resolved, discussion focused on the nature of the subject's experiences, The butterfly catheter was removed at 30 minutes postinjection, and the Hallucinogen Rating Scale (HRS, see below) was administered. After the subjects ate a snack or meal, they were discharged.

Subjects felt the onset of effects before the 45-second infusion was completed. Peak hallucinogenic effects of 0.4 mg/kg doses occurred usually before the first vital sign check at 2 minutes postinjection, a useful temporal reference point for subjects. Peak effects began resolving at 90 to 120 seconds postinjection. Subjects remained moderately intoxicated by the 0.4 mg/kg dose for another 5 to 15 minutes and felt relatively normal by 25 to 30 minutes postinjection.

The nonblind days served several purposes. They allowed assessment of the cardiovascular and subjective effects of both subclinical and fully hallucinogenic doses of DMT in a novel and potentially anxiety-provoking setting. These days also provided the research team and subjects an opportunity to become acquainted and familiar with each others' styles without the extensive blood drawing and use of the rectal thermistor that could exaggerate any potentially paranoid reactions. Subjects were also able to calibrate themselves to what to expect regarding minimal and maximal effects of the drug, one of the purposes of the double-blind study being to determine whether subjects could distinguish among incremental doses of DMT. Finally, these nonblind days provided subjects with the opportunity to drop out of the study before extensive data had been collected. None withdrew, although one subject was dropped because his diastolic blood pressure rose to >100 mm Hg after a low dose.

Double-blind study days were quite similar to the nonblind days. Two IV lines were inserted, the second for frequent blood drawing, as was a flexible reusable rectal thermistor for core temperature measurement. Pupil diameter was measured against a standard card with black circles of

1 millimeter gradations near the subject's face. DMT and sterile saline placebo dosages of 0.05, 0.1, 0.2, and 0.4 mg/kg were administered. Dosages of 0.4 mg/kg were assumed to be fully hallucinogenic, 0.05 mg would be barely distinguishable from placebo, and the intermediate doses were equally spaced on a log scale. Blood samples were drawn and later assayed for beta-endorphin, adrenocorticotropic hormone (ACTH), cortisol, prolactin, growth hormone, and melatonin. Pupil diameter and vital signs (VS) (i.e., heart rate and blood pressure) were measured 2, 5, 10, 15, 30, and 60 minutes after drug administration. Since the last blood sample was at 60 minutes postinjection, the HRS was administered 30 minutes later than for the nonblind sessions. This second 30-minute period was usually taken up by more relaxed conversation, although when possible, attention was repeatedly turned back toward the subjects' drug experience.

Study sessions occurred at an interval of at least 2 weeks from January to September, 1991. The one female subject was studied during the early follicular phase of her cycle. A negative result to a pregnancy test drawn the night before her study days was always ensured before drug administration.

SCIENTIFIC ISSUES

Serotonergic Mediation of Biologic and Behavioral Effects

Hallucinogenic drugs have multiple effects on central neurotransmission. The area currently of greatest interest is serotonin and its receptors, specifically the 5-HT_2, 5-HT_{1A} and 5-HT_{1C}, subtypes (Heym and Jacobs 1987). Noradrenergic (Horita and Hamilton 1969) and dopaminergic (Ahn and Makman 1979) effects of hallucinogens have been described but are not now receiving comparable attention.

Hallucinogenic drugs, as primarily serotonergic agonists or partial agonists, have diffuse and relatively well documented physiological effects. Growth hormone, prolactin, beta-endorphin, ACTH, and cortisol are all affected by serotonergic stimuli (Van de Kar 1991). Many of these hormones rise with the administration of hallucinogens in lower animals (Koenig et al. 1987) and humans (Demsich and Neubauer 1979) and are believed modulated by 5-HT_{1A}, 5-HT_{1C}, and/or 5-HT_2 receptor subtypes. Cardiovascular variables (McCall and Humphrey 1982), core temperature (Horita and Dillie 1954), and pupillary diameter (Greiner et al. 1958) also

are affected by serotonergic hallucinogens, with the majority of data suggesting 5-HT_2 mechanisms of action.

Studies in Animals. Animal behavioral studies use several models of hallucinogenicity to pharmacologically characterize known hallucinogens and to assess whether novel compounds have hallucinogenic properties. These models include drug discrimination (DD) (Appel et al. 1982) (consistent with 5-HT_{1A} [Spencer et al. 1987] and 5-HT_2/5-HT_{1C} [Glennon, this volume] effects); the serotonin syndrome (mediated by the 5-HT_{1A} subtype [Smith and Peroutka 1986]) and one of its components, head shakes (mediated by the 5-HT_2 subtype [Peroutka et al. 1981]); the abortive limb flick in cats (Jacobs et al. 1976); and the characteristic exploratory behavior patterns of rodents placed in a novel environment (Geyer, this volume). All these models, although generating much relevant data, lack requisite specificity. For example, apomorphine (Geyer et al. 1979) and quipazine (Kuhn et al. 1978) appear hallucinogenic in some of these models, and psilocybin (Koerner and Appel 1983) is sometimes not found to be so.

The 5-HT_2/5-HT_{1C} subtype is now of most interest regarding mediation of hallucinogenic drug effects. For example, ritanserin, a potent and selective 5-HT_2/5-HT_{1C} antagonist (Sahin-Erdemeli et al. 1991), blocks the discriminable effects of LSD in lower animals (Colpaert et al. 1985). It also blocks certain neuroendocrine (Lee et al. 1991) and subjective (Seibyl et al. 1991) effects of serotonergic agonists in humans, effects that may be mediated by 5-HT_2/5-HT_{1C} receptors. Finally, anecdotal reports indicate that ritanserin pretreatment blocks the hallucinogenic effects of LSD in humans. However, the 5-HT_{1A} receptor subtype also is important. DMT (Deliganis et al. 1991) and LSD (Pierce and Peroutka 1989) have almost equal affinities for the two subtypes.

An endogenous human hallucinogen, 5-MeO-DMT (Smythies et al. 1979), used in many animal studies of hallucinogenic drug effects, has greater affinity for the 5-HT_{1A} receptor than for the 5-HT_2 receptor (McKenna et al. 1990). Additionally, the discriminative effects of 5-MeO-DMT, which generalize to LSD (Young et al. 1982) and the selective 5-HT_2/5-HT_{1C} agonist l-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (Glennon et al. 1979; Glennon et al. 1986), are blocked by the 5-HT_{1A} antagonist pindolol (Spencer et al. 1987), which is essentially devoid of 5-HT_2/5-HT_{1C} effects (Hoyer 1988). The 5-HT_{1A} receptor may therefore contribute necessary effects that produce the *characteristic* hallucinogenic drug syndrome. Although DMT's

neuroendocrine and behavioral effects were inconsistently blocked by cyproheptadine (Meltzer et al. 1980), the lack of specificity of this antagonist makes assessment of relative contributions of the 5-HT_2 and 5-HT_1 subtypes difficult.

Experimental Results of DMT Administration. Full details of the effects of DMT administration on multiple biological variables have been presented (Strassman and Qualls 1994). Generally, DMT was physically well tolerated by the subjects participating in this study. There were no episodes of nausea or vomiting with the dose range used, something that cannot be always said for longer-acting drugs (Denber 1956). One subject's diastolic blood pressure rose to >100 mm Hg after a screening, nonblind dose of 0.04 mg/kg; that subject was withdrawn from further participation. As one other subsequent subject had to be dropped from further study after a similar rise in response to 0.05 mg/kg DMT, this test dose of drug is necessary if higher doses are to be used.

As expected, based upon lower animal and clinical data, there were dose-dependent elevations in several neuroendocrine variables in response to DMT. Beta-endorphin, ACTH, cortisol, and prolactin were stimulated. The rise in POMC peptides was nearly 4 to 5 times baseline, with peak effects occurring within 5 minutes of DMT administration. Growth hormone rose relative to placebo, but the effects of the 0.05 mg/kg dose were no different than those of the 0.4 mg/kg dose. Melatonin levels in six subjects whose 0.4 mg/kg dose blood samples were analyzed demonstrated no daytime stimulation of this pineal hormone. The negative melatonin data are inconsistent with DMT's biological effects being mediated by nonspecific stress rather than by selective serotonin receptor subtype activation. The rise in beta-endorphin seen in this study is more than twice that seen after a 28.5 mile mountain race in which daytime melatonin levels doubled (Strassman et al. 1989). The earlier study proposed that massive sympathetic drive (i.e., catacholamine release) was responsible for the observed rise in melatonin. In the present study, a greater rise in beta-endorphin was seen in circumstances eliciting no effect on melatonin levels. These two sets of data make it unlikely that a stress effect (i.e., purely sympathetic activation) drove the neuroendocrine variables; if that were the case, melatonin levels would have risen, which they did not.

Additionally, there were dose-dependent elevations in mean arterial blood pressure, heart rate, rectal temperature, and pupil diameter. For nearly all of these biological dependent variables, the dose at which statistically

significant differences could be seen between DMT and placebo was 0.2 mg/kg, the threshold dose for hallucinogenic effects.

Although sublingual nitroglycerin and IV diphenhydramine were available at the bedside for every session, there was never an occasion to use them for hypertensive or allergic reactions. However, the author believes these drugs and IV diazepam should be readily available during all hallucinogenic drug sessions.

Subjective Effects

The study protocol was designed to generate normative dose-response data for DMT in nonpsychiatrically ill volunteers. The data were to include biological and psychological variables. There was little difficulty deciding upon which biological variables to study, based on human and basic data regarding 5-HT regulation of multiple physiological processes.

Rating Scale. The author decided to develop a new rating scale to carefully assess the subjective effects induced by the powerful, short-acting hallucinogen DMT rather than using previously developed instruments. The goal was to devise a scale developed from a population different than that used to develop normative data for the standard drug effect questionnaire, the Addiction Research Center Inventory (ARCI) (Haertzen and Hickey 1987). The ARCI subjects were former narcotic addicts serving prison sentences for violations of narcotics laws, and they were not informed what drugs were administered or what effects were expected. The subjects were not necessarily experienced with, or prone to use hallucinogens.

Other LSD rating scales' development also suffered from both a lack of truly informed consent and subjects' lack of previous experience with hallucinogens (Linton and Langs 1962; Abramson et al. 1955). The author believed it important to develop a questionnaire based on what respondents got out of using DMT (and other hallucinogens). The LSD scale on the ARCI is also known as the dysphoria scale, and although users of hallucinogens usually experience some dysphoria while intoxicated, they do not take these drugs for their dysphoric effects. Thus, a less pathological approach to scale development was also thought useful.

Nineteen experienced DMT free base smokers, who were also experienced with a wide variety of other hallucinogens, were interviewed

to provide a detailed description of the physical, perceptual, emotional, and cognitive effects of smoked DMT. A draft of the new scale, the HRS, was developed. It was administered to subjects in 13 nonblind low dose (0.04 mg/kg) and 12 high dose (0.4 mg/kg) DMT sessions. One low dose subject did not receive a high dose because of his blood pressure response to 0.04 mg/kg DMT. The HRS was modified based on the experiences reported during these sessions and upon suggestions of the subjects, all but two of whom had college degrees. The final questionnaire contained 126 questions. It generally took 15 to 20 minutes to fill out. The HRS was administered to subjects after all drug effects had resolved; subjects usually would fill out the questionnaire while eating or relaxing in bed. The brief duration of drug effect and incapacitating nature of the higher doses precluded subjects answering the questionnaire during the acute intoxication.

Two methods were used to create a more manageable number of groups of questions. The first method for developing factors was the clinical method. Based upon initial interviews with DMT smokers, the subjects' narrative accounts of their experiences, and a mental status descriptive approach to mental functions, six clusters of conceptually coherent questions were created. These clusters were: (1) affect, (2) somatesthesia/interoception, (3) intensity, (4) perception, (5) cognition, and (6) volition.

The second method of developing clusters was based on principal component factor analysis with varimax rotation, allowing the computer to create relevant factors equal in number to the clinical clusters. A detailed description of the development of the HRS and the derivation of factors/clusters has been published previously (Strassman et al. 1994).

Results. These data were described in more detail by Strassman and colleagues (1994). Behaviorally, DMT caused no troublesome effects in the dose range 0.04 to 0.4 mg/kg. No subject dropped out after the nonblind screening administration of the low (0.04 mg/kg) and high (0.4 mg/kg) doses. Only one subject was withdrawn halfway through the study because of the development of a major depressive episode. However, he had a history of recurrent major depression, acute episodes being triggered by stressful personal circumstances similar to those he was experiencing while in the study. Followup of 11 of the 12 subjects has not revealed any delayed negative effects of participation. The one subject lost to followup had gone through the protocol with no difficulties.

A brief summary of each dose's effects follows.

0.4 mg/kg: All subjects described an intense, rapidly developing rush that was both pleasurable and transiently anxiety-provoking, felt throughout the body and mind. Visual effects, noted by all subjects, ranged from intensely colored, rapidly moving, concrete, formed, more or less recognizable images with eyes open or closed, to abstract geometric patterns that were not obviously representational. With eyes open, the visual field was overlaid by geometric patterns, with undulating movement and intensification of colors of objects. Auditory hallucinations were noted in more than half the subjects but were not clearly familiar (i.e., music or voices). Subjects described usually high-pitched, whining, chattering, crinkling/crunching, or at times comical noises, such as "boing" and "sproing" sounds heard in cartoons. Somatesthetic effects were of a highly stimulatory fear-response nature, although all subjects distinguished between their physical reaction to the drug and the less emotional subjective response to this reaction.

As effects resolved, the physical sensations became pleasant and relaxing. Some subjects (N=2) described a sexual effect of the highest dose, a sensation of heat developing in the genital area; no one experienced orgasm or ejaculated. After the initial anxiety associated with the onset of high dose effects, all but one subject (11/12) described exciting, euphoric, and highly positively charged feelings, often related to the visual hallucinatory display. One-third to one-half of the subjects also described the emotional valence as bland, impersonal, and journalistic in nature, the experience developing and resolving so quickly that there did not seem to be time for subjects to react or modify it in any way. Subjects described their thoughts and evaluative capacities as relatively unimpaired and likened the experience to a dream in which, although awareness of the outside world was abolished, they were alert and attentive to the hallucinatory effects, remembering them quite well and in detail.

The first nonblind high dose was more anxiety-ridden (particularly for the first 30 seconds postinjection) than was the subsequent high dose. Subjects were better prepared to lose control after having been in that state once before. Their understanding that the drug experience was physically safe and that they would not lose their minds was strengthened by having had the high dose before. Finally, their confidence in the research team to unobtrusively support their relatively helpless state grew as their participation in the study progressed.

0.2 mg/kg: This generally was the threshold dose for hallucinogenic effects. The majority of subjects had some visual hallucinations, but auditory hallucinations were rare. Some found this to be their dose of choice, being less disorienting and anxiety provoking in onset than the 0.4 mg/kg dose, but generating enough perceptual and affective effects to be interesting and pleasurable.

0.1 mg/kg: This dose was not enjoyed by about half of the subjects. They felt the somatesthetic sensations of excitation and a tense, dysphoric "drive to discharge" more striking than the perceptual or affective changes.

0.05 mg/kg: One-third of the subjects mistook this dose for placebo. Those who were able to distinguish the dose from saline remarked on its uniformly relaxing, comfortable, and warm physical effects. One former heroin user likened it to the "soft cotton batting" of heroin. There were no perceptual (auditory or visual) effects at this dose.

Either method of grouping questions, the clinical clustering or the principal components factor analysis, provided better statistical separation of doses than did the biological factors described above. Two factors, intensity and cognition, separated all five treatments. Two of the principal component factors also could distinguish among the five treatments. The clinical clusters were more capable of separating placebo from 0.05 mg/kg effects; the principal component factors were more sensitive in distinguishing between hallucinogenic doses (0.2 mg/kg) and nonhallucinogenic (0.1 mg/kg) doses.

SUMMARY AND AREAS FOR FUTURE RESEARCH

This study demonstrated that if experienced hallucinogen users are carefully screened, prepared, supervised, and followed up, the naturally occurring short-acting hallucinogen DMT can be safely administered repeatedly in a clinical research setting. Normative dose-response data for multiple biological variables were generated, including neuroendocrine, cardiovascular, and autonomic (temperature and pupil diameter) variables. Data generated from a new rating scale, the HRS, provide a detailed account of subjective effects using several factors/clusters that demonstrate better resolution of dose effects than did the biological variables. Statistically significant effects of DMT on biological variables relative to placebo were almost always associated

with hallucinogenic effects. These biological and psychological data will provide the bases for further pharmacologic characterization of hallucinogenic drugs' properties.

Selective Blockade

A logical series of followup studies involve selective blockade of 5-HT receptor subtypes before DMT administration. Ritanserin or a similar 5-HT_{1C}/5-HT_2 antagonist could be used in this manner, and effects on DMT's biological and psychological perturbations could be assessed. If ritanserin does block DMT's neuroendocrine and/or behavioral effects, basic neuropharmacologic data will be confirmed in humans regarding the primacy of the 5-HT_2/5-HT_{1C} receptor(s) in mediating hallucinogenic drug effects. Second, a selective and safe antagonist might be available for hallucinogenic drug crises (Sadzot et al. 1989). Third, if ritanserin pretreatment *enhances* any effects of DMT, as might be hypothesized based on the enhancement of neuroendocrine responses to L-tryptophan by ritanserin in humans (Charig et al. 1986), an understanding of the functional interactions between 5-HT_2/5-HT_{1C} and 5-HT_{1A} receptors in humans will be advanced. Fourth, less related to drug abuse but also of clinical import, would be justification for the previously mentioned use of ritanserin in endogenous hallucinatory states.

Pindolol, a beta-blocker with potent 5-HT_{1A} antagonistic effects, has been used in humans to block presumptive 5-HT_{1A} agonists' neuroendocrine and other biological effects (Anderson and Cowen 1992; Lesch et al. 1990). No data exist on whether pindolol blocks serotonergic agonists' psychological effects in humans. Modulation of DMT's biological or psychological effects would also provide data validating or refuting the role of the 5-HT_{1A} receptor in the mechanism of action of hallucinogens.

Tolerance

Repeated or chronic administration of behaviorally active drugs often results in decreased responsiveness to their acute effects. Tolerance to LSD's behavioral effects was established in the rat (Freedman et al. 1958) and behaviorally and physiologically in humans (Isbell et al. 1956). Current emphasis is on receptor downregulation, particularly with respect to the 5-HT_2 subtype, which can occur within 2 to 3 days (Buckholtz et al. 1990).

DMT is unique in human hallucinogenic drug research in that tolerance to its effects has never been demonstrated (Gillin et al. 1976). These investigators administered a fully hallucinogenic IM dose of DMT twice a day for 5 days and found no tolerance to psychological, cardiovascular, or pupillary effects. Additionally, DMT's relatively undiminished effects in subjects tolerant to LSD (Rosenberg et al. 1964) is puzzling and may be associated with differential 5-HT_{1A} versus 5-HT_2/5-HT_{1C} effects. Illicit users describe variable tolerance depending on frequency of smoked administration. In animals, heroic regimes may be necessary to induce tolerance (Kovacic and Domino 1976). DMT's electroencephalographic (EEG) effects, in fact, may be potentiated by repeated administration (Gillin et al. 1973). In addition, 5-MeO-DMT's prolactin-raising effects are enhanced with repeated administration (Simonovic and Meltzer 1979), in contrast to LSD's effect on rat corticosterone showing tolerance (Halaris et al. 1976). Clearly the situation is complex and requires human studies with special attention to dose and interval.

Gender Differences

Animal data suggest a role for sex steroids in modulating several facets of serotonergic neurotransmission. Female rats are more sensitive than males to 5-HT_2-mediated hyperthermia (Bigeon et al. 1979) and show' a more robust prolactin response to the hallucinogen 5-MeO-DMT (McBride et al. 1990)

Human data also suggest sex differences in several parameters of 5-HT function. However, many studies demonstrating effects of gender on serotonergic variables do not control for menstrual state; for example, womens' less robust cortisol response to 5-hydroxy-tryptophan (Maes et al. 1989). Recent data do suggest menstrual phase effects of responsivity to 5-HT agonists (O'Keene et al. 1991). Prevalence of hallucinogen use in males in all ethnic groups is two to three times that in females (see above). Is this due to unpredictable effects of hallucinogens in women because of differential sensitivity across the menstrual cycle? A study of hallucinogenic drugs' effects across the cycle will provide additional data relating human and animal psychopharmacology and neuroendocrinology. More importantly, it will also establish norms by which data on human male and female hallucinogenic drug responses can be compared.

Other Hallucinogens

Now that systematic dose-response data for the biological and psychological effects of DMT in experienced hallucinogen users have been generated, a similar approach may be taken with other drugs. These could include psilocybin, the primary hallucinogen in "magic" mushrooms (Beug and Bigwood 1982); LSD; and phenylethlyamines such' as DOM, DOB, and DOI. These latter compounds are generally included with the classical hallucinogens, but little or no systematically acquired human data have been published to support or refute this classification. The biological and psychological data obtained with DMT could be compared and contrasted to that generated by similar dose-response studies with other similar compounds.

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AUTHOR

Rick J. Strassman, M.D.
Associate Professor
Department of Psychiatry
University of New Mexico
2400 Tucker Avenue, NE.
Albuquerque, NM 87131

Serotonin Receptor Involvement in an Animal Model of the Acute Effects of Hallucinogens

Mark A. Geyer and Kirsten M. Krebs

INTRODUCTION

To facilitate the study of hallucinogenic drugs in the whole animal, investigators have explored a variety of animal behavioral measures that potentially could parallel hallucinogenic activity in humans (see Glennon, this volume), In the development of such models, d-lysergic acid diethylamide (LSD), the most well-known and potent of the hallucinogens, has been used most frequently as the prototype drug. Structurally an ergot alkaloid compound, LSD acts on the serotonin (5-hydroxytryptamine [5-HT]) system as a partial agonist at both 5-HT_{1A} and 5-$HT_{1C/2}$ receptors (Peroutka and Snyder 1979; Sanders-Bush et al. 1988). In attempting to model the behavioral effects of hallucinogens in humans, considerable research has focused on examining the responsiveness of animals to discrete phasic stimuli. Such studies have revealed that LSD reduces the rate of habituation of reflex responding (Geyer et al. 1978; Izquierdo 1975; Miliaressis and St. Laurent 1974) and produces alterations in both conditioned and unconditioned responding consistent with enhanced reactivity to irrelevant stimuli without direct alterations of sensory thresholds (Key 1964*a*,*b*; Key and Bradley 1960). Unfortunately, none of these effects has been demonstrated to be both common to a variety of hallucinogens and uncharacteristic of nonhallucinogens (Geyer et al. 1978; Stoff et al. 1978), criteria that are vital to an animal model of hallucinogenic activity.

More recently, researchers have attempted to use the elicitation by hallucinogens of animal behaviors that are not typically observed in normal animals as indicators of hallucinogenic action. While problems of nonselectivity still apply to most such behaviors, including increased initial startle responses, flat body posture, forepaw treading, and lower lip retraction (Arnt and Hyttel 1989; Berendsen and Broekkamp 1991; Braff and Geyer 1980; Johansson et al. 1990), other behaviors may be more specifically associated with hallucinogens. For example, behaviors such as head twitches and shakes have been elicited by hallucinogenic

compounds but not by nonhallucinogenic 5-HT agonists of the 5-HT_{1A} receptor such as 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) (Berendsen and Broekkamp 1991; Darmani et al. 1990*a,b*). However, most of these responses are elicited only by relatively high doses of hallucinogenic drugs and are difficult to quantitate by automated procedures. Further, such measures provide no information about the psychological processes influenced by the hallucinogens and are difficult to relate directly to the subjective effects of hallucinogens in humans.

Among the most widely used and successful approaches to assessing the hallucinogenic activity of drugs is the drug discrimination (DD) procedure discussed in more detail elsewhere in this volume (Glennon; Winter). While this procedure has demonstrated considerable predictive validity, the psychological nature of the effect(s) being detected is difficult to define. Furthermore, the procedure requires that the animals be treated repeatedly with the hallucinogen being studied. Such repeated administrations prevent the application of the procedure to the study of the acute effects of hallucinogens, which is important because of the rapidity with which tolerance occurs to the effects of hallucinogens in humans.

HALLUCINOGEN EFFECTS ON EXPLORATORY BEHAVIOR

Recent studies of the effects of acute administrations of hallucinogens on spontaneous behaviors of experimental animals have revealed consistent and specific effects on the normal behavioral repertoire of the animals that can be studied efficiently with automated procedures. The initial evaluations of the effects of LSD on locomotor activity yielded seemingly inconsistent results. Depending on the specific parameters used in each particular study, hallucinogens were found to increase, decrease, or produce no change in the amount of locomotor or investigatory behavior.

This apparent inconsistency is now understood as being a reflection of the fact that the effects of hallucinogens on spontaneous activity are demonstrably dependent on the size of the experimental chamber (Hughes 1973), the nature and degree of stimulation from the test environment (Cunha and Masur 1978), the animal's degree of familiarity with the test environment (Adams and Geyer 1985*a;* Tilson et al. 1975), and the manner in which the animals are handled prior to testing (Geyer and Light 1979). Thus, the changes in locomotor or investigatory

behavior produced by hallucinogenic drugs are critically dependent on the precise nature of the environmental context in which the animals are tested. Furthermore, this variability in the effects of hallucinogens on measures of locomotion suggests that locomotion *per se* is not directly affected by these drugs. Rather, the changes in locomotion appear to be secondary to the effects of hallucinogens on the animal's sensitivity to environmental stimuli. As in humans, hallucinogens do not lead to consistent effects on the level of arousal as reflected in measures of motor activity; rather, hallucinogens alter the manner in which the organism's behavior is influenced by the environment.

To examine this hypothesis more directly, several researchers have studied the effects of hallucinogens on the exploratory behavior of animals. One of the most common tests of exploration uses a holeboard, which is simply a chamber with holes placed in the floor and/or walls. These holes serve as specific stimuli that burrowing animals such as rats readily investigate. Hence, measures of the frequency or duration of "holepokes" are viewed as indices of the animal's investigatory tendencies (File and Wardill 1975). In an extensive series of studies, LSD and several other hallucinogens were found to produce dose-dependent alterations in the temporal distribution of holepokes, consisting of an initial reduction and subsequent increase during a 24-minute test (Geyer et al. 1979). This temporal distribution of responding was found to be unaltered by variations in preinjection time, suggesting that it was related to drug-induced alterations in responsiveness to the environment rather than to the time-course of drug action. Additional studies using the holeboard confirmed that the initial decrease in exploration was attributable to an interaction between the effects of the drug, the animal's exposure to the stimuli associated with handling, and the introduction of the rat to the novel chamber (Geyer and Light 1979). These effects were therefore interpreted as an LSD-induced enhancement of the animal's responsiveness to the test environment.

A BEHAVIORAL PROFILE OF HALLUCINOGEN EFFECTS

Subsequent studies have more thoroughly characterized the effects of hallucinogenic drugs on the responsiveness of rats to environmental stimuli by simultaneously monitoring both locomotor and investigatory responses in the behavioral pattern monitor (BPM) system. The BPM is a combination of an open-field activity monitor and a holeboard. By tracking the movements of the animals within a Cartesian coordinate

system of infrared photobeams, the BPM enables analyses of quantitative and qualitative changes in patterns of locomotor and investigatory activity (Geyer 1990). The BPM is a 30.5 x 61.0 centimeter (cm) black Plexiglas™ chamber illustrated schematically in figure 1. Ten 2.5 cm holes are placed in the chamber (three in each long wall, one in one short wall, and three in the floor). Photocells in each hole detect investigatory holepokes. A touchplate 15.2 cm above the floor allows detection of rearings when contact is made by the animal between the metal floor and the metal touchplate. A 4 x 8 grid of infrared photobeams detects the animal's position in an X-Y plane. Every 200 milliseconds (ms) the computer sampled the status of all the beams in each chamber, enabling reconstruction of the path taken by the animal and calculation of a variety of descriptive statistics that reflect changes in the amount or pattern of the activity.

When rats were tested by placing them directly into a novel BPM chamber (forced exploration), a consistent effect of LSD was a dose-dependent reduction of exploration in the first half of an hour-long test session (Adams and Geyer 1982). In order to ensure that the behaviors being studied were as natural as possible in a laboratory setting, all studies were conducted in the dark phase of the animal's day-night cycle when nocturnal animals such as rats are typically most active. To maximize the exploratory behavior of the control animals and minimize the influences of extraneous factors, the animals were tested in dark chambers that were kept scrupulously clean. In addition, it has proven essential that the animals be handled as gently and consistently as possible as exemplified by the demonstrated influence of handling on the behavioral effects of LSD (Geyer and Light 1979). During the week before testing, animals were also acclimated to the handling associated with transport to the laboratory and injections.

Under these controlled conditions the number of photobeam interruptions (movements) was decreased very consistently by reasonably low doses of LSD as illustrated in figure 2a (Adams and Geyer 1982, 1985*a*). At the same time both holepokes and rearings, two measures of investigatory behavior, were also decreased by LSD (figure 3). These results confirmed the findings of Geyer and Light (1979) and extended those findings to include activity and rearings as well as holepokes. These decreases were limited to the initial 30 minutes of the test session, although a variety of other drugs decreased activity throughout the hour-long test session (Mittman and Geyer 1989). Further, these decreases in locomotor and investigatory behavior were specifically

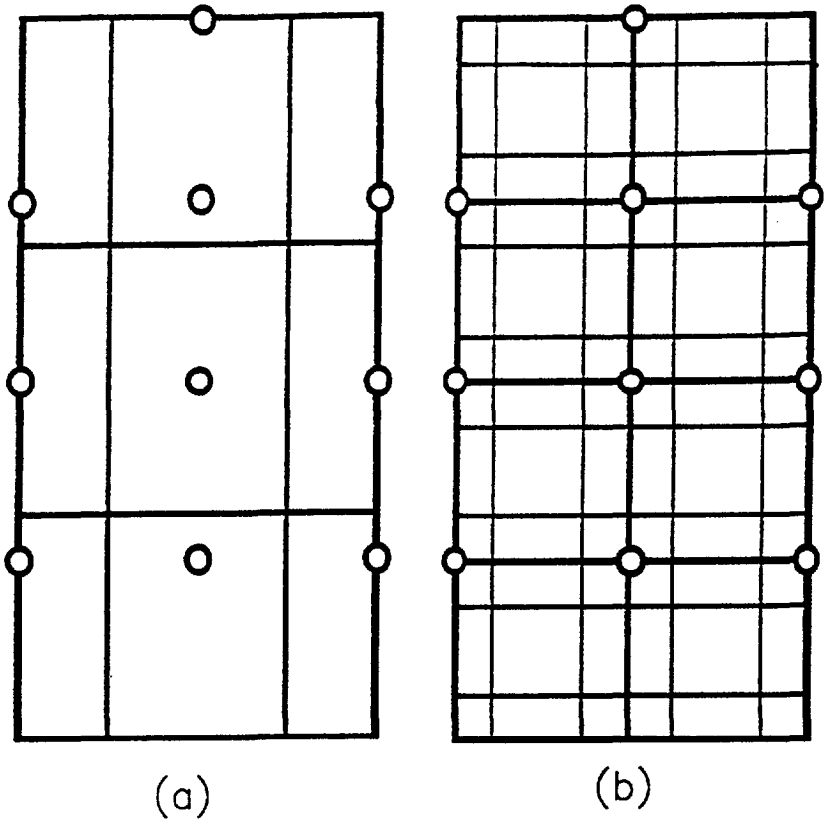

FIGURE 1. *Diagrammatic representation of the BPM chamber (30.5 x 61 cm). (a) shows black lines indicating regions, unequal areas used to define transitions from one region to another for assessment of locomotor patterns. (b) Infrared photobeams are represented here by thin black lines and are used to define movements, a measure of locomotor activity. Sectors, equal 15 cm squares, are indicated by thick black lincs and are used to define crossings, a measure of horizontal locomotion.Wall and floor holes are portrayed by open circles and are used to define investigatory holepokes.*

related to the animal's initial exploration of the novel chamber rather than the time-course of the drug's action. Hence, the effect of LSD is not limited to the investigation of discrete stimuli and cannot be described as generalized sedation, but appears to reflect a more pervasive influence of the drug on responsiveness to a wide range of environmental stimuli.

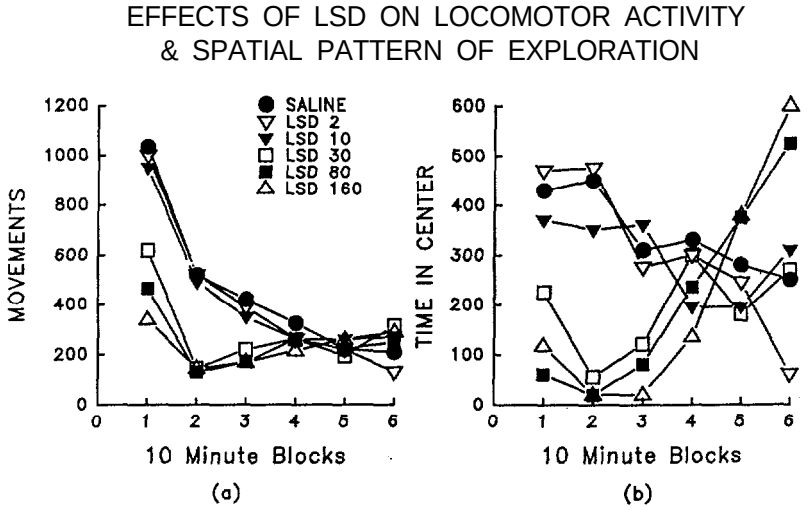

FIGURE 2. *Disruption of spatial pattern and amount of locomotor activity by LSD. Six groups of 10 male rats each were tested in the BPM chambers 10 min following injections (SC) of 0, 2, 10, 30, 80, or 160 ug/kg LSD. LSD dose-dependently decreased both (a) movements (total photobeam breaks in the BPM) and (b) the time the animals spent in the center of the chamber (refer to figure 1 for center region representation) in tenths of a second. (Values are group means per 10-min block of time, $p<0.05$). Redrawn from data presented in Adams and Geyer 1985a.*

One of the most robust and sensitive measures of the effect of LSD is the decrease in the amount of time that the animal spends in the center of the chamber, as illustrated in figure 2b. Because the tendency of rats to explore a novel environment is generally believed to compete for expression with the opposite tendency to avoid novel and open spaces (Berlyne 1966; Montgomery 1955), the pattern of effects produced by LSD suggests that the drug enhances the latter tendency. Analyses of the spatial patterns of locomotion reveal that LSD's initial suppression of activity and holepokes were best correlated with a reduction in entries into, and time spent in, the central region of the chamber (Adams and Geyer 1982, 1985*a).* As illustrated by computer-generated displays of locomotor patterns (figure 4), the effects of LSD on exploration of novel and central areas are profound (Adams and Geyer 1985*a*). It has long been recognized that normal rats tend to avoid large, open spaces (Barnett

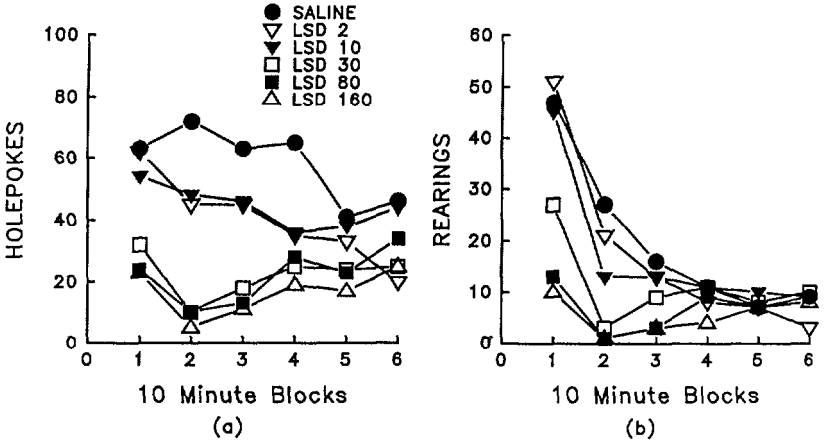

FIGURE 3. *Disruption of exploratory behavior by LSD. Six groups of male rats (n = 10) were tested in the BPM 10 min after receiving injections (sc) of 0, 2, 10, 30, 80, or 160 µ/kg LSD. LSD produced significant dose-dependent decreases in both (a) the number of holepokes made during testing and (b) the number of rearings against the wails. (Values are group means per 10-min block, $p<0.05$). Redrawn from data presented in Adams and Geyer 1985a.*

1963). As with the exaggerated response of hallucinogen-treated rats to the aversive aspects of the exposed central region of the chamber, there is also an increased tendency of hallucinogen-treated rats to avoid novel chambers altogether.

To further clarify the nature of this effect, animals were tested in a free exploration paradigm (Welker 1957) in which a familiar home cage is connected to the open field. When rats were allowed to enter and leave the novel chamber at will, LSD produced dose-dependent reductions in the amount of time spent in the novel chamber without an alteration in the overall rate of locomotor or investigatory responses while they were in the chamber (Adams and Geyer 1982) (figure 5). Hence, the effect is not attributable to sedation; rather it reflects an alteration in the responsiveness of the animal to the nature of the test chamber itself. In

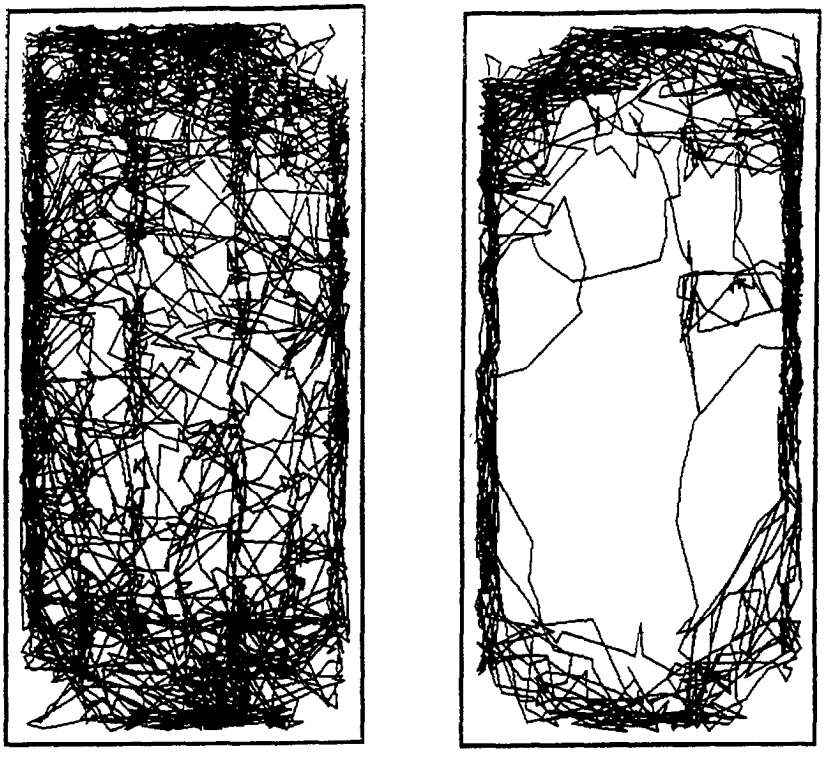

FIGURE 4. *Computer-generated display of the locomotor path produced during the initial 30 min of testing in the BPM by two different representative rats treated with (a) saline or (b) 60 μ/kg LSD. In contrast to the varied path the saline-treated rat takes, the LSD-treated animal makes few center entries, prefers one corner of the chamber, and circles the periphery of the BPM chamber.*

further studies, it was found that the initial suppression of activity induced by LSD was absent when animals were tested in a familiar environment (figure 6*a*). The fact that familiarizing an animal with the test environment attenuates the suppression of locomotor activity induced by hallucinogens suggests that LSD potentiates the normal neophobia exhibited by rats when confronted with a novel environment (Adams and Geyer 1985*a*).

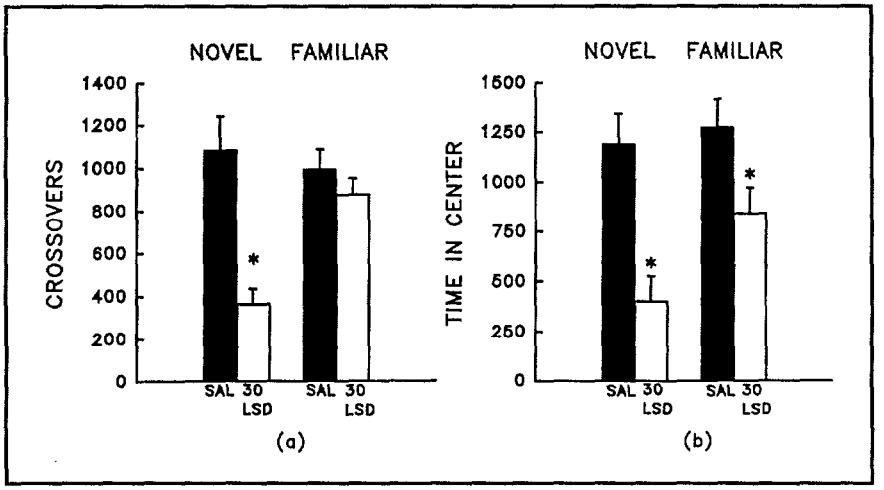

FIGURE 5. *Disruption of locomotor activity by LSD. Five groups of male rats (n = 8-10) were tested in a modified BPM chamber that allowed animals to move freely between the BPM and a home cage environment (free exploration). Ten min prior to testing, injections (SC) of 0, 2, 10, 30, or 80 µ/kg LSD were given. LSD significantly decreased the number of crossovers (movements between 15 cm square sectors) but had no effect on the rate of locomotor activity (crossovers/min). (Values are means from total counts or transforms performed on 60 min totals, $p<0.001$). Redrawn from data presented in Adams and Geyer 1982.*

By contrast, the decrease in time spent in the center of the chamber was still evident in the familiar environment (figure 6*b*), indicating that LSD potentiates the normal animal's response to aversive or threatening environmental stimuli, in this case the center of the chamber, even in a familiar environment. This effect has been characterized as an increase in agoraphobia. Thus, in the typical novel environment paradigm, LSD potentiates both neophobia and agoraphobia (Adams and Geyer 1985*a*).

