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RAUS

RESEARCH ANALYSIS
and
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Structure-Activity Relationships of the Cannabinoids

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration

Structure-Activity Relationships of the Cannabinoids

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Structure-Activity Relationships of the Cannabinoids

ACKNOWLEDGMENT

This monograph is based upon papers and discussion from the RAUS review on structure-activity relationships of the cannabinoids which took place on October 30 and 31, 1986, at Bethesda, Maryland. The review meeting was sponsored by the Office of Science and the Division of Preclinical Research, National Institute on Drug Abuse.

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Preface

The Research Analysis and Utilization System (RAUS) is designed to serve four functions:

- Collect and systematically classify the findings of all intramural and extramural research supported by the National Institute on Drug Abuse (NIDA);
- Evaluate the findings in selected areas of particular interest and formulate a state-of-the-art review by a panel of scientific peers;
- Disseminate findings to researchers in the field and to administrators, planners, instructors, and other interested persons;
- Provide a feedback mechanism to NIDA staff and planners so that the administration and monitoring of the NIDA research program reflect the very latest knowledge gleaned from research in the field.

Since the number of research topics that can be intensively reviewed annually is limited, four subject areas are chosen each year to undergo a thorough examination. Distinguished scientists are invited to participate. Each scientist is provided reports from NIDA-funded research and asked to add information derived from the literature and his or her own research and to prepare a comprehensive, state-of-the-art review paper on an assigned topic. These papers, together with a summary of the discussions which take place at the review meeting, make up a RAUS Review Report in the NIDA Research Monograph series.

In recent years a large number of reviews and books have appeared summarizing research advances in the chemistry, pharmacology, toxicology, metabolism, and distribution of cannabinoids and their synthetic analogs. Yet the volume of research on structure-activity relationships of cannabinoids appears to have decreased. In order to evaluate NIDA's current research program on cannabinoids, to rekindle interest in this critical area, and to focus attention on profitable avenues for future research, NIDA found it timely to bring together experts on the structure-activity relationships of cannabinoids for a comprehensive review.

Many cannabinoid analogs have been designed. Some have served as excellent probes for understanding the mechanism of action of cannabinoids and have generated optimism that in the near future potent therapeutic drugs that lack "cannabimimetic" activity can be developed. In this monograph, while emphasis is placed on structure-activity relationships and molecular mechanisms of action of cannabinoids. chapters added to broaden the subject area will increase the usefulness of this volume as a reference text for researchers in the chemistry and biology of the cannabinoids.

Drs. Rao S. Rapaka, Alexandros Makriyannis, and Sumner Burstein served as the scientific moderators of the RAUS review meeting. The introduction and the concluding overview chapter, by Drs. Makriyannis and Rapaka, provide a brief summary of the presentations and the discussions. Jacqueline P. Ludford, Chief, Research Analysis Branch, Office of Science, is the RAUS coordinator for NIDA.

Contents

Introduction

Rao S. Rapaka, Ph.D., and Alexandros Makriyannis, Ph.D.

Although a number of reviews on cannabinoids are held each year, reviews devoted entirely to structure-activity relationships of the cannabinoids are less frequent. Moreover, in recent years a slackening of interest appears to have occurred, both among academic scientists and scientists from drug houses, in undertaking studies of SAR and development of drugs based on the cannabinoid molecular skeleton. A number of related areas in the medicinal chemistry of the cannabinoids, such as development of new drugs, resolution of cannabimimetic activity from other bioactivities such as anticonvulsant action, and studies of receptors, require special attention. In order to evaluate the progress and to revitalize research in SAR and related medicinal chemistry aspects of cannabinoids, the National Institute on Drug Abuse felt that the time had come for a review and a publication on the structure-activity relationships of the cannabinoids.

The stereochemical requirements for cannablmimetic activity are one major issue. Dr. Mechoulam discusses the structure-activity relationships (SAR) with emphasis on stereochemical requirements. Dr. Mechoulam presents biological data to show that (-) enantiomers of cannabinoids are more active than the (+) enantiomers. Dr. Razdan, who was unable to attend the review, contributed an overview chapter on structure-activity relationshlp in cannabinoids, with special emphasis on SAR and drug development, and on SAR and the mechanism of action of cannabinoids. Dr. Arnold Martin discusses SAR of cannabinoids regarding anticonvulsant activity along with recent information on pinyl and carnyl analogs, simplified derivatives lacking the 4-isopropenyl group, and a variety of 1'-1' dialkylhexyl and 1'-1'-dialkeylheptyl analogs. Comparisons of anticonvulsant potency of delta-6-tetrahydrocannabinol, delta-6-THC-1', 1'-dimethylheptyl and related analogs are presented by Dr. Consroe. In addition, Dr. Consroe presents evidence for stereoselectivity for anticonvulsant and neurotoxic activities.

Drs. Melvin and Johnson review SAR of tricyclic and nonclassical bicyclic cannabinoids. Their studies demonstrate that some of the analogs are potent analgesics and exhibit enantiomeric potency. More importantly, their studies show that it is possible to achieve extreme biological potency in structures different from classical cannabinoid ring systems. This, in turn, gives the researchers optimism for further development of new analogs and potent analgesic compounds. Electrophysiological properties of cannabinoids, delta-9-THC, 11-hydroxy-delta-9--THC (the principal metabolite of THC) and cannabidiol are discussed by Dr. Turkanis. His studies demonstrate that different cannabinoids exhibit markedly different electrophysiological properties. An interesting presentation on the molecular parameters that determine the cannabimimetic activity is given by Dr. Reggio. One of the hypotheses presented by Dr. Reggio is that the basis of cannabimimetic activity is related to molecular properties conferred by a lone pair of electrons on the phenolic oxygen and to the orientation of the carboxyclic ring.

Another major point of focus is the molecular mechanisms of cannabinoid activity. Several researchers are currently involved in answering the question of "receptor" versus "membrane." Dr. Billy Martin has reviewed the area, and outlines the different points of view. Dr. Nye presents findings on the high affinity cannabinoid binding sites in the brain and on the isolation of an endogenous inhibitor of cannabinoid binding. This endogenous compound has been partially characterized and the role of this compound is not clear at this time. Dr. Makriyannis presents evidence to support the hypothesis that the cannabinoids exert their physiologic effects, at least in part, by interacting with the phospholipid component of the membrane. The dynamic and conformational changes in the bilayer that accompany the incorporation of cannabinoids has been studied using solid state NMR. Dr. Makriyannis shows that cannabinoid analogs orient in the membrane in such a manner that the phenolic hydroxyl group has an important role in determining the geometry of cannabinoid-membrane interaction. Dr. Burstein gives additional evidence for cannabinoid action at the membrane level. He also reports an important discovery that major cannabinoid metabolites antagonize the stimulatory action of THC by inhibiting a specific step in prostaglandin synthesis. A different view, which favors a specific protein site for cannabinoid action, is presented by Dr. Howlett.

Dr. Karler describes the problems involved in the bioassay of cannabinoids and points out the dramatic differences in cannabinoid activity among different species. Drs. Chiang and Rapaka make the monograph more comprehensive with their chapter on pharmacokinetics and disposition of cannabinoids. In addition, Dr. B.N. Dhawan of the Central Drug Research Institute, Lucknow, has contributed a chapter on drug abuse in India. This is especially timely as it is expected that, in the near future, there will be more scientific research collaboration between the two countries.

By bringing together the contributions of scientists having multidisciplinary research backgrounds, this monograph represents an effort to summarize the existing knowledge on the structure-activity relationships of cannabinoids and to discuss the implications of this information for further progress toward NIDA's research objectives.

Structure-Activity Relationships in Cannabinoids: An Overview

Raj K. Razdan, Ph.D.

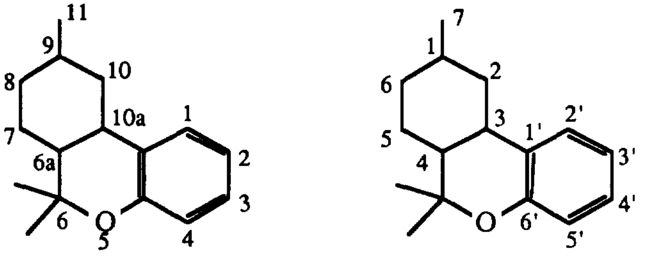
INTRODUCTION

The most important pharmacologically active member of this family of compounds is (-) delta-9-THC,6a,10a-*trans*-tetrahydrocannabinol (THC) which is the main active constituent of marijuana. Delta-9-THC is an optically active resinous material which is very lipid soluble and water insoluble, a property shared by numerous cannabinoids, which makes their pharmacological testing difficult. Both in man and animals effects produced by ingesting marijuana or delta-9-THC are unique. The gross effects produced cannot be classified pharmacologically as being primarily due to a stimulant, sedative, tranquilizer, or hallucinogen, although they share some properties with each of these. In general, the effects produced by delta-9-THC are dose dependent. It is not a narcotic and compared to many drugs, such as the opiates, barbiturates, etc., it does not produce physical dependence.

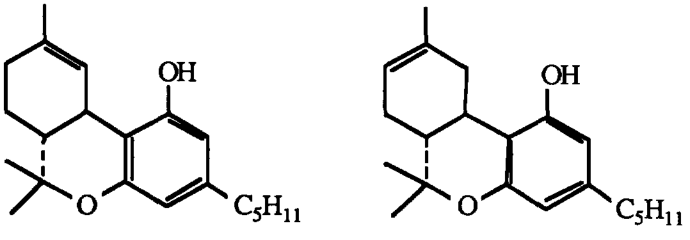
Cannabinoids is a term used for the typical C_{21} group of compounds present in *Cannabis saliva L.* and includes their analogs and transformation products. Two different numbering systems, dibenzopyran and monoterpenoid, are generally used for naming the cannabinoids, as shown in figure 1.