One of the most stringent tests of the specificity of any behavioral model of the effects of LSD involves comparison of the effects of LSD in the model to the effects of lisuride in the model. Lisuride is a behaviorally potent derivative of isolysergic acid that closely resembles LSD in both chemical structure and neurochemical effects. Nevertheless, in normal humans, lisuride appears to produce none of the perceptual changes or

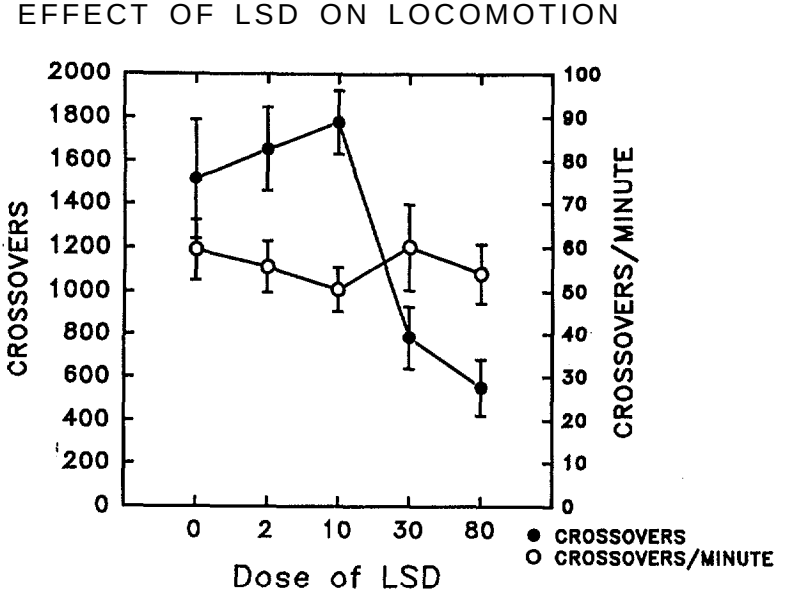

FIGURE 6. *Impact of familiarization with test chamber on LSD's effects on the spatial pattern and amount of locomotor activity. Four groups of male rats (n = 10-16) were tested in the BPM 10 min after receiving injections of either saline or 30 µ/kg LSD (SC). The novel groups were tested only once, while the familiar groups were tested after two previous exposures (without injections) to the BPM. In a novel environment, 30 µ/kg LSD significantly decreased (a) crossovers (movements between 15 cm square sectors) and (b) the time spent in the center of the chamber.However, in a familiar environment, the effects of LSD were attenuated on (a) crossovers, but not on (b) the time spent in the center of the chamber. (Values are group means for the first 30 min of testing, p<0.05). Redrawn from data presented in Adams and Geyer 1985a.*

intensifications of responsiveness that are characteristic of hallucinogens (Herrmann et al. 1977). In rats, low doses of lisuride produced sedation with no evidence of an enhanced avoidance of open or novel spaces, while higher doses produced stereotyped patterns of locomotor hyperactivity that were quite different from the effects of high doses of LSD (Adams and Geyer 1985*c*).

A further distinction between the behavioral effects of lisuride and LSD was revealed by the drugs' interactions with the neuroleptic haloperidol. Haloperidol blocked the hyperactivity produced by high doses of lisuride but failed to alter the effects of LSD on enhanced responsiveness to the environment (Adams 1983). Thus, this animal model of hallucinogenic activity was sensitive enough to discriminate easily between lisuride and LSD, two compounds that differ primarily with respect to their hallucinogenic effects.

One of the hallmarks of the effects of hallucinogens in humans is the rapidity with which tolerance occurs to their acute effects (Freedman 1981; Freedman et al. 1964; Isbell et al. 1956; Mandell and Geyer 1976). Hence, any behavioral effect in an animal model purporting to reflect the physiology underlying the defining effects of hallucinogens in humans is expected to exhibit rapid tolerance. As shown in figure 7, LSD's effects on locomotor activity in rats showed partial tolerance 24 hours after a single dose and complete tolerance following five daily injections. Tolerance was also observed to the effects of LSD on investigatory holepokes and rearing after five consecutive injections of LSD (Adams and Geyer 1985*a*). Thus, this profile of behavioral effects is especially sensitive only to the acute effects of LSD, which are presumed to be those most relevant to the subjective effects reported by humans.

While all the hallucinogens tested in this paradigm produced a behavioral profile similar to that of LSD, other psychoactive drugs do not. Table 1 lists the psychoactive drugs that have been examined in full dose-response studies using this paradigm. In addition to the ergot derivative LSD, similar effects have been observed with both phenylalkylamine hallucinogens such as mescaline and 2,5-dimethoxy-4-methylamphetamine (DOM) and indoleamine hallucinogens such as psilocin and N,N-dimethyltryptamine (DMT) (Adams and Geyer 1985*b;* Geyer et al. 1979).

By contrast, nonhallucinogenic congeners of both LSD and DOM fail to produce this behavioral profile. Furthermore, drugs from a variety of other categories produce no effects or different effects in this model. Among the common false positives that frequently occur in establishing the specificity of animal models of hallucinogens, lisuride and apomorphine are notable in that they are readily discriminated from the hallucinogens in this model (Adams and Geyer 1985*c;* Geyer et al. 1986). Quipazine is one drug that shares 5-HT_2/5-HT_{1C} agonist properties with

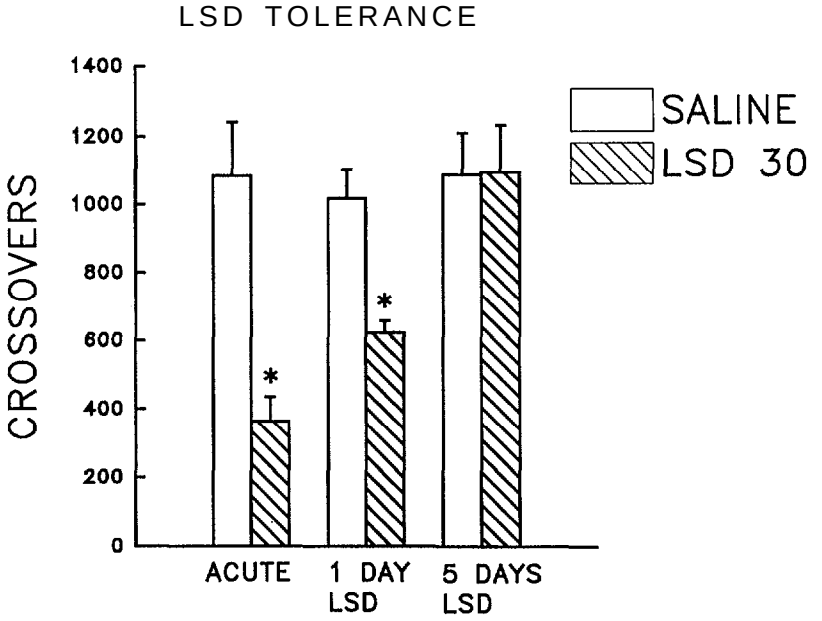

FIGURE 7. *Tolerance to the effects of LSD on locomotor activity. Six groups of male rats (n = 6) were pretreated chronically with either saline or LSD (30 µ/kg SC) daily. Two groups received five daily injections of saline and were tested 24 hrs later in the BPM 10 min after injections of either saline or 30 µ/kg LSD [ACUTE]. Two groups received four daily injections of saline and an injection of 30 µ/kg LSD on the fifth day, and then were tested on the sixth day after injections of either saline or LSD (30 µ/kg SC) [1 DAY LSD]. Two groups received five daily injections of LSD (30 µ/kg SC) and were tested the next day 10 min after injections of saline or 30 µ/kg LSD (n = 14) [5 DAYS LSD]. The acute effects of LSD, e.g. decreasing crossovers (movements between 15 cm square sectors), were partially blocked by a single injection of LSD and completely blocked by five days of chronic LSD treatment. (Values are group means for the first 30 min of testing, $p<0.05$). Redrawn from data presented in Adams and Geyer 1985a.*

TABLE 1. *This table summarizes data presented in: Adams and Geyer 1985a, b, c; Callaway et al. 1991; Geyer and Frampton 1988; Geyer et al. 1979, 1986; Gold et al. 1988; Lehmann-Masten and Geyer 1991; Mittman and Geyer 1989; Rempel et al., in press; and Wing et al. 1990.*

HALLUCINOGENS EXHIBITING PROFILE	DRUGS EXHIBITING DIFFERENT PROFILES	
LSD	8-OH-DPAT	AMPHETAMINE
DOM	BUSPIRONE	APOMORPHINE
DOET	GEPIRONE	COCAINE
DOPR	IPSAPIRONE	SCOPOLAMINE
MESCALINE	TFMPP, mCPP	NICOTINE
PSILOCIN	RU-24969	CAFFEINE
DMT	DMA, DOAM	CLENBUTEROL
5-MeO-DMT	MDMA, MDA	CLONIDINE
(QUIPAZINE)	MBDB, PCA	NADOLOL
	LISURIDE	MORPHINE
	METHYSERGIDE	PCPA
	CYPROHEPTADINE	FLUOXETINE
	HALOPERIDOL	CHLORIMIPRAMINE
	PROPRANOLOL	PHENCYCLIDINE
	RITANSERIN	DIZOCILPINE
	PINDOLOL	

the classical hallucinogens but that is not clearly established as being hallucinogenic in humans. As it produces a comparable behavioral profile, quipazine appears to be similar to the hallucinogens (Wing et al. 1990). Whether this drug represents a false positive cannot be easily determined without more adequate studies of its effects in humans.

In table 1, indirect serotonin agonists such as MDMA and MDA are listed as being different from the classical hallucinogens, despite the fact that they have been classified in legal terms together with hallucinogens and have been described as having related effects. As reviewed in detail elsewhere (Geyer and Callaway 1994), these compounds differ markedly from both typical psychostimulants and classical hallucinogens in terms of their behavioral effects and mechanisms of action and should be considered as a distinct class of drugs. In summary, the increased

avoidance of novel and open spaces induced by LSD in rats appears to satisfy several requirements for an animal model of hallucinogenic activity. First, the effect is common to all the hallucinogens tested at doses that generally correspond to the potencies of these drugs in humans. Second, this profile of effects has not been reproduced by any of the nonhallucinogenic drugs tested, including inactive congeners of hallucinogens. Third, tolerance to this increased avoidance effect of LSD develops at a rate is comparable to that observed in humans.

SEROTONERGIC MECHANISMS OF HALLUCINOGEN EFFECTS

In order to investigate the mechanisms of action of hallucinogens, a number of studies have examined the 5-HT receptor subtypes that contribute to the hallucinogen-induced behavioral profile of an increased avoidance of novel and open spaces. In the authors' laboratory, three approaches to this investigation have been used. First, selective 5-HT agonists have been tested. Second, 5-HT receptor antagonists, such as they are, have been tested for their ability to block the effects of the respective agonists. Third, due to the limited set of 5-HT antagonists, cross-tolerance paradigms have been used to examine the involvement of particular receptor subtypes in the behavioral effects of the agonists.

Serotonin Agonists

Of the phenalkylamine hallucinogens which are more selective for the 5-HT_{1C}/5-HT_2 receptor sites, mescaline and 2,5-dimethoxy-4-iodo-amphetamine (DOI) as well as other 5-HT_{1C}/5-HT_2 agonists produced dose-related decreases in the initial levels of exploratory activity in the same BPM paradigm used to characterize the effects of LSD (Wing et al. 1990). In each case, these decreases were accompanied by preferential decreases in entries into and time spent in the center of the chamber. As with LSD, this suppression of exploratory behavior was attenuated significantly when rats were familiarized with the testing chamber prior to the administration of DOI (Wing et al. 1990). Similarly, as shown in figure 8, acute administration of the 5-HT_{1C}/5-HT_2 agonist DOM suppressed locomotion and both entries into and time spent in the center of the BPM chamber (Adams and Geyer 1985*b).* Familiarization with the chamber diminished the effect of DOM on exploratory locomotion but not on the rats' tendency to enter the center of the chamber (figure 8).

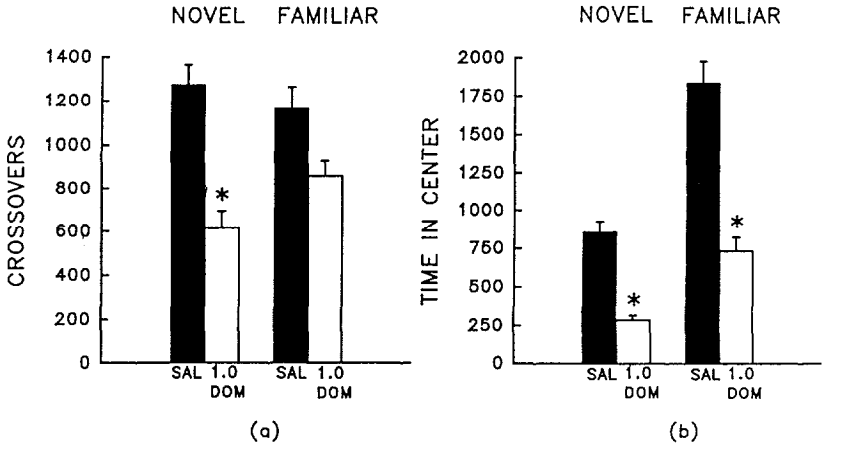

FIGURE 8. *Impact of familiarization with test chamber on DOM's effects on the spatial pattern and amount of locomotor activity. Four groups of 10 male rats each were tested in the BPM 10 min after receiving injections of either saline or 1.0 mg/kg DOM (SC). The novel groups were tested only once, while the familiar groups were tested after two previous exposures (without injections) to the BPM. In a novel environment, 1.0 mg/kg DOM significantly decreased both (a) crossovers (movements between 15 cm square sectors) and (b) the time spent in the center of the chamber. However, in a familiar environment, the effects of DOM were attenuated on (a) crossovers, but not on (b) the time spent in the center of the chamber. (Values are group means for the first 30 min of testing, $p<0.05$). Redrawn from data presented in Adams and Geyer 1985b.*

Thus, as with LSD, phenalkylamine hallucinogens such as mescaline, DOI, and DOM suppress exploratory locomotion and time spent in the center. As with LSD, the effects of DOI and DOM on locomotion are diminished when a familiar test chamber is used, although familiarization does not suppress the potentiation of agoraphobia as operationally measured by the time spent in the center of the chamber. Similarly, the indoleamine hallucinogen N,N-dimethyltryptamine (DMT) also preferentially suppressed both entries into the center and investigatory holepokes when rats were tested in a novel BPM chamber (Adams and Geyer 1985*b*). Thus, LSD and more selective 5-HT_{1C}/5-HT_2 agonists exaggerate the animal's normal responses to aversive sensory stimuli. In

the novel environment, this potentiation of sensory responsiveness is expressed as initial decreases in exploratory behavior. In a familiar environment, the effect is expressed in a more specific decrease in visits to the open area in the center of the chamber.

The 5-HT_{1A} agonists, which are not generally considered hallucinogenic, have also been tested in this behavioral paradigm. Buspirone, 8-OH-DPAT, gepirone, and ipsapirone all produced decreases in locomotor activity in a novel environment. The decreases were superficially similar to those produced by the 5-HT_{1C}/5-HT_2 agonists (Mittman and Geyer 1989). Unlike the mixed 5-HT_{1A} and 5-HT_{1C}/5-HT_2 agonist LSD, the more selective 5-HT_{1A} compounds produced decreases in center entries that were strictly proportional to the decreases in other measures of activity and were typically maintained throughout the test session. Furthermore, in contrast to the effects of LSD or phenalkylamine hallucinogens, the effects of the 5-HT_{1A} agonists were not attenuated when the animals were tested in a familiar environment (figure 9). These decreases were more indicative of a general suppression of motor behavior rather than of a specific potentiation of the animals' neophobic and agoraphobic responses to threatening stimuli (Mittman and Geyer 1989). Thus, hallucinogenic 5-HT agonists such as the 5-HT_{1C}/5-HT_2 agonists DMT, DOI, and mescaline were found to produce behavior similar to that produced by LSD, while 5-HT_{1A} agonists that were not hallucinogenic produced behavior more akin to a generalized sedation rather than the behavioral profile elicited by LSD administration.

Serotonin Antagonists

The effects of the 5-HT_{1C}/5-HT_2 and 5-HT_{1A} agonists in this paradigm can be distinguished pharmacologically as well as behaviorally. Mittman and Geyer (1991) showed that the effects of the 5-HT_{1C}/5-HT_2 agonist DOI on exploratory locomotion and time spent in the center were blocked effectively by the 5-HT_{1C}/5-HT_2 antagonist ritanserin as shown in figure 10. Similar effects were obtained with the 5-HT_{1C}/5-HT_2 antagonist ketanserin using a variety of 5-HT_{1C}/5-HT_2 hallucinogens (Wing et al. 1990). By contrast, the effects of the 5-HT_{1A} agonist 8-OH-DPAT were completely insensitive to ketanserin (Wing et al. 1990).

However, administration of the 5-HT_1 antagonist (and beta-adrenergic antagonist) propranolol did not yield receptor-specific effects (figure 11). When propranolol was used as an antagonist to the 5-HT_{1A} agonist

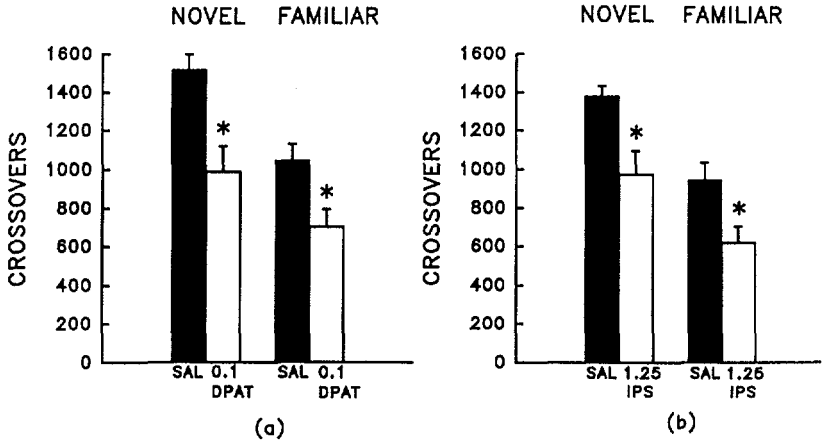

FIGURE 9. *Effect of familiarization with test chamber on 5-HT$_{1A}$ agonists' effects on locomotor activity. Rats were tested in the BPM 10 min after receiving injections of either saline or drµ. The novel groups were tested only once, while the familiar groups were tested after two previous exposures (without injections) to the BPM. (a) Four groups of 10 male rats each were tested 10 min after injections of either saline or 8-OH-DPAT (0.1 mg/kg SC). In a novel environment, 8-OH-DPAT significantly decreased crossovers (movements between 15 cm square sectors). In a familiar environment 8-OH-DPAT also significantly decreased crossovers. (b) Four groups of male rats (n = 11) were tested in the BPM 10 min after receiving SC injections of either saline or 1.25 mg/kg ipsapirone (IPS). In a novel environment, ipsapirone significantly decreased crossovers. In a familiar environment, ipsapirone also significantly decreased crossovers. Redrawn from data presented in Mittman and Geyer 1989.*

8-OH-DPAT and the 5-HT$_{1C}$/5-HT$_2$ agonist DOI, the hypoactivity induced by both 8-OH-DPAT and DOI was attenuated by administration in the first 10 minutes of testing in the BPM (figure 11). Thus, the effects of the 5-HT$_{1A}$ agonists are blocked by 5-HT$_1$ antagonists but not by 5-HT$_{1C}$/5-HT$_2$ antagonists, arguing for receptor-specific antagonism. However, the 5-HT$_{1C}$/5-HT$_2$ agonist DOI is blocked either by the 5-HT$_{1C}$/5-HT$_2$ antagonist or the 5-HT$_1$ antagonist, which indicates either a

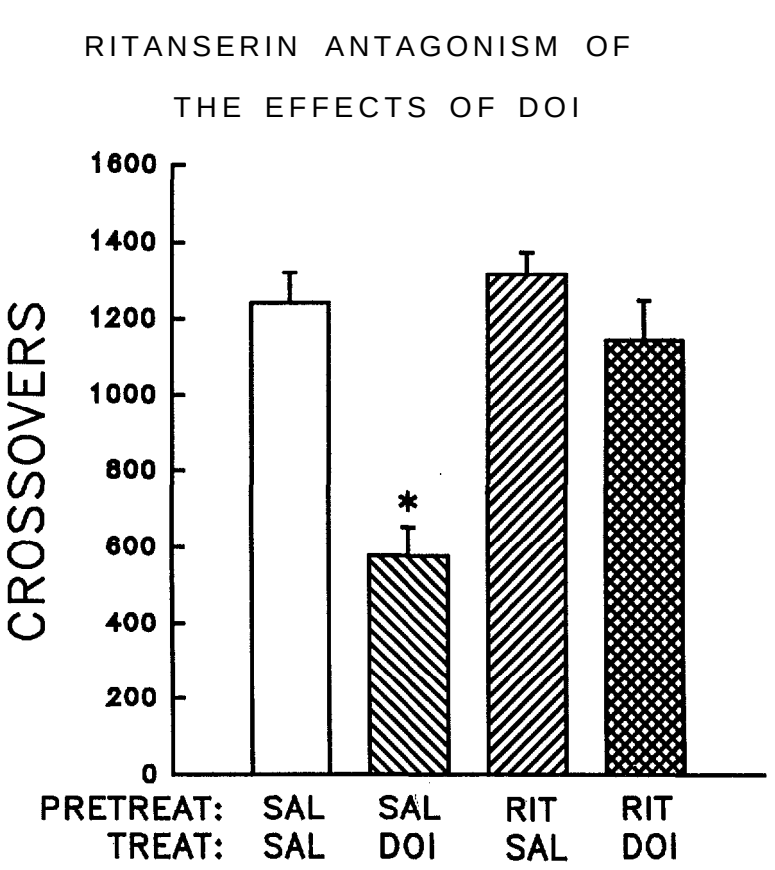

FIGURE 10. *Antagonism of 5HT-1C/2 agonist effects by a 5HT-1C/2 antagonist. Four groups of male rats (n = 9) were tested in the BPM 30 min after receiving pretreatment SC injections of either saline or 2.0 mg/kg ritanserin (RIT) and 10 min after receiving treatment injections of either saline or 0.27 mg/kg DOI (SC). DOI alone significantly decreased crossovers (movements between 15 cm square sectors); but the effects of DOI were antagonized when rats were pretreated with ritanserin. (Values are group means for the first 30 min, $p<0.005$). Redrawn from data presented in Mittman and Geyer 1991.*

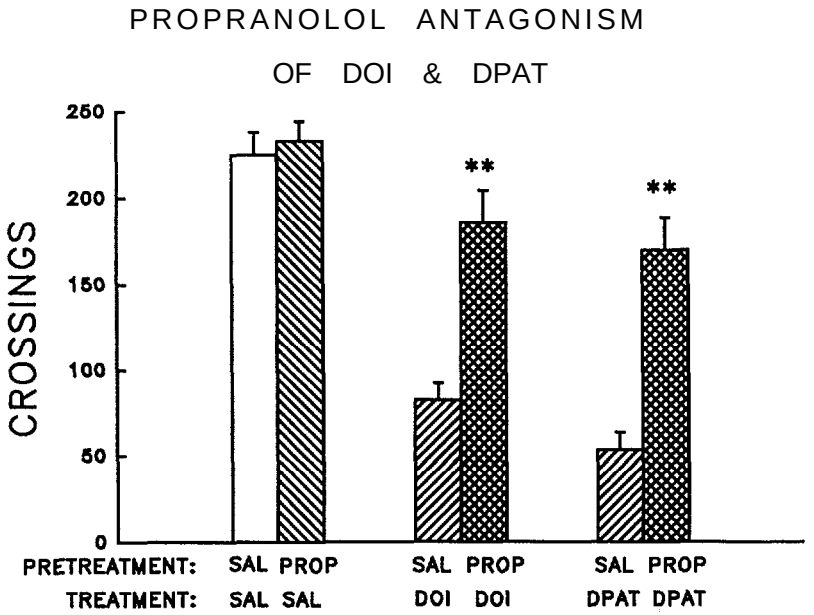

FIGURE 11. *Antagonism of 5-HT$_{1A}$ agonist and 5-HT$_{1C}$/5-HT$_2$ agonist effects by a 5-HT$_1$ antagonist. Six groups (n = 10-11) of male rats were tested in the BPM 30 min after receiving pretreatment injections of either saline or 10.0 mg/kg propranolol (SC) and 10 min after receiving treatment injections of either saline, DOI (1.0 mg/kg SC), or 8-OH-DPAT (DPAT) (0.5 mg/kg SC). Both DOI and 8-OH-DPAT significantly decreased crossings (movements between 15 cm square sectors) for the initial 10 min of testing; these effects were antagonized by pretreatment with propranolol. (Values are group means for the first 10 min of testing; $F(2,57) = 8.07$, $p<0.01$).*

nonspecific antagonism or an interaction between the 5-HT$_{1A}$ and 5-HT$_{1C}$/5-HT$_2$ receptors.

Similar studies using ritanserin and propranolol as pretreatments prior to the administration of LSD yielded interesting results. Recent speculation about the differential functions of the 5-HT$_{1A}$ and the 5-HT$_{1C}$/5-HT$_2$ receptors in LSD action has led to the hypothesis that the hallucinogenic action of LSD is due to its binding to the 5-HT$_{1C}$/5-HT$_2$ receptor complex. The prevailing view currently holds that hallucinogenic action

is due to actions mediated via the 5-HT_{1C}/5-HT_2 receptor complex. As evidence for this hypothesis, some studies have correlated the 5-HT_2 binding affinity of a drug with its hallucinogenic potency (Glennon et al. 1984; Titeler et al. 1988).

However, because of the paucity of dose-finding studies in humans, these studies have been unable to thoroughly examine the indoleamine and ergot alkaloid hallucinogens, although LSD and some indoleamines have been examined. These studies have focused instead primarily on the phenalkylamine hallucinogens such as mescaline, DOI, and DOM. LSD appears to be more closely related to the indoleamines, which include DMT and psilocybin (Mandell and Geyer 1976). Correlations between 5-HT_{1C}/5-HT_2 function and hallucinogenic potency have yet to be studied for the indoleamines or the ergots as groups. Therefore, the relative contributions of 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptors in hallucinogenic action have not been established definitively, especially for the indoleamine and ergot alkaloid hallucinogens.

Because the prevailing view is that classical hallucinogens act primarily via agonist actions at 5-HT_{1C}/5-HT_2 receptors, it was predicted that the 5-HT_{1C}/5-HT_2 antagonist ritanserin would block the effects of LSD, whereas the 5-HT_1 antagonist propranolol would not. Instead, ritanserin only partially blocked the effects of LSD, as did propranolol. Only the combination of ritanserin and propranolol completely blocked the effects of LSD (Mittman and Geyer 1991). These results suggest a role for the 5-HT_{1A} receptor as a contributor to the behavioral effects of LSD. Perhaps, as suggested by the antagonist studies, there is an interaction between the 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptors. To explore further the contributions of the receptors to the effects of LSD, an approach based on the apparent tolerance associated with 5-HT agonists was used.

TOLERANCE AND CROSS-TOLERANCE TESTS

The previous attempts to use antagonists to block specific receptor sites when testing LSD, a mixed 5-HT_{1A} and 5-HT_{1C}/5-HT_2 agonist, were problematic primarily because of the lack of selective 5-HT_{1A} antagonists. Unfortunately the common 5-HT_1 antagonist propranolol is an antagonist at both 5-HT_{1A} and 5-HT_{1B} sites and is a ß-adrenergic blocker as well. Without selective antagonists, it was difficult to assess the respective contributions of the receptor subtypes to the effects of LSD on locomotor and investigatory behavior.

Alternatively, another approach is that of tolerance. Chronic administration of a selective 5-HT_{1A} agonist such as 8-OH-DPAT produces tolerance to the acute effects of 8-OH-DPAT (Berendsen and Broekkamp 1991; Johansson et al. 1990; Larsson et al. 1990; Pranzatelli and Pluchino 1991). Similarly, tolerance has also been reported to the acute effects of DOI, a 5-HT_{1C}/5-HT_2 agonist, after repeated administration (Berendsen and Broekkamp 1991; Darmani et al. 1990*a;* Pranzatelli and Pluchino 1991). In the serotonin system, it appears that receptor regulation is not an adequate explanation for tolerance, as behavioral tolerance is seen in the absence of receptor change (Trulson 1985). Nevertheless, binding studies have demonstrated that chronic administration of both 8-OH-DPAT and DOI result in decreased numbers of 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptor sites, respectively (Buckholtz et al. 1988; Larsson et al. 1990; McKenna et al. 1989; Pranzatelli 1991).

It is well established that tolerance to LSD in humans is striking for the rapidity with which it occurs (Freedman et al. 1964; Isbell et al. 1956; Mandell and Geyer 1976). In animals, tolerance or partial tolerance to the acute effects of LSD administration has also been demonstrated for most behaviors (Adams and Geyer 1985*a;* Appel and Freedman 1968; Bridger 1975; Carter and Appel 1978; Geyer and Light 1979; Murray et al. 1977; Rech et al. 1975; Trulson 1985; Winter 1971), although some behavioral paradigms may be resistant to tolerance induction (Braff and Geyer 1980; Bridger 1975; Murray et al. 1977).

Cross-tolerance regimens were used to elucidate the contribution of 5-HT_{1A} receptor action to the effects of LSD on locomotor activity. These experiments were designed to test the hypothesis that the effects of LSD in the BPM model are influenced by 5-HT_{1A} function and by 5-HT_{1C}/5-HT_2 function, thus supporting a combination of 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptor action.

Tolerance to 8-OH-DPAT and DOI

First, it was determined whether tolerance to the selective agonists would exhibit cross-tolerance to a different selective agonist. The hypothesis, based on earlier research (Berendsen and Broekkamp 1991; Darmani et al. 1990*a;* Johansson et al. 1990; Nash et al. 1989; Pranzatelli and Pluchino 1991), was that the 5-HT_{1A} and 5-HT_{1C}/5-HT_2 agonists 8-OH-DPAT and DOI, respectively, would both show tolerance but that cross-tolerance between the two drugs would not occur.

The most robust acute effect of 8-OH-DPAT administration, that of decreasing the number of movements made by the rat in the BPM in the first 10 minutes of an hour-long test session, was attenuated when 8-OH-DPAT was administered repeatedly every 12 hours for 5 days (figure 12). That is, tolerance was observed to the effects of 8-OH-DPAT when it was given chronically.

A similar pattern of tolerance was observed with DOI (figure 12). A diminished effect of the challenge dose of DOI occurred in animals pretreated chronically with DOI. As predicted, the repeated administration of DOI produced no significant cross-tolerance to the effects of an acute dose of 8-OH-DPAT (figure 12). However, the chronic administration of 8-OH-DPAT produced some cross-tolerance to the effects of a challenge dose of DOI (figure 12). Thus, the expected tolerance to the effects of 8-OH-DPAT and DOI occurred. Although DOI produced no cross-tolerance to 8-OH-DPAT, some cross-tolerance was found when DOI was tested in rats pretreated with 8-OH-DPAT (Krebs and Geyer 1994). Thus, the tolerance regimens were sufficient to produce significant tolerance, but this tolerance was not completely specific pharmacologically to each receptor subtype.

These effects are similar to what was observed when the 5-HT_1 antagonist propranolol was able to block some of the effects of the 5-HT_{1C}/5-HT_2 agonist DOI (figure 11). The partial cross-tolerance to DOI after chronic 8-OH-DPAT administration may reflect an interaction between 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptors. Such an interaction has been suggested previously (Amt and Hyttel 1989; Darmani et al. 1990*b;* Krebs and Geyer 1994) and will require further studies to elucidate.

Tolerance to LSD and 8-OH-DPAT

A parallel study examined cross-tolerance between the effects of 8-OH-DPAT and LSD. Based on previous studies (Adams and Geyer 1985*a*), tolerance for the effects of LSD was expected. Based on its affinity to 5-HT_{1A} receptors, chronic LSD pretreatment was expected to produce cross-tolerance to the effects of 8-OH-DPAT treatment. The most important hypothesis was that 8-OH-DPAT pretreatment would produce cross-tolerance to the effects of LSD, suggesting either that 5-HT_{1A} receptors contribute to the effects of LSD or that 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptors interact functionally. In either case, reciprocal cross-tolerance between 8-OH-DPAT and LSD was hypothesized. As before, tolerance to the effects of 8-OH-DPAT was evident (figure 13).

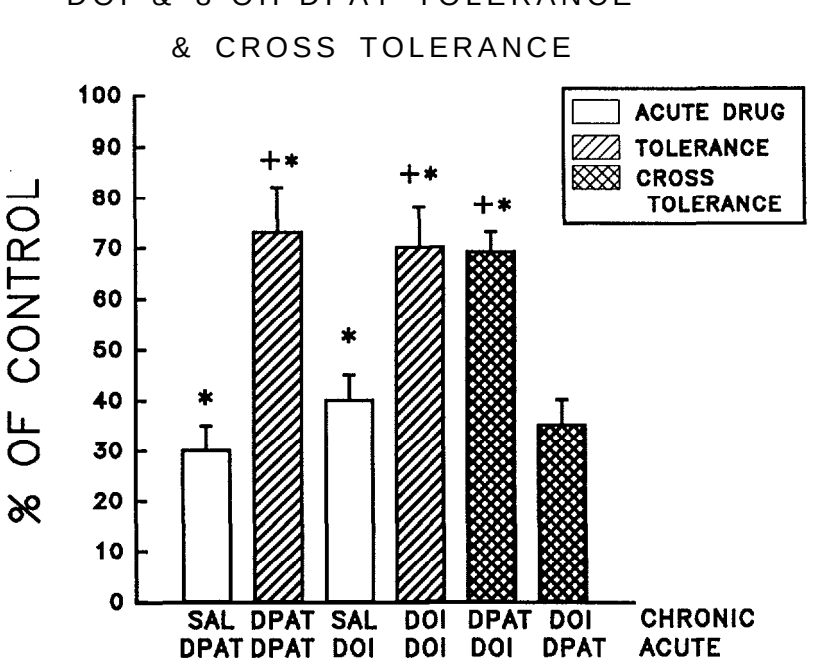

FIGURE 12. *Asymmetrical cross-tolerance between 5-HT_{1A} and 5-HT_{1C}/5-HT_2 agonists. Nine groups of male rats (n = 7-8) were pretreated chronically every 12 hrs for 5 days with either saline, DOI (1.0 mg/kg SC), or 8-OH-DPAT (0.5 mg/kg SC).Thirty-six hrs after the last pretreatment, rats were tested in the BPM 10 min after receiving treatment injections of either saline, 1.0 mg/kg DOI, or 0.5 mg/kg 8-OH-DPAT (DPAT). Both DOI and 8-OH-DPAT significantly decreased movements, a measure of global motor activity; these effects were significantly attenuated when the corresponding pretreatmeqts of DOI and 8-OH-DPAT were given, respectively. Also, cross-tolerance occurred to the effects of DOI when 8-OH-DPAT was the pretreatment, but no attenuation of 8-OH-DPAT's effects occurred when DOI was the pretreatment. (Values are percentages of group means of movements for corresponding control groups, for the first 10 min of testing, $p<0.05$). An * represents a significant difference from control, while a + represents a significant difference from the acute drμ group. Each symbol indicates significant tolerance or cross-tolerance, while both symbols together indicate partial tolerance or cross-tolerance. Redrawn from data presented in Krebs and Geyer 1994.*

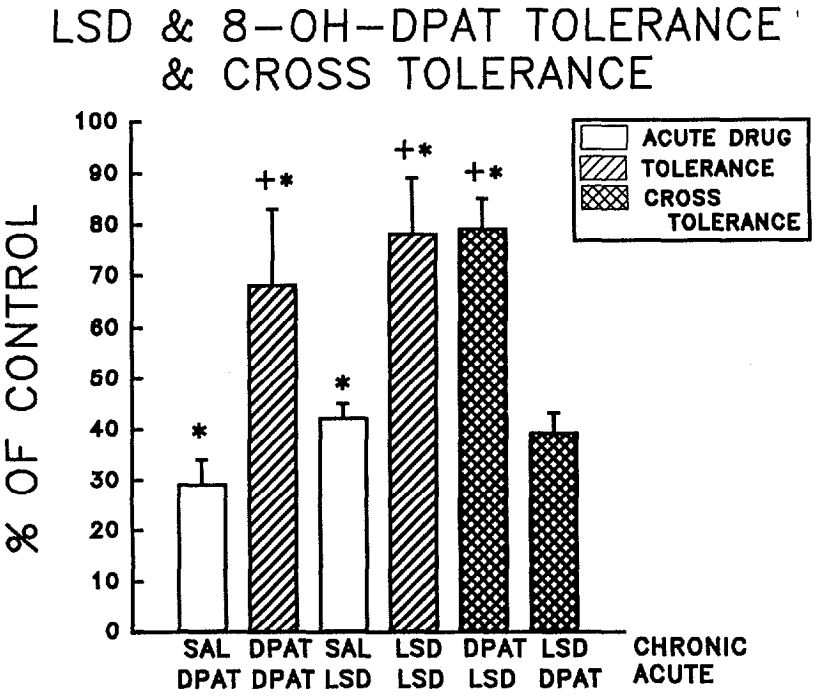

FIGURE 13. *Asymmetrical cross-tolerance between LSD and 5-HT$_{1A}$ agonist. Nine groups of male rats (n = 8) were pretreated chronically every 12 hrs for five days with either saline, 8-OH-DPAT (DPAT) (0.5 mg/kg SC), or LSD (60 µ/kg SC). Thirty-six hrs after the last pretreatment, rats were tested in the BPM 10 min after receiving treatment injections of either saline, 0.5 mg/kg 8-OH-DPAT, or 60 µ/kg LSD. Both LSD and 8-OH-DPAT significantly decreased movements, a measure of global motor activity; these effects were significantly attenuated when the corresponding pretreatments of LSD and 8-OH-DPAT were given, respectively, Also, cross-tolerance occurred to the effects of LSD when 8-OH-DPAT was the pretreatment, but no attenuation of 8-OH-DPAT's effects occurred when LSD was the pretreatment. (See figure 12 for explanation of values and symbols). Redrawn from data presented in Krebs and Geyer 1994.*

Also, the effects of LSD on movements showed tolerance after the chronic regimen was performed (figure 13). In animals chronically pretreated with 8-OH-DPAT every 12 hours for 5 days, the effects of LSD were significantly diminished. Thus, cross-tolerance to LSD was observed after the chronic administration of 8-OH-DPAT.

This result was consistent with the previous observation that propranolol partially blocks this effect of LSD, and further suggests that 5-HT_{1A}-related systems participate in these behavioral effects of LSD. However, even after chronic 8-OH-DPAT pretreatment a significant effect of LSD was still evident, indicating that the cross-tolerance was only partial. The reciprocal comparison did not evidence cross-tolerance. As seen in figure 13, animals pretreated chronically with LSD did not exhibit cross-tolerance to the effects of 8-OH-DPAT (Krebs and Geyer 1994). This nonreciprocal cross-tolerance is hypothesized to have occurred because the dose of LSD used did not occupy 5-HT_{1A} receptor sites as effectively as did the dose of 8-OH-DPAT, although the doses were equated in terms of the acute behavioral effects of the two drugs. The major finding of asymmetrical partial cross-tolerance between 8-OH-DPAT and LSD indicates that the effects of LSD on locomotor behavior, as defined by movements, are influenced by 5-HT_{1A} receptor function.

Tolerance to LSD and DOI

A parallel cross-tolerance study was performed with DOI and LSD. Because the previous study indicated that DOI tolerance was marginal when animals were treated every 12 hours for 5 days (figure 12), the chronic regimen was increased to injections every 12 hours for 8 days. As shown in figure 14, tolerance to the effects of both DOI and LSD was replicated. The effects of LSD were diminished somewhat in animals chronically pretreated with DOI, indicating that partial cross-tolerance to the effects of LSD occurred (figure 14). However, there was no reciprocal cross-tolerance when animals were chronically pretreated with LSD and challenged with DOI (Krebs and Geyer 1994). Therefore, the major finding of partial cross-tolerance to LSD after chronic DOI administration indicates that the effects of LSD on locomotion are influenced by the action of the 5-HT_{1C}/5-HT_2 system.