BACKGROUND

The first systematic study of structure-activity relationships (SAR) in cannabinoids was reported by Roger Adams in the early 1940s (Adams 1942). It was based on the characteristic dog ataxia test as developed and quantitated with estimates of error by Loewe (1947). At about the same period, Todd and others (Todd 1946; Avison et al. 1949) used the Gayer areflexia test (Gayer 1928) for suppression of rabbit corneal reflex to determine the potency of various extracts of cannabis and synthetic cannabinoids. They also reported SAR of cannabinoids based on this test. Both groups assumed that the activity in these tests was a quantitative measure of the behavioral or psychotropic activity of marijuana in man, although no controlled experiments to support this assumption were reported. Since that time, clinical evaluation of a number of natural and synthetic cannabinoids has shown that there is good correlation between the relative potency in the dog ataxia test and the psychoactive component of these compounds in man. The usefulness of the rabbit areflexia test has, however, been found to be limited and controversial; and this test is not presently used for screening cannabinoids (Razdan 1986).



Dibenzopyran Monoterpenoid



Δ^9-THC (Δ^1-THC) Δ^8-THC (Δ^6-THC)

FIGURE 1. Dibenzopyran and monoterpenoid numbering systems generally used for naming cannabinoids.

When pure delta-9-THC and other cannabinoids became available in the early 1960s, several other qualitative tests were developed to determine the "cannabis-like" activity of various compounds, for example, the cataleptic reaction in mice and the effects on motor activity and gross behavior in mice and rats. It was only after the first study was reported in 1968 on the effects of cannabinoids in monkeys (Scheckel et al. 1968) that the monkey behavioral test procedures were standardized and the SAR of cannabinoids were developed. This was largely due to the efforts of Grunfeld, Edery, and Mechoulam (Grunfeld and Edery 1969; Mechoulam et al. 1970; Edery et al. 1971, 1972). This test, which provides good correlation between potencies of various cannabinoids in monkeys and man, complements the dog ataxia test. Loewe's procedure for the dog ataxia test has been further refined and standardized by Martin, Dewey, and Harris (Martin et al. 1975, 1984a). The SAR based on both of these tests are very similar and provide a good foundation for the SAR of cannabinoids. However, it should be emphasized that these SAR are mainly reflective of the behavioral activity, rather than the other pharmacological properties, of the cannabinoids. From time to time, attempts have been made to introduce newer models for the bioassay of cannabinoids. These include the mouse ring immobility assay, the genetically unique tetrahydrocannabinol-seizure susceptible (THC-SS) rabbit, and spontaneous activity and body temperature in mice. Recently, the drug discrimination procedure has been used to study cannabinoids in rats and pigeons (Razdan 1986).

SAR IN MAN

On the basis of comparative data, delta-9-THC by smoking is considered to be 2.6 to 3 times more potent, and i.v.-administered delta-9-THC is about 10 times more potent than delta-9-THC ingested p.o. (Isbell et al. 1967; Hollister and Gillespie 1973). By either smoking or i.v., the effects of delta-9-THC appear within seconds or minutes, whereas with p.o. doses, the onset of symptoms is delayed from 30 minutes to 2 hours. The clinical syndrome, however, is very similar in all cases. Based on the various cannabinoids which have been evaluated clinically, the following SAR in man are suggested (Razdan 1986).

1. It appears that a benzopyran ring (figure 2) is a definite requirement for activity, since the ring-opened compound cannabidiol (CBD) is inactive. However, the benzopyran by itself does not confer activity, since cannabichromene is inactive. Moreover, the oxygen in the benzopyran ring can be substituted by a nitrogen without loss of activity as in levonantradol (figure 3).

2. Attachment of a *nonplanar* alicyclic ring (i.e., ring C) to the benzopyran ring in the 3,4-position is important for activity. However, a planar ring attachment as in cannabinol reduces activity (nearly inactive).

3. In place of a nonplanar ring attachment, a bulky substituent in the 4-position of the benzopyran ring can also confer activity to the molecule.

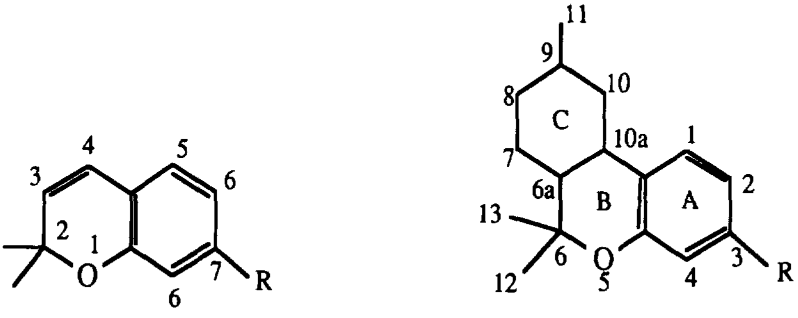
4. A variety of substituents can be introduced in the alicyclic ring C without loss of activity. Thus, the methyl in g-position as present in delta-9-THC is not essential and can be replaced by a hydroxyl (levonantradol), a hydroxymethyl (11-hydroxy-delta-9-THC), or a ketone (nabilone) without loss of activity. Even the presence of two different substituents in the alicyclic ring, such as a methyl group at 9-position and a double bond in the ring (e.g., delta-9, delta-8, delta-6a-THCs), or a hydroxyl in an S-position (8-hydroxy-THCs) retains activity. In the double-bond isomers, the position of the double bond at g-position appears optimum for activity (delta-9> delta-8> delta-6a-THCs).

5. The alicyclic ring attachment to the benzopyran ring can be substituted by a heterocyclic ring without loss of activity.

6. In the aromatic ring, esterification of the phenolic group retains activity.

7. The length of the aromatic side chain can be varied without loss of activity, but a three-carbon chain seems minimal for activity, and branching of the chain increases potency. Attachment of the side chain to the aromatic ring can also be via an oxygen atom (i.e., an ether as in levonantradol) without loss of activity.

It is noteworthy that, within the structural constraints present in the THC ring system, changing the position of the double bond in the alicyclic ring, the length and branching of the side chain, or producing hydroxy metabolites at the 11- or 8-positions *do not alter qualitatively the THC effects but may strongly affect potency.* Changing the double bond from delta-9- to delta-8- or delta-6a- reduces potency. Decreasing the length of the side chain by two carbons reduces potency by 75%. Increasing the length of the side chain with branching enhances potency several fold. Substitution of a hydroxyl group in the 11-position retains potency, while hydroxylation in the 8-position reduces potency by 80%.



Benzopyran ring system

THC ring system

FIGURE 2. Comparison of the structures of the benzopyran and THC ring systems.

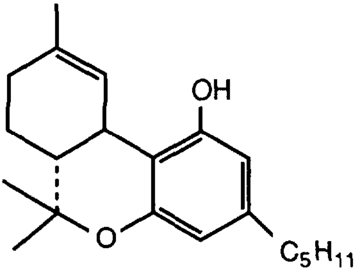
SAR IN ANIMALS

On the basis of recent data available from animals studies, the following conclusions are drawn regarding the SAR in cannabinoids. It should be emphasized that the SAR is developed on the basis of cannabis-like effects in animals, especially in the monkey and the dog. Some natural and synthetic cannabinoids are shown in figure 3.

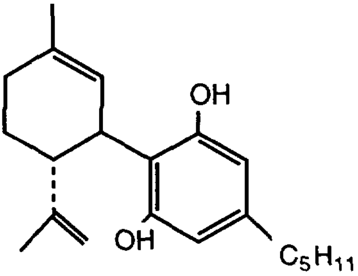
1. Essentially, a benzopyran structure with an aromatic hydroxyl group at C-l position and an alkyl or alkyl ether group at C-3 position is a requirement for activity. In general, changing the above substitution pattern leads to a major loss of potency (Avison et al. 1949; Loev et al. 1973). Although a benzopyran ring is a definite requirement for activity, the benzopyran by itself does not confer activity. This is based on the observation that the ring-opened compound CBD is inactive in both the monkey and the dog and does not generalize to delta-9-THC in the drug discrimination test in the rat. Similarly, cannabichromene, which has an intact benzopyran ring, is inactive.

2. The position and the environment around the aromatic hydroxyl group are very important for activity, viz., (a) the OH at position C-1 is in itself necessary for activity. Thus, the abnormal compounds where the hydroxyl and the side chain have been interchanged (i.e., side chain at C-1 and OH at C-3) are all inactive. Similarly, removal of OH at C-1 eliminates activity. (b) Esterification of the OH at C-1 retains activity. Etherification of the OH at C-1 eliminates activity. Replacement of the OH by NH, retains activity, and replacement by SH eliminates activity. (c) Since C-10 in the alicyclic ring C is in close proximity to the OH group, a methyl substituent at C-10 significantly alters the activity in the case of a planar five-membered C-ring.

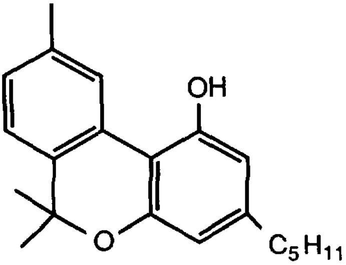
3. Substitution in the aromatic ring by electronegative groups like carboxyl, carbomethoxyl, and acetyl groups eliminates activity, whereas alkyl or hydroxyl groups at C-2 position retain, and in C-4 position reduce, activity.



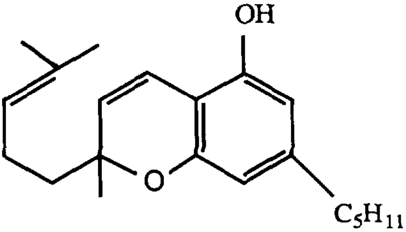
Δ^9-THC



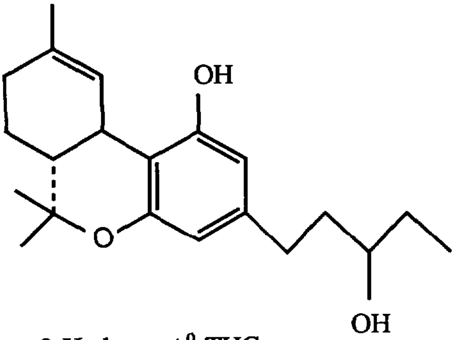
Cannabidiol



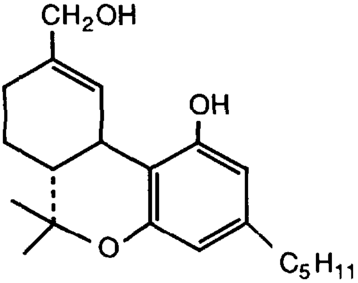
Cannabinol



Cannabichromene

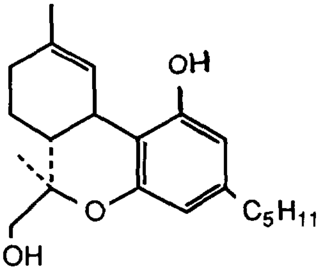


3-Hydroxy-Δ^9-THC

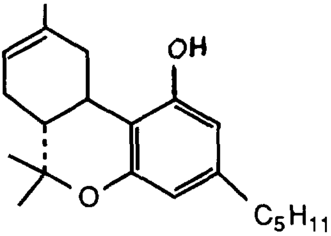


11-Hydroxy-Δ^9-THC

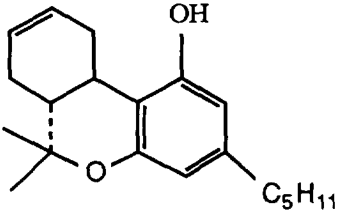
FIGURE 3. Some natural and synthetic cannabinoids.