Taken together, evidence of partial cross-tolerance between 8-OH-DPAT and LSD and between DOI and LSD indicates that the behavioral effects of LSD are influenced by a combination of 5-HT_{1A} and 5-HT_{1C}/5-HT_2

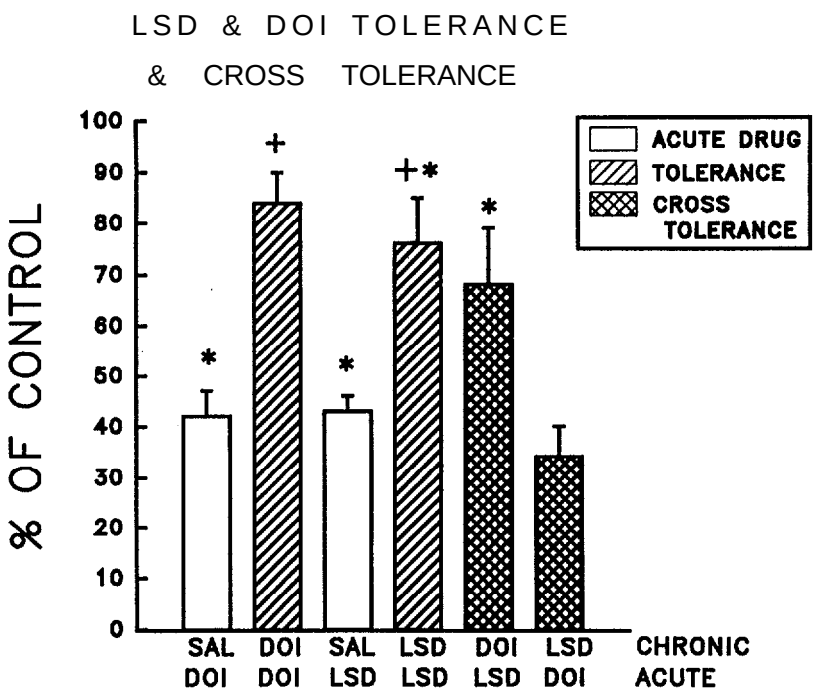

FIGURE 14. *Asymmetrical cross-tolerance between LSD and 5-HT$_{1C}$/5-HT$_2$ agonist. Nine groups of male rats (n = 7-8) were pretreated chronically every 12 hrs for eight days with either saline, DOI (1.0 mg/kg SC), or LSD (60 µ/kg SC).Thirty-six hrs after the last pretreatment, rats were tested in the BPM 10 min after receiving treatment injections of either saline, 1.0 mg/kg DOI, or 60 µ/kg LSD.Both LSD and DOI significantly decreased movements, a measure of global motor activity; these effects were significantly attenuated when the corresponding pretreatments of LSD and DOI were given, respectively. Also, cross-tolerance occurred to the effects of LSD when DOI was the pretreatment, but no attenuation of DOI's effects occurred when LSD was the pretreatment. (See figure 12 for explanation of values and symbols). Redrawn from data presented in Krebs and Geyer 1994.*

receptor action or that 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptors interact in the modulation of this behavior. Accordingly, the prevailing view that the action of the 5-HT_{1C}/5-HT_2 receptor is primarily responsible for hallucinogenic activity may be true for the phenalkylamine hallucinogens, but this interpretation cannot completely explain the action of the less selective 5-HT_{1A}/5-HT_{1C}/5-HT_2 ergot alkaloid LSD.

There is some evidence of an interaction between the 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptors. In one study it was found that the effect of the 5-HT_{1A} agonist 8-OH-DPAT was significantly increased when rats were treated simultaneously with DOI, a 5-HT_{1C}/5-HT_2 agonist. Interestingly, the effect of DOI on head twitches was inhibited by simultaneous treatment with 8-OH-DPAT (Arnt and Hyttel 1989). Moreover, the increased forepaw treading induced by cotreatment with 8-OH-DPAT and DOI was partially inhibited by high doses of either the 5-HT_2 antagonists, ritanserin and ketanserin, or the 5-HT_1 and ß-adrenoceptor antagonist (-)-alprenolol. Furthermore, the effects of the 5-HT_2 antagonists were synergistic with those of (-)-alprenolol in their ability to block the forepaw treading induced by the agonist combination (Arnt and Hyttel 1989). This synergistic relationship was interpreted as an asymmetrical interaction between the 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptors.

Another study has replicated some of these findings by demonstrating that the head twitch response induced by the 5-HT_{1C}/5-HT_2 agonist DOI was significantly inhibited by simultaneous administration of the 5-HT_{1A} agonist 8-OH-DPAT, but not by the 5-HT_{1B}/5-HT_C agonist 1-(3-trifluromethylphenyl)piperazine (TFMPP) (Darmani et al. 1990*b*). These researchers argue for a pharmacological interaction between the 5-HT_{1A} and 5-HT_2 receptors that manifests itself in a 5-HT_{1A} inhibitory action on 5-HT_2 receptor-modulated behavior (Darmani et al. 1990*b*).

These results, taken together with the asymmetrical cross-tolerance results (Krebs and Geyer 1994) and the propranolol antagonism of DOI discussed earlier, suggest that there may indeed be interactions between the 5-HT_{1A} and 5-HT_{1C}/5-HT_2 receptors. With further investigation, the BPM system may be able to shed light on the nature of this interaction by more thoroughly characterizing the effects of 5-HT_{1A} agonists and hallucinogens. With more detailed analyses of phenalkylamine, indoleamine, and ergot alkaloid hallucinogens, clarification of the mechanisms of action of these drugs should be possible.

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AUTHORS

Mark A. Geyer, Ph.D.
Professor of Psychiatry

Kirsten M. Krebs, M.A.
Department of Psychology

School of Medicine
University of California at San Diego
La Jolla, CA 92093-0804

The Stimulus Effects of Serotonergic Hallucinogens in Animals

Jerrold C. Winter

INTRODUCTION

Mankind has known of naturally occurring hallucinogens for thousands of years (Schultes and Hofmann 1980), but less than one-tenth of one millennium has passed since it could be said with confidence that a pure chemical of known structure is hallucinogenic (Heffter 1897; Spath 1919). That chemical was mescaline, a representative of the phenethylamine subclass of the serotonergic hallucinogens. The other subclass, the indoleamines, is perhaps best represented by lysergic acid diethylamide (LSD), a drug whose hallucinogenic properties were discovered by Albert Hofmann on April 16, 1943 (Hofmann 1959). That both the indoleamines and the phenethylamines are properly classified together as serotonergic hallucinogens has become apparent only in the last 2 decades.

The remarkable alterations in thought and perception produced by hallucinogens have been described by many. In some instances, carefully selected human volunteers have received these drugs in rigidly controlled studies (Isbell et al. 1959; Martin and Sloan 1977; Strassman, this volume). In others, investigators have told of the effects of hallucinogens following self-administration. These reporters have ranged from persons without scientific training (DeCurtis 1987) to distinguished scientists such as Hofmann (1959), Szára (1957), and Shulgin (this volume; Shulgin et al. 1986). But to seek out the most intimate mechanisms by which hallucinogens work their magic, and in the process to make discoveries of ultimate benefit to humanity, researchers are obliged to use other species.

In turning to animals to aid in understanding of hallucinogens, major ethical problems are avoided, but, concomitantly, there arise questions of relevance. Is there a suitable animal model of hallucination, a phenomenon thought by many to be a uniquely human experience? Most of those who work with animals find comfort in the knowledge that the

fundamental biochemical and physiological aspects of neuronal function are remarkably similar across species. On the other hand, hallucinations are primarily expressed in behavioral terms, and, in the absence of verbal communication, one is hard pressed to demonstrate the validity of animal models of complex human behavior. This chapter describes how the study of serotonergic hallucinogens as discriminative stimuli has already enlarged the knowledge of these drugs and how these studies in animals have led to hypotheses testable in humans.

STIMULUS CONTROL

A discriminative stimulus is any feature of an animal's environment that is correlated with a schedule of reinforcement (Kelleher and Morse 1968). Operant behavior that is reinforced only in the presence of a specified stimulus soon occurs with greater frequency in the presence of the stimulus than in its absence. The behavior is then said to be under the control of the stimulus. Discriminative stimuli may act on one or more of a variety of receptors, both external and internal.

In classic studies of visual discrimination, pigeons were trained to emit a particular response in the presence of light of a specified frequency. When an organism has been conditioned to respond to a particular stimulus, other similar stimuli also increase the probability of elicited response. This ability of stimuli other than the training stimulus to evoke a conditioned response is known as stimulus generalization. However, as the character of the tested stimuli becomes less and less like the training stimulus, the degree of generalization declines in a regular fashion; a generalization gradient is observed. Thus, for example, a pigeon that has been reinforced after pecking a key only when it is illuminated with yellow light will emit progressively fewer responses as the illuminating color is gradually shifted toward the red and violet ends of the spectrum, It is obvious that stimulus control offers an investigator the opportunity to ask questions about the perception of light by a nonverbal species in both normal and pathological states (Stebbins 1970).

If the correlation of a schedule of reinforcement with the falling of photons upon eye receptors can induce stimulus control, then might a similar correlation of reinforcement between drug interaction and its receptors likeu ise come to control behavior? The answer is yes. Beginning with the studies reported by Conger (1951), many hundreds of investigations have shown that a variety of drugs can induce stimulus

control. (For a recent review, see Colpaert and Balster 1988). By analogy with visual discriminations, drug-induced stimulus control offers the opportunity to ask questions, and occasionally to receive answers, regarding the effects of psychoactive drugs in nonverbal species. Because these stimuli are engendered, not in some totally mysterious fashion but by drug-receptor interactions, researchers may hope to learn not only about the nature of the stimuli but also about the biochemical and molecular processes from which they arise.

HALLUCINOGEN-INDUCED STIMULUS CONTROL

Hirschhorn and Winter (1971) reported that the prototypic serotonergic hallucinogens mescaline and LSD are able to induce stimulus control in rats (figures 1 and 2). Furthermore, it was found that the stimulus effects of mescaline and LSD differ not only from those of drugs from distinct pharmacological classes such as stimulants (Winter 1975*a*) and depressants (Hirschhorn and Winter 1975) but also from nonindoleamine, nonphenethylamine hallucinogens (Shannon 1981; Silverman and Ho 1978; Swedberg and Jarbe 1986).

In the United States and elsewhere, hallucinogens have traditionally been classified as drugs of abuse together with stimulants, depressants, and opiates. However, unlike the latter categories of drugs, hallucinogens do not function as reinforcers in animals (Deneau et al. 1969). For this reason hallucinogens are not amenable to study in animals using the very powerful operant techniques for self-administration. Against this background, the 'potential value of drug-induced stimulus control for the identification, characterization, and scheduling of potential hallucinogens prior to their use in human subjects is readily apparent (Winter 1974).

SEROTONERGIC HALLUCINOGENS

Soon after the discovery of LSD by Hofmann, it was recognized that the clinical syndromes produced by the phenethylamine hallucinogens, mescaline and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), are quite similar to those following indoleamine hallucinogens such as LSD and N,N-dimethyltryptamine (DMT) (Hoch et al. 1952; Hollister et al. 1969). That the phenethylamines and the indoleamines might be acting via a common mechanism and that the mechanism might be serotonergic in nature was suggested by a number of observations. In

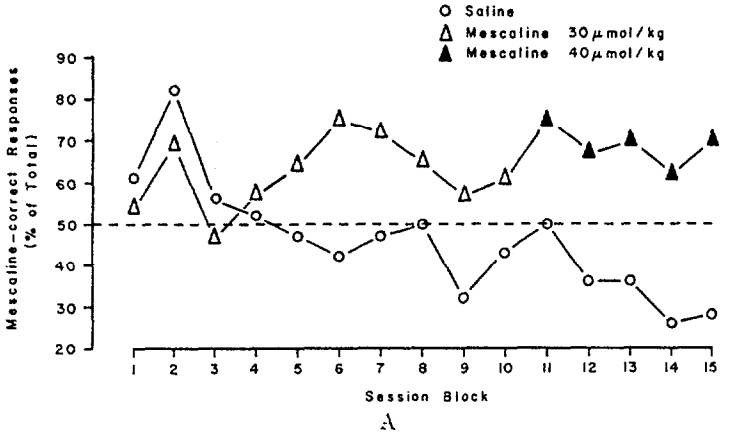

FIGURE 1. *Discriminated responding following the injection of mescaline or saline. Each point is the mean of 2 determinations in each of 4 animals. Circles represent experiments in which saline was injected 5 minutes before the session. Triangles represent identical experiments in which either 7.4 mg/kg (open triangles) or 9.9 mg/kg (closed triangles) of mescaline was given. Ordinate: number of responses on the mescaline-correct lever in the first 5 minutes of the session, expressed as a percentage of total responses. Abscissa: successive blocks of 4 sessions. From Hirschhorn and Winter 1971, by permission*

human subjects (Balistrieri and Fontanari 1959; Wolbach et al. 1962) as well as in animals (Appel and Freedman 1968; Winter 1971), cross-tolerance develops between LSD and mescaline. In addition, both groups of hallucinogens produce similar effects on the firing rate of serotonergic neurons (Aghajanian et al. 1970) and on the level and rate of turnover of serotonin in the brain (Tonge and Leonard 1969). Finally, it was known that serotonergic antagonists block some of the nonbehavioral effects of phenethylamine hallucinogens in animals (Cheng et al. 1973; Horita and Hamilton 1972; Huang and Ho 1972).

Antagonism of mescaline-induced stimulus control in rats by cinanserin, a serotonergic antagonist, was reported independently by Browne and Ho (1975) and by Winter (19753). This observation was then extended to

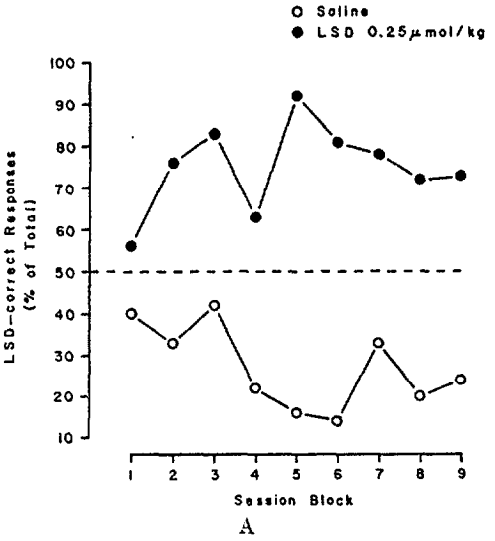

FIGURE 2. *Discriminated responding following the injection of LSD or saline. Closed circles: LSD [0.12 mg/kg]. Open circles: saline. All other details are as in figure 1.*

include other antagonists of serotonin and other hallucinogens including LSD, DOM, and DMT (Glennon et al. 1982; Kuhn et al. 1977; Winter 1978*a*). The phenomenon is illustrated in figure 3 for LSD and in figure 4 for DOM. Based upon data such as those shown, it appears appropriate to apply the term "serotonergic hallucinogens" to both the indoleamines and the phenethylamines.

PARTIAL AGONISTS

Because of the crucial role played by antagonists in the characterization of a variety of drugs including hallucinogens, it is important to examine certain assumptions regarding the antagonists to be used. This is especially important when drugs shown to be antagonists in one or another in vitro, isolated tissue, or biochemical assay systems are used in intact animals in behavioral experiments.

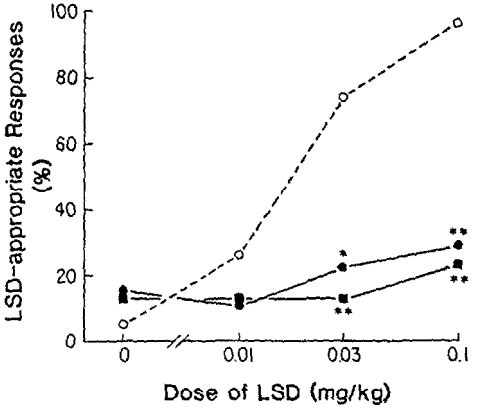

FIGURE 3. *The effects of LSD alone (open circles) and in the presence of either pizotyline (closed circles; 10 mg/kg) or pirenperone (closed squares: 0.16 mg/kg) in rats trained with LSD (0.1 mg/kg) as a discriminative stimulus. LSD and the antagonists were injected 15 min and 60 min, respectively, before testing. The values given at the zero dose level are the effects of saline, pizotyline, and pirenperone when given alone. Each is the mean of 2 determinations in each of 10 subjects. All other points represent the mean of I determination in each of 10 animals. Ordinate: Mean percentage of responses on the LSD-appropriate lever. Abscissa: dose plotted on a log scale. Statistical comparisons are with the value for LSD alone; *:p less than 0.05; **: p less than 0.01. From Winter and Rabin 1988 by permission*

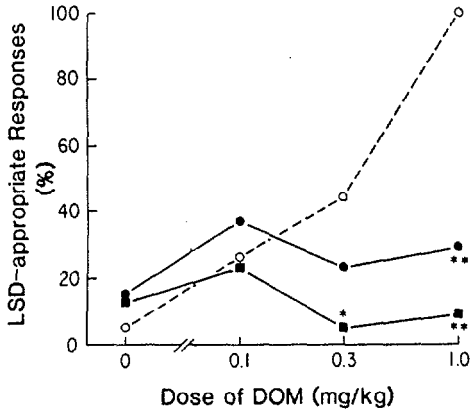

FIGURE 4. *The effects of DOM alone (open circles) and in the presence of either pizotyline (closed circles) orpirenperone (closed squares) in rats trained with LSD as a discriminative stimulus. DOM was injected 15 min before testing. All other details are as in figure 3.*

As has been noted above, blockade of the stimulus effects of indoleamine and phenethylamine hallucinogens by serotonergic antagonists has provided powerful evidence of an underlying mechanism mediated by 5-hydroxytryptamine (5-HT). However, stimulus control also permits the identification not only of full agonists and pure antagonists but also those drugs with mixed properties, the partial agonists. Data published by Colpaert and colleagues (1982) provide excellent examples.

Figures 5 through 7 show the agonistic effects (upper panels) and the antagonistic effects (lower panels) of a series of serotonergic antagonists in rats trained with LSD as a discriminative stimulus. It is obvious that a full spectrum of activity is represented. Methysergide, cyproheptadine, and mianserin are significantly LSD-like (figure 5, upper panel) and are, as would be expected from their agonistic effects, only marginally effective as antagonists (lower panel). Pizotifen (also known as BC-105

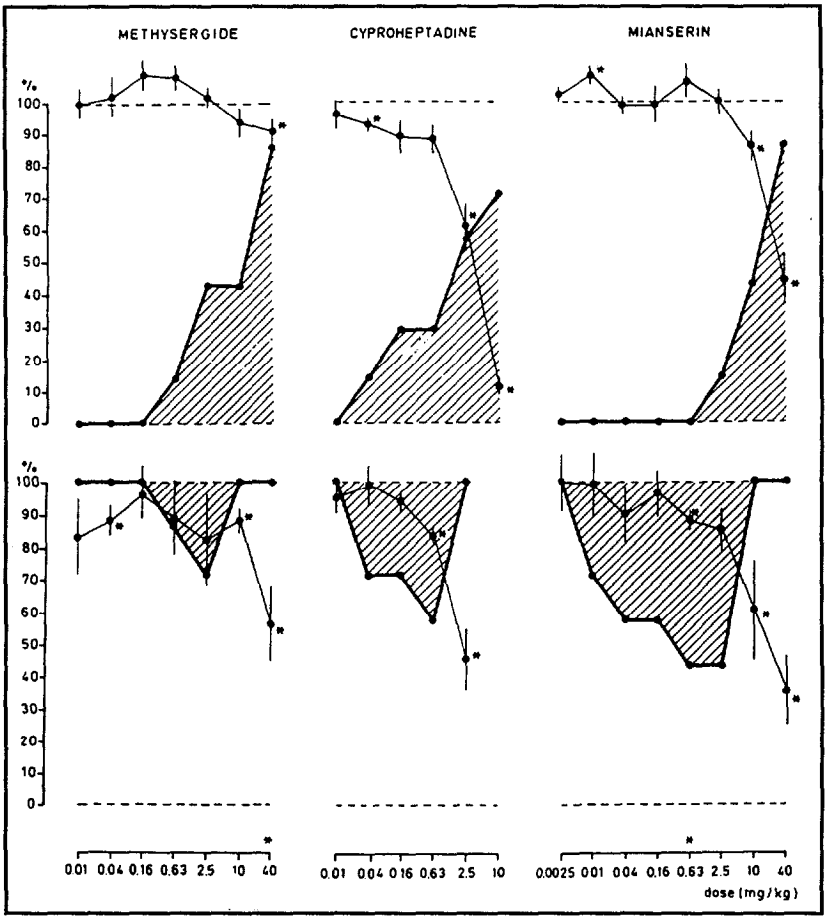

FIGURE 5. *Agonist and antagonist effects of methysergide, cyproheptadine, and minaserin in rats trained with LSD [0.16 mg/kg] and saline. Upper panel: agonistic effects [thick line] and suppression of response rate [thin line] by the test drug when given alone. Lower panel: antagonistic effects [thick line] and suppression of response rate [thin line] by the test drug when given prior to the training dose of LSD. Ordinate: percentage of rats choosing the LSD-appropriate lever [thick line] and response rate [thin line] expressed as a percentage of saline-control rate. Abscissa: dose of test drugs. From Colpaert et al., 1982, by permission*

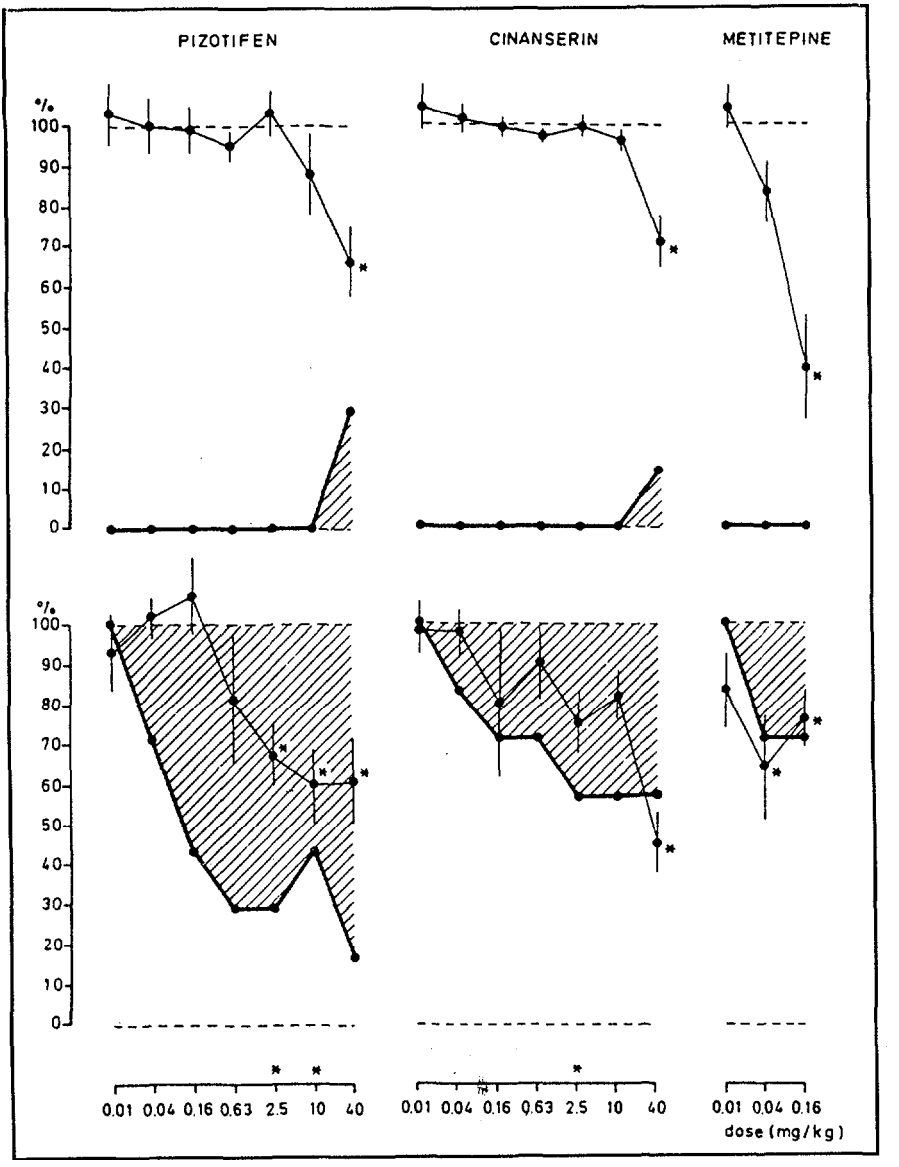

FIGURE 6. *Agonist and antagonist effects of pizotifen, cinanserin, and metitepine in rats trained with LSD [0.16 mg/kg] and saline. All other details are as in figure 5.*

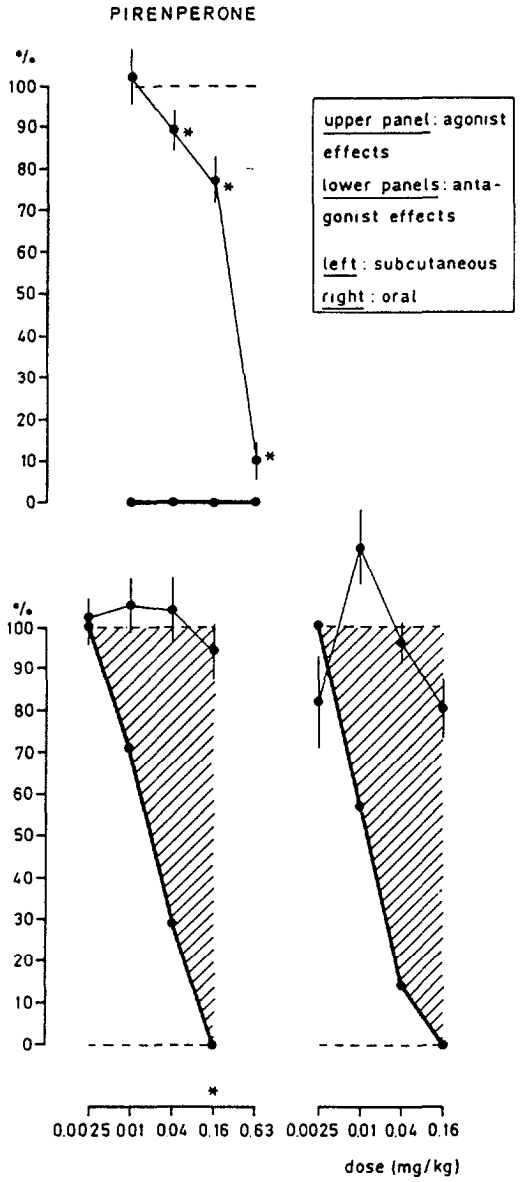

FIGURE 7. *Agonist and antagonist effects of pirenperone in rats trained with LSD [0.16 mg/kg] and saline. All other details are as in figure 5.*

and as pizotyline), cinanserin, and metitepine (figure 6) are clearly less agonistic, but only pizotifen approaches being a complete antagonist. Finally, figure 7 provides convincing evidence that pirenperone is devoid of LSD-like stimulus effects and is a complete antagonist of LSD at doses that have only modest effects upon rate of responding.

Of the three purported antagonists showing the highest degree of LSD-like stimulus effects in the rat, two-methysergide and cyproheptadine-are claimed to be sometimes associated with hallucinations in humans. It is clear that drug-induced stimulus control provides the means to assess full agonists, pure antagonists, and those drugs that exhibit intermediate degrees of activity. It is likely that extrapolation from animals to humans is most problematic for the last-named group of drugs.

INTERMEDIATE RESULTS AND THE BERRY-ATOR HYPOTHESIS

One of the most remarkable features of drug-induced stimulus control is that animals trained with a drug of one pharmacological class do not always exhibit drug-appropriate responses when tested with a drug of another class even at doses known to have stimulus properties. For example, rats trained with morphine sulfate (6 milligrams per kilogram [mg/kg]) versus saline emit primarily saline-appropriate responses when tested with ethanol at a dose of 630 mg/kg, despite the fact that stimulus control is readily established in rats trained with a 630 mg/kg dose of ethanol versus saline (Winter 1975*c*). However, observations such as these should not diminish the appreciation of what have been called "intermediate results" (Winter 1978*b*).

In the larger context of stimulus control, it was noted above that a generalization *gradient* is observed. As the tested stimulus becomes less and less like the conditioned stimulus, the probability of response emission becomes less and less. With respect to drug-induced stimulus control, a clear example of a stimulus gradient is seen when subjects trained with saline versus a specified dose of a drug are tested with a range of lesser doses of that drug. As expected, intermediate degrees of drug-appropriate responding occur.

Despite the ready acceptance of intermediate results in the context of dose-response relationships, there has been some reluctance to accept similar intermediate degrees of generalization when subjects trained with one drug are tested with other drugs. That such intermediate results may be informative is illustrated by an examination of the comparative stimulus properties of p-methoxyamphetamine (PMA) and LSD.

In studies that employed an animal model of hallucination based on patterns of conditioned avoidance responses, PMA was characterized as having a typical hallucinogenic profile similar to that of LSD (Smythies et al. 1967; 1970). However, rats trained with PMA as a discriminative stimulus and tested with LSD gave a maximum of only about 50 percent PMA-appropriate responses (Winter 1984). When rats were trained with LSD and tested with PMA, the results were as shown in table 1. At a dose of PMA of 1 mg/kg, 48 percent of the responses were LSD-appropriate. At 3 mg/kg the percentage rose to 75 percent, but only half of the tested animals responded.

As shown in figure 3, LSD-induced stimulus control is completely antagonized by the serotonergic antagonist pizotyline. As seen in table 2, the intermediate degree of generalization of LSD to a dose of 1 mg/kg PMA is likewise blocked by pizotyline. Is the conclusion to be that PMA-induced stimulus control is partially mediated by a serotonergic mechanism? As attractive as that conclusion may be, the data in table 2 argue against ‘it. The table shows that PMA-induced stimulus control is not significantly antagonized by a dose of pizotyline that completely blocks the partial substitution of PMA for LSD.

TABLE 1. *Effects of PMA in rats trained with LSD (0.1 mg/kg)*

Dose of PMA (mg/kg)	N	Percent LSD choice
0.3	10	11
1	10	48
3	5	75

NOTE: Ten animals were tested at each dose. N = the number which completed the test session. Redrawn from Winter (1984).

TABLE 2. *Effects of PMA alone and in combination with pizotyline in rats trained with either PMA or LSD*

Training drug (mg/kg)	Dose of PMA (mg/kg)	Dose of pizotyline (mg/kg)	N	Cross test
PMA	3	0	6	98
(3)	3	3	6	96
	3	10	6	90
LSD	1	0	6	52
(0.1)	1	3	6	16

NOTE: Six animals were tested at each dose. N = the number which completed the test session. Redrawn from Winter (1984).

An explanation for this apparent paradox is provided by what has come to be known as the Berry-Ator hypothesis. Specifically, asymmetrical generalizations are explained in terms of differential salience of certain elements of a compound discriminative stimulus depending on the training drug (Ator and Griffiths 1989). Although originally proposed to account for the observation that baboons trained with pentobarbital generalize to lorazepam, but lorazepam-trained subjects do not reliably generalize to pentobarbital, the Berry-Ator hypothesis is also applicable to the data for LSD and PMA shown in tables 1 and 2. Specifically, the intermediate results obtained with PMA in rats trained with LSD indicate a stimulus component common to both drugs. Blockade by pizotyline of both LSD and the intermediate effects of PMA in rats trained with LSD suggest that the shared component is serotonergic in nature. Nonetheless, the failure of pizotyline to antagonize PMA-induced stimulus control in rats trained with the drug indicates that the serotonergic component of the action of PMA is not essential for the induction of stimulus control. Furthermore, the serotonergic effect of PMA is manifest only when PMA is tested in subjects trained with a drug such as LSD whose major stimulus effects are mediated by 5-HT.

CORRELATIONS BETWEEN HUMANS AND ANIMALS

How much can a rat or other nonverbal species tell us about the human experience with hallucinogens? In the present context, what can drug-induced stimulus control by serotonergic hallucinogens tell us about their mechanisms of action in humans? Can drug-induced stimulus control provide the means to reliably predict whether a drug fresh from the synthetic chemist will produce hallucinations in human subjects? One approach to answering these questions is to examine the ability of animals to classify drugs correctly as being either hallucinogenic or nonhallucinogenic on the basis of their stimulus properties. A convenient way to pursue this approach is in terms of false negatives and false positives.

FALSE NEGATIVES

A false negative is a drug known to be hallucinogenic in man, yet fails to mimic other known hallucinogens *of the same class* trained as discriminative stimuli in animals. "Of the same class" is emphasized because of the well-established fact, noted above, that drugs such as LSD, phencyclidine, tetrahydrocannabinol, and atropine are all properly called hallucinogens, yet each is pharmacologically distinct. Thus, the false negatives to be considered at this time are those within the class designated as serotonergic hallucinogens.

In theory, false negatives have a finite probability of occurrence. For example, imagine two drugs, A and B, that share a common hallucinogen-inducing effect in humans. Further imagine that the shared effect of drugs A and B is reflected in a distinctive discriminative stimulus in animals. However, drug A does not generalize to drug B, because drug B suppresses behavior in a chosen animal species at doses lower than those required to elicit the hallucinogen-specific stimulus. The principle is clear: an animal in which all behavior has been obliterated cannot answer questions about discriminative stimuli.

In practice, the clearest example of a false negative among the serotonergic hallucinogens was provided by Koerner and Appel(1982). Rats trained to discriminate between 4-phosphoryloxy-DMT (psilocybin) and saline generalized to 4-hydroxy-DMT (psilocin) and LSD but failed to generalize to mescaline.

Koerner and Appel (1982) regarded their data as evidence of "the inability of existing drug discrimination procedures to detect 'hallucinogenicity' in drugs (p. 134)." This assessment may be too harsh in view of the numerous demonstrations of the similarities between the stimulus effects of various indoleamine and phenethylamine hallucinogens and, more specifically, the blockade of the stimulus effects of both psilocybin and mescaline by the serotonergic antagonist, cinanserin (Silverman and Ho 1978). A gentler interpretation is that, despite their similarities, each member of the class of serotonergic hallucinogens is a distinct pharmacological entity, and as such each would be expected to have distinctive features in its stimulus complex. Ironically, the more thorough the research, the more likely it is that such distinctive features will be detected. As a practical matter, one might wish to characterize an unknown drug against several serotonergic hallucinogens. It should be noted as well that in a subsequent study by Appel and Callahan (1989), mescaline-trained rats generalized fully to psilocybin.

FALSE POSITIVES

False positives are of greater concern than false negatives with respect to the discriminative power of drug-induced stimulus control, but at the same time they are of perhaps greater heuristic value. A false positive is a drug known to be devoid of hallucinogenic activity in humans but which nonetheless fully mimics known serotonergic hallucinogens in animals.

Before discussing false positives, it must be noted that interpretation of animal data is sometimes hampered by the inadequacy of available clinical data. For example, 2, 3, 4-trimethoxyphenethylamine (2,3,4-TMPEA) is a structural isomer of mescaline that was reported to have no psychotropic effects (Shulgin 1964; Shulgin and Shulgin 1991). On that basis it was used to test the hypothesis that the different pharmacologic properties of hallucinogens and nonhallucinogens in man are reflected in distinctive stimuli in rats. Only after it was found that rats appeared unable to distinguish between the effects of mescaline and 2,3,4-TMPEA (Winter 1973) was the author's attention drawn to a citation of 2,3,4-TMPEA hallucinogenic activity in schizophrenic patients (Slotta and Muller 1936). The proper interpretation of this set of observations or their implications for schizophrenia remains uncertain.

One may argue on theoretical grounds, as argued above for false negatives, that true false positives should occasionally arise. If one imagines two drugs, C and D, to possess a shared hallucination-inducing property and drug D to also be a very potent emetic agent, one may remain unaware of D's hallucinogenic activity for the simple reason that hallucinogenic doses are never reached in vomiting human subjects. On the other hand, if the emetic effect is species-specific, C and D would be expected to generalize to one another in an emesis-resistant species and thus yield a false positive.

Despite the foregoing caveats, false positives have been identified. Understanding them is essential to a valid judgment of the efficacy of drug-induced stimulus control as a practical tool for the study of serotonergic hallucinogens. Although a number of candidates present themselves, only lisuride and 2-(1-piperazinyl) quinoline maleate (quipazine) are discussed in detail in this chapter.

Lisuride is an ergot that is usually classified as a dopaminergic agonist. As such, it has a therapeutic role in the treatment of Parkinson's disease. Lisuride is generally regarded as being nonhallucinogenic and quite different from LSD in its actions. Despite their distinctive effects in humans, complete symmetrical generalization of LSD and lisuride has been reported (Holohean et al. 1982; White and Appel 1982*a*,*b*).

The observation that the stimulus effects of LSD and lisuride are differentially antagonized by serotonergic and dopaminergic antagonists (Cunningham and Appel 1987; White and Appel 1982*c*) is of obvious pharmacologic interest. However, it does not resolve the question of lisuride as a false positive. What is required is a demonstration that animals can behaviorally differentiate the two drugs. A series of experiments by Callahan and Appel (1990) provided just such a demonstration.

Rats were presented with three levers simultaneously. Each lever was associated with one of three conditions: saline, LSD, or lisuride. The doses of LSD and lisuride were chosen to be comparable in their stimulus effects when tested in animals trained with lisuride and LSD, respectively, versus saline. As shown in figure 8, the rats learned to discriminate between the three training conditions. Furthermore, when dose-response tests were done with a range of doses of LSD and lisuride, there was a graded distribution of responses between the saline-appropriate lever and the LSD- and lisuride-appropriate levers, respectively. Thus, in the

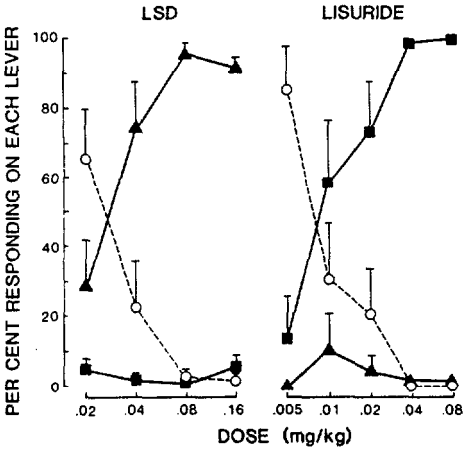

FIGURE 8. *Dose-response relationships for LSD [left panel] and lisuride [right panel] in rats trained to discriminate LSD [0.08 mg/kg] from lisuride [0.04 mg/kg] from saline. Responding on the LSD-appropriate lever is indicated by triangles, on the lisuride-appropriate lever by squares, and on the saline-appropriate lever by circles. Ordinate: percent responding on the indicated lever. Abscissa: dose of test drugs. From Callahan and Appel 1990, by permission*

three-lever choice trial, no evidence of cross-generalization between LSD and lisuride was seen; the issue of lisuride as a false positive is resolved.

The second false positive to be discussed is quipazine, a drug thought to act as an agonist at both central and peripheral serotonergic receptors. Stimulus control induced by quipazine was first reported by White and

colleagues (1977). They also observed complete generalization of quipazine to LSD. Furthermore, the generalization is symmetrical in that quipazine completely mimics LSD in rats trained with the latter drug (Winter 1979). A recent example of LSD generalization to quipazine is shown in figure 9. There it is obvious that quipazine's mimickry of LSD is not due to lisuride-like effects. Indeed, in keeping with a serotonergically mediated event, quipazine-induced stimulus control and substitution of quipazine for LSD are blocked by a variety of serotonergic antagonists (White et al. 1977; Winter 1979). It should be noted that in mescaline-trained subjects, only an intermediate level of generalization to quipazine was observed (Winter 1979).

When normal human volunteers were given 25 mg of quipazine by mouth in a double-blind cross-over study and tested using the Addiction Research Center Inventory (ARCI) (Hill et al. 1963), no LSD-like subjective effects were observed (Villarreal, personal communication, Dec. 12, 1977). However, at this dose there was a high incidence of nausea, flatulence, gastrointestinal discomfort, and diarrhea. Similarly, an anecdotal report of effects of quipazine in a single subject suggests "low dose mescaline-like effects followed by the onset of dysphoric effects including nausea!' (Daumier, personal communication, July 27, 1976). Interestingly, three of four monkeys given quipazine by intramuscular (IM) injection developed projectile vomiting while exhibiting behavioral signs similar to those induced by LSD (Schlemmer, Jr., personal communication, Jan. 14, 1977).

When the admittedly fragmentary data for quipazine in man, monkey, and rat are taken as a whole, there is some similarity to the hypothetical false positive described earlier in this section; that is, a drug which would induce hallucinations in man were it not for a limiting side effect that is present to a lesser degree in nonhuman primates and absent in the rat. Given the high affinity of quipazine for 5-HT_3 receptors and the known antiemetic effects of certain 5-HT_3 antagonists, the present hypothesis might be tested by determining the hallucinogenic activity of quipazine in humans pretreated with a drug such as ondansetron. (For a review of 5-HT_3 receptors and ligands, see Kilpatrick et al. 1990.)

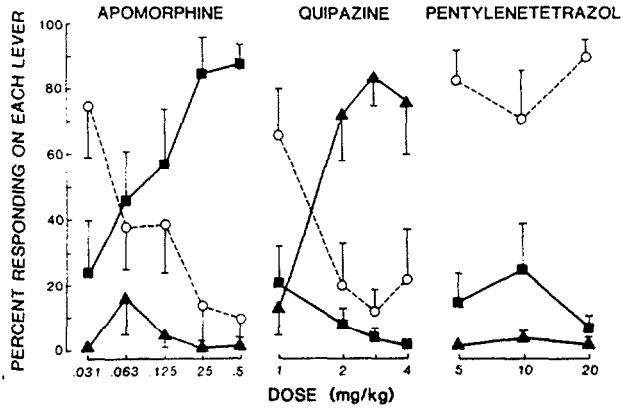

FIGURE 9. *Dose-response relationships for apomorphine, quipazine, and pentylenetetrazole in rats trained to discriminate LSD [0.08 mg/kg] from lisuride [0.04 mg/kg] from saline. All other details are as in figure 8.*

SEROTONERGIC RECEPTOR SUBTYPES

Behavioral pharmacology has elsewhere been referred to as a marriage of convenience between psychology and pharmacology (Winter 1978*b*). Nowhere are the difficulties of that marriage more evident than in the application of stimulus control to serotonergic hallucinogens. While behavioral techniques have been slowly maturing, there has been an exponential growth in the knowledge of the pharmacology, molecular biology, and functional significance of serotonergic receptors. The light cast by the discovery of each new receptor subtype permits—nay, requires—constant reexamination and reinterpretation of behavioral data. The serotonergic receptors designated 5-HT_2 and 5-HT_{1C} illustrate this point.

The role of the 5-HT_2 receptor in the actions of serotonergic hallucinogens appears well established (Glennon et al. 1985; Glennon, this volume). However, the discovery of the 5-HT_{1C} receptor subtype (Pazos et al. 1984) and the realization that there is often a close correlation between affinities for the 5-HT_2 and 5-HT_{1C} sites (Glennon 1990; Sanders-Bush and Breeding 1988; Titeler et al. 1988) have led to speculation that the 5-HT_{1C} receptor may play an independent or complementary role in-hallucinogenic activity (Sanders-Bush and

Breeding 1991; Titeler et al. 1988). This hypothesis is supported by the observation that LSD, but not its nonhallucinogenic congeners, is an agonist at 5-HT_{1C} receptors as indicated by stimulation of phosphoinositide hydrolysis (Burris et al. 199 1; Sanders-Bush, this volume.) It is obvious that each of these biochemical advances calls for the investigation of possible behavioral correlates. The investigation of the discriminative stimulus properties of drugs reputed to have selectivity for the 5-HT_{1C} receptor should prove informative. One such agent presently under study is *m*-chlorophenylpiperazine (MCPP) (Winter and Rabin 1992).

CONCLUSION

Although much has been learned regarding the stimulus effects of serotonergic hallucinogens, this area of investigation remains exciting in its promise. The observation that atypical antipsychotic drugs such as clozapine have high affinity for serotonergic receptors has given new life to the old idea that there may be a connection between exogenous hallucinogens and endogenous hallucinogenic processes. Increasing awareness of interactions between chemically distinct neurotransmitters encourages a broader mechanistic view. On an experimental level, researchers have perhaps become too set in their ways. For example, recent work in the author's laboratory (Fiorella et al., in press; Palumbo et al., in press) indicates that so simple a design feature as pretreatment time may have a significant influence upon the stability of DOM-induced stimulus control and, by inference, upon the nature of the cue which is trained. A continued effort is needed to identify those pharmacological features of hallucinogen-induced stimulus control in animals and their correlates in humans.

Twenty years ago, Winter (1972) stated, "It remains to be established whether study of the stimulus properties of drugs will permit the early identification of hallucinogens or will contribute in any significant way to our understanding of hallucinogens." (Winter 1974, p. 831). Evidence gathered during the intervening two decades, some of which is reviewed here and elsewhere in this volume, has answered both questions in the affirmative. Others appear to be encouraged as well: "Drug discrimination has proven to be a reliable, sensitive, and specific procedure that provides a powerful tool in the analysis of neuropharm-acological mechanisms underlying the behavioral effects of diverse classes of drugs." (Koek et al. 1992, pp. 97-98). With the long-awaited

resumption of tests in human subjects (Strassman, this volume) and the guidance provided by ever more sophisticated chemical, biochemical, and molecular techniques, the continued investigation of the stimulus properties of serotonergic hallucinogens promises to enhance the understanding not only of these agents as drugs of abuse, but also to elucidate the normal, pathological, and perhaps even supranormal functioning of the brain.

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AUTHOR

Jerrold C. Winter, Ph.D.
Professor of Pharmacology and Toxicology
Department of Pharmacology and Therapeutics
School of Medicine and Biomedical Sciences
State University of New York at Buffalo
Buffalo, NY 14214-3000

Electrophysiological Studies on the Actions of Hallucinogenic Drugs at 5=HT_2 Receptors in Rat Brain

George K. Aghajaniun

INTRODUCTION

There has been a longstanding interest in the role of the brain serotonin (5-hydroxytryptamine [5-HT]) system in the electrophysiological actions of psychedelic hallucinogenic drugs such as d-lysergic acid diethylamide (LSD) and mescaline. Early studies focused on the actions of LSD on serotonin-containing (serotonergic) neurons in the raphe nuclei of the brain stem. LSD and other indoleamine hallucinogens were found to have potent *direct* inhibitory effects upon serotonergic raphe neurons (Aghajanian et al. 1972). However, it was soon discovered that mescaline and other phenethylamine hallucinogens failed to share this action (Haigler and Aghajanian 1973). Later, it was shown that the direct inhibitory effect of LSD and other indoleamine hallucinogens on serotonergic neurons is mediated by 5-HT_{1A} receptors, and that this action is shared by a number of selective 5-HT_{1A} agonists that are not hallucinogenic (Sprouse and Aghajanian 1987, 1988). Thus, there appears to be no correlation between the activity of various drugs at 5-HT_{1A} receptors in the raphe nuclei and the presence or absence of hallucinogenic properties.

In contrast, a good correlation exists between the affinity of both indoleamine and phenethylamine hallucinogens for 5-HT_2 receptors and the potency of these drugs as hallucinogens in humans (Glennon et al. 1984; Titeler et al. 1988) As described below, electrophysiological studies in a number of brain regions show that indoleamine and phenethylamine hallucinogens have common electrophysiological action at 5-HT_2 receptors. There has, however, been some debate on whether the hallucinogens act as agonists, partial agonists (Glennon 1990; Sanders-Bush et al. 1988; Sheldon and Aghajanian 1990) or antagonists (Pierce and Peroutka 1990) at 5-HT_2 receptors. Recent electrophysiological studies bearing on this issue are described in this chapter.

The discovery of the structural and functional similarities between the 5-HT_2 and 5-HT_{1C} receptors (Hartig 1989) has also led to studies examining the effect of the hallucinogens at 5-HT_{1C} sites. Recent data from studies in the choroid plexus showed that the hallucinogens act as partial agonists at 5-HT_{1C} receptors that mediate an activation of phosphoinositide turnover (Burris et al 1991; Sanders-Bush and Breeding 1991). The electrophysiological studies are reviewed from the standpoint of how they may distinguish between actions at 5-HT_2 and 5-HT_{1C} receptors.

Quantitative autoradiographic studies show high concentrations of 5-HT_2 sites in the forebrain including the neocortex (layers IV/V), piriform cortex, claustrum, nucleus accumbens (NACC), and olfactory tubercle (OT) (Pazos et al. 1985). In contrast, with the exception of a few areas (e.g., facial nucleus and the n. tractus solitarius), relatively low concentrations of 5-HT_2 receptors are found in the brain stem. Single-cell studies on the physiological properties of 5-HT_2 receptors in several representative brain regions are described in the following sections.

PHYSIOLOGICAL ACTIONS OF HALLUCINOGENS

Locus Coeruleus

The systemic administration of indoleamine and phenethylamine hallucinogens (e.g., LSD and mescaline) results in decreased spontaneous activity of noradrenergic cells in the locus coeruleus (LC) of anesthetized rats but, paradoxically, a facilitation of the activation of these cells by sensory stimuli (Aghajanian 1980; Rasmussen and Aghajanian 1986). These effects in the LC are of theoretical interest since, in contrast to the raphe nuclei (see introduction), they are shared by the two major classes of psychedelic hallucinogens. Mediation by 5-HT_2 receptors is suggested by the fact that effects of the hallucinogens on LC neurons can be reversed by low intravenous (IV) doses of selective 5-HT_2 antagonists such as ritanserin, LY-53857 (figure 1) (Rasmussen and Aghajanian 1986), and ketanserin (Gorea and Adrien 1988). Antipsychotic drugs with affinity for 5-HT_2 binding sites are also able to reverse the actions of hallucinogens in the LC independently of their actions at dopamine (DA) and adrenergic receptors (Rasmussen and Aghajanian 1988). The relative potencies of antipsychotic drugs in blocking the actions of hallucinogens in LC neurons correlates highly with their affinity for 5-HT_2 receptors (Rasmussen and Aghajanian 1988). Of particular note is the fact that at

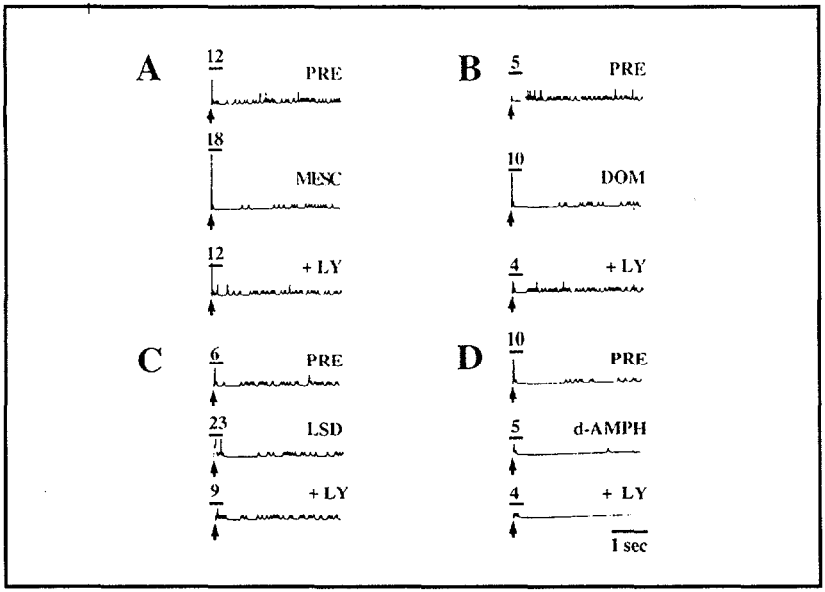


FIGURE 1. *Poststimulus time histograms during locus coeruleus cell recordings in vivo before and after administration of A, mescaline (MESC); B, DOM; C, LSD; or D, (+)-amphetamine (D-AMPH); and again after the administration of LY 53857 (LY).*

Each histogram was generated from 10 sweeps initiated by electrical stimulation of the sciatic nerve (arrows). Evoked spikes occurred during the first 250 milliseconds of each histogram (bars). The total number of evoked spikes occurring during this period is given above the bars. Note both the increased number of spikes evoked by sciatic nerve stimulation (initial 1.50 milliseconds of histogram) and the decrease in spontaneous activity (remainder of histogram) after LSD, DOM, and mescaline administration. In addition, following hallucinogen ddministration, the increased number of spikes evoked by sciatic nerve stimulation, combined with the decrease in spontaneous activity, leads to a prolonged period of postactivation inhibition. Also, note the reversal of these effects after LY 53857 administration. After (+)-amphetamine administration, both the spontaneous activity and the number of evoked spikes are decreased; however, the subsequent administration of LY 53857 does not reverse either of these effects. From Rasmussen and Aghajanian 1986.

extremely low doses spiperone, which has almost a thousandfold greater affinity for 5-HT_2 than 5-HT_{1C} receptors, completely blocks the effects of the hallucinogens. Thus, the effects of hallucinogens on the LC appear to be mediated by 5-HT_2 rather than 5-HT_{1C} receptors.

It should be noted that the effects of hallucinogens in the LC are not direct since they are not mimicked by local iontophoretic application of the drugs onto LC cell bodies (Aghajanian, unpublished observations). Moreover, the systemic administration of mescaline or LSD does not enhance the excitation of LC neurons evoked by microiontophoretically applied acetylcholine, glutamate, or substance P (Aghajanian 1980). These results imply that the hallucinogens are acting indirectly, perhaps via afferents to the LC. Thus, the LC is not useful as a model for studying the *direct* cellular actions of hallucinogens. Nevertheless, the effects of the hallucinogens upon the LC are of interest because of the unique role of this nucleus as a nodal point in processing a convergence of sensory information, both somatosensory and visceral, and relaying this information to virtually all parts of the central nervous system (CNS).

Facial Nucleus

In contrast to LC neurons, facial motoneurons have a high density of 5-HT_2 receptor binding sites, and they also express a high level of 5-HT_2 receptor messenger ribonucleic acid (mRNA) (Mengod et al. 1990*b*). The microiontophoretic application of 5-HT or norepinephrine (NE), while not by itself inducing firing in quiescent facial motoneurons, facilities the excitatory effects of iontdphoretically applied glutamate (McCall and Aghajanian 1979). Intracellular recordings in vivo (VanderMaelen and Aghajanian 1980, 1982) and in brain slices (Aghajanian and Rasmussen 1989; Larkman et al. 1989) reveal that 5-HT induces a slow depolarization in facial motoneurons by decreasing a resting potassium conductance. Similar effects of 5-HT on potassium conductance have been described in spinal motoneurons (White and Fung 1989) and in the NACC (North and Uchimura 1989).

The action of iontophoretically applied 5-HT in the facial nucleus can be blocked by the classical 5-HT antagonists metergoline, methysergide, cyproheptadine, and cinanserin (McCall and Aghajanian 1980*b*), which have varying degrees of selectivity for the 5-HT_2 receptor. More recent studies show that the selective 5-HT_2 antagonist ritanserin blocks the excitatory effects of 5-HT in facial motoneurons (Rasmussen and

Aghajanian 1990). The selective 5-HT_{1A} agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) increases facial motoneuron excitability when given in vivo by the systemic route (Rasmussen and Aghajanian 1990). However, this effect is presumably due to an indirect action, since 8-OH-DPAT has no effect when applied locally either by microiontophoresis or bath application in brain slices (Garratt et al. 1993; Rasmussen and Aghajanian 1990). These results suggest that 5-HT_{1A} receptors are probably not directly involved in the excitatory actions of 5-HT on facial motoneurons.

Since behavioral as well as binding techniques have shown that both indoleamine and phenethylamine hallucinogens interact with 5-HT_2 receptors (Glennon et al. 1983, 1984; Heym et al. 1984; Mokler et al. 1985), it is not surprising that members of both classes of hallucinogens have direct effects in the facial motor nucleus. The iontophoretic administration of LSD, mescaline, or psilocin, although at low doses having relatively little effect by themselves, enhance the facilitiation of facial motoneuron excitation produced by ionophoretically applied 5-HT and NE (McCall and Aghajanian 1980*a*). Curiously, the enhancement can persist for several hours after only a single brief application. Intracellular studies in brain slices show that LSD produces an increase in the electrical excitability of facial motoneurons; however, even at maximal concentrations, this is associated with only a small depolarization of facial motoneurons compared to that produced by 5-HT itself (figure 2) (Garratt et al. 1993).

The phenethylamine hallucinogen l-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) produces effects similar to those of LSD, but with a slightly greater maximal depolarization. Since the depolarizing effect of 5-HT is mainly due to a decrease in potassium conductance, LSD and DOI would appear to have low intrinsic activity relative to 5-HT in this transduction pathway. This observation parallels studies of the effects of hallucinogens on phosphoinositide hydrolysis (Sanders-Bush et al. 1988; Barker et al. 1991). In those studies, 5-HT caused the greatest increase in phosphoinositide hydrolysis, whereas 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB), 2,5-dimethoxy-4-methylamphetamine (DOM), and LSD acted as partial agonists with LSD having the least intrinsic activity.

Recently, it was found that in addition to producing a decrease in resting potassium conductance, 5-HT produces an enhancement of the non-

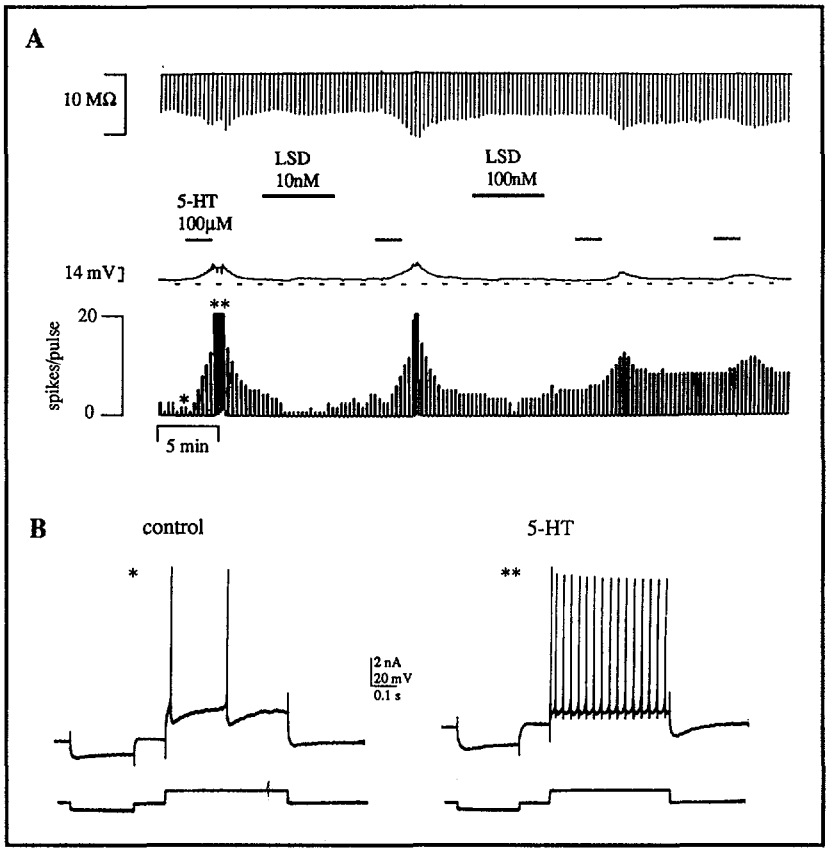


FIGURE 2. *A. Effects of bath application of 5-HT and LSD on input resistance (upper trace), membrane potential (middle trace) and electrical excitability (lower trace) recorded simultaneously in a rat facial motoneuron from an in vitro brain slice preparation.*

Bath application of 5-HT (100μM) produces an increase in apparent input resistance, a depolarization (14 mV), and an increase in electrical excitability of the cell (i.e., an increase in spikes induced by a constant current pulse); note that the ratemeter trace goes off scale at the peak of the response. Application of LSD (10 nM) produces little change in apparent input resistance or membrane potential; however, there is a clear increase in electrical excitability, which has a slow onset of action. Interestingly, LSD at this dose slightly suppressed the response of the cell to 5-HT.

FIGURE 2. (cont.) *A. Effects of bath application of 5-HT and LSD on input resistance (upper trace), membrane potential (middle trace) and electrical excitability (lower trace) recorded simultaneously in a rat facial motoneuron from an in vitro brain slice preparation.*

A higher concentration of LSD (100 nM) again produces little change in apparent input resistance or membrane potential. There is, however, a further increase in the electrical excitability which also has a slow onset of action and very long duration (>2 hrs., not shown). At this dose there is further suppression of the response of the cell to 5-HT. Electrical excitability is measured by injecting the cell with a depolarizing pulse (+1.5 nA, 400 ms duration) which elicits one or two spikes under control conditions. Periods of drug application are indicated by horizontal bars.

B . Oscilloscope traces showing the effect of 5-HT on the electrical excitability of the facial motoneuron shown in A.

The spikes were evoked by a depolarizing pulse (+1.5 nA); the cell is otherwise silent (resting potential, -72 mV). The left hand panel shows that under control conditions two spikes are elicited by the depolarizing pulse. Single asterisk denotes time point that sweep was taken in (A). In the presence of 5-HT (100 μM) (in the right hand panel), the same pulse elicits 16 spikes (double asterisk in A), thus indicating that 5-HT increases the electrical excitability of the cell. Also, note the increase in input resistance (i.e., greater voltage deflection in response to the hyperpolarizing pulse) following 5-HT.Prom Garratt et al. 1993

specific hyperpolarizing activated cationic current (I_h) which also increases the excitability of facial motoneurons (Garratt et al. 1993).

Surprisingly, both LSD (figure 3) and DOI produce a greater maximal enhancement of I_h than does 5-HT (LSD > DOI > 5-HT). Moreover, in contrast to 5-HT, DOI and LSD have a slow onset and very long duration

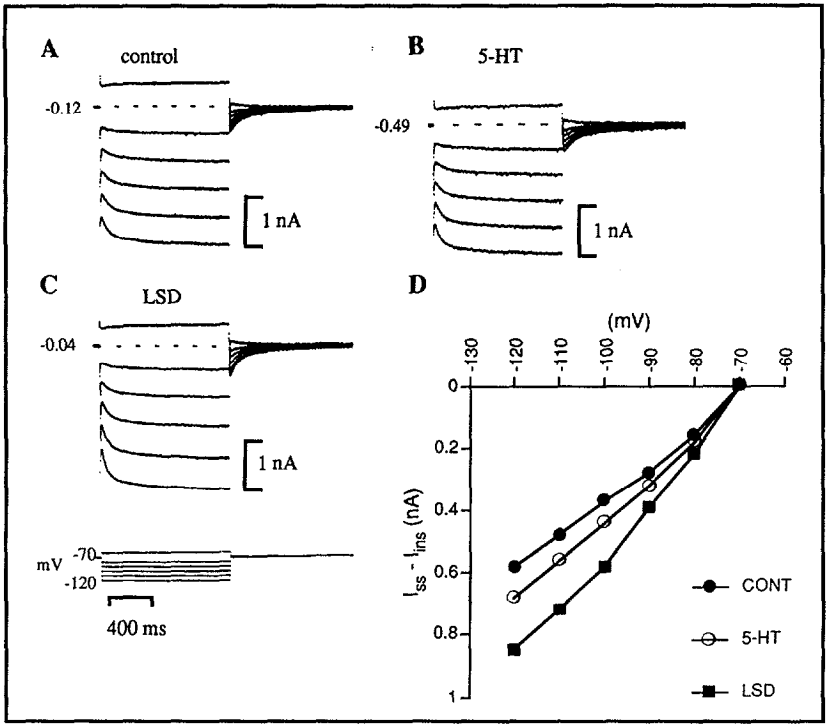


FIGURE 3. *Effects of bath application of 5-HT and LSD on the hyperpolarization-activated cation current (I_h) in facial motoneurons.*

A, B, C are oscilloscope traces showing intracellular voltage-clamp recordings from facial motoneuron. (A) shows a progressing increase in the size of the hyperpolarization-activated cation current (I_h) in response to increased hyperpolarizing steps from -70 mV to -120 mV. (B) shows the enhancement of I_h in the presence of 5-HT (100 µM).(C) shows that LSD (100 nM) produces a greater increase in I_h than 5-HT. (D) is a current-voltage plot obtained from A, B, and C showing the difference between steady state and instantaneous currents in response to voltage clamp commands in 10 mV steps from a holding potential of -70 mV to -120 mV. Note that LSD produces a greater increase than 5-HT in I_h at all voltage steps. From Garratt et al. 1993.

of action (> 2 hours). The enhancement of I_h by LSD and DOI is reversed by application of both the 5-HT_2/5-HT_{1A}/5-HT_{1A} antagonist spiperone and the 5-HT_2/5-HT_{1C} antagonist ritanserin, suggesting that the increase in I_h is mediated by receptors. This appears to be the first example of a direct 5-HT_2-mediated electrophysiological response in the brain in which hallucinogens have greater efficacy than 5-HT (Pierce and Peroutka 1990).

The finding that LSD has high efficacy in increasing I_h and low efficacy in decreasing resting potassium conductance could be explained most simply by supposing that the 5-HT_2 receptor couples to the two different channels via two different guanosine triphosphate binding proteins (G proteins). Thus, the same type of 5-HT_2 receptor could couple to potassium channels via one type of G protein and to I_h channels via another type of G protein, yielding two different receptor/G-protein complexes that would have differential intrinsic activity with respect to the different agonists. A second possibility is that there are two or more different 5-HT_2 receptor subtypes that independently confer differential intrinsic activity to 5-HT and the hallucinogens.

Regardless of the mechanism, the high efficacy of LSD relative to, 5-HT on the I_h current in rat facial motoneurons raises the interesting possibility that such an action elsewhere in the brain could be involved in mediating hallucinogenic processes. Neurons in several brain regions have been shown to have an I_h current that is enhanced by 5-HT (Bobker and Williams 1989; McCormick and Pape 1990; Nedergaard et al. 1991; Takahashi and Berger 1990). It would be interesting to see if LSD produces a greater enhancement of I_h relative to 5-HT in these or other brain regions, especially in cases when the effect on I_h may be mediated by a 5-HT_2 receptor.

Cerebral Cortex

The electrophysiological effects of 5-HT at 5-HT_2 receptors have been studied in several cortical regions. In vivo, the 5-HT_2 agonist DOI has been reported to have an overall inhibitory effect on the firing of unidentified neurons in the prefrontal cortex (Ashby et al. 1989). Curiously, the inhibitions produced by DOI but not by 5-HT itself are blocked by 5-HT_2 antagonists (Lakoski and Aghajanian 1985). In brain slices, pyramidal cells in various regions of the cerebral cortex have been found to respond to 5-HT by a small hyperpolatization, depolarization, or no change in potential (Davies et al. 1987). It was suggested that the

depolarizations are mediated by 5-HT_2 receptors since they can be blocked by 5-HT_2 antagonists.

Recently, the author and coworkers have observed a novel effect of 5-HT in pyramidal cells of the piriform cortex: the enhancement of spontaneous inhibitory postsynaptic potentials (IPSPs) (Sheldon and Aghajanian 1990). The IPSPs are blocked by the gamma-aminobutyric acid (GABA) antagonist bicuculline, suggesting that GABAergic interneurons are excited by 5-HT, giving rise to the increase in IPSPs in the pyramidal cells. In accord with this expectation, a subpopulation of interneurons at the border of layers II and III were found that are excited by 5-HT. The 5-HT_2/5-HT_{1C} antagonist ritanserin blocks both the 5-HT-induced activation of interneurons and the associated IPSPs in pyramidal cells (Sheldon and Aghajanian 1990). Interestingly, the hallucinogens LSD and DOM behave as partial agonists in this system, producing a modest activation by themselves but occluding the full effect of 5-HT (figure 4).

Another effect of 5-HT in the piriform cortex is a direct depolarization of pyramidal cells that is also blocked by ritanserin. However, blockade of the 5-HT-induced activation of interneurons by ritanserin occurs much more readily than the blockade of pyramidal cell depolarization (Sheldon and Aghajanian 1990). Since ritanserin has a nearly tenfold higher affinity for the 5-HT_2 receptor than for the 5-HT_{1C} receptor (Hoyer 1988), it is possible that the action of 5-HT on these interneurons might be through 5-HT_2 receptors, and that the action of 5-HT on the pyramidal cells might be through 5-HT_{1C} receptors. This hypothesis is consistent with recent in situ hybridization studies that show that mRNA for the 5-HT_2 receptor is expressed in cortical interneurons (Mengod et al. 1990*b*), while that for the 5-HT_{1C} receptor is expressed in pyramidal cells (Mengod et al. 1990*a*).

To test this hypothesis further, the author and coworkers studied the effects of the 5-HT antagonist spiperone on the actions of 5-HT in both interneurons and pyramidal cells (Sheldon and Aghajanian 1991). The activating effect of 5-HT on interneurons is blocked by bath application of low concentrations of spiperone (100 nanomolars [nM]), while in pyramidal cells the direct excitatory effects of 5-HT are unaffected by 100 nM to 1 micromolar (μM) spiperone but are blocked by 10 μM ritanserin. These findings support the hypothesis that in the piriform cortex the excitatory action of 5-HT on interneurons is mediated by 5-HT_2 receptors while the excitatory action of 5-HT on pyramidal cells is

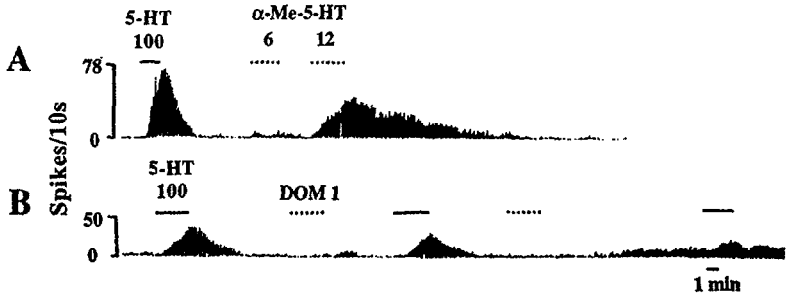


FIGURE 4. *Note the prolonged action of DOM following the second application. Concentrations in mM. Agonist actions of 5-HT, α-Me-5-HT, and DOM on putative interneurons in piriform cortex. (A) The 5-HT_2 agonist α-Me-5-HT mimics the action of 5-HT in a dose-dependent manner. (B) The 5-HT_2 agonist DOM acts as a partial agonist, having a small activating effect of its own and blunting the efect of 5-HT. From Sheldon and Aghajanian 1990*

mediated by 5-HT_{1C} receptors. Thus, the effects of 5-HT and hallucinogens in the cerebral cortex are likely to be complex, involving actions at multiple 5-HT receptors differentially expressed by interneurons and pyramidal cells.

5-HT RECEPTORS: SIGNAL TRANSDUCTION MECHANISMS

The role of G proteins in mediating the 5-HT_2-induced slow inward current in facial motoneurons was evaluated by using the hydrolysis-resistant guanine nucleotide analogs GTPγS and GDPßS (Aghajanian 1990). Due to a persistent activation of G protein, the 5-HT-induced inward current becomes largely irreversible in the presence of intracellular GTPγS. The inward current is reduced by intracellular GDPßS, suggesting mediation by G proteins since the binding of GDPßS to the G protein renders it resistant to activation by agonist. These electrophysiological results are consistent with binding studies which show that guanosine triphosphate (GTP) analogs induced a downward shift in the affinity of a radiolabeled 5-HT_2, agonist for brain membranes (Lyon et al. 1986). This GTP dependency is consistent with the structural analysis of a cloned 5-HT_2 receptor from a rat brain carrier deoxyribonucleic acid (cDNA) library, demonstrating seven transmembrane regions

typical of G protein coupled receptors (Pritchett et al. 1988). The identity of the G proteins that couple to the 5-HT_2 receptor remains to be determined.

Serotonin stimulates phosphatidylinositol hydrolysis in the brain through 5-HT_2 and 5-HT_{1C} receptors (Conn and Sanders-Bush 1986). LSD and DOM act as partial agonists of this effect (Sanders-Bush et al. 1988). Phosphatidylinositol hydrolysis yields at least two major second messengers: inositol trisphosphate (IP_3) and diacylglycerol. The latter activates protein kinase C (PKC), thereby potentially affecting many long-term cellular responses through protein phosphorylation. The author and coworkers have tested the effect of protein kinase inhibitors on the response of facial motoneurons to serotonin (Aghajanian 1990). In concentrations that have no effect of their own (100 and 10µM, respectively), two protein kinase inhibitors with different mechanisms of action, 1-(5-isoquinolylsulfonyl)-2-methylpiperazine (H7), a nonselective protein kinase inhibitor, and sphingosine, a selective PKC inhibitor, both markedly enhance and prolong the excitation of facial motoneurons induced by 5-HT. Conversely, phorbol esters that are known to activate PKC reduce the excitatory effect of serotonin. These results suggest that activation of phosphatidylinositol turnover, perhaps through PKC-induced receptor phosphorylation, has a negative feedback effect on 5-HT-induced excitations in the facial nucleus. These observations are consistent with studies on 5-HT_2 receptor-mediated responses in rat aorta, which indicate that the activation of PKC desensitizes 5-HT_2 receptors (Roth et al. 1986).

An interesting implication of the negative feedback model is the possibility that the partial agonist (Sanders-Bush et al. 1988) or even antagonist (Pierce and Peroutka 1988) properties of hallucinogens with respect to 5-HT-stimulated phosphatidylinositol hydrolysis may contribute to, rather than interfere with, their electrophysiological actions. Thus, the greater enhancement of $I_h$in facial motoneurons by hallucinogens relative to 5-HT could be explained by a combination of two factors: LSD has high efficacy in this transduction system, and by not markedly activating phosphatidylinositol hydrolysis, LSD does not provoke a large negative feedback reduction in response.

SUMMARY AND CONCLUSIONS

Receptor Specificity

This review has considered the electrophysiological effects of hallucinogens in three different brain regions: the LC, the facial motor nucleus, and the cerebral cortex. The results of those studies, together with findings from earlier studies in the raphe nuclei, make it clear that the indoleamine and phenethylamine hallucinogens have common electrophysiological actions on 5-HT_2 but not 5-HT_{1A}, receptors. Furthermore, in each of these three regions, the effects of the hallucinogens are blocked by spiperone, which has almost a thousandfold higher affinity for 5-HT_2 than 5-HT_{1C} receptors. Thus, it may be concluded that the electrophysiological effects of hallucinogens, at least those examined to date, are likely to be mediated primarily by 5-HT_2 rather than 5-HT_{1C} receptors, although the latter may have a contributory role.

Signal Transduction Mechanisms

The net effect of 5-HT_2 receptor stimulation is to produce an increase in neuronal excitability through a G protein-coupled mechanism. The ionic mechanism underlying increased excitability involves a reduction in resting potassium conductance and/or enhancement of the hyper-polarizing-activated cationic current I,. In addition, biochemical studies show that both 5-HT_2 and 5-HT_{1C} receptors are coupled to PKC and IP_3 mechanisms through the phosphoinositide signal transduction pathway. Altogether it would appear that 5-HT_2 receptors are coupled to at least three different transduction systems: potassium channels, cationic I_h channels, and phosphoinositide hydrolysis. The coupling could be through a single G protein or through multiple G proteins and multiple 5-HT_2 receptor subtypes, one for each transduction pathway. Several hypothetical models for 5-HT_2-receptor/G protein coupling mechanisms are presented in figure 5.

It might be supposed that the electrophysiological effects of 5-HT and hallucinogens acting upon 5-HT_2 receptors are mediated through the phosphoinositide pathway. However, it has been found, mainly from studies in the facial motor nucleus, that activators of PKC suppress, while inhibitors of PKC enhance, 5-HT_2-induced increases in neuronal excitability. Thus, the phosphoinositide second messenger system may

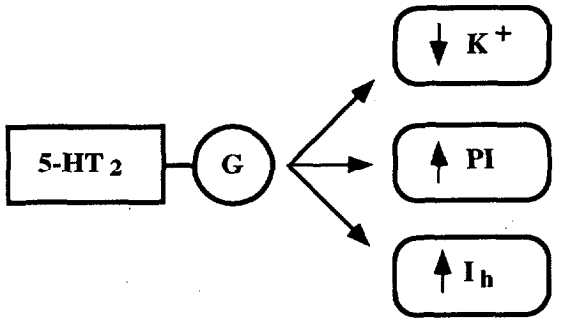
serve as a negative feedback system with respect to the electrophysiological effects produced by 5-HT_2 receptor activation. The fact that hallucinogens are only partial agonists at stimulating phosphoinositide turnover may enhance rather than diminish their electrophysiological effects.

Behavioral Correlations

As yet, the interrelationships between electrophysiological and behavioral actions of hallucinogenic drugs have not been examined in detail. Nevertheless, certain intriguing links are emerging. For example, the enhancement of sensory responsivity in LC neurons may contribute to the characteristic intensification of certain kinds of sensory experience produced by hallucinogens in humans. The increase in motoneuronal excitability producted by hallucinogens could explain the hyperreflexia induced by these drugs. Finally, the persistent activation of a subpopulation of 5-HT_2-expressing interneurons in the cerebral cortex may underlie the various cognitive and perceptual distortions produced by the hallucinogenic drugs.

FIGURE 5. *(facing page) Hypothetical models for the coupling of 5-HT_2 receptors to three different effector mechanisms: decreased K^+ conduc-tance, increased phosphoinositide (PI) turnover, and increased I_h. (A) A single class of 5-HT_2 receptors couples via one type of G protein to the three different effectors. (B) A single class of 5-HT_2 receptors couples to three different G proteins (G, G', and G"), one for each effector system. (C) Three 5-HT_2 receptor variants (5-HT_2, 5-HT_2', 5-HT_2") couple separately to the three effectors, each via a different G protein.*

A



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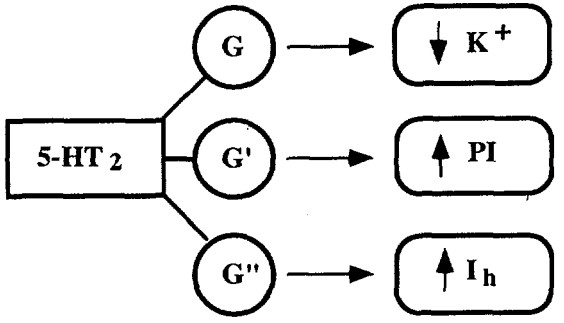

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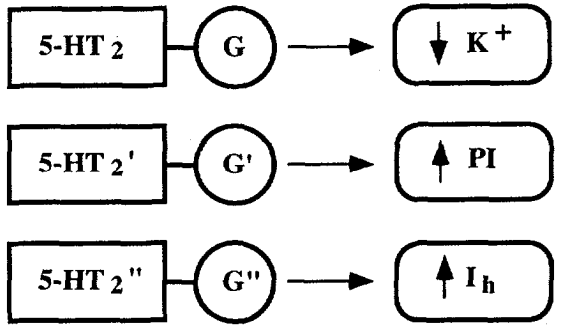

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AUTHOR

George K. Aghajanian, M.D.
Professor of Psychiatry and Pharmacology
Departments of Psychiatry and Pharmacology
Yale University School of Medicine and the
Connecticut Mental Health Center
34 Park Street
New Haven, CT 06508

Neurochemical Evidence That Hallucinogenic Drugs Are 5=HT_{1C} Receptor Agonists: What Next?

Elaine Sanders-Bush

INTRODUCTION

Behavioral, electrophysiological, and neurochemical evidence points to the serotonin$_2$(5-HT_2) receptor as an important site of action for hallucinogenic drugs (Glennon, this volume). Several years ago, the hypothesis was developed that hallucinogenic drugs might also be 5-HT_{1C} receptor agonists. This hypothesis was based on the significant sequence homology in the deduced amino acid structure of the 5-HT_2 and 5-HT_{1C} receptors, their pharmacological similarities, and the evidence that both receptors are linked to the same signal transduction pathway: phosphoinositide hydrolysis. In the past 4 years, the author and colleagues at Vanderbilt University have accumulated compelling evidence in support of this hypothesis.

A more profound question remaining unanswered is the relative roles of the 5-HT_2 and 5-HT_{1C} receptors in the various actions of these drugs, in particular, hallucinations. Studies of this question are just beginning and are based on three strategies: a neurochemical effect important to hallucinations should be a common property of all hallucinogenic drugs; close structural analogs of hallucinogenic drugs that do not elicit hallucinations should also not elicit the neurochemical response; and tolerance should develop to the behavioral effects of hallucinogenic drugs. Results of these studies are summarized in this chapter.

IS 5-HT_{1C} RECEPTOR ACTIVATION A COMMON PROPERTY OF HALLUCINOGENIC DRUGS?

In the choroid plexus, 5-HT_{1C} receptors are positively coupled to the phosphoinositide hydrolysis signaling cascade (Conn et al. 1986). The phosphoinositide hydrolysis response was therefore used to characterize the properties of hallucinogenic drugs at 5-HT_{1C} receptors. The first study examined (+) lysergic acid diethylamide (LSD), the most potent

hallucinogen known. (+)LSD potently activated phosphoinositide hydrolysis in the choroid plexus (median effective concentration [EC_{50}] = 10 nanomolars [nM]), although the maximum response was less than that produced by serotonin (figure 1). (+)LSD produced an incomplete blockade of the effect of serotonin, suggesting that it is a partial 5-HT_{1C} receptor agonist (Sanders-Bush et al. 1988).