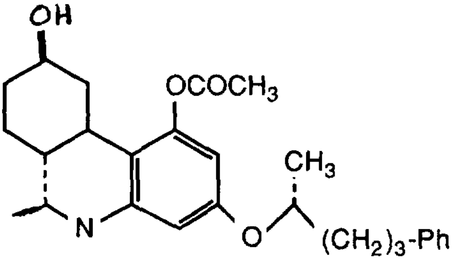
12-ß-Hydroxy-Δ^9-THC



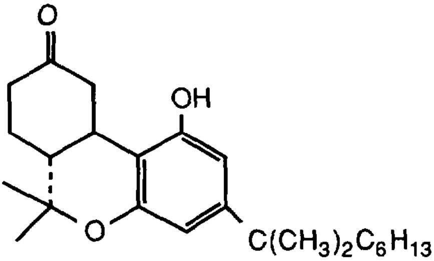
Δ^8-THC



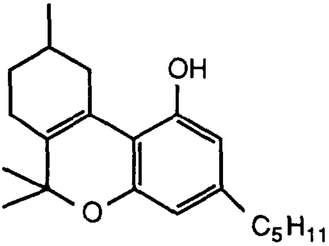
9-Nor-Δ^8-THC



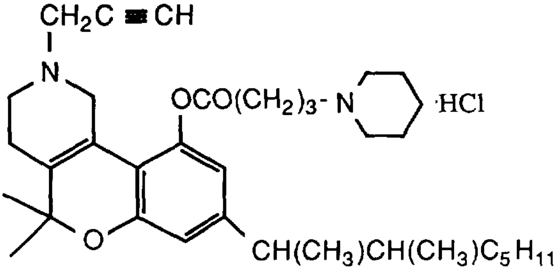
Levonantradol



Nabilone



$\Delta^{6a,10a}$-THC



Nabitan

4. A minimum length of the aromatic side chain is necessary to elicit activity, and the branching of the alkyl side chain increases potency. Thus, 1',2'-dimethylheptyl or 1', 1'-dimethylheptyl gives the most potent compounds. Similarly, *p*-fluorophenyl alkyl and side chains as shown below retain good activity:

—O—$CH(CH_3)(CH_2)_3$-Ph,—$CH_2CH{=}CHCOC_5H11$,and—$CH_2CH_2CH(OH)C_2H_5$

5. On ring B, removal of the gem dimethyl group at C-6 decreases activity. Replacement of one of the geminal methyl groups at C-6 by a hydroxymethyl group retains activity (12 beta-hydroxy-delta-9-THC is as active as delta-9-THC). Replacement of pyran O by N and ring expansion of ring B by one carbon can retain activity.

6. In the alicyclic ring C, compounds with the double bond in delta-9-, delta-8-, or delta-6a,10a-position are active. It is noteworthy that compounds with a double bond in the delta-10,10a-position are also active, but only a few examples are available at present. A 6a,10a-*trans* junction increases, and a *cis* junction decreases, activity. The natural THCs are active in the 10a R and 6a R series only. A methyl at C-9 increases and is optimum for activity, but metabolism to the 11-hydroxy-methyl is not a prerequisite for THC activity, since g-nor-delta-8-THC is active. Compounds are active even when no double bonds are present in the ring, e.g., the HHCs. In these compounds, an equatorial group at C-9 increases activity, and an axial group decreases activity. Furthermore, the activity is retained even when a diverse group is present, such as an epoxide, a ketone, or an alcohol.

7. The C-ring can be substituted by a variety of nitrogen- and sulfur-containing rings without loss of activity. With the nitrogen and sulfur analogs, the most active central nervous system (CNS) agents are obtained when the heteroatom is in a phenethyl orientation, e.g., inserted in place of C-7 or C-9.

8. Planarity of the C-ring is not a necessary criterion for activity.

9. In both carbocyclic and heterocyclic analogs, opening the pyran ring generally decreases activity.

SAR AND DRUG DEVELOPMENT

It is noteworthy that the active constituent of marijuana, delta-9-THC, and a closely related synthetic analog, nabilone, have been recently approved for marketing in the United States as an antinauseant in cancer chemotherapy treatment. This may mark the beginning of therapeutic drug development from this field. It is quite obvious that the future development of clinically useful drugs from this field will undoubtedly depend on the success achieved by synthesis in introducing structural changes in the THC molecule, which will lead to selectivity of pharmacological action. This presupposes the availability of reliable animal models for screening this class of drugs. The shortcomings in this area of pharmacological evaluation are numerous.

There is the difficulty of initial screening, i.e., the selection of compounds prior to the more expensive and more sophisticated behavioral tests in dogs and monkeys. Generally, the activity cage rotorod, the hypothermia, and the popcorn tests in mice and rats are used for initial screening. What relevance these tests have in identifying potential therapeutic candidates is presently not clear. Primarily, these tests only give an indication that the compound is "cannabis-like" and will show a similar profile. There is need for the development of other rodent models, which will provide a lead to selectivity of pharmacological action.

For more advanced screening, the presently available pharmacological models for cannabinoids, such as the dog ataxia and the monkey behavioral tests, do provide a good indication of the psychoactive component of these compounds, since there is a good correlation in extrapolating from animal data to humans. But a problem arises in quantifying the extent of separation of psychoactive and other CNS side effects from therapeutic effects.

Unless progress is made in this direction, one would be left with no recourse but to rely on the importance of early studies in humans. The extent of separation of undesirable CNS side effects from clinically useful effects could only be meaningfully gauged in human studies. This point is well illustrated by the results obtained in the clinical evaluation of analgesics. Nabitan and levonantradol, two synthetic nitrogen analogs of THC, were both developed as analgesics in cancer and postoperative pain, respectively, but the incidence of side effects was higher than the animal data indicated (Razdan and Howes 1983).

In drug development from this field, the above clinical experience highlights the point that, so far, very few cannabinoids have been studied in the clinic, and the baseline data generated are not sufficient to provide proper guidelines for extrapolation of animal data to humans in terms of dosages and side effect to therapeutic ratios. When more compounds have been clinically evaluated, the interpretation of animal data will become more meaningful.

It appears that further modifications in the structures of nabitan and levonantradol will be necessary to develop useful analgesics from this field. It should be emphasized that the potential exists for development of novel analgesics from this field, as cannabinoids act at different receptors than the opiates (analgesic action is not antagonized by naloxone), do not have the physical dependence liability and respiratory depression properties of the strong opiates, and are orally active with a long duration of action.

Anticonvulsants are one area where animal studies have provided a good indication for a clinical candidate from this field (Razdan and Howes 1983 and references cited therein). Of all the natural constituents of marijuana studied for antiepileptic activity, CBD was proposed as the agent of choice. This is primarily because in animal screens it was found to be devoid of marijuana-like CNS effects, a finding that was confirmed in humans. In the only clinical study (Razdan and Howes 1983 and references cited therein), CBD was reported to be effective. This bears out the animal model data. Further confirmation of these results and more clinical studies will no doubt be required before CBD’s place in the treatment of epilepsy can be judged.

There are a few other instances (Adams et al. 1942; Hartley et al. 1978; Leite et al. 1982) where animal models have indicated a clear separation or dissociation of cannabinoid (psychotropic effects) from other therapeutic effects. However, it remains to be seen if they can be substantiated in the clinic.

SAR AND MECHANISM OF ACTION OF CANNABINOIDS

Recent SAR studies have been directed toward elucidation of the mechanism of action of cannabinoids. It is well known that in spite of extensive knowledge of the pharmacological actions of cannabinoids, the mechanisms involved in producing these effects have not been established (Dewey 1986; Martin 1986). The effects of cannabinoids on the CNS are numerous and include CNS depression, ataxia, psychoactive effects, hypothermia, analgesia, cardiovascular effects, etc. They are known to alter neurotransmitter functionality, enzyme activity, prostaglandin synthesis, membrane perturbation, etc. It is therefore highly likely that multiple mechanisms are involved in producing the cannabinoid behavioral syndrome.

Currently, two mechanisms are proposed which may account for the central effects of cannabinoids, namely, general membrane perturbation, and direct interaction with a specific cannabinoid receptor. If cannabinoids are producing their effects solely by perturbation of membranes, there should not be a specific antagonist but there may be a correlation between activity and membrane (lipid) solubility. On the other hand, if they are interacting with a specific receptor and binding sites, then there should be a strict SAR as well as a specific antagonist.

Strict structural requirements for cannabinoid behavioral activity are supported by SAR studies. Stereospecificity has been demonstrated for delta-9-THC since (-)-*trans*-delta-9-THC is many times more potent than the (+)-isomer in a variety of tests (Mechoulam and Edery 1973; Jones et al. 1974; Martin et al. 1981). In the 3'-hydroxy-delta-9-THC series, the S-isomer is much more active than the R-isomer (Martin et al. 1984b). Furthermore, greater potency is found in cannabinoids with the *trans* ring junction (6a,10a) as compared to the *cis* junction; the *cis* isomers are much less active (Martin et al. 1981).

Recent studies on the evaluation of cannabinoids for antagonistic properties have shown that 10-N-ethylamino-CBD is a partial antagonist of delta-9-THC-induced antinociception but does not produce an antagonism of delta-9-THC's effects on spontaneous activity and rectal temperature in mice. In addition, it does not alter the behavioral effects of delta-9-THC in dogs (Jorapur et al. 1985; Martin et al., this volume). Therefore, the attenuation of delta-9-THC's effects by this compound appears to be limited to mouse antinociception only. Another compound which has shown interesting properties in this regard is the exocyclic analog, delta-9,11-THC. Pretreatment with delta-9,11-THC in mice did not attentuate any of the effects of delta-9,11-THC. The study of antagonistic effects of this compound in the dog was hampered by the fact that it had some depressant properties in this behavioral test. However, in the monkey test, pretreatment with delta-9,11-THC attentuated the delta-9-THC-induced ptosis, sedation, ataxia, and operant behavior. The delta-9-THC-induced operant suppression was decreased by as much as 25 times. This suggests a lead for a possible antagonist and points out that the monkey may be a better animal model suited for characterizing cannabinoid antagonistic activity than other behavioral tests (Beardsley et al., in press; Martin et al. this volume).

Based on the work discussed above and additional SAR developed recently, Martin and coworkers have proposed that the pharmacological properties unique to the cannabinoid class of drugs are produced by the interaction of delta-9-THC with specific receptors, while cannabinoid-induced CNS depression is a general phenomenon produced by nonspecific membrane interactions (Martin et al. this volume). Examination of the pharmacological profile in mice, dogs, and monkeys of various cannabinoids, including the recently synthesized novel 11-, 12 beta-, and side-chain functionalized THCs, have led Martin's group to classify cannabinoids as follows: Analogs which produce the entire spectrum of cannabinoid effects are grouped in class 1. CBD and many of its analogs appear to be devoid of cannabinoid effects, but they are capable of producing weak CNS depression and are grouped in class 2. Whereas, class 3 compounds are potent CNS depressants that appear to lack other effects that are unique to cannabinoids, and analogs in class 4 appear to exert selective CNS depression (Martin et al. this volume). Therefore, recent studies indicate that structural modifications in the cannabinoid molecule can lead to compounds which show either a selective or a variety of pharmacological actions.

CONCLUSION

Despite the extensive knowledge about the pharmacological actions of cannabinoids, the mechanisms involved in producing these effects have not been established. Difficulties are encountered in correlating animal activity with activity in man for this class of compounds, and this makes the task of clinical evaluation and determination of therapeutic utility very complex. This is borne out by the fact that the two most promising therapeutic areas for the cannabinoids, antiemetic and antiglaucoma activities, were discovered serendipitously without any preclinical pharmacology.

In spite of difficulties and complexity in the evaluation of these compounds, progress is being made. Lately, several novel behavioral and drug discrimination tests have been designed and introduced to test these compounds, and a separation of several specific pharmacological actions has been noted in different analogs. A basis for the pharmacological action of cannabinoids has been proposed. Hopefully, with the continued progress in this field and the extended clinical usage of delta-9-THC and nabilone, other areas of therapeutic use will become evident, and the potential of this class of novel drugs will be realized.

REFERENCES

Adams, M.D.; Earnhardt, J.T.; Martin, B.R.; Harris, L.S.; Dewey, W.L.; and Razdan, R.K. A cannabinoid with cardiovascular activity but no overt behavioral effects. Experientia 33:1204-1205, 1977.

Adams, R. Marijuana. Harvev Lect 37:168-197, 1942.

Avison, A.W.D.; Morrison, A.L.; and Parkes, M.W. Tetrahydrodibenzopyran derivatives isomeric with tetrahydrocannabinols. J Chem Soc (Lond) 952-955, 1949.

Beardsley, P.M.; Scimeca, J.A.; and Martin, B.R. Studies on the agonistic activity of delta-9,11-tetrahydrocannabinol in mice, dogs and rhesus monkeys and its interactions with delta-9-tetrahydrocannabinol. J Pharmacol Exp Ther, in press.

Dewey, W.L. Cannabinoid pharmacology. Pharmacol Rev 38:151-178, 1986.

Edery, H.; Grunfeld, Y.; Ben-Zvi, Z.; and Mechoulam, R. Structural requirements for cannabinoid activity. Ann NY Acad Sci 191:40-53, 1971.

Edery, H.; Grunfeld, Y.; Porath, G.; Ben-Zvi, Z.; Shani, A.; and Mechoulam, R. Structure-activity relationships in the tetrahydrocannabinol series. Arzneimittelforsch 22:1995-2003, 1972.

Gayer, H. Pharmakologische Wertbestimmung von Oriontatischem Hashish und herba. Cannabis, Indicae. Arch Exp Pathol Pharmacol 129:312-318, 1928.

Grunfeld, Y., and Edery, H. Psychopharmacological activity of the active constituents of hashish and some related cannabinoids. Psychopharmacologia. 14:200-210, 1969.

Hartley, J.P.R.; Nogrady, S.G.; Seaton, A.; and Graham, J.D.P. Bronchodilator effect of delta-9-THC. Br J Clin Pharmacol 5:523-524, 1978.

Hollister, L.E., and Gillespie, H.K. delta-8- and delta-9-Tetrahydrocannabinol. Comparison in man by oral and intravenous administration. Clin Pharmacol Ther 14:353-357, 1973.

Isbell, H.; Gorodetsky, C.W.; Jasinski, D.; Clauseen, U.; Spulak, F.V.; and Korte, F. Effects of (-) delta-9-*trans*-tetrahydrocannabinol in man. Psychopharmacologia 11:184-188, 1967.

Jones, G.; Pertwee, R.G.; Gill, E.W.; Paton, W.D.M.; Nilsson, I.M.; and Agurell, S. Relative pharmacological potency in mice of optical isomers of delta-1-tetrahydrocannabinol. Biochem Pharmacol 23:439-446, 1974.

Jorapur, V.S.; Khalil, Z.H.; Duffley, R.P.; Razdan, R.K.; Martin, B.R.; Harris, L.S.; and Dewey, W.L. Hashish: Synthesis and central nervous; activity of some novel analogs of cannabidiol and oxepin derivatives of ^{9}-tetrahydrocannabinol. J Med Chem 28:783-787, 1985.

Leite, J.R.; Carlini, E.A.; Lander, N.; and Mechoulam, R. Anticonvulsant effects of the (-) and (+) isomers of cannabidiol and their dimethylheptyl homologs. Pharmacoloay 24:141-146, 1982.

Loev, B.; Bender, P.E.; Dowalo, F.; Macko, E.; and Fowler, P.J. Cannabinoids. Structure-activity studies related to 1,2-dimethylheptyl derivatives. J Med Chem 16:1200-1206, 1973.

Loewe, S. Bioassay by direct potency estimation. Science 106:89-91, 1947.

Martin, B.R. Cellular effects of cannabinoids. Pharmacol Rev 38:45-74, 1986.

Martin, B.R.; Dewey, W.L.; Harris, L.S.; Beckner, J.; Wilson, R.S.; and May, E.L. Marijuana-like activity of new synthetic tetrahydrocannabinols. Pharmacol Biochem Behav 3:849-853, 1975.

Martin, B.R.; Balster, R.L.; Razdan, R.K.; Harris, L.S.; and Dewey, W.L. Behavioral comparison of the stereoisomers of tetrahydrocannabinols. Life Sci 29:565-574, 1981.

Martin, B.R.; Harris, L.S.; and Dewey, W.L. Pharmacological activity of delta-9-THC metabolites and analogs of CBD, delta-8-THC, and delta-9-THC. In: Agurell, S.; Dewey, W.L.; and Willette, R.E., eds. The Cannabinoids; Chemical, Pharmacologic, and Therapueutic Aspects. Orlando, FL: Academic Press, Inc., 1984a. pp. 523-544.

Martin, B.R.; Kallman, M.J.; Kaempf, G.F.; Harris, L.S.; Dewey, W.L.; and Razdan, R.K. Pharmacological potency of R- and S-3'-hydroxy-delta-9-tetrahydrocannabinoh Additional structural requirements of cannabinoid activity. Pharmacol Biochem Behav 21:61-65, 1984b.

Mechoulam, R., and Edery, H. Structure activity relationships in the cannabinoid series. In: Mechoulam, R., ed. Marijuana: Chemistrv, Pharmacology, Metabolism, and Clinical Effects. New York: Academic Press, Inc., 1973. pp. 101-136.

Mechoulam, R.; Shani, A.; Edery, H.; and Grunfeld, Y. Chemical basis of hashish activity. Science 169:611-612, 1970.

Razdan, R.K. Structure-activity relationships in cannabinoids. Pharmacol Rev 38:75-149, 1986.

Razdan, R., and Howes, J.F. Drugs related to tetrahydrocannabinol. Med Res Rev 3:119-146, 1983.

Scheckel, C.L.; Boff, E.; Dahlen, P.; and Smart, T. Behavioral effects in monkeys of racemates of biologically active marijuana constituents. Science 160:1467-1469, 1968.

Todd, A.R. Hashish. Experientia 2:55, 1946.

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Stereochemical Requirements for Cannabimimetic Activity

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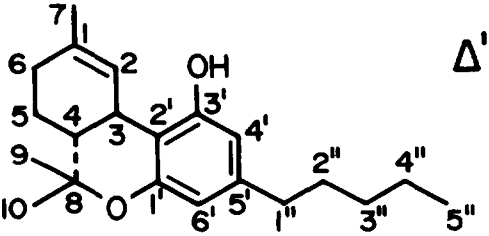
About 15 years ago, we formulated some tentative rules for cannabimimetic structure-activity relationships (SAR) (Edery et al. 1971; Mechoulam and Edery 1973). These rules were based on work our group had done since 1964, when we isolated and elucidated the structure of delta-1-tetrahydrocannabinol (delta-1-THC) (Gaoni and Mechoulam 1964), as well as on research by numerous other groups. Most of our work was done with rhesus monkeys; much of the work by the other groups was done with either rodents or dogs. However, gratifyingly, the results obtained with different species were qualitatively comparable.

Most of the rules have withstood the erosion of time, although exceptions have been noted and certain refinements have to be made.



Δ^1-THC

The generalisations listed below apply today:

1. A dihydro-benzopyran type structure with a hydroxyl group at the 3’ aromatic position and an alkyl group on the 5’ aromatic position seems to be a requirement. Opening of the pyran ring generally leads to complete loss of activity.