Consistent with this interpretation, the 5-HT_2 and 5-HT_{1C} antagonists mianserin and ketanserin antagonized the agonist effect of LSD on phosphoinositide hydrolysis whereas spiperone, a selective 5-HT_2 antagonist, was without effect (figure 2). Since these original studies, a number of hallucinogens of different chemical classes have been examined, and all were found to act as agonists—some full, some partial—at 5-HT_{1C} receptors (Sanders-Bush and Breeding 1991). Comparison of the properties at 5-HT_{1C} and 5-HT_2 receptors shows that the hallucinogenic drugs are nearly equally effective at the two receptors.

The studies of Sanders-Bush and Breeding (1991) were the first to show that hallucinogenic drugs are 5-HT_{1C} receptor agonists. Because this property is common to all hallucinogens tested, this action may be important in the behavioral effects of these drugs. The challenge now is to formulate testable hypotheses, given the limitations of studies of hallucinogenic properties in animals. The most obvious and straightforward approach is to determine whether 5-HT_{1C} receptor antagonists block the behavioral effects of hallucinogenic drugs in animal models that are predictive of human activity (e.g., the drug discrimination [DD] paradigm). Such studies are not feasible, however, because there are no suitable antagonists (i.e., drugs that block 5-HT_{1C} but not 5-HT_2 receptors or vice versa). In vitro studies have relied on spiperone, which blocks 5-HT_2 but not 5-HT_{1C} receptors. However, behavioral studies of spiperone are not possible because of its potent neuroleptic action. Definitive behavioral studies await the development of specific 5-HT_2 and 5-HT_{1C} receptor antagonists.

WHAT ARE THE PROPERTIES OF CLOSELY RELATED STRUCTURAL ANALOGS OF LSD THAT ARE NOT HALLUCINOGENIC?

Recent studies have focused on three drugs: (-)LSD, 2-bromo LSD (BOL), and lisuride, all of which are close chemical relatives of (+)LSD

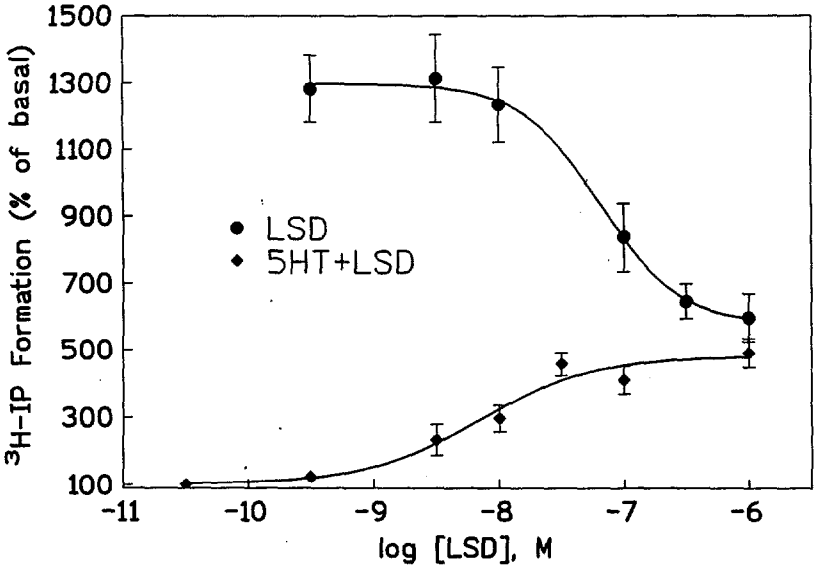

FIGURE 1. *Partial agonist effect of LSD at 5-HT$_{1C}$ receptors in rat choroid plexus. [^{3}H]-Inositol-labeled choroid plexi were incubated for 45 minutes with lithium chloride, pargyline, and increasing concentrations of (+)LSD with and without 100 nM serotonin. [^{3}H]-Inositol-monophosphate (IP) formation is expressed as percentage of the basal radioactivity. The circles represent a dose-response for LSD alone (n=4). The diamonds show the effect of 100 nM serotonin plus increasing concentrations of LSD alone (n=4). The maximum [^{3}H]-IP formation in the presence of 1 µM serotonin was 1363 ± 143% of basal. Mean basal [^{3}H]-IP formation was 729 ± 117 cpm.*

SOURCE: Sanders-Bush et al. 1988.

(figure 3) but apparently do not mimic its effects in humans (White 1986). The properties of these drugs at both 5-HT$_2$ and 5-HT$_{1C}$ receptors were examined. (-)LSD and BOL were pure antagonists of these receptors, with no evidence of agonist properties (Burris et al. 1991).

The effects of lisuride on the 5-HT$_2$ and 5-HT$_{1C}$ receptors are illustrated in figure 4. As shown, lisuride had no apparent 5-HT$_{1C}$ receptor agonist activity, and it completely blocked the response to 100 nM serotonin. In

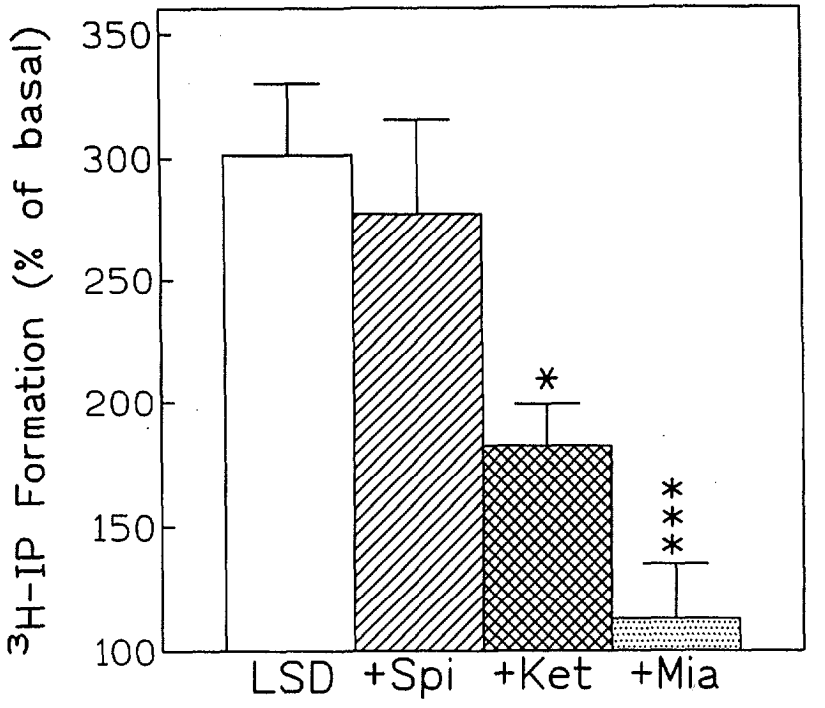

FIGURE 2. *Antagonism of (+)LSD-stimulated phosphoinositide hydrolysis in rut choroid plexus. Antagonists (1 µM) were added 15 minutes before the addition of 20 nM (+)LSD. Results are expressed as percent of basal, which was 2,751±219 cpm (n=21). Antagonists alone did not alter basal. *p<0.05, ***p<0.001. Spi = Spiperone; Ket = Ketunserin; Mia = Mianserin.*

contrast, lisuride was an agonist at the 5-HT_2 receptor, eliciting a strong phosphoinositide hydrolysis signal that was somewhat less than that of serotonin. Like LSD, lisuride partially blocked the effect of serotonin consistent with a partial agonist action at 5-HT_2 receptors. Therefore, lisuride, a nonhallucinogenic congener of LSD, appears to mimic LSD's agonist properties at the 5-HT_2 receptor. Lisuride does not, however, reproduce the effects of LSD at the 5-HT_{1C} receptor, consistent with the hypothesis that the 5-HT_{1C} receptor may be involved in the hallucinogenic action of LSD. On the other hand, the finding that 5-HT_2 receptor activation is a property shared by (+)LSD and lisuride is not consistent with a critical role for the 5-HT_2 receptor in the production of such au action.

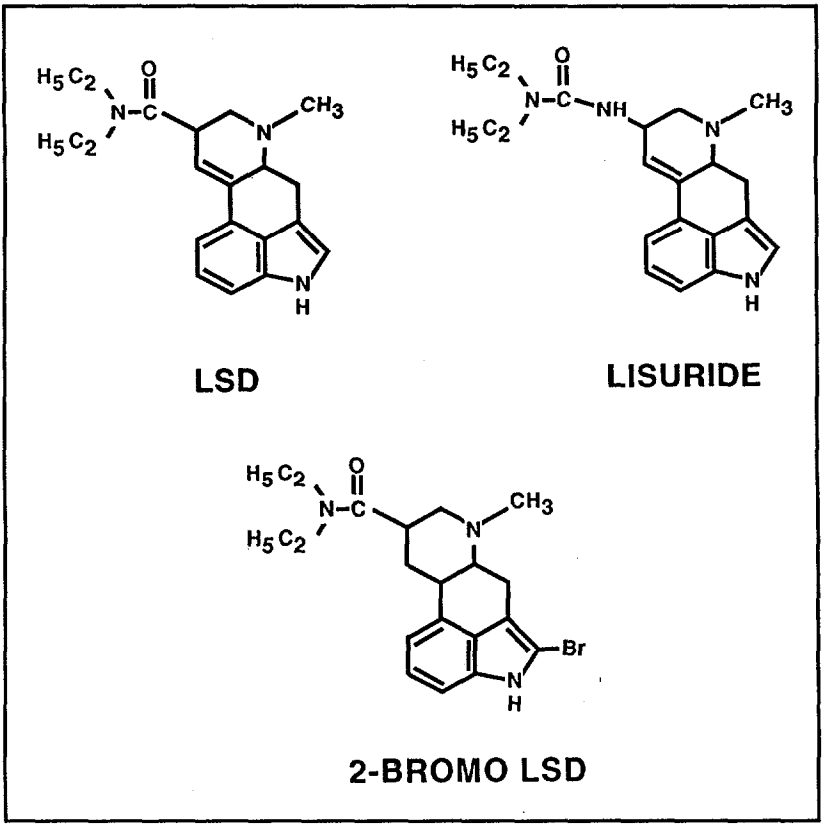

FIGURE 3. *Chemical structures of LSD, lisuride, and 2-bromo-lysergic acid diethylamide.*

DOES TOLERANCE DEVELOP TO ACTIVATION OF 5-HT$_{1C}$ RECEPTORS BY HALLUCINOGENS?

Early clinical studies showed that a profound and rapid tolerance occurred after the administration of LSD in humans (Abramson et al. 1956; Isbell et al. 1959). This tolerance has since been confirmed innumerous animal studies of hallucinogenic drugs (Adams and Geyer 1985; Carvey et al. 1989; Darmani et al. 1990; Leysen et al. 1989). One of the most common mechanisms of tolerance is a reduction in the density of receptors for the drug (i.e., down-regulation). Down-regulation of 5-HT$_2$ receptors is found in rat brain after chronic administration of LSD and hallucinogenic amphetamines (Buckholtz et al. 1990; Leysen et al. 1989; McKenna et al. 1989). This neurochemical

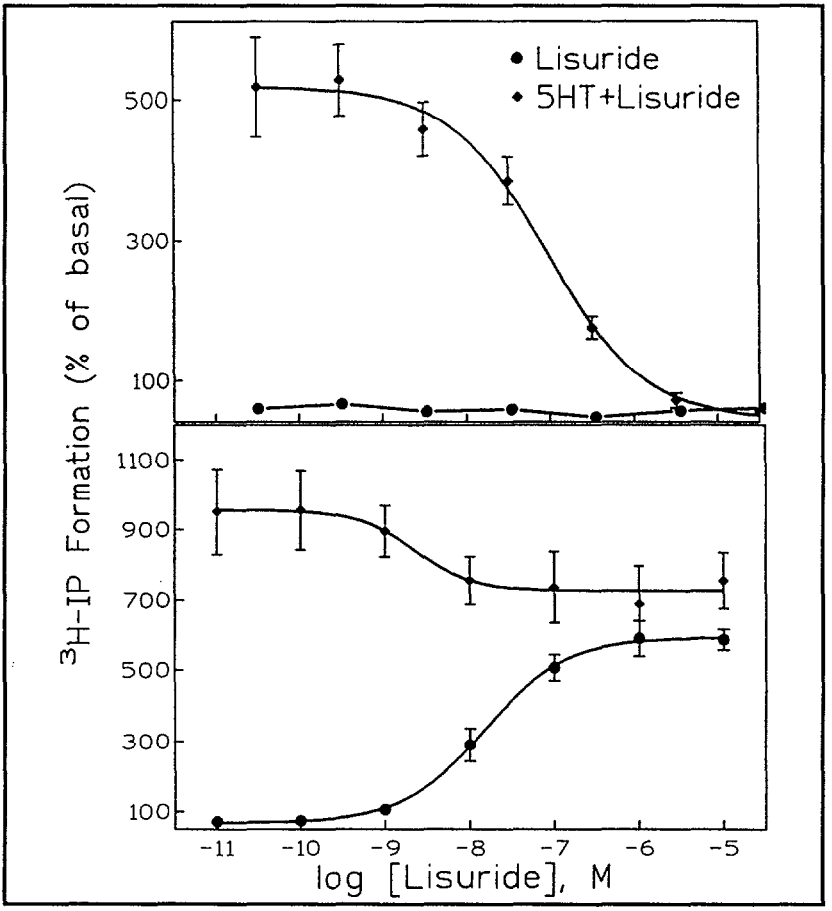

FIGURE 4. *Effect of lisuride at 5-HT_{1C} receptors in choroid plexus (upper panel) and 5-HT_2 receptors in transferred cells (lower panel). [^{3}H]-Inositol-labeled tissue was incubated for 45 minutes with lithium chloride, pargyline, and increasing concentrations of lisuride in presence and absence of 100 nM serotonin. [^{3}H]-Inositol-monophosphate (IP) formation is expressed as percentage of the basal radioactivity. The circles represent a dose-response for lisuride alone (n=6). The diamonds show the effect of 100 nM serotonin plus increasing concentrations of lisuride. Mean basal [^{3}H]-IP formation was 195±21 cpm in choroid plexus and 257±36 cpm infibroblasts transfected with 5-HT_2 receptor cDNA. Julius et al. 1990*

response may be involved in the tolerance that occurs to the behavioral effects of hallucinogens.

Recent studies have begun to explore the question of whether a down-regulation of 5-HT_{1C}, receptors is also produced after chronic treatment with hallucinogenic drugs. These studies have been done in choroid plexus epithelial cells that express the 5-HT_{1C} receptor. Chronic exposure to 1 micromolar (μM) of (-)1(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB) elicited a delayed decrease'in the density of 5-HT_{1C} receptors (Barker and Sanders-Bush 1993). This loss of receptors presumably will blunt the response to 5-HT_{1C}, receptor agonists such as hallucinogenic drugs; however, this effect has not yet been demonstrated. A blunted response would be consistent with the interpretation that 5-HT_{1C} receptors may be involved in the behavioral effects of hallucinogenic drugs.

WHAT IS DIFFERENT ABOUT 5-$HT_{2/1}$ AGONISTS THAT ARE HALLUCINOGENIC VERSUS THOSE THAT ARE NOT?

This author advocates the opinion that actions shared by lisuride and LSD, such as 5-HT_2 receptor activation, are probably not important in mediating hallucinations. This is not a new idea; for example, it was eloquently defended several years ago by White (1986). An alternative interpretation is that although LSD and lisuride, as well as other nonhallucinogenic 5-HT_2 agonists (e.g., quipazine), are capable of eliciting a receptor signal, they do so by different mechanisms (e.g., by interacting with different functional domains of the receptor). This possibility is especially interesting in light of the suggestion that LSD is an allosteric antagonist of the 5-HT_2 receptor (Kaumann 1989; Xu and Purdy 1989). Since the antagonist and agonist properties of a partial agonist such as LSD presumably reflect the same binding event, this may indicate that LSD has unique agonist properties (i.e., it may interact with a domain that is different from the serotonin binding domain). Conversely, lisuride and other nonhallucinogenic 5-HT_2/5-HT_{1C} agonists may not interact in an allosteric fashion.

Recent investigations have explored the putative allosteric interactions of LSD in cells transfected with the 5-HT_2 receptor (Burris and Sanders-Bush 1992). LSD produced a noncompetitive blockade of 5-HT-mediated phosphoinositide hydrolysis; high concentrations of serotonin were not able to overcome the LSD blockade. One possible

interpretation of these results is that LSD interacts at a site on the 5-HT_2 receptor that is different from the site where serotonin binds (i.e., an allosteric site). An alternative interpretation is that LSD binds tightly to the active site of the receptor in an irreversible or pseudoirreversible manner. To investigate the latter possibility, the dissociation rate of LSD was determined in membranes isolated from cells expressing the cloned 5-HT_2 receptor carrier deoxyribonucleic acid (cDNA).

In the initial studies of ^{3}H-LSD binding by Bennett and Snyder (1975), ^{3}H-LSD dissociated slowly, but since a whole brain was used and LSD is nonspecific, it is not clear which 5-HT receptor was involved. This is not a problem with fibroblasts transfected with the 5-HT_2 receptor cDNA, since only one 5-HT receptor subtype is expressed: the 5-HT_2 receptor (Julius et al. 1990). In membranes from these cells, ^{3}H-LSD dissociated slowly, with a half life (t l/2) of 20 minutes. Consistent with this slow off-rate, LSD caused an apparent loss of receptors in the usual ^{3}H-ketanserin binding assay, where the incubation time was 30 minutes. However, this receptor loss was not found when ^{3}H-ketanserin was incubated with the LSD-pretreated membranes for 60 minutes (Burris and Sanders-Bush 1992). Therefore, it is likely that the apparent blunting of the phosphoinositide hydrolysis response, as well as the blunting of serotonin-elicited contractions in smooth muscle (Kaumann 1989; Xu and Purdy 1989), are explained by the fact that LSD dissociates slowly from the 5-HT_2 receptor. With short incubation times, equilibrium is not reached.

These data suggest that LSD is not an allosteric agonist/antagonist, but rather that it binds tightly to the 5-HT_2 receptor and dissociates very slowly. This property of slow dissociation could be important. It would be interesting to compare the rates of dissociation of hallucinogenic versus nonhallucinogenic agonists from 5-HT_2 and 5-HT_{1C} receptors.

CONCLUSION

Hallucinogens of different chemical classes activate the 5-HT_{1C} receptor whereas nonhallucinogenic congeners interact with the receptor as antagonists. The numerous behavioral studies of hallucinogens that utilize 5-HT_2 receptor antagonists should be reevaluated in light of this finding, especially because currently available 5-HT_2 receptor antagonists also block 5-HT_{1C} receptors (Sanders-Bush and Breeding 1988). These neurochemical studies are consistent with a role for the 5-HT_{1C} receptor

in the psychoactive effects of hallucinogenic drugs, but they are far from conclusive. In the future new pharmacological tools that discriminate between 5-HT_2 and 5-HT_{1C} receptor subtypes may become available and can be used to explore this possibility in greater detail.

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AUTHOR

Elaine Sanders-Bush, Ph.D.
Professor of Pharmacology
Vanderbilt University School of Medicine
432 Medical Research Building
Nashville, TN 37232-6600

Autoradiographic Approaches to Studying Hallucinogens or Other Drugs

Nathan M. Appel

INTRODUCTION

The technique of autoradiographic localization of neurotransmitter and drug binding sites, often referred to as receptor autoradiography, has become a frequently used approach to investigate drug effects in the central nervous system (CNS). The technique enables the researcher to reveal the anatomic locus of actions of drugs and can be used to evaluate consequences of drug treatment. It is a rare issue of *Current Contents* in which the keyword "autoradiography" does not yield relevant citations.

This chapter provides brief descriptions of the theory and practice of in vitro and in vivo receptor autoradiography, provides examples of how this approach has been used to study hallucinogens and other drugs in experimental animals, describes how the technique of receptor autoradiography has been extended to study hallucinogens in a clinical setting using positron emission tomography (PET), and describes a novel autoradiographic approach for assessing fatty acid incorporation that is a promising research and clinical tool for studying the response to and effects of hallucinogens and other classes of drugs or stimuli.

There are many excellent review articles and books that address the theoretical basis, practical aspects, and limitations of the receptor autoradiographic technique. For the reader who is interested in a more comprehensive treatment of the subject, the chapters on receptor autoradiography by Kuhar are an excellent "first read" (Kuhar 1985; Kuhar and Unnerstall 1990).

RECEPTOR AUTORADIOGRAPHY

Receptor autoradiography is essentially a receptor binding assay. The assay is performed on an intact, instead of homogenized, tissue sample. Thus, the anatomic integrity of the tissue under investigation is preserved.

Binding of the radiotracer follows the law of mass action. Radiotracer binding is saturable and stereospecific and displays nanomolar affinities. For a particular radiotracer, the optimum incubation time (ligand association) and the optimum rinse times (separation of bound drug from free) are determined. Thus, binding kinetics can be determined, as can orders of potency of competing drugs, sensitivity to ions, nucleotides temperature, and other factors.

In practice, the differences between the techniques are manifested in how the tissue of interest is radiolabeled and how the quantity of bound radiolabel in tissue is determined. To perform in vitro receptor autoradiography, tissues are labeled by incubating slide-mounted tissue slices (microtome sections) instead of homogenized tissue with a radiolabeled drug. Nonspecific binding is detected by incubating tissues with the radiolabeled drug in the presence of excess (typically thousandfold) unlabeled ("cold") competing drugs. For in vivo receptor autoradiography, tissues are labeled by injecting test animals with a radiolabeled drug. By using in vivo receptor autoradiography, nonspecific binding can be determined in animals treated with excess unlabeled competing drug prior to injection of the radiotracer. An alternative method for illustrating specific binding using the in vivo approach is to express binding as the ratio of radioactivity in a region of interest and another area where few receptors are known to exist, for example, the ratio of spiperone-labeled dopamine (DA) receptors in caudate putamen and cerebellum (Kuhar 1985; figure 1).

With receptor autoradiography, the binding of radiotracer is quantified by determining the intensity of the image that results from exposing the radiolabeled tissue to a photosensitive medium. In contrast, for a homogenate receptor binding assay, the radioactivity accumulated on a filter or pelleted at the bottom of a test tube is counted. The photosensitive medium is typically photographic emulsion. It can be directly applied to the radiolabeled tissue, apposed to the radiolabeled tissue in the form of an emulsion-coated coverslip, or apposed to the radiolabeled tissue in the form of commercially available autoradiographic film.

An exciting new development is filmless autoradiography in which autoradiographic images are acquired by apposing radiolabeled specimens to screens coated with a storage phosphor. A latent image of the pattern of labeling forms within the storage phosphor and is visualized by scanning the screen with a laser that allows the image to be

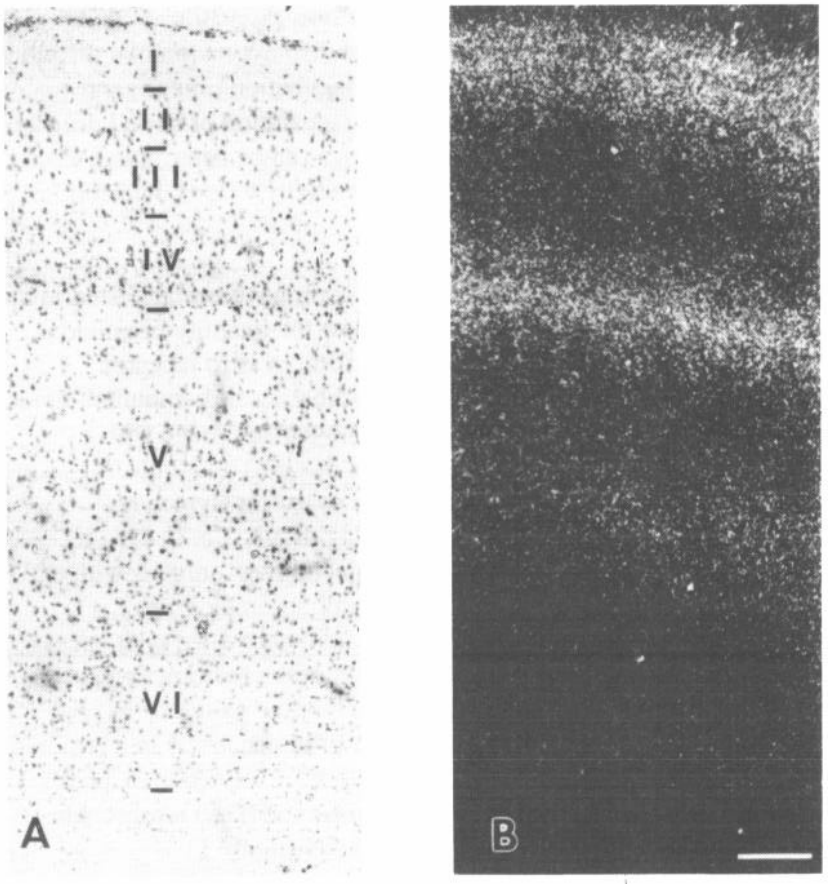

FIGURE 1. *Distribution of [^{125}I] DOI-labeled serotonin, (5-HT$_2$) receptors in cerebral cortex. A: Bright-field photomicrograph of a Nissl-stained horizontal section through parietal cortex. Lamination is annotated with Roman numerals and delineated by the short black horizontal bars. B: Dark-field photomicrograph of the "coverslip" autoradiograph, generated by the section shown in A (same magnification), depicting ([^{125}I]DOI) 1-(2,5-dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane) binding in cerebral cortex. Note that silver grains are most concentrated in laminae I and IV. Magnification bar (lower right-hand corner) = 200 μm.*

SOURCE: Appel et al. 1990*a*

detected electronically using a photomultiplier. The image then is stored on a computer for subsequent analysis (Appel et al. 1991; Kuhar et al. 1991). The image pattern (autoradiographic distribution) depicts the spatial distribution of binding sites in the tissue.

Regardless of the photographic medium used, the optical density or intensity of the image is related to the quantity of bound drug and, by extension, of binding sites in a particular region of interest. Thus, the principal advantage that receptor autoradiography provides over homogenate binding assays is increased anatomic resolution. For example, using a homogenate binding assay, it can be determined that binding of a certain drug is greater in the cerebral cortex than in the cerebellum. Using autoradiography, it can be said with certainty that binding is greater in cerebral cortex layer V than layer VI but less than in layers I or IV (e.g., figure 1). For an exact quantitative assessment of regional binding, regional optical densities can be measured by densitometry and compared with the optical density produced by calibrated radioactive standards coexposed with the experimental material. Several computer-assisted digital image analysis systems are available commercially to facilitate quantification of autoradiographs.

Another advantage of receptor autoradiography over homogenate binding assays is increased sensitivity. In a homogenate binding assay, the ability to detect binding is limited if the density of a particular binding site or the specific activity of a labeled drug is low. Using autoradiography, one can increase the duration the photosensitive medium is exposed to the radiolabeled tissue to overcome these impediments.

IN VITRO RECEPTOR AUTORADIOGRAPHY TO STUDY POTENTIAL SITES OF ACTION OF HALLUCINOGENS

The exact criteria that categorize a substance as a hallucinogen remain unresolved (Szára, this volume). However, three classes of compounds constitute the majority of what typically are referred to as hallucinogenic drugs (see chapters by Glennon; Pfaff et al.; and Jacob and Shulgin, this volume). They are indolealkylamines (e.g., dimethyltryptamine [DMT]), ergolines (e.g., lysergic acid diethylamide [LSD]), and phenylalkylamines (e.g., 2,5-dimethoxy-4-methylamphetamine [DOM]). They have in common a high affinity for serotonin$_2$ (5-HIT$_2$) receptors. Drug discrimination studies, other behavioral tests, and homogenate receptor binding assays have related hallucinogenic potency to binding affinity of

5-HT_2 receptor agonists (Glennon and Rosecrans 1982; Glennon et al. 1984; Heym and Jacobs 1987, 1988; Titeler et al. 1988). Moreover, a significant positive correlation has been demonstrated between 5-HT_2 binding affinities of drugs and their human hallucinogenic potencies (Glennon et al. 1984). In consideration of these data, drugs that label 5-HT^2 receptors would, by extension, label at least some sites in the brain where hallucinogenic effects of these drugs may be manifested.

In an early study in which binding sites for a hallucinogen were demonstrated, autoradiographic distribution of 5-HT binding sites was demonstrated using [^{3}H]LSD. However, unlabeled serotonin (5-HT) was unable to displace [^{3}H]LSD labeling in all areas (Meibach et al., 1980). The recognition of subtypes of 5-HT receptors, as distinguished by differential binding of [^{3}H]5-HT, [^{3}H]LSD, and [^{3}H]spiroperidol (spiperone), would point the way toward using more selective ligands to study the now apparent different classes of 5-HT receptors (Peroutka and Snyder 1979). Thus, anatomic distribution of 5-HT_2 receptors has been studied autoradiographically using radioligands such as [^{3}H]spiperone (Altar et al. 1985; De Souza 1986; Palacios et al. 1981; Pazos et al. 1985) and [^{3}H]ketanserin (Fishette et al. 1987; Pazos et al. 1985; Slater and Patel 1983). However, both these radioligands are antagonists: [^{3}H]spiperone potently labels DA type 2 (D_2) receptors (Niznik et al. 1985; Stefanini et al. 1980), and [^{3}H]ketanserin labels nonserotonergic sites in striatum (Leysen et al. 1987).

The autoradiographic distribution of 5-HT_2 receptors also has been studied using another radioiodinated ligand, [^{125}I]-LSD (Altar et al. 1986; De Souza 1986; Engel et al. 1984; Nakada et al. 1984). However, because [^{125}I]-LSD binds D_2 receptors with nanomolar affinity (Engel et al. 1984; Hartig et al. 1985*a*), one must perform [^{125}I]-LSD autoradiography for 5-HT_2 receptors in the presence of unlabeled D_2 ligands to ensure that only 5-HT_2 receptors are being visualized. There is another congener of LSD, *N*1-methyl-2-[^{125}I]-LSD, that is more selective for 5-HT_2 receptors than D_2 receptors. It also has been used to autoradiographically localize 5-HT_2 receptors (Hoffman et al. 1987).

The hallucinogenic potency of drugs appears to be related to their 5-HT_2 receptor binding affinities and their being agonists at this binding site (Glennon and Rosecrans 1982; Glennon et al. 1984; Heym and Jacobs 1988; Titeler et al. 1988). However, evidence has been presented suggesting that 5-HT_2 receptors exist in two states-one state having high affinity for agonists and one state having low affinity for agonists-and

that antagonists (such as ketanserin and spiperone) do not discriminate between these two states. Moreover, the density of the high-affinity state of 5-HT_2 receptors is only a small fraction of the total 5-HT_2 receptor binding sites (Lyon et al. 1987; Teitler et al. 1990). Thus, to examine autoradiographically the distribution of the high-affinity state of the receptor, and by extension a hallucinogen binding site, would require an agonist 5-HT_2 receptor radioligand with high specific activity so that it could be detected in spite of the low density of these sites. A drug satisfying these criteria is [^{125}I]DOI. It is a potent agonist at 5-HT_2 receptors and has high specific activity (~2200 curies per millimole [Ci/mmol]) (Glennon et al. 1988; Johnson et al. 1987; Teitler et al. 1990). These pharmacological and radiochemical properties make [^{125}I]DOI well suited for studying this state of 5-HT_2 receptors.

[^{125}I]DOI has proven to be an excellent ligand to autoradiographically label 5-HT_2 receptors (Appel et al. 1990*b*; McKenna and Saavedra 1987; McKenna et al. 1989). Patterns of autoradiographs of [^{125}I]DOI binding to 5-HT_2 receptors were similar to those seen when 5-HT_2 receptors were labeled with [^{3}H]ketanserin or [^{125}I-LSD (Appel et al. 1990*a*; McKenna et al. 1989). High densities of [^{125}I]DOI labeling were present in the olfactory bulb, anterior regions of cerebral cortex, claustrum, caudate putamen, globus pallidus, ventral pallidum, islands of Calleja, mammillary nuclei, and inferior olive. Binding in the hippocampus, thalamus, and hypothalamus generally was sparse (see figures 1 and 2). The choroid plexus, a site rich in 5-HT_2 receptors, had a high density of [^{125}I]DOI binding sites, but [^{3}H]ketanserin binding in this region was low (figure 2). [^{125}I]DOI binding on autoradiographs also displayed appropriate pharmacology for agonist-labeled 5-HT_2 receptors, in that it was inhibited by guanyl nucleotides but not adenosine triphosphate (Appel et al. 1990*a*; Battaglia et al. 1984; Lyon et al. 1987).

Such data allow speculation on possible circuits mediating hallucinogenic effects of drugs. For example, the serotonergic innervation of the brain is widespread and not uniform (Kosofsky and Molliver 1987; Steinbusch 1981). In particular, the cerebral cortex displays a distinctive, highly organized laminar pattern of innervation (for review, see Audet et al. 1989). Similarly, [^{125}I]DOI binding in the cerebral cortex has a laminar pattern. [^{125}I]DOI binding was especially enriched in layer IV in this study, with additional relative enrichment of binding sites also seen corresponding to layers I and V (see figure 1). On the other hand, [^{125}I]DOI binding in the claustrum was especially dense, but the serotonergic innervation of this brain region was not (Steinbusch 1981).

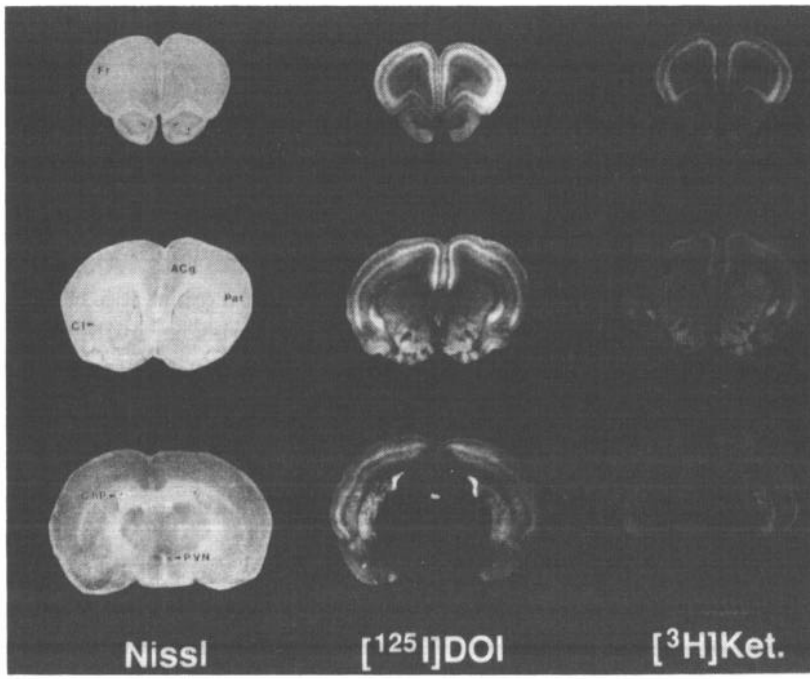

FIGURE 2. *Autoradiographs comparing the distribution of [^{125}I]DOI- and [^{3}H]ketanserin-labeled 5HT$_2$ receptors in coronal sections of rat brain. Left-hand column: Nissl-stained sections sampled adjacent to sections used for autoradiography and annotated to denote anatomical structures. Center and right-hand columns: Distribution of 5-HT$_2$ binding sites as revealed using [^{125}I]DOI and [^{3}H]ketanserin, respectively. These images were generated by using the autoradiographs (Ultrofilm ^{3}H) as photographic negatives; thus, lighter areas in individual autoradiographs correspond to brain regions displaying higher densities of binding. For [^{125}I]DOI autoradiographs, tissues were labeled with 200 pM [^{125}I]DOI. For [^{3}H]ketanserin autoradiographs, tissues were labeled with 2 nM [^{3}H]ketanserin.*

KEY: ACg = anterior cingulate cortex; ChP = choroid plexus; C1 = claustrum; Fr = frontal cortex; Par = parietal cortex; PVN = paraventricular nucleus.

SOURCE: Appel et al. 1990*a*

Similar enrichment of [^{3}H]ketanserin- and [^{125}I]-LSD-labeled 5-HT$_2$ receptors has been noted in the claustrum (Appel et al. 1990*a*; McKenna et al. 1989). McKenna and colleagues (1989) suggested that the claustrum may play an important, but as yet unrecognized, role in mediating the effects of hallucinogens via its extensive connections with the cerebral cortex and the limbic system (Pearson et al. 1982; Wilhite et al. 1986).

Relatively high densities of [^{125}I]DOI binding sites also have been noted in mammillary nuclei, which are richly innervated by serotonergic afferents (Steinbusch 1981). Similar to the claustrum, mammillary nuclei may play an important, but as yet unrecognized, role in mediating effects of hallucinogens via their extensive connections with the limbic system (Bleier and Byne 1985; MacLean 1985). Likewise, the ventral pallidum, which displays high levels of [^{125}I]DOI binding sites and is richly innervated by serotonergic afferents, may mediate effects of hallucinogenic 5-HT$_2$ agonists via its extensive connections with the limbic system and basal ganglia (Heimer et al. 1985; Steinbusch 1981).

The choroid plexus, a site rich in 5-HT$_2$ receptors, has a high density of [^{125}I]DOI binding sites (Appel et al. 1990*a*; McKenna et al. 1989). Competition studies on autoradiographs demonstrated that [^{125}I]DOI was binding at 5-HT$_2$ receptors (figure 3). Using [^{125}I]DOI autoradiography, the distribution of 5-HT^2 receptors now can be demonstrated in the brain outside of the choroid plexus (figure 4). In situ hybridization histochemistry studies have demonstrated that 5-HT$_2$ receptor messenger ribonucleic acid mRNA) is widespread in rat brain (Hoffman and Mezey 1989; Molineaux et al. 1989). Such observations take on added significance in the context of accumulating evidence that hallucinogenic drugs may be acting at 5-HT$_{1C}$ *receptors* (Burris et al.1991; Sanders-Bush, this volume; Sanders-Bush and Breeding 1991; Titeler et al. 1988).

IN VITRO RECEPTOR AUTORADIOGRAPHY TO STUDY EFFECTS OF HALLUCINOGENS ON STRUCTURAL INTEGRITY OF NEURONS

Receptor autoradiography need not be limited to studying neurotransmitter or drug binding sites. A candidate for receptor autoradiography essentially requires a saturable and specific binding at a biologically relevant site and the availability of a selective ligand with

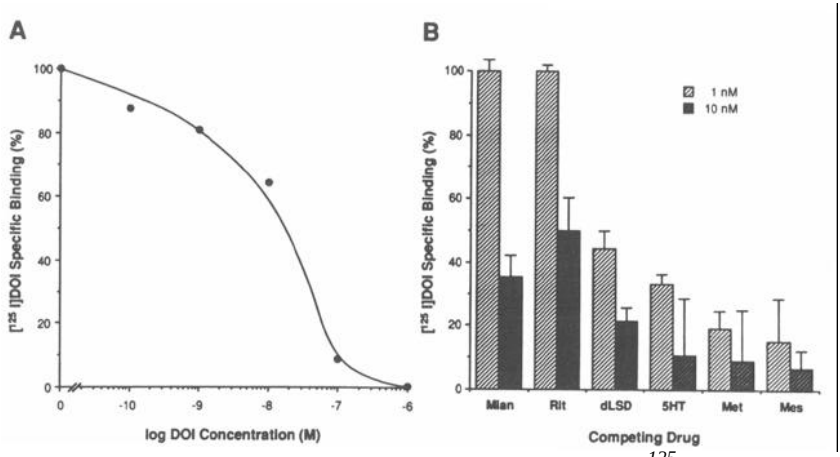

FIGURE 3. *Pharmacological characterization of [^{125}I]DOI binding to 5-HT$_{1C}$ receptors in rat choroid plexus. Slide-mounted sections (2/slide, 10 µm thick) of rat brain were labeled with 200 pM [^{125} I]DOI in the presence of spiperone (100 nM, to block [^{125}I]DOI binding to 5-HT$_2$ receptors) and varying concentrations of drugs. Nonspecific binding was determined in the presence of 1 µM mianserin. A: Displacement of [^{125}I]DOI binding to 5-HT$_{1C}$ receptors in rat choroid plexus by (±)DOI. Median effective concentration (IC$_{50}$) is approximately 12 nM. B: Potencies of various drugs that displace [^{125}I]DOI binding to 5-HT$_{1C}$ receptors in rat choroid plexus.*

SOURCE: Appel et al. 1990*a*

sufficient specific activity to facilitate its detection. To date, several cellular components in the brain have been studied using principles of receptor autoradiography. They include ion channels, neurotransmitter reuptake sites, second messenger molecules, metabolic enzymes, and hexose transporters, among others (Appel and De Souza, in press; Kuhar and De Souza 1989).