However, several major exceptions have been found, such as compounds (1) and (2) and some of their derivatives (Johnson and Melvin 1986). Hopefully, future work will throw light on these discrepancies,

2. The aromatic hydroxyl group has to be free or esterified. Blocking of the hydroxyl group as an ether inactivates the molecule. It is possible that the esters are actually inactive but undergo hydrolysis in vivo to the free phenols. Thus, delta-1-THC acetate, when tested in vitro, shows negligible activity in biochemical reactions in which delta-1-THC is active (Banerjee et al. 1975).

3. An all carbon side chain on C-5’ is not an absolute requirement. The side chain may contain an etheric oxygen (e.g., compound 3) (Loev et al. 1973).

4. 7-Hydroxy THCs, which are major metabolites, are very potent cannabimimetics. Monohydroxylation on other position of the terpene ring also usually leads to active derivatives. Dihydroxylation generally causes loss of activity. Further oxidation of the C-7 hydroxyl group to a carboxyl group causes inactivation.

5. Hydroxylation on the C-1" of the side chain abolishes activity. Hydroxylation at the other side chain carbons retains activity with hydroxylation on C-3" potentiating activity. Some of these hydroxylated compounds have been detected as major metabolites.

6. Alkylation on the C-4’ aromatic position retains activity; alkylation on the C-6’ position eliminates activity. Electronegative groups, such as carbonyl or carboxyl, at either C-4’ or C-6’ eliminate activity.

7. The double bond isomers of THC show the following order of activity in humans: delta-1-THC> delta-6-THC> delta-3-THC (Hollister 1974). Delta-5-THC and delta-7-THC are inactive in animal tests, while delta-2-THC and delta-4-THC have not been tested yet.

8. The 7-methyl group is not an absolute requirement for activity: 7-nor-delta-1-THC (4) and 7-nor-delta-6-THC (5) are active in dogs (Wilson and May 1974).

9. The terpenoid ring may be exchanged by some heterocyclic systems (Pars et al. 1977; Lee et al. 1983).

10. The 1,1- or 1,2-dimethyl heptyl (DMH) side chain strongly potentiates the cannabimimetic activity of compounds which have low activity in the n-pentyl series.

The above rules are, in reality, approximations. In the cannabinoid series, for example, compounds which are considered inactive in some tests frequently show low activity in other cannabimimetic tests.

The above SAR findings do not cover stereochemical requirements for cannabimimetic activity. In the present summary, we will bring up to date the SAR in this area, with emphasis on as yet unpublished material from our laboratories.

C-1 EPIMERS

Reduction of the double bond in delta-1- or delta-6-THC leads to the formation of two C-1 epimers (Gaoni and Mechoulam 1966). The equatorial epimer, in which the methyl group on C-1 is almost in the plane of the hexahydrocannabinol molecule (compound 6a), is about as active in the rhesus monkey as delta-6-THC and is about 20 times more active than the axial epimer (7a), in which the methyl group is below the plane (Mechoulam et al. 1980).

The same relationship is observed when the C-1 substituent is an acetoxymethyl group: epimer (6b), in which the C-1 substituent is equatorial, is active in monkeys at doses of 0.5 mg/kg while the axial epimer (7b) is inactive at doses 10 times higher (Mechoulam et al. 1980).

The same trend is observed in drug discrimination tests. Rats and pigeons trained to discriminate between the presence and absence of the effects of delta-1-THC (3 and 0.56 mg/kg, respectively) were tested for generalization with the two epimeric 7-hydroxy-hexahydrocannabinols (6c and 7c). Both epimers generalized with delta-1-THC in rats as well as in pigeons (table 1) (Jarbe et al. 1986). As expected, the equatorial isomer (6c) was considerably more active than the axial one (7c). Surprisingly, compound (6c) was only 5 to 7 times more active in the rat than (7c), while in the pigeon, compound (6c) was 85 times more active. Apparently, species specificity in this test is very pronounced.

TABLE 1

Discrimination of 7-OH-Hexahydrocannabinols (6c and 7c) to delta-1-THC

Hrs	Δ^1 THC	Compound (6c) (equat)	Compound (7c) (axial)
		Rats (i.p.)	
0.5	0.85 (0.94)	0.24 (0.95)	1.58 (0.98)
1.5	1.07 (0.94)	0.44 (0.997)	2.16 (0.96)
4.5	4.78 (0.79)	-	-
		Pigeons (i.m.)	
0.5	0.53 (0.92)	0.03 (0.99)	2.60 (0.58)
1.5	0.16 (0.91)	0.02 (0.87)	1.72 (0.97)
4.5	0.25 (0.94)	-	-
9.0	1.21 (0.70)	-	-

Median dose (mg/kg) effect estimates, ED_{50}, according to logarithmic regression analysis and within brackets corresponding fits (r) for the regressions are given. Absence of an estimate (-) means no value in the dose-generalization curve determination was above 50% drug appropriate responding (% RDP). (Jarbe et al. 1986)

The above experimental data point out that, in the absence of other molecular changes, the planarity at the C-1 position determines cannabimimetic activity.

The above tentative rule does not necessarily apply to other biological activities. Thus, the axial 1-alpha-7-dimethylamino-hexahydrocannabinol (8) causes intense yawning in monkeys, while the equatorial 1-beta-7-dimethylamino-hexahydrocannabinol (9) is inactive. Neither (8) nor (9) are cannabimimetic (Edery et al. 1984).

SUBSTITUTION ON C-1, C-2 VERSUS SUBSTITUTION ON C-1, C-6

Two groups of THC-type cannabinoids have been synthesized which differ only in that the chemical groupings in one of them at C-1, C-2 are situated at C-1, C-6 in the other (but retain their stereochemistry). They show almost equivalent cannabimimetic activity.

Thus, 2-alpha-hydroxy-1-beta-hexahydrocannabinol (10) is essentially inactive, as is 6-alpha-hydroxy-1-beta- hexahydrocannabinol (11); 2-beta-hydroxy-1-alpha- and 6-beta-hydroxy-1-alpha- hexahydrocannabinol (12 and 13, respectively) are also inactive. However, both 2-alpha-hydroxy-1-alpha-hexahydrocannabinol (14) and 6-alpha-hydroxy-1-alpha-hexahydro-cannabinol (15) are active at the same dose levels (Mechoulam et al. 1980).

A biochemical rationalization of the above data is not obvious at present.

SUBSTITUTION ON C-6 AND C-5

Several groups have compared the cannabimimetic activity of 6-beta-hydroxy-delta-1-THC (16) and 6-alpha-hydroxy-delta-1-THC (17) (for a review, see Narimatsu et al. 1985). In man, the 6-beta-epimer (16), in which the hydroxyl group is axial, is more active than (17); but the reverse has been observed in mice and rabbits, and they are equiactive in rhesus monkeys. Recently, the alpha-epimer (17) was again found to be less active than the 6-beta-epimer (16) in a mouse catalepsy test but more potent in a test for hypothermia and equipotent in barbiturate synergism.

5-beta-Hydroxy-delta-6-THC (18) and 5-alpha-hydroxy-delta-6-THC (19) have been tested in rhesus monkeys only (Mechoulam et al. 1972). The 5-beta-epimer (18), in which the hydroxyl group is equatorial, was six to eight times less active than the 5-alpha-epimer (19), in which the hydroxyl group is axial.

The only conclusion that can be drawn (though somewhat fatuous) is that the level of cannabimimetic activity of THCs hydroxylated at some allylic positions seems to depend on the test used.

SUBSTITUTION ON THE SIDE CHAIN

A Pfizer group (Milne et al. 1980; Johnson and Melvin 1986) has found that diastereoisomer A (20) is at least 10 times more potent than its side chain counterpart, diastereoisomer B (21). in analgetic tests. As analgetic activity in this series parallels cannabimimetic activity, one can presume that the same ratio of activity will be observed in cannabimimetic tests.

Martin et al. (1984) have reported that (3"S)-3"-hydroxy-delta-1-THC (22) is circa (ca) five times more active than delta-1-THC and is about seven times more active than the R epimer (23) in THC discrimination in rats. Approximately the same ratio of activity was observed in dog ataxia tests; in hypothermia, (22) and (23) are surprisingly equiactive. However, Martin and coworkers pointed out that "calculation of exact potency ratios is complicated somewhat by the lack of absolute purity of the isomers."

3,4-CIS-THCs

Racemic 3,4-cis-delta-1-THC (24), racemic 3,4-cis-delta-6-THC (25), and (+)-3,4-cis-delta-1-THC appear to be essentially inactive in several animal tests (Edery et al. 1971; Uliss et al. 1975; Martin et al. 1981).

CANNABINOID ENANTIOMERS

Delta-3-THC

This cannabinoid in its racemic form was synthesized in the early 1940s. Although attempts were made then to obtain the enantiomers in a pure form, they were only partially successful. Recently, we were able to synthesize both (1S)-delta-3-THC (26), [alpha]D - 117° and (1R)-delta-3-THC (27). [alpha]D + 114° with absolute optical purity (Srebnik et al. 1984). These enantiomers have now been tested in human volunteers (Hollister et al., in press). Subjects received progressively increasing intravenous doses of (1S)-delta-3-THC (26) and (lR)-delta-3-THC (27), beginning with 1 mg (in 1 ml ethanol) followed by progressive doubling of doses until. definite effects were observed. Cannabimimetic effects with the (1S) epimer were noted at doses of 8 mg or higher. No effects were noted with the (1R) epimer. (1S)-delta-3-THC (26) is thus estimated to have a potency from one-third to one-sixth that of delta-1-THC.

The behavioral cannabimimetic effects observed with (lS)-delta-3-THC (26) were indistinguishable from those caused by delta-1-THC. As with delta-1-THC, an increase in the pulse rate was also noted. These experiments are the first ones with THC enantiomers in human subjects.

(-)-(3R,4R)-THCs versus (+)-(3S,4S)-THCs

The natural delta-1-THC and delta-6-THC have a (3R,4R) configuration and a negative rotation. The synthetic route, which was developed by our laboratory for delta-1-THC and delta-6-THC nearly 20 years ago (Mechoulam et al. 1967), makes possible also the synthesis of the unnatural (3S,4S) enantiomers as the starting material; verbenol (28) is available in both enantiomeric forms. This sequence has been widely used for the preparation of (+) cannabinoids and apparently is the only one still practical for the synthesis of (+)-delta-1-THC. (+)-Verbenol on conden- sation with olivetol leads to (29), which on ring opening leads to (+)-(3S,4S)-delta-6-THC (30), which can be converted with ease into (+)-(3S,4S)-delta-1-THC (31).