Detection of nonneurotransmitter binding sites by autoradiography can be used as an approach for studying the anatomic locus of structural or functional consequences of drug treatment. One approach is to assess changes in the density of presynaptic markers as an index of the neurotoxic potential of a drug. For example, in vitro assessment of

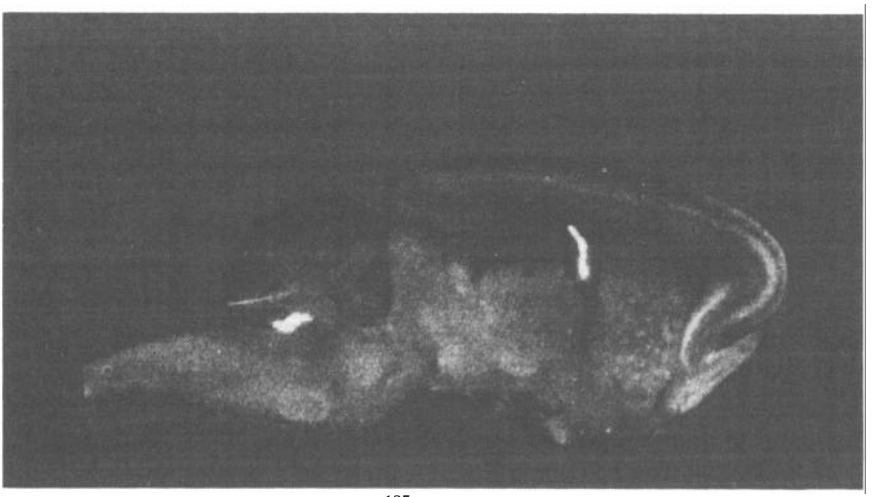

FIGURE 4. *Distribution of [^{125}I]DOI-labeled 5-HT$_{1C}$ receptors in a sagittal section of rat brain. The tissue was incubated in 200 pM [^{125}I]DOI in the presence of 100 nM spiperone to block [^{125}I]DOI binding to 5-HT$_2$ receptors. Under this condition the fractional occupancy of [^{125}I]DOI at 5-HT^2 receptors is <0.1 percent. This image was generated by using the autoradiograph (Ultrofilm ^{3}H) as a photographic negative; thus, lighter areas correspond to brain regions displaying higher densities of binding.*

monoamine uptake sites can be used as an index of brain monoamine neuron structural integrity following a drug treatment. The validity of this approach has been established (D'Amato et al. 1987; De Souza and Kuyatt 1987; Javitch et al. 1985). In vitro autoradiography of 5-HT uptake sites as labeled by [^{3}H]paroxetine can serve as an index of structural integrity of brain serotonergic axons following fenfluramine treatment. Serotonin uptake sites are highly concentrated on 5-HT-containing nerve terminals (Kuhar and Aghajanian 1973); thus, a decrease in the density of [^{3}H]paroxetine binding would reflect loss of 5-HT axons following drug treatment. By extension, the same approach can be used to examine drug effects on catecholamine axons by looking for alterations in density and distribution of catecholamine uptake sites as labeled by [^{3}H]mazindol.

The ring-substituted phelylalkylamines 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymeth-amphetamine (MDMA) have received much notoriety recently because of their use as recreational

drugs. (For a comprehensive review, see Asghar and De Souza 1989.) Although these compounds differ in structure by only a single methyl group, evidence suggests that, whereas MDA is hallucinogenic, MDMA is not (Glennon 1989; Nichols and Oberlender 1989). Evidence has been presented that MDA and MDMA are neurotoxic and cause diffuse and prolonged decreases in brain 5-HT markers (Battaglia et al. 1987; Commins et al. 1987; Insel et al. 1989; Ricuarte et al. 1985).

Effects of these compounds on serotonergic neurons have been assessed using immunohistochemistry (O'Hearn et al. 1988). Widespread loss of 5-HT-like immunoreactivity was observed in the cerebral cortex, hippocampus, caudate putamen, hypothalamus, and other forebrain areas. Decreases in immunostaining appeared to be more profound following MDA treatment than following MDMA treatment. In addition, some remaining 5-HT-like immunoreactive axons displayed morphology reminiscent of that seen following treatment with the 5-HT neurotoxin 5,7-dihydroxytryptamine. Such axons appeared swollen, fragmented, and intensely stained. They suggested also that, in particular, axons derived from the dorsal raphe nucleus, which are morphologically distinct from axons derived from the median raphe nucleus (Kosofsky and Molliver 1987), were selectively vulnerable to these drugs. Serotonin-like immunoreactive cell bodies did not appear to be affected. When treated tissues were immunostained for tyrosine hydroxylase, a marker of monoaminergic neurons, immunostaining appeared unaffected by treatment with either drug. O'Hearn and colleagues (1988) thus concluded that MDA, a purported hallucinogen, and MDMA were exerting neurotoxic effects on dorsal raphe-derived serotonergic axons.

Although these immunohistochemical data strongly supported the conclusion that MDA and MDMA were neurotoxic, a shortfall of interpreting immunohistochemical data is that detection techniques depend on the presence of the antigen (in this case, 5-HT). Thus, if drug treatment causes depletion of the antigen of interest, lack of or decreased immunostaining could mistakenly be interpreted as an actual loss of axons. Furthermore, immunohistochemistry is limited from the perspective that related changes in staining are difficult to assess quantitatively.

To address this question with respect to MDA and MDMA, [^{3}H]paroxetine autoradiography was used to assess the potential neurotoxic effects of these drugs by examining changes in a presynaptic marker, 5-HT uptake sites (Battaglia et al. 1991; De Souza and Kuyatt

1987). Both the hallucinogen MDA and MDMA caused widespread decreases in regional [^{3}H]paroxetine binding that correlated with loss of 5-HT-like immunoreactivity noted by O'Hearn and coworkers (1988).

Furthermore, regional decreases in [^{3}H]paroxetine binding in MDMA-treated rats were greater 2 weeks after, rather than immediately after, cessation of drug treatment. This suggested that there was a continuing degenerative process occurring during this time interval (Battaglia et al. 1991).

To confirm the neurochemical specificity of this effect, Battaglia and colleagues (199 1) performed parallel experiments using [^{3}H]mazindol to assess the structural integrity of catecholaminergic nerve terminals. [^{3}H]Mazindol is a marker for DA and norepinephrine (NE) uptake sites (Javitch et al. 1985). [^{3}H]Mazindol binding was unaffected by either MDA or MDMA treatment, confirming the specificity of the neurotoxic effect of these drugs to 5-HT monoaminergic neurons.

Such autoradiographic assays also have been used to investigate the neurotoxic potential of other drugs structurally derived from phenylalkylamines. Regional decreases in 5-HT-uptake site density have been noted following treatment with the CNS stimulant methamphetamine using [^{3}H]cyanoimipramine binding as a marker (Kovachich et al. 1989). Fenfluramine is a substituted phenylalkylamine derivative structurally similar to MDA and MDMA. Unlike methamphetamine, MDA, or MDMA, it is devoid of undesirable psychostimulant effects. Racemic fenfluramine and its dextrorotary (D) isomer are used therapeutically as anorectics to treat obesity (McTavish and Heel 1992; Rowland and Carlton 1986). Short-term administration of racemic fenfluramine at high doses results in a pattern of 5-HT immunoreactivity similar to that seen with MDA, suggesting neurotoxic effects of this drug on 5-HT axons using this treatment protocol (Appel et al. 1989; Molliver and Molliver 1990).

This hypothesis was supported by autoradiographic studies using [^{3}H]paroxetine and [^{3}H]mazindol as markers for 5-HT and catecholamine uptake sites, respectively (Appel et al. 1990*b*). Decreased paroxetine binding parallels decreases in 5-HT-like immunostained axons following an identical fenfluramine treatment protocol. Catechol-aminergic axons as assessed by [^{3}H]mazindol autoradiography were unaffected. These data suggest that as a class, phenylalkylamine derivatives may have increased potential for neurotoxic side effects regardless of their being

hallucinogenic and may reinforce the utility of autoradiographic approaches as a tool for studying such effects of drug treatment.

PRECLINICAL AND CLINICAL APPLICATIONS OF IN VIVO RECEPTOR AUTORADIOGRAPHY IN THE STUDY OF HALLUCINOGENS

As discussed earlier, receptor autoradiographic technique lends itself to being applied in vivo. The obvious advantage is that assessment can be made in situ in a living subject. Furthermore, using tracer drugs labeled with positron-emitting isotopes such as ^{11}C, ^{18}F, ^{15}O, and ^{13}N, dynamic mapping studies can be accomplished in animal or, potentially, human subjects using PET. In PET, the spatial distribution of positron-emitting isotopes in the brain or any other body region is detected using a circular array of detectors that surrounds the subject. Precise spatial localization is realized by accepting as the signal pairs of simultaneously emitted 511-kiloelectron volt (keV) gamma rays that are detected 180" apart from each other. The gamma rays are the result of positron annihilation that occurs when a positron interacts with a negatively charged electron to form a short-lived positronium that is subsequently converted into electromagnetic radiation in the form of two photons (gamma rays) (Frost 1990; Ter-Pogossian 1985). With high probability, the gamma rays represent the product of a single disintegrating positron. An obvious advantage of PET over postmortem (in vitro) receptor autoradiography is that it permits measurements several times from the same subject. Thus, dynamic studies during, as well as longitudinal studies following, hallucinogen administration are possible.

Several preconditions limit whether a drug is useful as an in vivo marker. As a minimum, the tracer drug cannot be toxic to the host. Other basic requirements summarized by Kung (1990) are that the radiotracer:

- Be labeled with a short-lived isotope and be quickly prepared (minutes);
- Possess high specific activity;
- Be able to penetrate the intact blood-brain barrier following intravenous (IV) injection;
- Be relatively resistant to in vivo metabolism;

- Have a high affinity for the binding site of interest (nM K_d);
- Possess a high target-to-nontarget ratio after IV injection; and
- Can be modeled to quantitate receptor density based on kinetic information obtained by imaging.

For the most part, in vivo studies performed with radiotracers in experimental animals and having the potential for improving understanding of hallucinogens have been focused not so much toward imaging binding sites in that setting as toward determining whether a particular tracer is of potential clinical use. For this purpose, regional binding under different experimental conditions is quantified by solubilizing dissected regions of the brain and counting bound radioactivity in a scintillation counter. As discussed earlier, ligands that label 5-HT_2 binding sites would theoretically label these binding sites in living brain and, by extension, identify initial sites of action of hallucinogenic drugs. Drugs tested for this use include derivatives of spiperone (spiroperidol) and ketanserin (Frost 1990; Kuhar et al. 1986). However, these drugs are 5-HT_2 receptor antagonists and do not label 5-HT_2 receptors exclusively (Leysen et al. 1987; Niznik et al. 1985; Stefanini et al. 1980). A tritiated naphthosultam derivative, RP 62203, also is being used as an in vivo probe for 5-HT_2 receptors. This drug is also a 5-HT_2 receptor antagonist but is reported to have greater 5-HT_2 receptor selectivity than spiperone or ketanserin (Fajolles et al. 1992).

Derivatives of LSD also have been used as in vivo probes for 5-HT_2 receptors. They include [^{125}I]-LSD (Hartig et al. 1985*b*), *N*1-methyl-2-[^{125}I]-LSD (Hoffman et al. 1987), and D-(+)-(N1-[^{11}C]methyl)-2-Br-LSD ([^{11}C]MBL) (Lever at al. 1989). [^{11}C]MBL has been used to image 5-HT_2 receptors in living baboon and human brains by PET (Wang et al. 1987). In the baboon brain, specific binding of [^{11}C]MBL to 5-HT_2 receptors in vivo was demonstrated by comparing binding ratios between the frontal cortex and cerebellum in the presence and absence of ketanserin. In a human subject, widespread uniform labeling of brain structures not associated with 5-HT_2 receptors, such as the cerebellum, was seen soon after administration of [^{11}C]MBL. However, this most likely reflected blood flow delivery of the tracer because persistent labeling was seen subsequently in the frontal cortex and temporal poles, sites endowed with 5-HT_2 receptors. No pharmacological effects of drug administration were noted in any subjects receiving [^{11}C]MBL. However, MBL may be an antagonist at 5-HT_2

receptors (Ginzel and Mayer-Gross 1956). As discussed earlier, theoretical arguments support using 5-HT_2 agonists as probes for sites of action of hallucinogens rather than anatagonists (Lyon et al. 1987; Teitler et al. 1990). One such compound under investigation is 1-[2',5'-di-methoxy-4'- (ß-fluoroethyl)phenyl]-2-aminopropane, a phenylalkylamine (Gerdes et al. 1988).

As discussed earlier, presynaptic markers are useful probes to assess structural integrity of neurons. By using this approach, the regional distribution of neurotoxic effects of the hallucinogenic phenylalkylamine MDA and its nonhallucinogenic chemical congener fenfluramine have been reported using radiotracers selective for serotonin uptake sites in vitro (Appel et al. 1990*b*; Battaglia et al. 1991). Radiotracers with in vivo selectivity for serotonin uptake sites also have been reported, including radiolabeled derivatives of paroxetine, quipazine, imipramine, chlorimipramine, cyanoimipramine, citalopram, fluoxetine, and sertralme. However, all these compounds have limitations that compromise their usefulness as radiotracers in a clinical setting (Scheffel et al. 1992).

Recently, radiolabeled congeners of another class of compound have appeared that label cocaine binding sites. For example, RTI-55 is a parasubstituted cocaine analog derived from WIN 35,065-2 that labels DA and serotonin uptake sites (Boja et al., 1992; Madras et al. 1989; Scheffel et al. 1992). In vivo studies in mice using [^{125}I]RTI-55 have yielded satisfactory autoradiographs demonstrating regional binding patterns that reflect the distribution of serotonin and DA uptake sites (Cline et al. 1992). By labeling 5-HT uptake sites in vivo with [^{125}I]RTI-55, investigators have been able to detect regional changes in densities of 5-HT uptake sites following fenfluramine treatment in rats (Scheffel et al. 1992). Fenfluramine effects on 5-HT uptake sites are similar to those seen following MDA treatment (Appel et al. 1990*b* Battaglia et al. 1991). These data suggest that RTI-55 might be useful in a clinical setting to assess potential long-lasting adverse effects on the CNS in individuals who may have been abusers of phenylalkylamine hallucinogens such as MDA.

AUTORADIOGRAPHIC ASSESSMENT OF IN VIVO FATTY ACID INCORPORATION TO STUDY HALLUCINOGENS OR OTHER DRUGS

In addition to its use in receptor or binding site mapping applications, the in vivo autoradiographic approach has been used to study brain metabolism. The best-characterized and most well-known example of this approach is metabolic mapping by assessing incorporation of [^{14}C]deoxyglucose (2-deoxy-D- [1-^{14}C]glucose). 2-Deoxyglucose is transported bidirectionally from blood to brain by the same hexose transport protein as glucose. Glucose is the primary metabolic substrate of the brain in the fed state. The functional activity of the brain in vivo has been correlated with regional cerebral glucose utilization and with regional cerebral blood flow. By assessing incorporation of [^{14}C]deoxyglucose into the brain, regional glucose consumption and, by extension, regional cerebral metabolic activity can be estimated (for a review, see Sokoloff 1985). Using this approach, Freo and colleagues (1991) observed decreased incorporation of [^{14}C]deoxyglucose into rat hippocampus and prosencephalic regions (cingulate; prefrontal; precentral medial; and motor, auditory, and visual cortices) following high (25 milligrams per kilogram [mg/kg]) intraperitoneal (IP) doses of DOI. Another in vivo autoradiographic metabolic mapping approach under development is assessment of local cerebral [^{14}C]leucine incorporation as an index of regional brain protein synthesis (Smith et al. 1984; Sokoloff 1985). However, this model may underestimate actual protein synthesis (Smith et al. 1988).

The Laboratory of Neurosciences of the National Institute on Aging has developed in vivo autoradiographic methods to quantify local cerebral rates of incorporation of radiolabeled long-chain fatty acids into brain phospholipids (DeGeorge et al. 1989; Rimes et al. 1983; Noronha et al. 1990). A partial model underlying this approach has been described (Robinson et al. 1992). The technique is based on infusing radiolabeled fatty acids, such as [^{3}H]palmitic, [^{14}C]arachidonic, or [^{14}C]docosa-hexaenoic acids, into conscious rats. Regional accumulated radioactivity in the brain and integrated plasma fatty acid radioactivity (integrated over the time course of the experiment) are determined. Currently, data are expressed as normalized regional activities (k) having units of milliliters per second times grams (ml/sec x g). However, exact quantification of ‘fatty acid incorporation will not be possible until the kinetics of protein binding of fatty acids and the contributions of different precursor pools

and metabolic pathways have been determined (Robinson et al. 1992). Nevertheless, the technique has proven useful in that regional brain changes in fatty acid incorporation can be correlated with alterations in regional structural integrity and functional activation.

When infused in vivo, [^{3}H]palmitic acid is incorporated into brain membrane phospholipids, with the majority incorporated into phosphatidylcholine. This allows its use as a marker for synthesis and turnover of this phospholipid and, by extension, as a marker for neuronal structural integrity (Gnaedinger et al. 1988; Noronha et al. 1990). Increased [^{14}C]palmitate or [^{3}H]palmitate incorporation has been demonstrated in regions where cell division or proliferation is taking place, such as in active tumors or in areas of glial reaction and phagocytic invasion of the brain having extensive cell death secondary to carotid occlusion (Nariai et al. 1991*a*; Tone et al. 1987). In contrast, regional brain [^{3}H]palmitate incorporation is not altered in response to chemical stimulation of neurotransmitter receptors (DeGeorge et al. 199 1; Freed et al. 1991).

When infused in vivo, [^{14}C]arachidonic acid is incorporated into brain phospholipids primarily as phosphatidylinositol and phosphatidylcholine. Turnover of arachidonate in brain inositol phosphoglycerides, which is catalyzed by phospholipase C, is a metabolic pathway used for signal transduction by several neurotransmitters, including alpha, adrenergic receptors, cholinergic muscarinic receptors, excitatory amino acid receptors, H_1 histaminergic receptors, and 5-HT_2 and 54-HT_{1C} serotonin receptors (Fisher and Agranoff 1987; Sanders-Bush et al. 1990). Thus, incorporation of in vivo administered [^{14}C]arachidonic acid could act as an index of regional functional activation in response to neurotransmitter, drug, or other stimulation whose transduction is coupled to phosphatidylinositol. [14C]Arachidonic acid incorporation is increased in response to muscarinic cholinergic receptor activation with arecoline. In contrast, [^{3}H]palmitate incorporation is unaffected (DeGeorge et al. 1991; Nariai et al. 1991*b*). Similarly, visual stimulation to unilateral enucleated rats resulted in increased [14C]arachidonic acid incorporation in brain structures afferent to the intact orbit when compared with incorporation in the brain structures afferent to the enucleated orbit. No differences between sides were noted for [^{3}H]palmitate incorporation (Wakabayashi et al. 1992). These data support the validity of using fatty acid incorporation as indices of structural integrity and functional activation of neurons.

The fatty acid incorporation technique also has been applied to studying effects on rats treated with hallucinogens. DOI (10 mg/kg IP) resulted in increased [^{14}C]arachidonic acid incorporation in frontal cortex layer IV, claustrum, medial cortex, and motor cortex. In contrast, [^{3}H]palmitic acid incorporation was unaffected in these brain regions assayed (Freed et al. 1991). These brain regions have high densities of 5-HT_2 serotonin receptors (Fishette et al. 1987; Pazos et al. 1985; Slater and Patel 1983) and are strongly labeled by [^{125}I]DOI, which binds both 5-HT_2 and 5-HT_{1C}, serotonin receptors (Appel et al. 1990*a*; McKenna et al. 1989). Activation of 5-HT_2 and 5-HT_{1C} serotonin receptors results in phosphatidylinositol turnover (Sanders-Bush et al. 1990).

Evidence has been presented that hallucinogens are likely acting at 5-HT_2 (Glennon and Rosecrans 1982; Glennon et al. 1984; Heym and Jacobs 1987, 1988; Titeler et al. 1988) and possibly 5-HT_{1C} serotonin receptors (Burris et al. 1991; Sanders-Bush, this volume; Sanders-Bush and Breeding 1991; Titeler et al. 1988). The Freed and colleagues (1991) data demonstrate brain regions that appear to be specifically activated in response to administration of a hallucinogen. Moreover, these data offer further evidence that fatty acid incorporation can be used to study receptors linked to brain lipid metabolism. This approach should be a powerful tool for studying functional aspects of hallucinogen administration.

The fatty acid technique also lends itself to a clinical setting. Fatty acids have been labeled with ^{11}C and detected using PET (Channing et al. 1991). Thus, in the future, it may be possible to study dynamic functional activation of human brain in response to hallucinogen administration to provide a better understanding of how these drugs produce their psychotropic effects.

SUMMARY

Autoradiography provides a powerful tool whereby an investigator can study different aspects of hallucinogens in the laboratory and the clinic. Receptor autoradiography can be performed in vitro to map binding sites of hallucinogens or to assess potential neurotoxic sequelae of hallucinogen treatments. Similarly, such studies can be performed in vivo to the same end. Receptor autoradiography can be performed in a clinical setting using PET to study acute dynamic binding properties of hallucinogens in humans or for long-term followup studies. In vivo

autoradiography of metabolic markers appears useful in the laboratory and potentially in the clinic to help researchers understand not only where, but also the manner in which, the brain responds functionally to hallucinogens.

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AUTHOR

Nathan M. Appel, Ph.D.
Biologist
Division of Research and Testing
Office of Research Resources
Center for Drug Evaluation and Research
Food and Drug Administration
8301 Muirkirk Road
Laurel, MD 20708

Hallucinogens Acting at 5-HT Receptors: Toward a Mechanistic Understanding at Atomic Resolution

Harel Weinstein, Daqun Zhang, and Juan A. Ballesteros

INTRODUCTION

The cumulative scientific evidence, including new data discussed at the National Institute on Drug Abuse (NIDA) technical review meeting and presented elsewhere in this chapter, suggests that entire classes of compounds which elicit the complex array of psychotomimetic responses classifying them as hallucinogens shark specific actions on 5-hydroxy-tryptamine (5-HT) receptors and bind to 5-HT binding sites (for some additional recent reviews see Glennon 1990; Glennon et al. 1991; Peroutka 1991; Weinstein and Osman '1990*a* ; Weinstein et al. 1987). The correlation between affinity for 5-HT_2-type binding sites and receptors and the doses required to elicit hallucinogenic responses in humans has been demonstrated repeatedly (Glennon 1984, 1990). Of special interest in this context are similarities and differences in the pharmacological profiles of compounds belonging to the various families of chemical structures that yield hallucinogenic agents, that is, tryptamine (TRYP) derivatives (including the hallucinogenic N,N,-dimethyltryptamine), ergolines (including d-lysergic acid diethylamide [d-LSD]), and derivatives of phenylethylamine and phenylisopropylamine (including mescaline, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane [DOM], and methylenedioxymethamphetamine [MDMA]). These families of compounds are active both on the 5-HT_{1A} and 5-HT_2 receptor systems (Weinstein and Osman 1990*a*), but hallucinogenic action has been connected with partial agonism on 5-HT_2 receptors (Sanders-Bush et al. 1988) and more recently on 5-HT_{1C} receptors (Burris et al. 1991).

If one can understand what in the structures of the ligands is most directly involved in receptor activation and what receptor activation involves in terms of specific changes in molecular structure resulting from ligand binding, then one can design drugs to target such receptors and activate or inactivate them, as required. The actions of any other receptor ligands, the hallucinogens among #em, then could be understood and

countermanded by such specifically targeted and designed drugs. In addition to novel understanding of cellular signaling processes at the molecular level, the practical significance of this insight is therefore inherent to the potential it offers for targeted molecular design. Such designed compounds should provide new tools for investigation of the mechanisms of actions of hallucinogenic compounds and a promise of new therapeutic and preventive modalities.

The design of effective therapeutic agents with predetermined actions at specific receptors requires solid information on the pharmacology of the target compounds. A detailed understanding of the processes of drug-receptor interaction and the mechanisms involved in the evolution of pharmacological responses elicited by ligand-receptor interactions is also required (Jensen et al. 1990). All of these requirements are essential elements in rational design (Weinstein and Osman 1989*a*, 1989*b*, 1990*b*; Weinstein et al. 1987) and are subjects of the authors' studies (Weinstein and Osman 1990*a*; Weinstein et al. 1988). The resulting insights are summarized elsewhere; recent developments are reviewed briefly in this chapter. These insights and developments constitute the first steps in a novel understanding of the relationships between ligand structure, receptor structure, and receptor response.

This novel understanding is achieved at an atomic level of resolution from the analysis, modeling, and simulation of molecular mechanisms of drug-receptor interaction. Such computational simulations have become possible because of new information available on the molecular and structural biology of 5-HT receptors (Adham et al. 1992; Demchyshyn et al. 1992; Guan et al. 1992; Hen 1992; Julius et al. 1988, 1989; Shih et al. 1991) combined with insights from the molecular pharmacology of hallucinogens and other 5-HT receptor ligands (Mylecharane et al. 1989; Peroutka 1991).

An initial focus of the authors' work in the molecular and structural biology of the 5-HT receptors has been the computational modeling of the transmembrane helix bundle of the serotonin receptors of the 5-HT_2 subtype. This chapter describes success in the first steps of constructing macromolecular models of the membranal portions of the receptors because of a redefinition and refinement of structural criteria and functional considerations. These models are used to simulate computationally the effects of some receptor ligands binding inside the structure of the model receptor proteins under various conditions in order to explore the mechanisms of receptor activation.

The first aim of these computational simulations is to investigate the structural correlates of properties that affect the receptor proteins function. The current literature contains ample examples for simulations of this kind, usually performed on cytosolic proteins and water-soluble systems and leading to targeted experimental probing (e.g., through mutation studies) (Aqvist and Warshel 1992; Karplus and Petsko 1990; Levitt and Sharon 1988; Pascual-Ahuir et al. 1991; van Gunsteren et al. 1992; Weinstein 1986; Weinstein and Mehler 1992; Weinstein and Osman 1989*b*, 1990*b*).

The computational exploration of membrane protein systems is more complex because of methodological difficulties and uncertainties related to the representation of the protein in the membrane environment and the nature of the embedding of the entire system in a surrounding aqueous solvent (Ahlstrom et al. 1989; Milik et al. 1992; Stigter et al. 1992; Wendoloski et al. 1989; Xing and Scott 1992). Nevertheless, cautious computational studies performed on the transmembrane bundle components of the receptor structures, combined with the experimental probing, can be used as described below to define molecular correlates of pharmacological efficacy. In the context of computational simulations of receptor function, these insights can be evaluated further with known ligands exhibiting a range of efficacies at the 5-HT receptor subtypes.

Difficulties in the accurate description of the biophysical mechanisms further increase the importance of experimental validation of the inferences emerging from the computational simulations. Such validation depends on a concomitant probing of all the resulting pharmacological models and predictions in collaborative efforts, including experimental determination of the pharmacological profiles of compounds acting at the 5-HT receptor subtypes. As illustrated by examples from recent publications, the pharmacological assays used in the authors' collaborative studies have included measurements of responses mediated by the 5-HT_1A, receptor coupledto adenylate cyclase in membrane preparations from rat hippocampus (DeVivo and Maayani 1990), the contraction of the isolated rabbit aorta mediated by 5-HT_2 receptors (Ben-Harari et al. 1991; Christ et al. 1990; Mahle et al. 1990), and the response to 5-HT_1C receptors coupled to the hydrolysis of phosphatidyl inositol in selected areas of the brain (Burris et al. 1991). In addition, the collaborative experimental studies probe the modulation of agonist binding in various brain sections by receptor-guanosine triphosphate binding protein (G-protein) interactions to learn about the relations between receptor selectivity in vitro and the likelihood that the agonists

will elicit responses on multiple receptors in the brain (Yocca et al. 1990). The results are analyzed for clues on the chemical determinants required for recognition (binding) and activation (efficacy) for each of the two receptors.

The combination of computational simulation studies and experimental verifications is expected to yield a quantitative relationship between the molecular structures of such ligands and their efficacy in activating the receptor. Such a mechanistic understanding of structure-function relationships will lead to new molecular structures that will be selective for 5-HT receptor subtypes and have a predicted degree of activity on these receptors. The following sections provide a brief overview of recent results from such studies.

CONSTRUCTION AND EXPLORATION OF MOLECULAR MODELS FOR THE TRANSMEMBRANE PORTION OF 5-HT RECEPTORS

Identification of an Appropriate Template

The construction of molecular models for the transmembrane region of the 5-HT receptor subtypes by homology modeling and energy refinement requires specific structural templates. The molecular architecture of bacteriorhodopsin (BR) and the photosynthetic reaction centers (PRCs) is commonly regarded as a model for membrane proteins (Rees et al. 1989). In particular, BR has been offered as a structural template for the three-dimensional (3-D) structure of membrane receptors that are functionally coupled to regulatory guanine nucleotide-binding proteins (G-proteins) (Findlay and Eliopoulos 1990).

More recently, specific molecular models of such G-protein coupled receptors (GPCRs) were constructed on the basis of the functional and structural relation of rhodopsin to BR and the sequence homology between rhodopsin and the GPCR (Dahl et al. 1991; Hibert et al. 1991). Such models of GPCRs suffer from the apparent lack of any significant degree of sequence homology between the seven transmembranal helices (TMHs) of BR and the portions in the sequence of the various GPCRs that are considered their transmembrane domain (TMDs). However, strong arguments have been proposed in favor of the structural relation between BR and the opsins and, hence: the GPCR (Lefkowitz 1991).

These arguments prompted the authors' investigation of the possibility that the true sequence homology, including any existing similarity in the distribution of kink-inducing proline (Pro) among the helices, might have been obscured by the assumption that the TMHs maintained their sequential order from BR in the evolution of the mammalian proteins. With a definition of the TMHs in the neurotransmitter GPCRs guided by hydropathicity predictions and additional criteria used to define the span of each helix, an optimal alignment is achieved for each pair of sequences calculated with no gaps allowed in the matching (Pardo et al. 1992). This proposed alignment reveals considerable homology between the TMHs in BR and those in GPCRs, if the sequential order of the helices is ignored. The findings point to the possibilities of modifying the BR template to account for the correct packing of the helices in the tertiary structures of GPCRs.

This strategy was followed in the construction of a 3-D model of the neurotransmitter GPCR of 5-HT_2 on the basis of specific interhelical interactions such as those observed in BR and in PRC as well as on additional criteria. The main advantage of the resulting modified template for the modeling of GPCRs based on the nonsequential homology to BR is that it is possible to incorporate several additional criteria in the construction of the helix bundle at the level of protein sequence comparisons as well as at the level of helix-helix and helix-membrane interactions. Such considerations (described in the next section) should improve the quality of 3-D models of GPCRs and facilitate inferences on the mechanisms by which these proteins perform their biological functions.

ANALYSIS AND REFINEMENT OF THE CRITERIA FOR PREDICTING THE STRUCTURE AND RELATIVE ORIENTATION OF TRANSMEMBRANE HELICES IN MODELS OF THE NEUROTRANSMITTER RECEPTOR

The membrane-spanning domain of the serotonin GPCRs is considered to share at least the general topology of a seven-transmembrane helix bundle observed in BR. As a result of the significant homologies that were found (as described above, by allowing for a rearrangement of the helix-bundle template), the strategy for building the model for the 5-HT_2 receptor has been to construct hypotheses concerning helix-helix interactions, their orientations, and their arrangement in bundles surrounded by lipid based on inferences and observations from the

3.5 angstrom (Å) resolution structure of BR (Henderson et al. 1990). Criteria resulting from such considerations are being tested against the 2.3 Å resolution Structure of the PRC from *Rhodobacter viridis* (Deisenhofer and Michael 1989).

These comparisons led to a reevaluation of some of the methods available for the prediction and relative orientations of membrane-embedded α-helices (Ballesteros and Weinstein 1992*a*). The authors found that methods used in the construction of helical transmembrane domains could be misleading; e.g., by assuming that the arginine (Arg) and lysine (Lys) residues that appear preferentially at the cytoplasmic end of the transmembrane helices of BR and PRC must be inside the cell because of their polar nature. More careful structural considerations would place only the ends of these residues close to the phospholipid headgroups of the membrane, with the rest of the residues inside the lipid portion. Thus, the intramembrane helix "gains" 1 to 1.5 turns, and the span is redefined by 3 to 7 residues (Ballesteros and Weinstein 1992*a*). Because the orientation of the p-carbon atom positions the Arg-Lys side chains in a direction pointing from the C-terminus toward the N-terminus of the helix, the inaccuracy in determining the helix span caused by the Arg-Lys residues affects the helices traversing the membrane from inside to outside (i.e., N-terminal to C-terminal) more than it affects those oriented inward.

Consequently, the inaccuracy can lead to significant errors in packing the helices against each other in the transmembrane bundle and thus induce errors in the construction of models for these structures. Therefore, these considerations will have a significant effect on the results of methods commonly used in constructing such models, including the criteria based on hydrophobicity profiles, hydrophobic moments, helix amphiphilicity, and charge neutralization.

Another consideration centers on the structural role of the kinks induced by the presence of Pro residues in the a-helix. (For some recent reviews of these structural properties of Pro-containing helices and their possible consequences for protein function, see MacArthur and Thornton 1991 and Williams and Deber 1991.) Because the region of the Pro-induced kink does not have a 3.6 amino acids/turn periodicity, one would expect a twisting of the faces before/after the Pro-kink compared with the case of a straight helix. Such an effect would vitiate the profiles for amphiphilicity and hydrophobic moments of Pro-containing helices because the

algorithms for the calculation of these properties assume that the helices are regular and straight.

Yet, such profiles are commonly used for the prediction/modeling of transmembrane helices without special attention to the effects of Pro-induced kinks. Consequently, the modeling approaches will require modification according to the authors' findings. The proposed refinements from the considerations presented above overcome some of the shortcomings inherent in the use of the above-mentioned methods; they constitute an additional guide for identifying the cytoplasmic end of a transmembrane helix. Importantly, such criteria can be explored quantitatively from computational simulations after a specific mechanistic model has been proposed.

The authors have recently described such a model for a dynamic activation mechanism in the function of a transmembrane protein based on the structural properties induced by the presence of Pro residues in key transmembrane helices (Ballesteros and Weinstein 1992*a*,*b*). The model takes advantage of the following properties of the kink region: A Pro residue within a helix disrupts two 1-4 backbone H-bonds: the $C{=}O_{i\text{-}4}...CN_i$ due to the imide functional group replacing the amine and the $C{=}O_{i\text{-}3}....HN_{i+1}$ due to the axial kink. This can generate up to three putatively H-bond-free reactive sites around the kink region, the C=Os being unusually exposed (Woolfson and Williams 1990). Most charged and polar side chains in transmembrane domains are likely to be involved in extensive H-bonding because of the low dielectric constant of the transmembrane domain. Consequently, the three types of reactive sites in the backbone should be expected to make up a significant percentage of the total number of free H-bonding donors or acceptors within the transmembrane region.

The involvement of such available polar sites in the function of the proteins has been illustrated recently in the structure of the bacterial aspartate receptor where the crystal structure of the binding domain shows the ligand binding site to include the -C=O groups exposed by a Pro-induced kink (Milburn et al. 1991) and by the structure of a bacterial Ca^{2+}-channel crystallized in nonpolar solvents (Karle et al. 1991).

The putative gating mechanism for a channel formed by the transmembrane arrangement of a bundle of helical leucine (Leu)-zervamicin peptides that the authors proposed recently (Ballesteros and Weinstein 1992*b*) is based on the likely dynamic properties of the

structure containing Pro-induced kinks in a transmembrane helix. These dynamic properties are induced by the Pro-kink which, as shown below from a molecular dynamics simulation of the 5-HT_2 receptor model, is likely to be involved in the propagation of the structural response of the receptor protein to ligand binding. Thus, the dynamic mechanism supported by the special structure around a Pro site in a helix is likely to be involved in the relay of information about ligand binding into the transmembrane structure connected to the effector (i.e., a G-nucleotide binding regulatory protein). This mechanism requires a low activation energy as illustrated by the structure and dynamics of the Pro-containing helix of melittin (Bazzo et al. 1988; Pastore et al. 1989) and thus provides a reasonable mode for the transduction of a signal produced by ligand binding. Such mechanistic hypotheses for receptor activation, anchored in the structural and dynamic properties of a 3-D model of the receptor protein, are amenable to exploration by the methods of theoretical biophysics used in the computational simulations described briefly below.

A Three-Dimensional Structural Model of the 5-HT_2 Receptor

The authors have constructed a complete 3-D model of the TMD of the 5-HT_2 receptor at atomic resolution. Preliminary molecular dynamics simulations also have been carried out on the receptor model and its complexes with ligands, including full agonists, partial agonists, and antagonists. The results suggest an activation mechanism of the receptor based on the structural response of the receptor to the binding of ligands. Both the local effects at the recognition site, which produce the activation trigger, and the distal effects, which are propagated from the recognition site into the receptor region responsible for coupling to the effector, are identifiable from the analysis of the dynamic behavior of the ligand-receptor complex.

The 5-HT_2 receptor model was constructed based on specific criteria from the following considerations: ligand, structure-affinity relationships developed for pharmacologically well-characterized ligands, structural features of membrane proteins for which structures are available at atomic resolution (BR and PRC-see above), and results of studies on the molecular biology of GPCRs.

The construction of the 5-HT_2 receptor model and the first computational simulation of the dynamic behavior of the transmembrane helix bundle in the presence and absence of ligand bound at the putative recognition site are described in detail elsewhere (Zhang and Weinstein 1993). The main

considerations in the construction of the model and the qualitative features of the results from the computational simulations are outlined briefly here.

Ligand Binding Site. As discussed in detail elsewhere (Weinstein and Osman 1990*a*), the nature of the interaction between ligands and recognition sites in the various subtypes of 5-HT receptors is expected to be *different*, consonant with the differences in ligand selectivity of the receptor subtypes. The main molecular property related to recognition at 5-HT_{1A} receptors was shown to be the directional character of the electrostatic potential generated by the ligands in the molecular region corresponding to the indole in 5-HT. The corresponding recognition site in the receptor was predicted to have properties of a positively charged (imidazolium) form of the side chain of a histidine residue (Mercier et al. 1988, 1989; Weinstein and Osman 1989*b*, 1990*a,b*).

From studies of model proteins incorporating the type of residues proposed for the recognition site in the structural arrangement required to be selective for 5-HT_{1A} ligands, the mechanism of recognition at the 5-HT_{1A} receptor was shown to be electrostatic and conducive to a triggering of the receptor response through the change in the electronic structure of the imidazolium recognition site when it interacts with an activating ligand (agonist) (Weinstein and Osman 1990*u*). This effect was shown to induce a proton transfer from the ring to a neighboring residue to which it can be hydrogen bonded in the resting state (Mercier et al. 1988, 1989; Weinstein and Osman 1990*b*).

However, a different model was proposed for selective recognition at the 5-HT_2receptors based on structural details of 5-HT-binding peptides (Weinstein and Osman 1990*a*). The recognition site in the 54-HT_2 receptor was thus proposed to consist of two aromatic residues separated by a hydrophilic residue.

In contrast to the model for 5-HT_{1A}, the recognition was considered to be based on the interaction of neutral molecules with the stabilization provided by dispersion forces (Weinstein and Osman 1990*a* and references therein). These inferences from structure-activity relationships (SAR) and specific calculations of molecular complexes (see Weinstein and Osman 1990*a* and references therein) were incorporated in the construction of the receptor model.

Putative residues of the ligand binding site in the 5-HT_2 receptor sequence were determined according to the requirements for matching recognition sites in the ligand binding pocket based on specific models proposed from SAR studies (Glennon et al. 1991; Weinstein and Osman 1990*a*); the specific sequence of amino acids in the receptor subtype and its relation to BR (Pardo et al. 1992) and the other 5-HT receptors (Adham et al. 1992; Hartig 1989; Hen 1992), and published results of GPCR structures modification using methods of molecular biology, including point mutations and chimeric receptors of various types (Guan et al. 1992; Shih et al. 1991; Strader et al. 1989). The positions of the putative residues of the ligand binding site were used as constraints in positioning the TMHs relative to one another.

The ligand binding sites are assumed to be aspartic acid (Asp) 133 (TMH3) to interact with the protonated amine group of the ligand; side chains of phenylalanine (Phe)-218 and Phe-222 (TMH 5) to interact with the aromatic rings of the ligand; methionine (Met)-313 (TMH 6) to match the region of the ligand corresponding to N1, C7 of the indole ring in 5-HT; and serine (Ser)-350 (TMH 7) to interact with the region corresponding to the 5-OH of 5-HT.

Assembly of the Helix Bundle. With 5-HT as the template of ligands, TMH 3,5, 6, and 7 were positioned so that the residues in the putative ligand binding site could interact with the key groups of 5-HT as mentioned above. These initial positions of the TMHs were refined by the guideline of maximizing interactions between conserved residues. The position of TMH 2 is determined further by possible interaction between conserved residues, specifically Asp-98 (TMH 2), Ser-140 (TMH 3), and asparagine (Asn)-354 (TMH 7). The position of TMH 1 is determined by allowing for an interaction between Asn-79 (TMH 1) and histidine (His)-143 (TMH 3), which also interacts with Asp-98 (TMH 2). Such a positioning of TMH 1 relative to TMH 2 is based on the assumption that conserved residues in a receptor sequence play an important role in determining the structure and function of the receptor and that their interaction is essential for accomplishing this function. Consequently, the conserved residues, like the polar residues, were placed in the interior of the assembled helix bundle to the fullest extent possible.

Molecular Dynamics Simulations. After the assembly of the seven TMHs, energy minimization and dynamics simulation were run to optimize the structure of the model. Average structures were obtained

from the simulations, energy was minimized, and then the average structures were used as the starting structures for studies on the interaction between the receptor model and ligands of the receptor. Ligands were roughly docked into the putative ligand binding site with nuclear overhauser effect (NOE)-type constraints to the ligand recognition sites. Molecular dynamics simulations were applied again to obtain the structure of model/ligand complexes and to study the interactions between ligands and the model.