In several tests for cannabimimetic activity (Edery et al. 1971; Jones et al. 1974; Martin 1986), (+)-delta-1-THC (31) was ca 13 to 230 times less active than the (-)-isomer. These results indicate pharmacologic enantiomeric preference rather than absolute stereoselectivity. Indeed, Martin pointed out that, "while cannabinoid SAR supports the concept of a specific cannabinoid receptor, a disconcerting element is the apparent lack of greater stereoselectivity in some animal models." However, because the starting material (+) verbenol was not necessarily stereochemically pure, this conclusion is tentative at best.

Recently in our laboratories, we were able to prepare a pair of crystalline enantiomeric cannabinoids (compounds 32 and 33) which could be recrystallized to absolute enantiomeric purity. Their synthesis is based on myrtenol, which can be obtained in both enantiomeric forms. The primary allylic group is first blocked as a pivalate ester (reaction a) (compound 34). If the blocking group is the more mundane acetate, the penultimate reaction (reaction e) proceeds in an undesirable fashion leading to the benzofuran (36). Oxidation of (34) leads to the crystalline ketone (35) which can be highly purified by crystallization. The synthetic sequence then follows the standard procedure described above, leading ultimately to the crystalline (+)-(3S,4S)-7-hydroxy-delta-6-THC-DMH (33). The configuration at C-3 and C-4 depends on the absolute configuration of myrtenol (-) mrytenol leads to the (-) enantiomer (32); (+) myrtenol leads to the (+) enantiomer (33).

Compounds (32) and (33) were tested in several independent cannabimimetic tests in Jerusalem, in Uppsala, and in Tucson. In all tests, the (-) enantiomer (32) exhibited potent cannabimimetic activity; the (+) enantiomer was inactive.

In Jerusalem, the compounds were examined in the mouse ring test. The (-) enantiomer (32) was ca 100 times more active than natural delta-6-THC: the ED_{50} of delta-6-THC was 5 mg/kg; the ED_{50} of (32) was 0.05 mg/kg. The (+) enantiomer (33) was inactive up to 20 mg/kg. In Tucson, the same type of results were observed in the rotorod neurotoxicity test in rats (see table 2). The (-)-enan-tiomer (32) was ca 260 times more potent than natural (-)-delta-6-THC; the (+)-enantiomer (33) was inactive at doses ca 2,000 times higher than those of the ED_{50} of the (-)-enantiomer. In Uppsala, the compounds were tested in generalization tests with rats and pigeons. (-)-7-OH-delta-6-THC-DMH (32) was ca 87 times more active than natural delta-1-THC in the rat and ca 73 times more active in the pigeon (see table 3). The (+)-enantiomer (33) was inactive at doses ca 1,000 times and ca 4,500 times (for rats and pigeons, respectively) higher than those of the ED_{50} of the (-) enantiomer (32).

TABLE 2

Dose-Response Parameters of THC Stereoisomers in Rotorod (ROT) Neurotoxicity Tests in Rats[a]

Drug	ROT-TD_{50} (95% CL)
(-)-7-OH-delta-6-THC-DMH (32)	0.007 (0.005-0.009)
(+)-7-OH-delta-6-THC-DMH (33)	Not active[b]
(-)-delta-6-THC	1.85 (0.98--3.45)
(+)-delta-6-THC	21.66 (19.81-23.68)
(-)-delta-6-THC-DMH	0.034 (0.031-0.040)
(+)-delta-6-THC-DMH	0.53 (0.37-0.76)

[a] Median toxic (TD_{50}) dose and 95% confidence limits (CL) are in mg/kg.

[b] At 1.0, 7.0, and 15.0 mg/kg; higher doses were not tested due to insufficient quantity of drug.

TABLE 3

Generalization Tests with Various Doses of (-)-delta-1-THC, (-)-7-OH-delta-6-THC-DMH, and (+)-7-OH-delta-6-THC-DMH in Rats and Pigeons Trained to Discriminate Between the Presence and Absence of the Effects Induced by delta-1-THC

Compound	n	Dose Range (mg/kg)	Interval Range (hr)	(mg/kg)	r
(-)-delta-1-THC (R)	14	0.30-5.6	0.5-6.5	0.85	0.94
(-)-delta-1-THC (P)	7	0.10-0.56	0.5-9.0	0.16	0.91
(-)-7-OH-delta-6-THC-DMH (32) (R)	11	0.003-0.03	0.5-6.5	0.0098	0.99
(-)-7-OH-delta-6-THC-DMH (32) (P)	5-7	0.0001-0.0056	0.5-9.0	0.0022	0.99
(+)-7-OH-delta-6-THC-DMH (33) (R)	4-9	3.0- 10.0	0.5-6.5	>10.0	-
(+)-7-OH-delta-6-THC-DMH (33) (P)	4	3.0-10.0	0.5-9.0	>10.0	-

The animals, rats (R) and pigeons (P), were trained to discriminate between (-)-delta-1-THC (3 mg/kg, rats; and 0.56 mg/kg, pigeons) and vehicle administered 0.5-hr i.p. (rats) or 0.5-hr i.m. (pigeons) prior to session onset. The ED_{50} values are based on the % RDP (percentage of responding to drug, THC, associated position) during the test probe yielding the highest % RDP, i.e., the lowest ED_{50} value. n = the number of observations on which data points are based. ED_{50} values refer to logarithmic regression analysis, and r refers to the correlation coefficient for the regression. The solubilization vehicle consisted of 5% (v/v) of propylene glycol, 2% Tween-80 (v/v), and 93% saline. In some cases, 4% Tween-80 and 91% saline were used.

The above results show that (-)-7-hydroxy-delta-6-THC-DMH (32) is one of the most active cannabimimetic substances prepared so far and that, apparently, cannabimimetic activity resides exclusively in the (-)-(3R,4R)-enantiomer. We assume that the results with the enantiomers (32) and (33) are also valid for the enantiomers of delta-1-THC, i.e., that our results are of a general nature. This assumption is based on the well-documented increase of cannabimimetic activity on the exchange of the pentyl chain with a DMH chain and on hydroxylation of the C-7 methyl group, which are the two features present in (32) and (33) and absent in delta-1-THC.

The results of animal tests of the types described above with delta-1-THC, delta-6-THC, and other cannabinoids have been shown to parallel activity in man

TABLE 4

(+) 7-Hydroxy- Δ^{6}-THC-DMH (33) and Morphine: Comparative Doses Producing 50 Percent of the Maximum Possible Analgesic Effect

MPE_{50} (mg/kg sc) at Time of Estimated Peak Activity of Compound[a,b]

Compound	Mouse (Sabra Strain)				Bat (Sabra Strain)	
	Paw[c] Clamp	Acetic Acid[d] Writhing	Hot[e] Plate	Tail[f] Clamp	Tail[f] Clamp	Hot[e] Plate
(+) 7-OH- Δ^{6}-THC-DMH (33)[g]	0.9	0.2	0.1	0.8	4.9	0.3
Morphine HCL[h]	4.2	1.3	1.6	4.3	5.5	2.0

[a] All morphine injected animals observed 1 hr postinjection.

[b] All (+) 7-OH- Δ^{6}-DMH injected animals observed 2 hr postinjection.

[c] Haffner et al. (1929); an adaptation - 30 sec cutoff.

[d] Sophia et al. (1973) - 20 min observation.

[e] Woolfe and MacDonald (1944) - 30 sec cutoff.

[f] Bianchi and Franceschini (1954) - 30 sec cutoff.

[g] Dissolved in 5% EtOH, 5% emulphor 620, and 90% ddH_2O, with $5x10^{-4}M$ $CuCl_2$ added subsequently.

[h] Dissolved in 0.9% saline.

(Hollister 1974). Hence, we assume that the results with (32) and (33) likewise indicate parallel activity in man. If this correct, cannabimimetic activity in man has a strict stereochemical requirement, which indicates a probable interaction with a chiral biological system (enzyme, receptor site, etc.) and not just an unspecific action due to the high lipid solubility of the cannabinoids.

ANALGETIC ACTION OF THE (+) ENANTIOMER (33)

As mentioned above, the cannabimimetic SAR does not necessarily parallel the SAR of cannabinoids for other biological or therapeutic effects. Indeed, we have found now that enantiomer (33), which (as noted above) does not cause cannabimimetic effects, is a potent analgetic particularly in the presence of cupric ions. In table 4, we present the results of several analgetic tests. In all tests, (33) shows activity at (or above) the potency level of morphine. However, as previously noted with delta-1-THC and other cannabinoids, the dose-response curve is not sharp (as with morphine) but flat. Surprisingly, the level of analgetic potency is retained over a period of 3 to 4 days. In some tests, activity actually falls beyond a certain concentration, the dose-response curve assuming an inverted U shape.

The above results show that it is possible to achieve complete dissociation between cannabimimetic effect and analgetic action.

These preliminary results may be of considerable therapeutic value because cannabinoids generally lack many of the side effects of opiates, such as high addiction liability and respiratory depression.

SUMMARY

The SAR of cannabimimetic activity in the cannabinoid series are reviewed with emphasis on the stereochemical requirements. Some new results are presented. The most important are that a, in humans, (-)-(1S)-delta-3-THC is much more active than (+)-(1R)-delta-3-THC; and b, with the 7-OH-delta-6-THC DMH enantiomers (32) and (33), the activity in several animal species resides completely in the (-)-(3R, 4R) enantiomer (32). the difference between the two enantiomers being up to several thousand times. The (3R,4R)-enantiomer (32) is much more active than delta-1- or delta-6-THC in animal tests, the exact level of activity depending on the test employed. The cannabimimetically inactive (+)-(3S,4S) enantiomer (33) was shown to be a potent analgetic in several animal tests. Thus, a complete dissociation between the cannabimimetic and the analgetic effects in a cannabinoid has been achieved, apparently for the first time.

REFERENCES

Banerjee, S.P.; Snyder, S.H.; and Mechoulam, R. Cannabinoids: Influence on neurotransmitter uptake in rat brain synaptosomes. J Pharmacol Exp Ther 194:74-81, 1975.

Bianchi, C., and Franceschini, J. Experimental observations on Haffner's method for testing analgesic drugs. Br J Pharm 9:280-284, 1954.

Edery, H.; Grunfeld, Y.; Ben-Zvi Z.; and Mechoulam, R. Structural requirements for cannabinoid activity. Ann NY Acad Sci 191:40-53, 1971.

Edery, H.; Porath, G.; Mechoulam, R.; Lander, N.; Srebnik, M.; and Lewis, N. Activity of novel aminocannabinoids in baboons. J Med Chem 27:1370-1373, 1984.

Gaoni, R., and Mechoulam, R. Isolation, structure and partial synthesis of an active constituent of hashish. *J Med Soc* 86:1646, 1964.

Gaoni, Y., and Mechoulam, R. The isomerization of cannabidiol to tetrahydrocannabinols. *Tetrahedron* 22:1481-1488, 1966.

Haffner, F. Experimentelle Prufung schmerzstillender Mittel. *Deutsch Med Wschr* 55:731-733, 1929.

Hollister, L.E. Structure-activity relationships in man of cannabis constituents and homologs and metabolites of delta-9-THC. *Pharmacology* 11:3-11, 1974.

Hollister, L.E.; Gillespie, H.K.; Mechoulam, R.; and Srebnik, M. Human pharmacology of 1S and 1R enantiomers of delta-3-tetrahydrocannabinol. *Psychopharmacology,* in press.

Jarbe, T.U.C.; Hiltunen, A.J.; Lander, N.; and Mechoulam, R. Cannabimimetic activity (delta-1-THC cue) of cannabidiol monomethylether and two stereoisomeric hexahydrocannabinols in rats and pigeons. *Pharmacol Biochem Behav* 25:393-399, 1986.

Johnson, M.R., and Melvin. L.S. The discovery of nonclassical cannabinoid analgetics. In: Mechoulam, R., ed. *Cannabinoids as Therapeutic Agents.* New York: CRC Press, 1986. pp. 121-145.

Jones, G.; Paton, W.D.M.; Nilsson, I.M.; and Agurell, S. Relative pharmacological potency in mice of optical isomers of delta-1-tetrahydrocannabinol. *Biochem Pharmacol* 23:439-446, 1974.

Lee, C.M.; Zaugg, H.E.; Michaels, R.J.; Dren, A.T.; Plotnikoff, N.P.; and Young, P.R. New azacannabinoids highly active in the central nervous system. *J Med Chem* 26:278-280, 1983.

Loev, B.; Bender, P.E.; Dowalo, F.; Macko, E.; and Fowler, P.J. Cannabinoids. Structure activity studies related to 1,2-dimethyl heptyl derivatives. *J Med Chem* 16:1200-1206, 1973.

Martin, B.R. Cellular effects of cannabinoids. *Pharmacol Rev* 38:45-74, 1986.

Martin, B.R.; Balster, R.L.; Razdan, R.K.; Harris, L.S.; and Dewey, W.L. Behavioral comparisons of the stereoisomers of tetrahydrocannabinols. *Life Sci* 29:565-574, 1981.

Martin, B.R.; Kallman, M.J.; Kaempf, G.F.; Harris, L.S.; Dewey, W.L.; and Razdan, R.K. Pharmacological potency of R- and S-3'-hydroxy-delta-9-tetrahydrocannabinol: Additional structural requirement for cannabinoid activity. *Pharmacol Biochem Behav* 21:61-65, 1984.

Mechoulam, R., and Edery, H. Structure activity relationships in the cannabinoid series. In: Mechoulam, R., ed. *Marijuana Chemistry, Metabolism, Pharmacology and Clinical Effects.* New York: Academic Press, 1973. pp. 101-136.

Mechoulam, R.; Braun, P.; and Gaoni, Y. A stereospecific synthesis of (-)-delta-1- and (-)-delta-6-tetrahydrocannabinols. *J Am Chem Soc* 89:4552-4554, 1967.

Mechoulam, R.; Lander, N.; Varkony, T.H.; Kimmel, I.; Becker, O.; Ben-Zvi, Z.; Edery, H.; and Porath, G. Stereochemical requirements for cannabinoid activity. *J Med Chem* 23:1068-1072, 1980.

Mechoulam, R.; Varconi, H.; Ben-Zvi, Z.; Edery, H.; and Grunfeld, Y. Synthesis and biological activity of five tetrahydrocannabinol metabolites. *J Am Chem Soc* 94:7930-7931, 1972.

Milne, G.M.; Koe, B.K.; and Johnson, M.R. Stereospecific and potent analgetic activity for nantradol - A structurally novel, cannabinoid related analgetic. In: Harris, L.S., ed. *Problems of Drug Dependence, 1979.* National Institute on Drug Abuse Research Monograph 27. DHEW Pub. No. (ADM) 80-901. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1980. pp. 84-92.

Narimatsu, S.; Matsubara, K.; Shimonishi, T.; Watanabe, K.; Yamamoto, I.; and Yoshimura, H. Pharmacological activities in the mouse of delta-9-tetrahydro-cannabinol metabolites oxidized at the 8-position. Chem Pharm Bull 33:392-395, 1985.

Pars, H.G.; Razdan, R.K.; and Howes, J.F. Potential therapeutic agents derived from the cannabinoid nucleus. In: Simmonds, A.B., ed. Advances in Drug Research. Vol II. New York: Academic Press, 1977. pp. 97-189.

Sofia, R.D.; Nalepa, S.; Harakel, J.; and Vassar, H.B. Anti-edema and analgesic properties of delta-9-tetrahydrocannabinol (THC). J Pharmacol Exp Ther 186:646-655, 1973.

Srebnik, M.; Lander, N.; Breuer, A.; and Mechoulam, R. Base catalysed double bond isomerizations of cannabinoids: Structural and stereochemical aspects. J Chem Soc Perkin Trans I 2881-2886, 1984.

Uliss, D.B.; Razdan, R.K.; Dalzell, H.C.; and Handrick, G.R. Hashish: Synthesis of dl-delta-1,6-cis-tetrahydrocannabinol. Tetrahedron Lett 4369-4372, 1975.

Wilson, R.S., and May, E.L. 9-Nor-delta-8-tetrahydrocannabinol, a cannabinoid of metabolic interest. J Med Chem 17:475-476, 1974.

Wilson, R.S.; May, E.L.; Martin, B.R.; and Dewey, W.L. 9-Nor-9-hydroxyhexahydrocannabinols, synthesis, some behavioral and analgesic properties. J Med Chem 19:1165-1167, 1976.

Woolfe, G., and MacDonald, A. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J Pharmacol Exp Ther 80:300-307, 1944.

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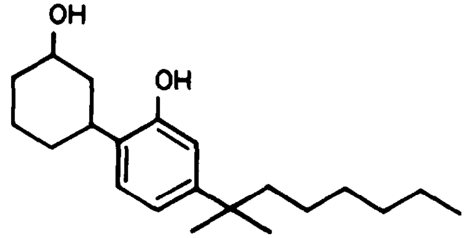

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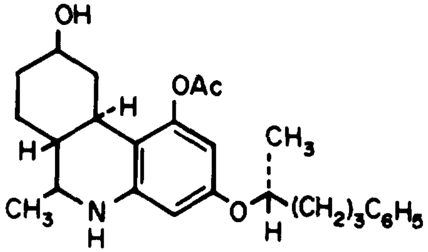

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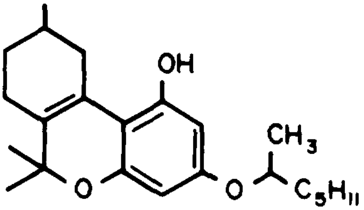

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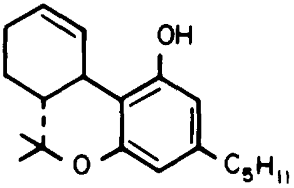

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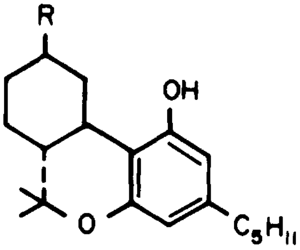

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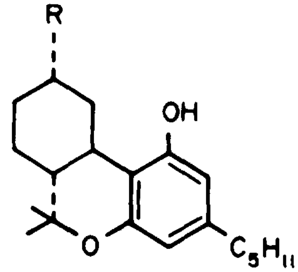
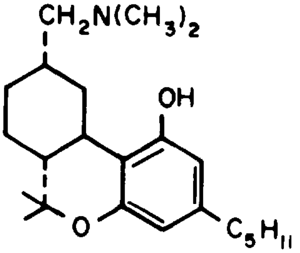


(7)

a, R = CH_3
b, R = CH_2OAc
c, R = CH_2OH

a, R = CH_3
b, R = CH_2OAc
c, R = CH_2OH



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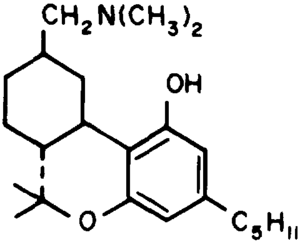

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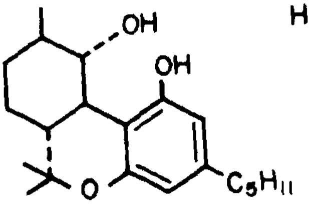

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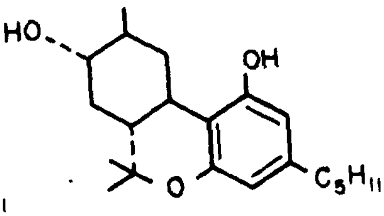

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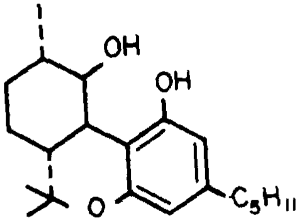

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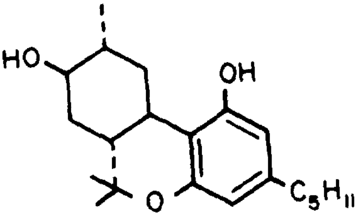