All the molecular dynamics simulations on the model were performed with the CHARMm program, following protocols similar to those described in other publications (Mehler and Weinstein 1990; Pascual-Ahuir et al. 1991) but with a fixed dielectric permittivity constant of $\epsilon = 4$. The energy-minimized average (more than 20 picoseconds [ps]) of a final equilibrated structure was taken as a starting structure for studies of the interaction between the receptor model and various ligands.

The detailed analysis of the ligand-receptor complexes is expected to yield information on the mode of ligand recognition expressed as the physicochemical nature of the complex between the ligand and the recognition sites as well as on the mechanism by which the response of the receptor to ligand binding is triggered and then propagated toward the effector. The resulting activation mechanism from such a mode of ligand recognition and binding is based on a structural rearrangement propagated from the recognition site into the receptor protein structure connected to the portion that interacts with the effector protein. These inferences were explored with the computational simulations of the dynamics of the modeled transmembranal region of the 5-HT_2 receptor.

Dynamics of the Receptor Response to Ligand Binding. The conformational changes of the receptor model induced by the interaction with ligands were analyzed from the root mean square (rms) deviation of the alpha-carbon (C-α) atoms of the complexes with respect to the starting structures to obtain information on the structural response of the receptor to the binding of the ligand. The preliminary results indicate that the patterns of the distribution of rms are different for the three complexes of full agonists, partial agonists, and antagonists, suggesting that different types of the ligands induce different types or degrees of conformational changes of the receptor model (Zhang and Weinstein 1993). In the results from initial simulations, these conformational changes were found to be consistent with experimental results, most

significantly by their concentration in the region connecting TMHs 5 and 6.

Mutation experiments and the pharmacology of chimera from different' receptor subtypes have indicated that the beginning and ending regions of the third intracellular loop are important for GPCRs to interact with G-proteins and that the regions that affect the binding of the activating ligands are the extensions of TMH 5 and TMH 6 in the intracellular side (Strader et al. 1989; Kjelsberg et al. 1992).

Therefore, it is reasonable to expect that the binding of an agonist would induce the most significant conformational changes in these two helices and that the binding of a partial agonist also would induce conformational changes similar to, but less significant than, those induced by an agonist. The binding of an antagonist should not induce conformational changes of this kind. All these expectations were met by the first results from the computational simulations (Zhang and Weinstein 1993).

Thus, figure 1 illustrates a comparison between the averaged structures obtained from a simulation of the receptor model and a simulation of the receptor with a 5-HT ligand in the recognition site described above. The initial position of the 5-HT molecule is indicated in the same shade as the structure of the receptor without the ligand (light grey), but the molecular dynamics run had been performed in the absence of ligand. The structures of the helices and the ligand outlined in solid black represent the result of the simulation of the receptor-ligand complex. It is evident that the position of 5-HT in the recognition pocket changes significantly during the simulation (compare the position of the initial [gray] structure of 5-HT with that of the final black one).

Although the interaction with Asp-133 is maintained at the same distance, there is considerable rearrangement around the flexible side chain with a resulting strengthening of the interactions indicated in the description of the recognition site. However, the overall orientation of the indole ring remains similar to the starting point, and the planes are nearly parallel. This finding is in striking contrast to the results obtained with TRYP as a ligand (figure 2), where a "flip" of the indole ring positions the bond dipole of the N-H group in the indole in roughly the same position relative to the recognition site that the -OH group occupies in the complex with 5-HT (Zhang and Weinstein 1993).

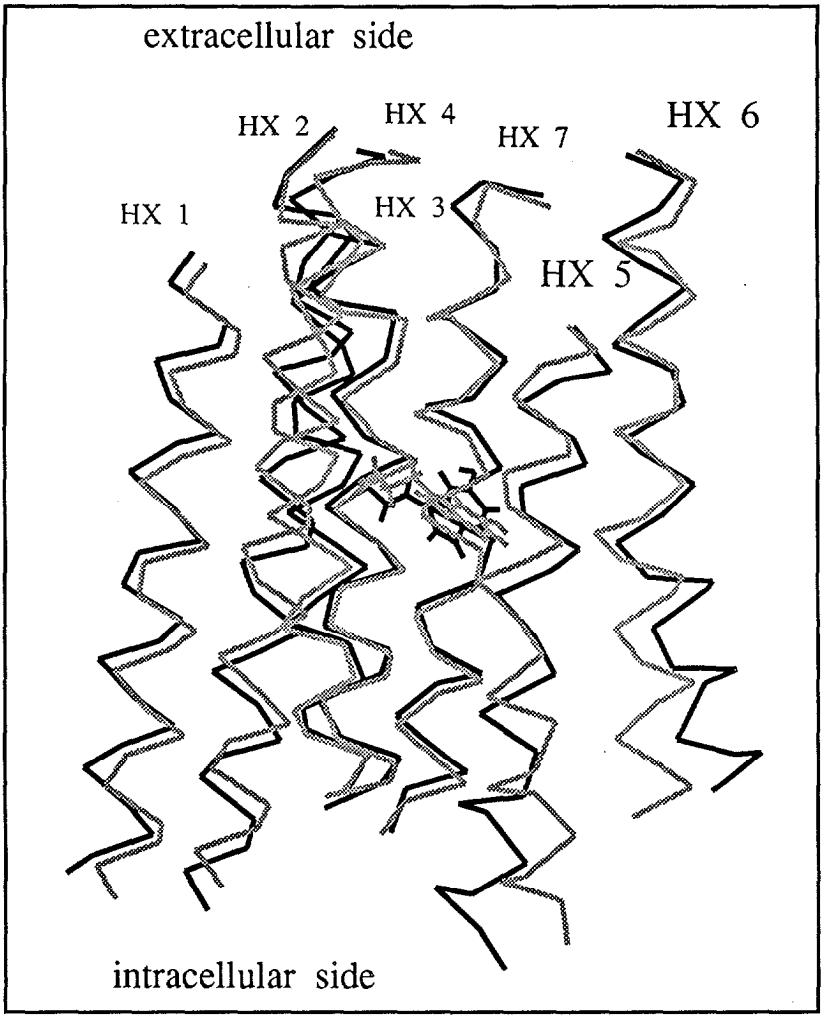

FIGURE 1. *Comparison of the structures of the molecular model of the transmembrane helix bundle of the 5-HT$_2$ receptor in the absence and presence of 5-HT. The C-a ribbon structures of the helices are shown in a view parallel to the membrane bilayer. The structures represent the results from molecular dynamics simulations (see text) of the receptor model without ligand (40 picosecond average structure representing 215 picosecond run, in shaded gray) and in the presence of 5-HT bound in the recognition site (snapshot at 65 picoseconds, in solid black). The initial position of 5-HT in the receptor is indicated in the gray shade; the position after dynamics simulation is shown in solid black.*

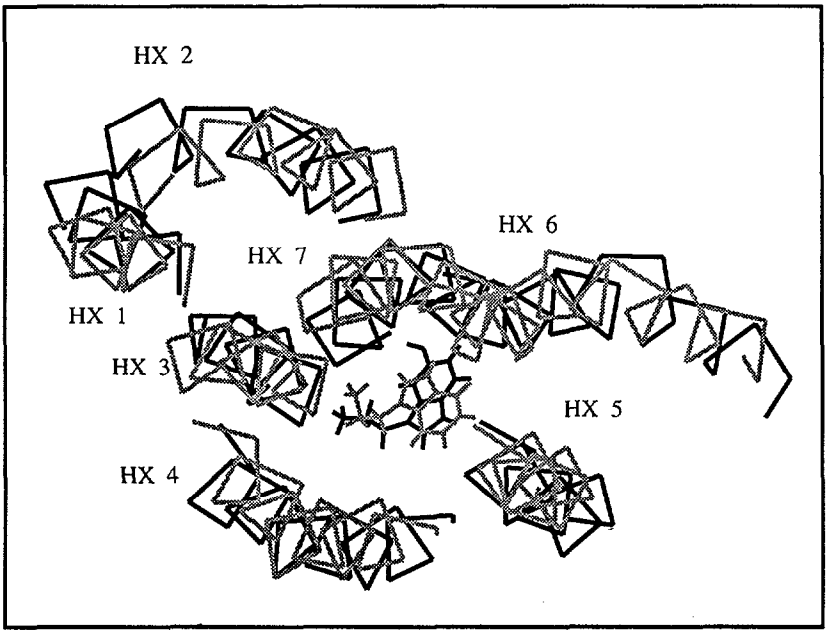

FIGURE 2. *Comparison of the structure of the 5-HT_2 receptor model in the presence of different ligands bound in the recognition site: 5-HT (snapshot at 65 picoseconds, in solid black) and TRYP (snapshot at 115 picoseconds, shaded gray). The view is perpendicular to the plane of the membrane, from outside the cell. See text and figure I legend for other details.*

The structural rearrangement in the helix bundle also is identifiable qualitatively from the comparison in figure 1. The Cα ribbons of helices 1, 2, 3, 4, and 7 are similarly arranged, whereas those of helices 5 and 6 show clear differences in the lower half pointing toward the cell interior. It is noteworthy that the major rearrangements in the structures of the two helices occur after the Pro-kink that exists in both. All the helices move during the dynamics simulation, but the major changes occur only in helices 5 and 6.

The differences in the structures of these helices in the presence and absence of the ligand are recognized in the dynamic averages from the results of the simulation, showing that the binding of the ligand has induced a force that causes the distortion in the positions of these two helices more than for the others in the bundle. The involvement of only

helices 5 and 6 at this level of comparative analysis (for more extensive details and analyses see Zhang and Weinstein 1993) is significant because these helices are the intramembrane ends of the cytoplasmic Loop III that constitutes the major structural element in the interaction of the receptor with the effector system (Kjelsberg et al. 1992; Strader et al. 1989).

As a probe of the validity of this receptor model, it is most gratifying that the main structural response to ligand binding seems to be in the region required for transmission of the signal to the effector system. The strong correlation between the structure of this region and ligand binding has been demonstrated from mutation studies in the general area of the loop (Strader et al. 1987) and has focused more specifically on one residue in that region (Kjelsberg et al. 1992).

Further support for the relation between the structural rearrangement caused by the ligand binding in the recognition site and the mechanism of receptor activation toward interaction with the effector is provided by the correlation the authors observed between the extent of the rearrangement and the known pharmacological efficacy of the ligand (Zhang and Weinstein 1993). This is illustrated in figure 2, which shows the comparison between the receptor helix bundle in the presence of the full agonist 5-HT and the partial agonist TRYP. The view of the helix bundles in figure 2 is perpendicular to the plane of the membrane. Careful inspection reveals that the bundles representing complexes with 5-HT (solid black) and TRYP (gray) differ little with the exception of helix 6. In fact, helix 6 in the TRYP-bound receptor is not significantly distorted from its position in the free receptor. Because 5-HT distorts helix 6, the superposition shown in figure 2 identifies a difference in that region. On the other hand, helix 5 superimposes well from the dynamics of the receptors with 5-HT and with TRYP, showing that the distortion produced by 5-HT (see above) is similar to that induced by the binding of TRYP. Overall, TRYP induces less distortion in the structure of the Loop III region than 5-HT does, consonant with its lower efficacy indicated by the pharmacological property of partial agonism.

A computational simulation of the receptor complex with the structurally related 5-HT_2-receptor antagonist gramine (not shown here) is remarkable in that ***no distortion*** in the average structure of the receptor bundle is observed in the key regions of helices 5 and 6 (Zhang and Weinstein 1993). Similar results are being obtained in preliminary studies with the 5-HT_2 antagonist ketanserin and with the hallucinogenic DOM, which behaves in this simulation in accordance with its pharmacological profile

as a partial agonist. Therefore, it is clear that a coherent outline of the molecular mechanism of ligand recognition and receptor response to ligand binding is emerging from the computational simulations with the present model of the transmembrane helix bundle of the 5-HT_2 receptor. The qualitative features of this mechanism of receptor function at atomic resolution are in good agreement with the molecular pharmacology of the receptor.

CONCLUSION

The major work of probing against experimental data the inferences obtained here for the first time from the simulation of the dynamic response of a receptor model to the binding of structurally related ligands is still ahead. To validate the model and the inferences to produce a reliable description of the molecular pharmacology of hallucinogens, it is necessary to extend these exciting preliminary findings to agonists, partial agonists, and antagonists in different chemical classes and to relate the structural effects of ligand binding to a reliable mechanistic hypothesis at atomic resolution. Such developments would permit specific physicochemical testing of the mechanisms and examination in detail of the behavior of hallucinogenic compounds in models of this type for other relevant receptor subtypes (e.g., 5-HT_{1C}).

These aims constitute only some of the exciting tasks that can be addressed by computational simulations with the 3-D receptor model. Such projects offer a comprehensive analysis of fundamental aspects of mechanisms of drug-receptor interactions and receptor function described at atomic resolution and also offer arguably the best hope of interfering with and/or exploiting the properties of hallucinogenic substances based on rational mechanistic means and specifically designed medications.

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AUTHORS

Harel Weinstein, D.Sc.
Professor and Chairman

Daqun Zhang, B.Sc.
Graduate Student

Juan A. Bahesteros, B.Sc.
Graduate Student

Department of Physiology and Biophysics
Mount Sinai School of Medicine of the City University of New York
New York, NY 10029-6574

Molecular Modeling of the Interaction of LSD and Other Hallucinogens With 5-HT_2 Receptors

Richard B. Westkaemper and Richard A. Glennon

INTRODUCTION

Multiple populations of serotonin (5-hydroxytryptamine [5-HT]) receptors have been identified, including 5-HT_{1A}, 5-HT_{lB}, 5-HT_{1c}, 5-HT_{1D}, 5-HT_2, 5-HT_3, and 5-HT_4 subtypes. The 5-HT_2 and possibly the 5-HT_{1C} receptors have been implicated as the probable sites of action of classical hallucinogenic agents (Glennon 1990). The goal of this chapter is to identify potential modes of interaction of hallucinogens with 5-HT_2 receptors,

In general terms, there are two ways to embark on a program designed to elucidate drug-receptor interactions. These have been referred to as the ligand-ligand and ligand-receptor approaches (Westkaemper and Glennon 1991). In the first approach, indirect information about specific receptor structural features and their locations in three dimensions is inferred indirectly from the structures of ligands. Typically, common features of structurally related ligands are graphically superimposed to illustrate common and disparate features that aid formulation of hypotheses related to specific drug-receptor interaction.

For example, (+)lysergic acid diethylamide (LSD) contains many structural features in a rigid polycyclic template that are important for 5-HT_2 receptor binding. Figure 1 shows one way in which the structures of LSD and the more flexible hallucinogenic agent 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB) can be related. For additional examples of the application of this approach to serotonergic agents, see Glennon and colleagues (199 1).

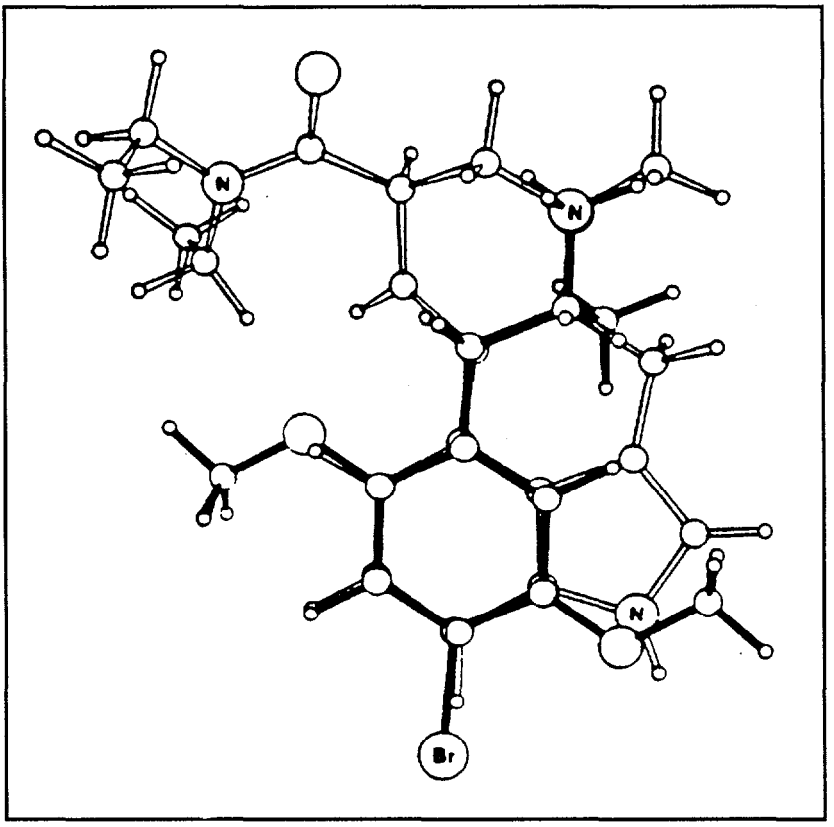

FIGURE 1. *DOB (solid bonds) superimposed on the structure of (+)LSD.*

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The ligand-receptor approach has been used extensively for biological macromolecules for which high resolution x ray structures are available. Until recently, this approach has not been feasible for neurotransmitter receptors because the three-dimensional (3-D) structures of neurotransmitter receptors have yet to be determined. Some of the earliest approaches to modeling ligand-receptor interactions utilized

simulations employing fragments that might be present at the ligand-binding site of a receptor.

Kier and Aldrich (1974) investigated ligand-receptor interactions using semiempirical molecular orbital methods to calculate the interaction energies between a drug and a small molecule thought to mimic a portion of the receptor binding site in a process known as receptor mapping using model interaction energy calculations. Later, this paradigm was applied to hallucinogenic phenethylamines (Di Paolo et al. 1978). In these studies, interaction energies between ring-substituted phenethylamines and methylindole, a presumed tryptophan surrogate, were found to correlate with hallucinogenic potency.

Weinstein explored a similar approach by calculating interaction energies between an imidazolium ion as a receptor model and 5-HT (Weinstein and Osman 1989). Although the implications of such studies are intriguing, the receptor models do not incorporate what is known about the molecular architecture of proteins. Later, more elaborate models, based on known protein structures not related to neurotransmitters, were used to provide a framework for the incorporation of ligand and receptor-like features (Weinstein and Osman 1990).

The construction of more complete receptor models had not been possible because information pertinent to 3-D structure, such as deduced amino acid sequence, was not available, Although several 5-HT receptors have now been cloned and their sequences reported (Fargin et al. 1988; Hamblin and Metcalf 1991; Hartig 1989; Hartig et al. 1990*a*,*b*; Huang and Julius 1991; Julius et al. 1988, 1990; Pritchett et al. 1988; Shih and Chen 1990; Voigt et al. 1991), their 3-D structures remain unknown.

On the other hand, the 3-D structure of the light-sensitive proton pump from bacteria, bacteriorhodopsin (BRH), has been determined to near atomic resolution (Henderson et al. 1990; Henderson and Schertler 1990). Although BRH is not a guanosine triphosphate binding protein (G-protein) receptor, it may be possible to deduce the structures of neurotransmitter receptors from it. BRH consists of seven membrane-spanning α-helices linked by extracellular and intracellular loops. The N-terminus is extracellular, and the C-terminus is located within the cell. The amphiphilic helices are found to form a pore in the membrane, with lipophilic sides oriented toward the membrane and with the more hydrophilic residues located in the central cavity.

The mammalian visual pigment rhodopsin, which is G-protein coupled, is functionally different from BRH, but both are light- and retinal-dependent (Henderson and Schertler 1990). There is little sequence homology between the mammalian visual pigments and BRH (Dohlman et al. 1987). However, evidence suggests that the overall topology for the mammalian protein is similar to that of BRH: both consist of seven membrane-spanning α-helices (see Westkaemper and Glennon 1991).

There is a significant homology between rhodopsin and the G-protein-coupled neurotransmitter receptors and an even greater homology between the neurotransmitters. Studies utilizing secondary structure-predicting methods, the analysis of the distribution of hydrophobic spans of amino acids, and the fact that the G-protein receptors have the greatest degree of sequence homology in the putative helical segments suggest that the architecture of G-protein neurotransmitter receptors is similar to that observed for BRH and to that presumed for rhodopsin (Dohlman et al. 1987).

If sequence homology between BRH and serotonin receptors were good, construction of 3-D receptor models could begin by graphically changing the amino acid side chains of the experimentally determined structure to those of the receptor of interest. Because there is no unambiguous alignment of the transmembrane portions of the 5-HT receptors and BRH sequences, model building is much more tenuous. Given that the helical segments of the serotonin receptors can be identified, placement of these in three dimensions requires the establishment of helix connectivity, vertical placement of each helix (perpendicular to the membrane), rotational alignment (in the plane of the membrane), and side chain geometry, none of which can be known with absolute certainty.

Given these ambiguities, abbreviated receptor models in which only a single helix is represented were chosen for investigation (Westkaemper and Glennon 1991). Docking studies performed with 5-HT ligands and models of helix 3 and helix 2 of 5-HT_2 (and other) receptors indicated that helix 3 is the more probable primary ligand-binding site; this has been recently verified by site-directed mutagenesis (Shih et al., this volume). Calculated binding energies for 5-HT ligands and the helix 3 model of 5-HT_2 receptors correlated qualitatively with experimentally determined affinities for a wide range of structural types, indicating that the features of helix 3 can account for ligand binding.

Despite this success, more complete receptor models are necessary for detailed understanding of ligand-receptor interaction and, in particular, linkage between agonist binding and second messenger coupling.

Hibert and coworkers (1991) recently described the first seven-helix model of a serotonin receptor. A potential sequence alignment between the helical segments of multiple G-protein receptors and BRH was established. The helix connectivity (both in the Hibert model and in the model presented below) of BRH was preserved; that is, helix 1 of the 5-HT receptor corresponds with helix 1 of BRH and so on. Alternative connectivity schemes have been proposed (Weinstein et al., this volume). Although not described, it appears that the final receptor configuration was arrived at by rotational adjustment of helices guided by the principles that highly conserved and charged amino acids should be oriented toward the receptor pore (central to the aggregate of helices). Consideration also was given to site-directed mutagenesis data for ß-adrenergic receptors (ß-AR).

The authors have taken a somewhat different approach and have attempted to identify sequence alignments between the 5-HT receptors and BRH that could be used to construct serotonin receptor models by mutating amino acid side chains without manual modification of helix disposition. White and Jacobs (1990) recently have shown that lengths and centers of helical spans in the photosynthetic reaction center and BRH can be accurately predicted (helical centers within 0.2 amino acids and helix ends within 1 to 3 residues of the experimentally determined values) using a modified hydrophobicity index, and that the helix turn potential has a minimum value at helix centers. Figure 2 shows the results of such an analysis for the 5-HT_2 receptor sequence.

The authors have chosen to evaluate the hypothesis that helical centers of serotonin receptors and BRH (see figure 3 for amino acid sequence of BRH) are structurally coincident. Once such a coincidence and sequence alignment are established, 3-D models of serotonin receptors can be constructed, subjected to molecular mechanics geometry optimization, and evaluated for reasonableness with respect to important criteria: helix packing, disposition of polar and hydrophobic residues, orientation of highly conserved residues, and accessibility of side chains known to interact with ligands.

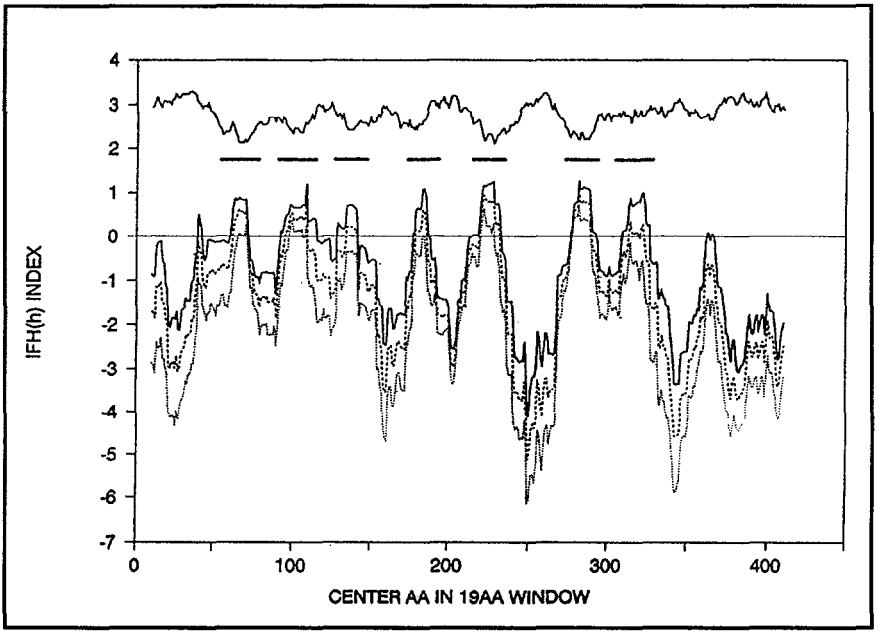

FIGURE 2. *Hydrophobiciy and turn potential analysis of the 5-HT_2 receptor sequence. Horizontal bars indicate helical segments. The upper trace represents turn potential. The lower traces are the hydrophobicity indexes for interfacial hydrophobicity scale where h=0, 0.5, 1.0.*

RECEPTOR MODEL FEATURES

As determined by alignment, the disposition of the ammoniumion-binding aspartate of helix 3 with respect to the rest of the aggregate is perhaps the most important single issue in the model-building process. Unfortunately, identification of the helix center is the least certain in the case of helix 3, perhaps because of the presence of several highly hydrophilic residues. Needleman and Wunsch (1970), using either physical properties or identity as the similarity criteria, produced an alignment shown in figure 4, panel A (identity of 23 percent, alignment score 0.6+0.08; 0.46 for jumbled alignments) which is within the range of possibilities identified using the method of White and Jacobs (1990). The second and third alignments (figure 4, panels B and C, respectively) are also consistent with helix center identification but result only in 4 percent identity. The first alignment places the helix 3 aspartate near the interface

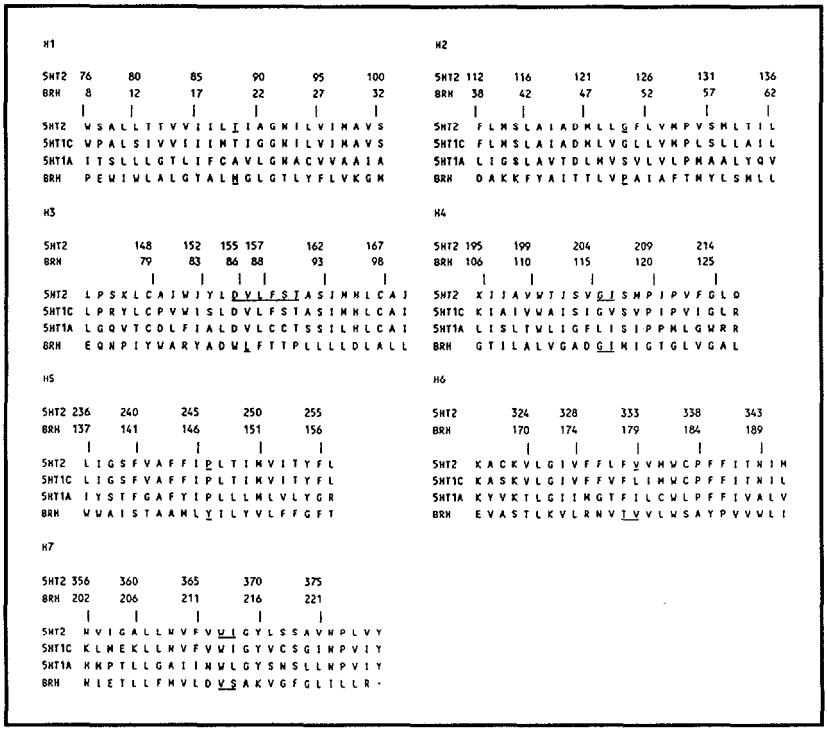

FIGURE 3. *Amino acid sequences for the* seven *transmembrane helices of 5-HT$_2$, 5-HT$_{1C}$, and 5-HT$_{1A}$ receptors and BRH. Underlined residues indicate the most probable locations of helix centers.*

between helix 3 and helix 2. The third alignment places the helix 3 aspartate inaccessible to ligands at the interface between helix 3 and helix 4 and closer to the membrane than to the pore of the receptor. Although identity is poorer than that for the first alignment, the second alignment has been evaluated most extensively because it places the aspartate of the helix in the central pore.

The 5-HT$_2$ receptor was generated by mutation of the BRH side chains according to the alignment shown in figure 3, retaining as much of the original side chain geometry as possible. The entire structure was subjected to molecular mechanics geometry optimization (Weiner et al. 1984) after removing obviously bad side chain contacts. The model of the 5-HT$_2$ receptor constructed in this manner (figures 5 and 6) is only one of several possibilities. Because of limited sequence homology, this

and all models should be considered provisional; nevertheless, several interesting points emerge from the model.

```
A   BRH          PSKLCAIWIYLDVLFSTASIMHLCAIS
    5-HT2        EQNPIYWARYADWLFTTPLLLLDLALL

B   BRH          LPSKLCAIWIYLDVLFSTASIMHLCAI
    5-HT2        EQNPIYWARYADWLFTTPLLLLDLALL

C   BRH          PLPSKLCAIWIYLDVLFSTASIMHLCA
    5-HT2        EQNPIYWARYADWLFTTPLLLLDLALL
```

FIGURE 4. *Several potential alignments of the helix 3 portions of the 5-HT_2 receptor and BRH.*

- The overall packing geometry of BRH is retained in the energy-minimized 5-HT_2 receptor model. The total calculated energy of the receptor model is comparable to the calculated energy of BRH. Helix-helix interactions observed in the model are qualitatively consistent with the experimental structures of adjacent proteins with nearly parallel α-helices.

- Conserved residues generally are located in the central cavity, as are most of the polar amino acid side chains. Exceptions occur at helix termini.

- The ammonium ion-binding aspartate of helix 3 is located in the central cavity nearer to the extracellular side than to the interior of the cell. It is central to two potential ligand binding cavities, one formed from helices 4, 5, and 6 (site I) and a second formed by helices 1, 2, and 7 (site II) (figure 7, panel A).

- Phenylalanine (Phe)-89 and tryptophan (Trp)-82 flank aspartic acid (Asp)-86 of helix 3 (see figure 8) and are in positions that would be accessible for interaction with a bound ligand.

- The hydroxy group of tyrosine (Tyr)-216 of helix 7 forms a hydrogen bond with Asp-86 (figure 8). This structural finding is particularly interesting given the observation that it is conserved only in G-protein receptors that have ammonium ion ligands (Hulme et al. 1990).

- Valine (Val)-212 of helix 7 is near the helix 3 aspartate. An asparagine at the analogous position in the 5-HT_{1A} receptor may modulate ligand selectivity (Surryanarayana et al. 1991).

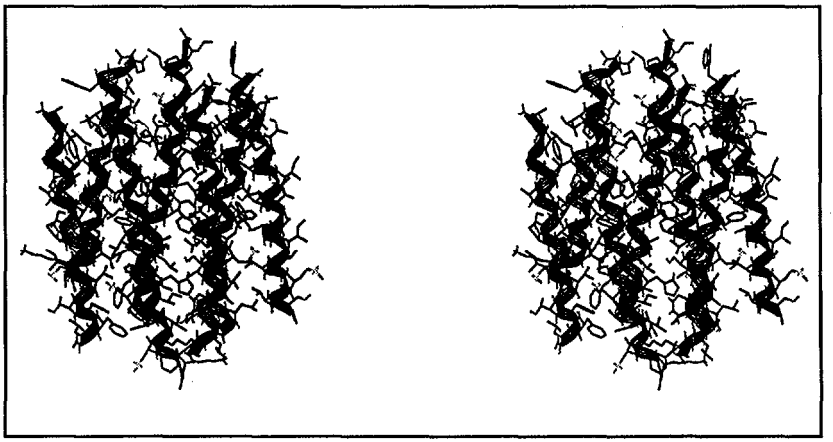

FIGURE 5. *Stereoscopic view of a 5-HT_2 receptor model as viewed from the plane of the cell membrane.*

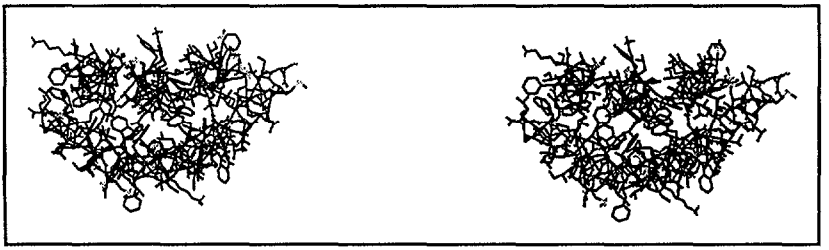

FIGURE 6. *Stereoscopic view of a 5-HT_2 receptor model shown in figure 5 as viewed from the extracellular (N-terminal) side.*

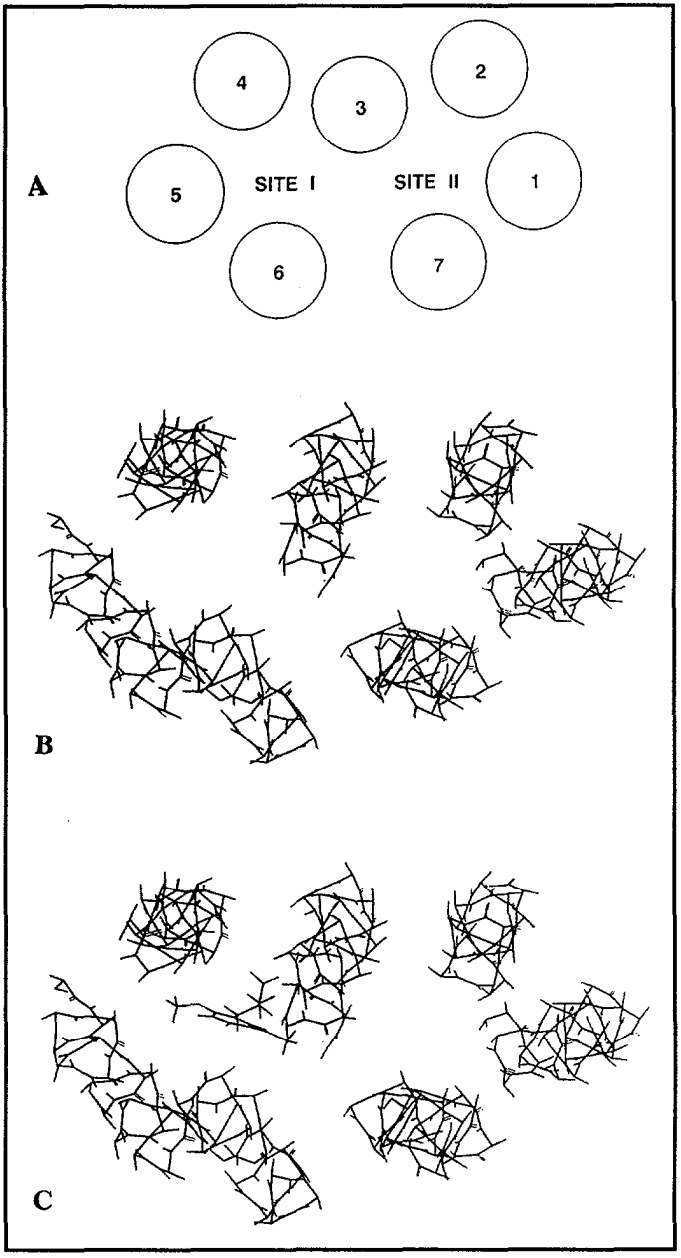

FIGURE 7. *A schematic view of sites I and II formed by helices 4, 5, and 6 and by helices 2, 1, and 7, respectively (A). A view of sites I and II showing only the helix backbone of the $5HT_2$ receptor model (B). A representation of DOB bound in site I of the $5HT_2$ receptor model (C).*

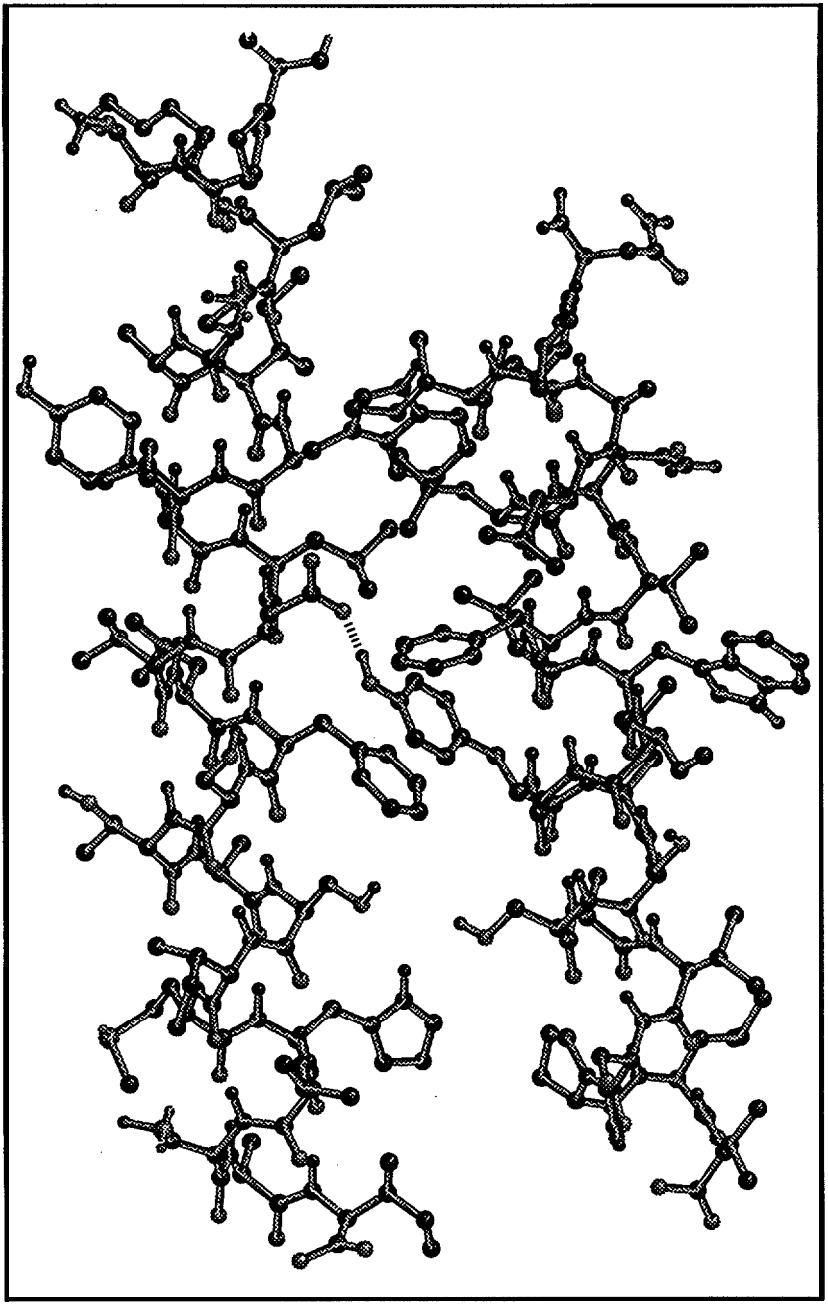

FIGURE *8.* *Closeup of the region surrounding Asp-86 (helix 3) and Tyr-216 (helix 7) of the 5-HT_2 receptor model. Note the hydrogen bond between Asp-86 and Tyr-216.*

One major difference between the model reported here and that reported by Hibert and colleagues (1991) is the rotational orientation of helix 5. In the present model, one of the same two serine (Ser) residues that may be involved in ligand-receptor interactions for the ß-AR (Strader et al. 1989) appears at the interface between helix 4 and helix 5.

LIGAND-RECEPTOR DOCKING

Several structurally similar 5-HT2 receptor models have been developed. One way to evaluate potential receptor models is to investigate modes of ligand-receptor interactions. This process requires selection of an appropriate ligand. Ideally, the ligand should be conformationally rigid and sterically demanding. Table 1 depicts the relative merits of dihydroergotamine (DHE), LSD, and DOB as ligands. LSD and DOB, both of which are hallucinogenic, were selected for initial docking studies. Preliminary studies using the program DGEOM (Blaney et al. 1990) for the automated docking of LSD and a 5-HT_2 receptor model indicated that, for steric reasons, the only reasonable orientation of the rigid polycyclic structure was in sites I or II (figure 7, panel A), with the plane of the aromatic nucleus of LSD more or less parallel to the helical axes. The program GRID (Goodford 1985) was used to identify receptor features that could provide energetically favorable noncovalent interactions. Probe species included an aromatic carbon, water, a methoxy group, an ammonium ion, a carbonyl moiety, and a bromine The information from structure-activity relationship (SAR) studies of ring-substituted phenylisopropylamines such as DOB also was used to guide docking studies (Seggel et al. 1990). The ligand binding site of the receptor must account for the fact that the 2-methoxy group of DOB is critical for high affinity. The 5-methoxy substituent is also important for 5-HT_{1C} and 5-HT_2 affinity, and may play a recognition role analogous to the indole nitrogen of serotonin and LSD. The substituent at the 4-position (e.g., the Br of DOB) modulates affinity over a 50,000-fold range. Typically, nonpolar substituents enhance affinity, the most effective being the halogens (H<F, Cl, Br, I) and alkyl groups (e.g., methyl, ethyl, propyl, butyl, t-butyl, amyl, hexyl, octyl). The receptor recognition site must accommodate alkyl groups as large as 4-octyl and 4-(3-phenylpropyl). In addition, primary amine alkylation decreases affinity, whereas stereochemistry and the presence of an a-methyl group have little effect on receptor affinity.atom. Manual docking was performed using GRID contours as a guide.

TABLE 1. *Potential ligands for receptor model docking.*

	DHE	LSD	DOB
Steric demand	+	+	-
Conformationally restricted	+	+	-
Subtype selectivity			+
SAR data			+
Hallucinogenic		+	+

KEY: DHE = dihydroergotamine; DOB = 1-(4-bromo-2,5-dimethoxyphenyl)-2aminopropane; SAR = structure-activity relationships

Both sites I and II can accommodate the steric bulk of DOB. Both sites have numerous polar, hydrogen-bond-donating side chains, but the side chains are somewhat more numerous in site II. Both sites have aliphatic amino acid side chains in the receptor pore, but site I is slightly richer in aromatic residues at the level of the helix 3 aspartate. Several potential modes of DOB-receptor binding have been evaluated, but only one is discussed in this chapter. There is some evidence that the analogous site I receptor features may be the important ligand recognition region for G-protein receptors other than serotonin receptors (Strader et al. 1989).

The first fact that becomes evident (partially from DGEOM docking experiments) is that the helix 3 aspartate must move away from its hydrogen-bonded, unligated conformation (figure 8) with rotation about the Cα–CTJ bond and move toward site I to allow the bound ligand access to functionally significant side chains. This process is shown schematically in figure 9. Disruption of the resting receptor conformation, in which the helix 3 aspartate and helix 7 Tyr hydrogen bond is intact, may prove to be the event initiating a conformational change that may be responsible for receptor activation.

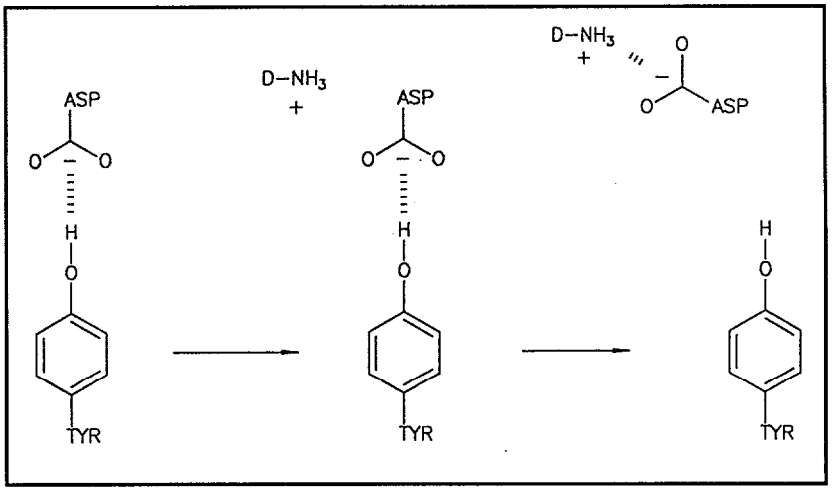

FIGURE 9. *Schematic representation of the possible sequence of events that occurs on the interaction of a ligand (D-NH_3+) with the helix 3 aspartate of a 5-HT_2 receptor model.*

DOB was found to bind favorably in site I (figure 7, panel C), with the aromatic ring approximately parallel to the helical axis, with the primary amine pointed toward the extracellular side and the 4-Br group downward toward the intracellular side. The helix 3 Asp-86 forms an ionic bond with the primary ammonium ion (figure 10). Ser-118 of helix 4 donates a hydrogen bond to the 2-metboxy group, and Ser-90 of helix 3 donates a hydrogen bond to the 5-methoxy substituent. Two aromatic residues (Phe-145 and Phe-185) form an edge-to-face hydrophobic interaction with the phenyl ring of DOB. Aliphatic side chains complete the remainder of the phenyl ring binding site. Trp-182 of helix 6 appears near the bottom of the DOB phenyl ring near the 4-Br group (figure 10), suggesting that this residue may form part of the hydrophobic region that can accommodate a variety of hydrophobic substituents. Further in the intracellular direction, the 4-position of DOB is surrounded by aliphatic and aromatic residues in a way that results in the formation of a short hydrophobic tunnel that can be expected to accommodate several substituents, even long chain aliphatic ones. A serine analogous to Ser-118 (figure 10) is uniformly conserved in the serotonin receptors as well as in the receptors for catecholamines.

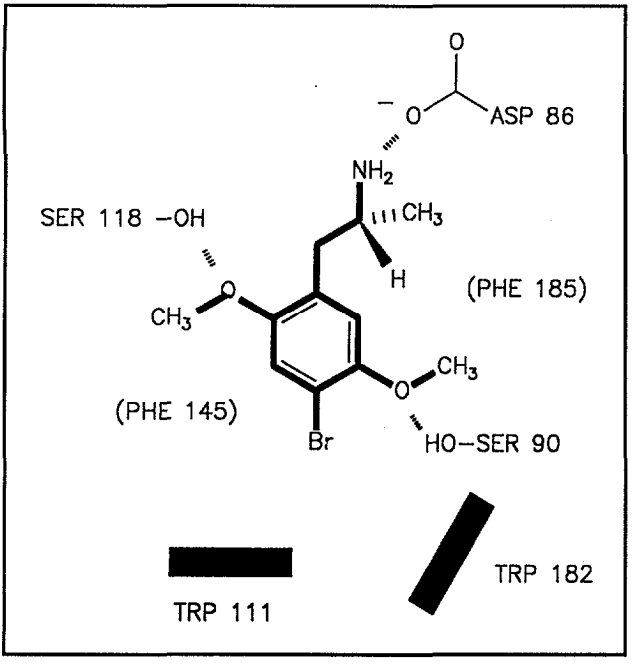

FIGURE 10. *Schematic of possible interactions between DOB and features of a 5-HT$_2$ receptor model as derived from figure 6, panel C*

The neurotransmitters for these receptors all have a phenolic hydroxy group in the position analogous to the 2-methoxy group of DOB (e.g., the 5-hydroxy group of 5-HT). Receptors for acetylcholine, which do not have an aromatic hydroxyl group, have a tryptophan residue at this position. Among the serotonin receptors, a Ser-90 at helix 3 occurs only for the 5-HT$_2$ and 5-HT$_{1C}$ receptors. This position bears a cysteine (Cys) in all other serotonin subtypes. Although a Cys can donate a hydrogen bond, the bond is considerably less energetically favorable than that formed by the Ser hydroxy group. This finding is consistent with the observation that groups analogous to 5-methoxy of DOB are not necessary for 5 HT$_{1A}$ binding. Taken together, these observations indicate that the mode of interaction described is feasible but is not proven.

LSD can bind to the receptor in a fashion analogous to DOB. Ser-118 of helix 4 may form a hydrogen bond with the amide carbonyl or with the C10-C11 double bond of LSD, and Ser-90 interacts with the indole

nitrogen (figure 11). Aromatic residues Phe-185, Phe-145, and Trp-182 can interact with the aromatic ring system of LSD as shown in figure 11. Other closely situated residues may participate in ligand-receptor interaction.

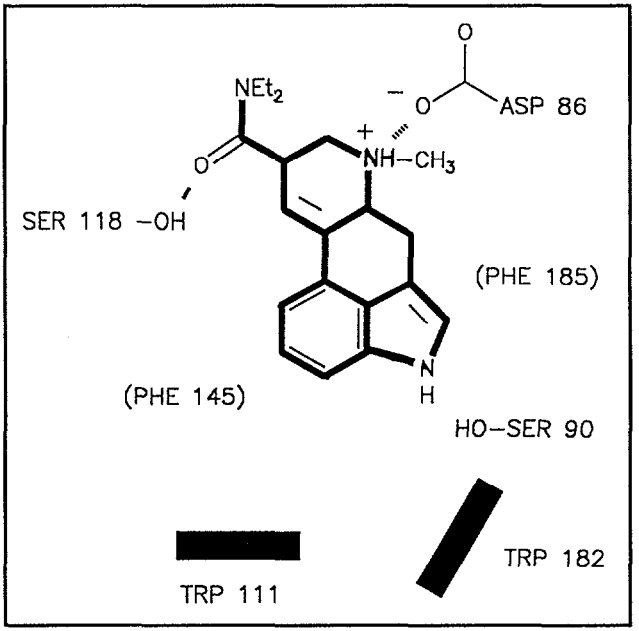

FIGURE 11. Schematic *of possible interactions between (+)LSD and features of a 5-HT$_2$ receptor model*

SUMMARY

Current evidence suggests that classical hallucinogens act via a 5-HT$_2$ (possibly 5-HT$_{1C}$) agonist mechanism. To gain further insight into the mechanism of action of these agents, the authors have attempted to model this drug-receptor interaction. Because of the unavailability of the 3-D structure of neurotransmitter receptors, it was necessary first to develop a hypothetical 5-HT$_2$ receptor model. By analogy to the structure of BRH and by utilizing pertinent information from other neurotransmitters, several 5-HT$_2$ receptor models were developed; one of these is shown in figures 5 and 6.

Using this model, the authors attempted to dock the structures of DOB and (+)LSD using DGEOM and GRID to aid this process. Docking also was guided by known SAR. (That is, the validity of any model rests with its ability to account for what is known about the binding of hallucinogens at 5-HT_2 receptors.) Two potential binding domains were identified (i.e., site I and site II, see figure 7). Several different modes of binding are possible within each of these sites. Figure 7, panel C shows one way in which DOB can bind at site I of the 5-HT_2 receptor model. A closeup view of the specific amino acid residues that may be involved in binding is shown as a schematic (figure 10). The proposed mode of interaction also accounts for the binding of (+)LSD and is consistent with established SAR. The proposed mode of interaction between drug and receptor is neither inflexible nor static. (That is, ligands with slightly different aromatic substitution patterns may still interact in an analogous manner with the receptor features that have been identified.) Furthermore, it must be emphasized that figures 10 and 11 represent one potential mode of binding of DOB and (+)LSD at site I of a single multiple-helix 5-HT_2 receptor model. Other modes of binding-indeed, other 5-HT_2 receptor models-are possible. Although the present drug-receptor model is certainly reasonable, not all other possible drug-receptor models have been fully evaluated yet.

The objective is to identify the most likely drug-receptor models using site-directed mutagenesis. For example, all models utilize the helix 3 aspartate as the ammonium ion binding site; thus, this aspartate must play a critical role in binding, and its elimination or replacement should result in reduced affinity. In collaboration with Dr. Shih, the authors now have demonstrated that replacement of this aspartate (but not the aspartate of helix 2) by asparagine (Asn) results in a dramatic decrease in affinity for various 5-HT_2 ligands. Similar studies are required to test other potential binding features consistent with the different models.

The classical hallucinogens bind at 5-HT_{1C} receptors with affinities comparable with the affinities for 5-HT_2 receptors. Indeed, there is less than a tenfold difference in 5-HT_{1C} versus 5-HT_2 affinities for a large series of DOB-related analogs (Glennon et al. 1992). Thus, any drug-receptor model developed for 5-HT_2 receptors should possess those (or structurally analogous) features found in 5-HT_{1C} receptors. In contrast, because classical hallucinogens (with the exception of (+)LSD) typically bind with low affinity at 5-HT_{1A} receptors, structural features common to 5-HT_2 and 5-HT_{1A} receptors would likely be unable to account for selectivity. One of several possible drug-receptor 5-HT_2 models is

described here in detail. The model accounts for, and is consistent with, much that is known about classical hallucinogens. Studies are under way to further validate this (and other) models. These studies, in addition to being relevant to the investigation of hallucinogenic agents, go well beyond the present limited scope of study and can have a significant influence on the understanding of drug-receptor interactions in general.

NOTE

All sequence numbers refer to the BRH numbering system for purposes of comparison and uniformity. The actual numbering system for the amino acid sequence of 5-HT_2 receptors is shown in figure 3.

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AUTHORS

Richard B. Westkaemper, Ph.D.
Associate Professor

Richard A. Glennon, Ph.D.
Professor

Department of Medicinal Chemistry
Medical College of Virginia
School of Pharmacy
Virginia Commonwealth University
Richmond, VA 23298

Structure and Function of Serotonin 5-HT_2 Receptors

Jean C. Shih, Kevin Chen, and Timothy K. Gallaher

INTRODUCTION

One of at least seven known subtypes of serotonin (5-hydroxytryptamine [5-HT_2]) receptors is the 5-HT_2 receptor. The molecular cloning of these subtypes has unequivocally defined receptor subtypes and confirmed the existence of 5-HT receptor subtypes found by earlier pharmacological, biochemical, and physiological studies. The 5-HT_2 receptors are integral membrane proteins that elicit a cellular response to serotonin in conjunction with a guanosine triphosphate binding protein (G-protein) and an effector enzyme (Shih et al. 1991). The 5-HT_2 receptors are known to activate the inositol triphosphate (IP_3)/diacylglycerol second messenger system via enzymatic cleavage of polyphosphoinositol by phospholipase C, which ultimately results in increased calcium ion levels in the cell (Conn and Sanders-Bush 1986; Pritchett et al. 1988).

The 5-HT_2 receptors are found in the brain cortical regions and may be involved in depression and suicide (Meltzer and Lowy 1987; Sternbach 1991). In addition, 5-HT_2 receptors are targets for hallucinogenic drugs that also implicate 5-HT_2 receptors as being important in mental health (Glennon, this volume; Pierce and Peroutka 1989; Sadzot et al. 1989). Outside the brain, 5-HT_2 receptors are found in platelets and smooth muscle tissue and play a role in blood pressure and in hypertension (Vanhoutte 1982). The 5-HT_2 receptors are of great interest for their multifaceted actions in the body and the importance of their function for mental and physical health.

This chapter presents the background of the research leading up to the identification and cloning of 5-HT_2 receptors and the current molecular knowledge of the 5-HT_2 receptor, with an emphasis on the structure and functions of these receptors.

BACKGROUND

Many fields of research contributed to the formation of receptor theory and the identification of 5-HT_2 receptors. Studies of hallucinogenic drugs and antipsychotic drugs provided information that led directly to the identification and cloning of 5-HT_2 receptors. Hallucinogenic drugs have been used for millennia in human culture, and scientific analysis of their chemical properties and functions began in this century. In 5-HT receptor research, lysergic acid diethylamide (LSD) has made an invaluable contribution. Hofmann first synthesized LSD and discovered its profound psychological properties (Hofmann 1975). The effects of LSD were recognized to be related to the effects of mescaline, a compound from the peyote cactus that has been used traditionally by Native Americans in religious ceremonies (Schultes 1938, 1972). LSD and mescaline present two basic structural types of hallucinogenic drugs: indoleamines (LSD) and phenylalkylamines (mescaline). Hofmann later isolated psilocybin and psilocin, the active ingredients of hallucinogenic mushrooms, and discovered that these compounds also were indolealkylamines (Schultes and Hofmann 1973). The shared indolealkylamine structure of hallucinogenic drugs and serotonin suggested that they acted through identical or similar physical mechanisms that were unknown at the time.

Concurrent with the early studies of hallucinogenic drugs was the development of a class of compounds known as antipsychotics. These drugs, exemplified by chlorpromazine and haloperidol, greatly reduced psychosis in mental patients and also were able to counteract LSD-induced psychosis (Shoichet and Solursh 1969). The observations of the structure and actions of hallucinogens and the actions of antipsychotics produced information indicating that specific functions of the, brain can be altered by specific drugs. The question then was how the drugs exert these effects.

Now it is clear that the actions of these drugs are explained by the existence of specific receptors at the neuronal surface. Early studies of receptors used isolated tissue systems such as the guinea pig ileum, where two types of serotonin receptors were identified: the D- and M-type receptors (Gaddum and Picarelli 1957). Much work also was done using electrophysiological methods to measure electrical responses of neurons to neurotransmitters and drugs such as LSD and chlorpromazine. The breakthrough technique of radioligand binding analysis occurred in the early 1970s and led to detailed pharmacological analyses of receptors and

identification of different receptor subtypes based on pharmacological specificities of individual receptors. Peroutka and Snyder (1979) first identified 5-HT_1 and 5-HT_2 receptors using [^{3}H]serotonin, [^{3}H]LSD, and [^{3}H]spiperone as radiolabels. Since that time, at least eight different 5-HT receptor subtypes have been identified by pharmacological analysis. The classification of 5-HT receptors by pharmacological methods now has been confirmed, and the pharmacological definitions are known to have been extremely accurate.

MOLECULAR CLONING OF 5-HT RECEPTORS

Today all 5-HT receptors that were identified by radioligand analysis have been cloned. (Table 1 summarizes the cloned G-protein-coupled 5-HT receptors and some of their molecular and functional properties.) The 5-HT_{1C} receptor was the first serotonin receptor cloned and identified (Julius et al. 1988). This receptor was cloned by using messenger ribonucleic acid (mRNA) extracts in conjunction with the *Xenupus* oocyte expression system to provide the basis for a functional assay. The 5-HT_2 receptor was cloned next, using a hybridization approach with a probe derived from the 5-HT_{1C} receptor (Pritchett et al. 1988).

The 5-HT_{1A} receptor was cloned by screening a genomic deoxyribonucleic acid (DNA) library with a probe derived from the ß-adrenergic receptor (P-AR). The isolated clone was called G-21, and its identity was unknown; it was later identified as the 5-HT_{1A} receptor (Fargin et al. 1988). The list of cloned receptors now includes the 5-HT_{1D}, 5-HT_{1B}, 5-HT_{1E} (also cloned as S31), and three 5-HT receptors from ***Drosophila.*** Another 5-HT receptor that has been cloned is the 5-HT_3 receptor (Maricq et al. 1991). It is not a member of the G-protein-coupled receptor family but a member of the ligand-gated ion channel receptor family.

The gene structures of several of the serotonin receptors have been determined, including the 5-HT_{1A}, 5-HT_{1D}, 5-HT_2, and ***Drosophila*** 5-HT receptors. Of considerable interest is that 5-HT_{1A}, receptors, 5-HT_{1D}, receptors, and two of the three *Drosophila* receptors are intronless genes, whereas the 5-HT_2 receptor and one of the *Drosophila* receptors contain multiple introns. These observations raise questions concerning the relationship and evolution of serotonin receptors and G-protein receptors in general.

TABLE 1. *Biochemical properties of cloned G-protein-coupled 5-HT receptors*

Receptor	No. Amino Acids	No. Introns	Second Messenger	Species
5-HT_{1A}	421 (human) 422 (rat)	0	cAMP ↓, K^+ channel, IP_3	Human, rat
5-HT_{1B}	390 (human) 386 (rat, mouse)	0	cAMP ↓	Human, rat, mouse
5-HT_{1D}	377	0	cAMP ↓	Human, dog
5-HT_{1E} (S31)	365	0	cAMP ↓	Human
5-HT_{1C}	459 (mouse), 460 (rat)	Has introns; no. not available	IP_3	Human, rat, mouse
5-HT_2	471	2	IP_3	Human, rat, mouse, hamster
5-HT-Dro_1	564	0	cAMP ↑	*Drosophila*
5-HT-Dro_{2A}	834	0	cAMP ↓	*Drosophila*
5-HT-Dro_{2B}	645	4	cAMP ↓	*Drosophila*

Traditionally, classification of receptors has been along lines of shared ligands, for example, the serotonin, acetylcholine, or gamma aminobutyric acid (GABA) receptors. However, molecular cloning of receptors has demonstrated clearly that protein structural relations are not reflected in ligand specificities of receptors. For example, 5-HT acts on the G-protein-coupled receptors and the 5-HT_3 receptor coupled with an ion channel. The observation that a receptor's functional and evolutionary relationship to other receptors cannot be determined by shared ligands is reinforced by the demonstration of the intronless nature of some 5-HT receptors.

Further evidence arose from the molecular cloning of the human and mouse 5-HT_2 receptor genes (Chen et al. 1992; Yang et al. 1992). The 5-HT_2 receptor genes contain three exons for the coding region of the receptor (figure 1). This was the first demonstration of an intron containing the 5-HT receptor gene: The 5-HT_{1A}, 5-HT_B, 5-HT_D, and 5-HT_E receptors are intronless. The 5-HT_{1C}, (Yu et al. 1991) and 5-HT_2 receptors have a high degree of sequence homology, and both contain introns. On the other hand, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D}, receptors are intronless, as is the ß-AR. The intronless 5-HT receptors are clearly more closely related to the P-AR than to the 5-HT_{1C}, and 5-HT_2, receptors, based on sequence homology and gene structure, but share activating ligand 5-HT with the 5-HT_{1C}, and 5-HT_2, receptors, not the more closely related P-AR.

Thus, in many cases the use by two receptors of the same activating ligand is seen as a convergent evolution that does not reflect a common ancestral source gene. More anomalies are presented by the structures of *Drosophila* 5-HT receptor genes when evolutionary relationships are examined. Three *Drosophila* 5-HT receptors have been cloned. The primary structures of these genes indicate that they are more closely related to the intronless 5-HT/ß-AR group, but one of these three genes in *Drosophila* contains introns. The intron organization is different from that of the 5-HT_2 gene. The questions concerning 5-HT gene structure and functional and evolutionary relationships—when and where introns were acquired or lost-are open and amenable to further study.

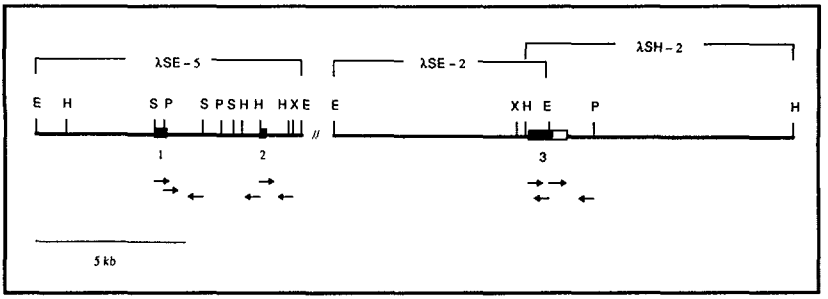

FIGURE 1. *Partial structural map of the human 5-HT$_2$ receptor gene. λ-Clones designated λSE-5, λSE-2, and λSH-2 were isolated from λ-phage genomic DNA libraries. The filled boxes are the three exon coding regions, and the open box is the untranslated region. '//' represents the intron gap between the genomic clones. Arrows indicate the start sites and direction of sequencing. The restriction enzyme sites are as follows: E = EcoRI; X = XbaI; S = SmaI; P = PstI; H = HindIII.*

SOURCE: Chen et al. 1992. Copyright 1992 by Elsevier Science Publishers, New York.

FUNCTIONAL STRUCTURE OF 5-HT RECEPTORS

Except for the 5-HT$_3$ receptor, 5-HT receptors are members of the G-protein-coupled family of integral membrane receptor proteins. These receptors show seven hydrophobic regions that are believed to traverse the lipid bilayer seven times analogously to bacteriorhodopsin (BR) and rhodopsin. This topological structure acts as the major determinant for the functional three-dimensional (3-D) structure. The receptors are classified into three main domains: the extracellular domain, which includes the amino terminal and three extracellular loops between membrane spanning regions; the transmembrane domain (TMD), consisting of the seven hydrophobic α-helices that span the membrane; and the intracellular domain, comprising the carboxy terminal and three cytoplasmic loops between the TMDs.

Experimental evidence indicates that the TMDs form the binding sites for agonists and antagonists and that certain TMD residues are necessary for allbsteric activation of the G-protein and signal transduction. The cyroplasmic loops two and three are implicated in G-protein coupling and

are necessary for activation of the G-protein and conferring the specific G-protein/receptor coupling. Most of the knowledge of the structure and function of the G-protein receptors comes from studies of adrenergic and muscarinic receptors. The general principles of their structure and function mentioned above are expected to be true also for 5-HT receptors and other G-protein receptors, but as yet little work has been done on the 5-HT receptors. The authors' laboratory has begun an analysis of the rat 5-HT_2 receptor using site-directed mutagenesis and the known primary structures of 5-HT_2 receptors from different species.

MUTAGENESIS OF ASPARTIC ACID RESIDUES IN THE 5-HT_2 RECEPTOR-G-PROTEIN COUPLING

Three aspartic acid (Asp) residues of the rat 5-HT_2 receptor were mutated to asparagine (Asn) residues to determine the role of the negatively charged aspartate on receptor function. The mutated residues were Asp- 120, Asp-l 55, and Asp- 172. The effects of the mutations were analyzed by using [^{125}I]-LSD for radioligand binding studies to determine agonist and antagonist binding properties and by measuring agonist-stimulated [^{3}H]polyphosphoinositol formation in the mutant and wild-type receptors.

The results indicate that Asp-120 is necessary for the activation of second messengers through the G-protein. Mutation of this residue in the second TMD abolishes 5-HT-stimulated phosphoinositol (PI) turnover. This mutation also confers a loss of affinity for 5-HT and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) binding to the receptor, comparable with the loss of affinity seen in wild-type receptors caused by the presence of guanosine triphosphate (GTP) that induces uncoupling of the receptor G-protein complex and a lower affinity state for agonist. The effect of GTP on the binding of 5-HT and DOI is greatly reduced in this mutation. Little difference is seen in DOI and 5-HT binding in the presence of GTP, whereas the wild-type receptor affinity is greatly reduced because of the presence of GTP. These results are consistent with Asp- 120 being necessary for G-protein/receptor allostericity where the receptor activates the G-protein and the G-protein/receptor complex confers the high-affinity agonist binding state to the receptor. When uncoupled because of GTP, the receptor exhibits lower affinity agonist binding. The Asp-120 mutation is seen as an uncoupled mutant that is unable to activate second messenger production because of its inability to allosterically modulate the G-protein. This uncoupling may be caused by

a loss of allosteric effects conferred by the loss of the negatively charged aspartate, rather than the loss of a direct interaction between Asp-120 and the G-protein, because the position of Asp-120 in the lipid bilayer TMD of the protein makes a direct contact unlikely.

The result of this mutation correlates well with mutagenesis of the P-AR, α-adrenergic receptor (α-AR), and muscarinic receptors where the cognate Asp in the TMD of these receptors also played the same role. The conserved structure of G-protein receptors where an aspartic acid occurs in the hydrophobic second TMD suggests that this is an allosteric feature retained by all members of the G-protein-coupled receptor family.

AGONIST-BINDING SITE

Mutagenesis implicates Asp-155 in the third TMD as being necessary for high-affinity agonist and antagonist binding. Mutation of this residue to Asn causes a profound loss of binding affinity for the agonists 5-HT and DOI, as determined by competition for [^{125}I]-LSD binding, and also decreases antagonist affinity. Phosphoinositide turnover is not abolished in this mutant, and GTP-sensitive binding is retained. The role of this negative amino acid in the hydrophobic third TMD is presumably to act as a counter ion for the amine group of the ligands. As a negative amino acid, Asp-155 acts as one of the epitopes for ligand binding but not as a determinant for G-protein coupling.

These observations parallel those seen for the adrenergic receptors and the muscarinic receptor. The conserved nature of the Asp residue in the third TMD in receptors for amine-containing ligands (e.g., adrenergic, muscarinic, dopaminergic, and serotonergic receptors) and its absence in receptors whose ligands do not contain aliphatic amines, support a conserved role in binding in the aliphatic amine receptors such as the 5-HT$_2$ receptor.

Another putative binding epitope for 5-HT association with the 5-HT$_2$ receptor is serine (Ser)-239 in the fifth TMD. The difference in primary structure of the rat and human 5-HT$_2$ receptors has been advantageous in examining this possibility. The human 5-HT$_2$ receptor and rat receptor are highly homologous in their amino acid structure. Both contain Ser-239, but one of the three differences in the TMD sequence is seen at position 242 in the fifth TMD, where the human receptor expresses a Ser and the rat receptor expresses an alanine (Ala). The Ser-239 is predicted

to act as a hydrogen-bonding site for serotonin's 5-hydroxyl group in both rat and human 5-HT_2 receptors.

Ser-242 of the human receptor presents another possible hydrogen bond donor, whereas Ala-242 of the rat receptor does not. The helical structure of the fifth TMD indicates that a hydroxy group at the 4-position of the indole ring could interact with Ser-242 of the human receptor with the same geometry as 5-HT interacting with Ser-239. Therefore, a 4-hydroxyl serotonin analog should bind with higher affinity to the human receptor than to the rat receptor.

The hallucinogenic drugs 4-hydroxydimethyltryptamine (psilocin) and 5-hydroxydimethyltryptamine (bufotenin), both amino-dimethylated analogs of 5-HT, were used to examine this possibility. Psilocin bound to human receptors with a fifteenfold higher affinity than to rat receptors, whereas bufotenin bound to both species' receptors with equal affinity (less than twofold difference in dissociation constants). These results indicate the hydoxyl substituents serve as binding epitopes and that Ser-239 associates with 5-hydroxy groups as in serotonin.

The human receptor with the Ser-242 can bind 4-hydroxytryptamines such as psilocin with high affinity, but other 5-HT_2 receptors that express Ala-242 do not. These results are also consistent with observations concerning the adrenergic receptors and their interactions with ligand hydroxy groups. The conservation of hydroxyl-containing amino acids in the fifth TMD of receptors for agonists, with hydroxyl substituents (e.g., serotonin), but not in those without, also supports this analysis.

TOPOLOGICAL STRUCTURE

The role of Asp-172 at the interface of the third TMD and the second cytoplasmic loop is not clearly defined by mutagenesis to asparagine. This mutation reduces the binding affinity of agonists and antagonists; decreases the magnitude of GTP-induced binding affinity changes, but does not abolish it; and does not abolish 5-HT-stimulated PI production. These results could be interpreted to indicate that Asp-172 may serve as an epitope for the ligand amine groups as suggested for Asp-155. However, the inconsistency of mutagenesis results in other G-protein receptors indicates Asp-172 may serve another role in 5-HT_2 receptor structure. When mutated, cognate aspartic acids sometimes abolish second messenger stimulation and sometimes do not, depending on the,

receptor. Asp-172 and its cognates are found in virtually all G-protein receptors, regardless of the structure of their ligand, in a three amino acid motif of Asp-Arg-Tyr (aspartic acid-arginine-tyrosine [DRY]). Some opsin proteins express the functionally analogous motif of Glu-Arg-Tyr (ERY).

It is possible that this motif serves as a topological determinant for the positioning of the third TMD in the lipid bilayer. Charged amino acids have been shown to serve this function in other membrane proteins (Boyd and Beckwith 1989). Mutation of Asp-172 would thus result in a changed topography and affect the global 3-D structure of the receptor. This mutation would account for lowered affinities seen for ligand binding and can account for the loss of G-protein coupling in some receptors but not others. The global changes induced by this mutation would slightly change the binding site geometry, conferring the observed lower affinity binding. In some receptors the topological difference would affect G-protein coupling, whereas in others such as the 5-HT_2 receptor the change would not affect such a coupling. Different receptors couple with different G-proteins, so this interpretation is consistent with the known properties of G-proteins and the nature of membrane protein topological determinants.

FUTURE

It is evident that 5-HT_2 receptors and other G-proteins are structurally complex and that the mutagenesis studies of these receptors and proteins have just begun to address their structure-function relationship. Many more studies combining mutagenesis with biophysical investigations can reveal the molecular mechanisms of their function. Currently, mutagenesis yields the most information regarding the structure of the receptors and provides the most easily accessible, although indirect but powerful, method to study the structure and function of 5-HT receptors.

Biophysical studies that can provide direct information concerning structure and function are hampered by the unavailability of purified receptor preparations. For example, fluorescent ligand studies using purified reconstituted ß-AR have shown the binding site to reside in the hydrophobic TMD of the receptor, a finding consistent with the results of mutagenesis. Because of the low percentage of 5-HT_2 receptor protein expressed in brain tissues, the receptors are difficult to purify in any large quantity. To overcome this problem, alternate expression systems need

to be developed in microorganisms such as yeast or bacteria to provide ample source materials for purification.

Detailed knowledge of the structure and function of the 5-HT_2 receptor obtained by a variety of methods will provide a basis for designing new therapeutic agents for treating diseases associated with 5-HT_2 receptors.

The isolation of the carrier deoxyribonucleic acid (cDNA) and genomic clones of 5-HT_2 receptors provides more than just a basis for the study of their molecular structure and function. The possibility that polymorphisms in 5-HT_2 receptor genes may contribute to disease states can be examined. Any polymorphisms in the gene can be examined using cDNA or gene probes. If a polymorphism is found to be associated with a disease state, then the DNA sequences can provide possible diagnostic tools for these states.

The most exciting future project is the regulation of the 5-HT_2 receptor gene expression. The promoter region of a gene consists of the core promoter, enhancer, and repressor. The core promoter is the region controlling the transcription of the gene and is regulated by enhancers or repressors. These DNA sequences bind specific protein transcription factors to fine-tune gene expression. An example of such gene expression regulatory factor is SP-1 proteins known to be active in the regulation of monoamine oxidase A gene expression,

Researchers are at an exciting point where they can use molecular biological techniques to investigate the mechanisms for regulation of the 5-HT_2 receptor gene. It is now possible to investigate whether hallucinogenic agents play a role in the gene expression by influencing either cis-element or *trans*-element.

Moreover, it is interesting to note that there may be tissue-specific elements in the 5-HT_2 receptor promoter. Once brain-specific elements are identified, it would be possible to manipulate the expression of the receptor only in the brain to better understand the function of brain 5-HT_2 receptors and their relation to hallucinogenic effects. This new knowledge will help researchers to design therapeutic agents that are brain specific. Any mutations in this region may affect the expression of the receptor and contribute to diseases. The molecular cloning of the 5-HT_2 receptor gene allows many possibilities to be examined and can contribute to the knowledge and treatment of mental disease or other serotonin-related illnesses.

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AUTHORS

Jean C. Shih, Ph.D.
Boyd and Elsie Welin Professor

Kevin Chen, Ph.D.
Research Associate Professor

Timothy K. Gallaher, Ph.D.
Research Associate

Department of Molecular Pharmacology and Toxicology
University of Southern California School of Pharmacy
1985 Zonal Avenue
Los Angeles, CA 90033

Summary

Richard A. Glennon

It is perhaps appropriate that the National Institute on Drug Abuse (NIDA) sponsored a technical review on hallucinogenic agents during the 500th anniversary of Columbus' sailing to the New World, because it is reportedly Columbus who wrote the first account of the use of psychoactive (presumably tryptamine- or ß-carboline-containing) plants in this hemisphere (Holmstedt and Lindgren 1967; Wassen 1967).

This meeting was the first technical review devoted solely to classical hallucinogens and covered the exciting progress being made in researchers' understanding of this class of agents. Just before coming to this meeting, the author reread the monographs from the NIDA technical reviews of hallucinogenic agents held in 1976 and 1978. An enormous amount of progress has been made on some fronts, whereas more needs to be done on others. The earlier two meetings were primarily concerned with the identification of new chemical entities and the development of novel pharmacological techniques and animal models to investigate hallucinogenic agents. For example, both of those reviews were held before the widespread use of radioligand binding and autoradiography, the discovery of multiple populations of central serotonin (5-HT) receptors, elucidation of second messenger systems, molecular graphics, and receptor cloning. Consequently the present meeting addressed topics and issues and described technologies that were essentially unknown at the time of the prior meetings. Serotonin, (5-HT_2) receptors were originally identified in 1979 and were first implicated as playing a mechanistic role in the actions of hallucinogens in 1983. Much of the focus of the present meeting was on 5-HT_2 receptors.

There has been, and continues to be, dissatisfaction with the term *hallucinogen;* not all classical hallucinogens produce frank hallucinations. This point was emphasized by the first several speakers, including Szára, Glennon, and Shulgin. Szára presented a historical perspective of the different phases of research with classical hallucinogens over the years and concluded with the suggestion that these agents be referred to as *psychoheuristic* agents. Many of the attendees agreed that a new terminology would be useful, but there was no consensus on any specific term.

After an introductory overview on classical hallucinogens by Glennon, Shulgin and Strassman presented papers on the human evaluation, including qualitative and quantitative aspects, of hallucinogens. Shulgin's presentation was broad in scope, of a review nature, and covered many examples of the classical hallucinogens. In contrast, Strassman described in depth his recent clinical studies on one specific agent, N,N-dimethyltryptamine (DMT). Both speakers commented on a need for the availability of more human data; this feeling was shared by many of the participants.

During the overview, it was mentioned that structure-activity relationships (SAR) have been formulated for certain subclasses of the classical hallucinogens but that such information is rather limited with regard to ergoline derivatives. Nichols presented the results of his timely SAR studies on ergolines, with specific emphasis on the nature of substituents at the 6- and 8-positions.

The next two speakers, Geyer and Winter, presented new data and reviewed some of the older results using several animal techniques that have been employed to study a large number of classical hallucinogens and structurally related agents. It might be noted that these techniques were scarcely mentioned during the 1976 and 1978 meetings.

Aghajanian outlined his work on the electrophysiology of hallucinogens and addressed the issue of whether these agents act as agonists, antagonists, or partial agonists. Depending upon what cells are examined, it appears that hallucinogens behave as agonists or partial agonists; in some cells, there is evidence that certain hallucinogens appear to have a significantly greater intrinsic activity than 5-HT itself. Sanders-Bush also addressed this issue and further described her second messenger studies attempting to find differences in the actions of agents at 5-HT_2 and 5-HT_{1C} receptors. Although hallucinogens bind at both populations of receptors, it is not yet known which (or whether both) are involved in their mechanism of action. Sanders-Bush showed that (+)LSD is a partial agonist, and the non-hallucinogenic 2-bromo-LSD (BOL) is a pure antagonist both at 5-HT_2 and 5-HT_{1C} receptors, whereas lisuride is a pure antagonist at 5-HT_{1C} receptors but a partial agonist at 5-HT_2 receptors. Lisuride could become a useful tool to further study the role of 5-HT_{1C} versus 5-HT_2 receptors.

The locus of action of hallucinogens in the brain is unknown. With the recent availability of radiolabeled hallucinogens and hallucinogen

analogs, it should eventually be possible to identify such loci. Appel described his (and others') work using autoradiography, positron emission tomography (PET) scanning, and other imaging techniques to label 5-HT_2 and 5-HT_{1C} receptors, and apprised the participants of new techniques currently being developed.

After discussion about human and animal evaluations and studies at the molecular, receptor, and second messenger level, the concluding session was devoted to investigations at the atomic level. Weinstein and Westkaemper presented their attempts to develop molecular graphics models of 5-HT_2 receptors. Both models begin with the known structure of bacteriorhodopsin, but each investigator described a unique approach to the problem. Weinstein presented a model to account for the actions of agonists, antagonists, and partial agonists, whereas Westkaemper identified several specific amino acid residues that could be important for ligand binding. One such key residue is the aspartate of transmembrane helix III. The models, although hypothetical, provide a framework for future investigation. Indeed, both models have been recently revised (Westkaemper and Glennon 1993; Zhang and Weinstein 1993). Shih outlined her work with the cloning of 5-HT_2 receptors and with site-directed mutagenesis. Mutation of the above mentioned aspartate to arginine reduces significantly the affinity of 5-HT_2 ligands, supporting a role for this aspartate moiety in a binding interaction, Additional site-directed mutagenesis is required to further test the receptor models.

Again, it should be emphasized that the present meeting was very timely. Certain philosophical issues such as terminology and explicit definition of the actions of classical hallucinogens remain undefined but, while important, have not detracted from other scientific studies. It is realized that animal models have severe limitations but provide useful data; this is particularly true when human data are hard to come by. Nevertheless, there was a consensus that there is an urgent need for new human testing. There is also a realization that certain agents are enigmatic in that they are routinely identified as being "active" in certain animal models even though human data (scant as they may be in some instances) suggest that they are not hallucinogenic. Hopefully, these agents will be used to challenge other animal models, and, conversely, the agents themselves may become the subject of additional investigations.

The identification of 5-HT_2 (perhaps 5-HT_{1C}) receptors as possibly being involved in the action of hallucinogens has provided a focal point for new studies. State-of-the-art techniques and methodologies have been brought

to bear to investigate this possibility. There is no question that hallucinogens interact at both populations of receptors (regardless of whether this is their site of action); thus, hallucinogens are providing a stimulus for the investigation of these, and other, populations of receptors.

Legitimate human investigation with classical hallucinogens was severely curtailed about 25 years ago. During the ensuing period, a significant body of information has been accrued primarily on the basis of animal studies. Novel agents have been identified, mechanisms of action have been proposed, new animal models have been developed, and means to antagonize the effects of classical hallucinogens have been described. New clinical data are now required to challenge or validate the results of these studies.

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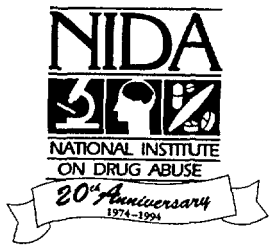

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