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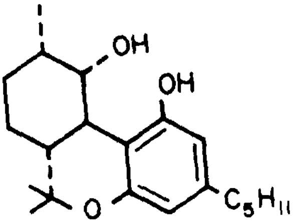

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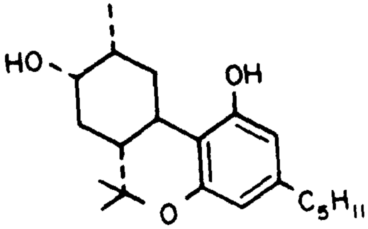

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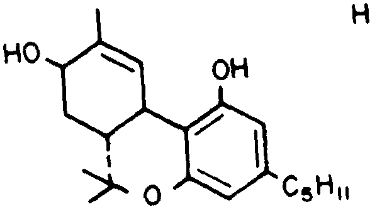

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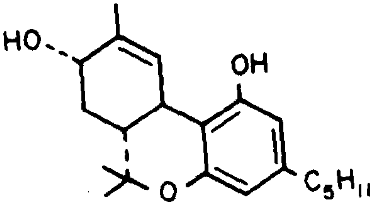

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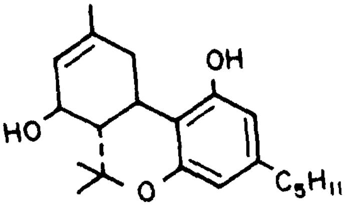

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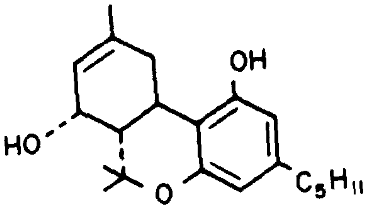

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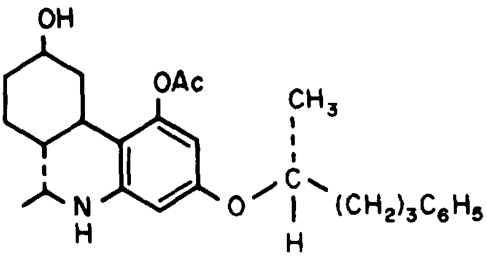

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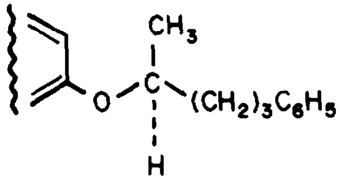

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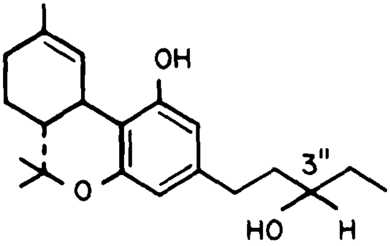
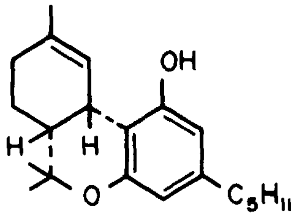


(22), 3"S (23), 3"R



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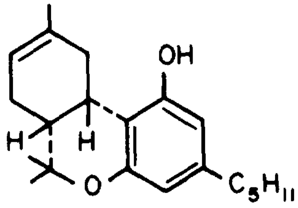

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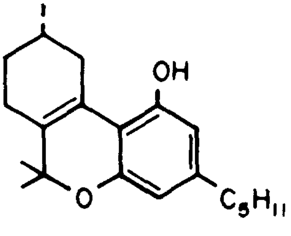

(1S)-Δ^3-THC

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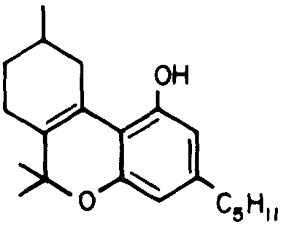
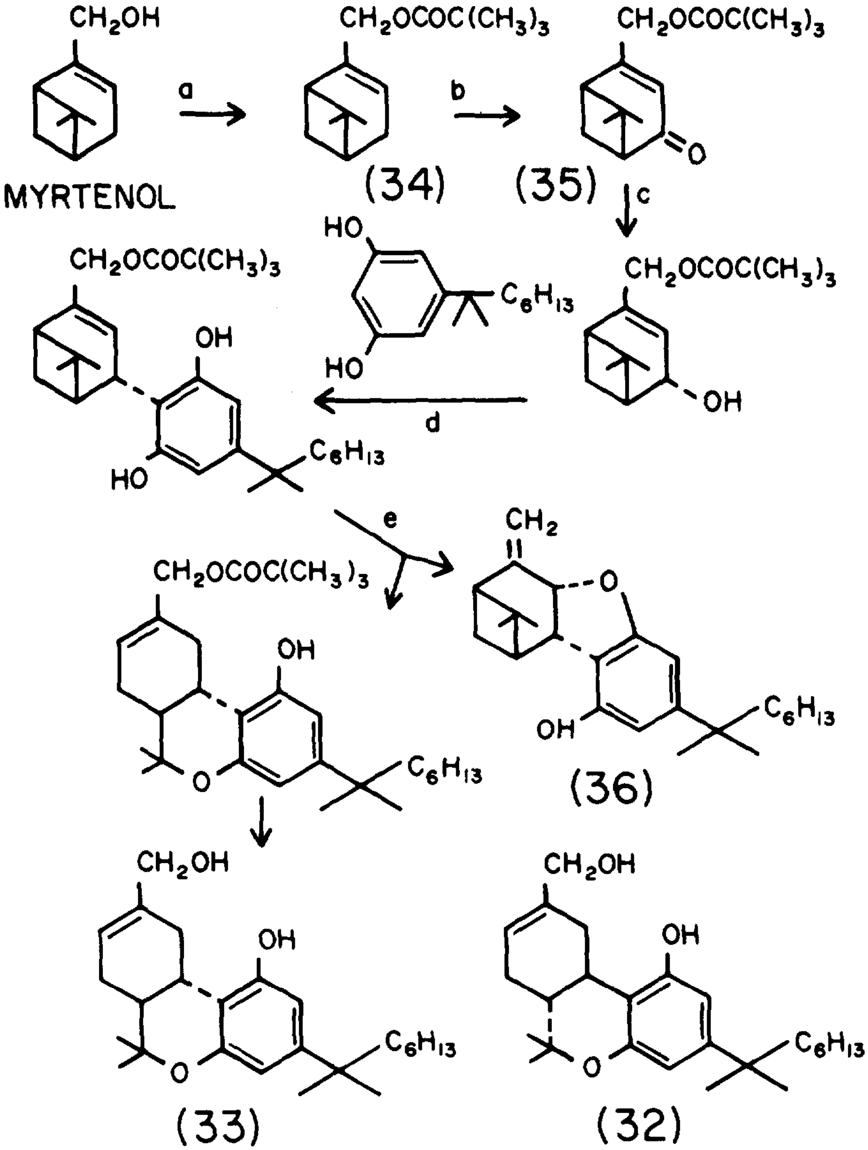


(1R)-Δ^3-THC

(27)

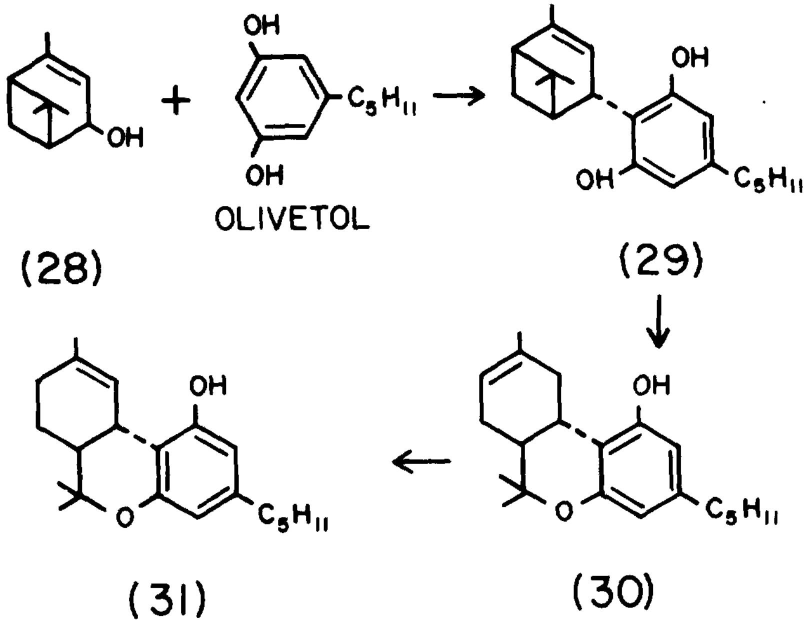
SYNTHESIS OF 7-OH-Δ^6-THC-DMH



m. p. 141-2°
$[\alpha]_D$ + 238°

m. p. 141-2°
$[\alpha]_D$ -236°

SYNTHESIS OF (+)-Δ^{1}-THC

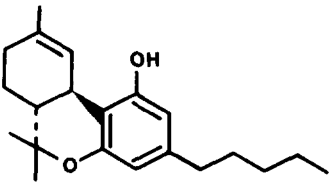


Structure-Activity Relationships of Tricyclic and Nonclassical Bicyclic Cannabinoids

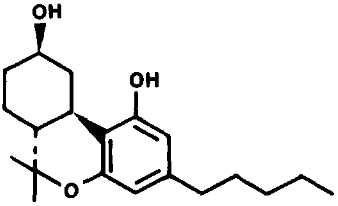
Lawrence S. Melvin, Ph.D., and M. Ross Johnson, Ph.D.

INTRODUCTION AND SUMMARY

The usefulness of marihuana has contributed to folklore and medicine for many centuries (Li 1974; Lemberger 1980). However, a good medicinal chemical rationale for the mechanism of action and structure-activity relationships (SAR) of Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**) has not been forthcoming. Our interest in attempting to dissect out the many therapeutic indications reported for Δ^9-THC led us to question the chemical dogma of cannabinoids that the tricyclic pyran-containing ring system of Δ^9-THC was an absolute necessity for biological activity. After the reported (Wilson et al. 1976) enhanced analgetic activity of 9-nor-9β-hydroxyhexahydrocannabinol (HHC, **2**), we became especially interested in locating and defining the pharmacophore for analgesia in the Δ^9-THC and HHC structures.



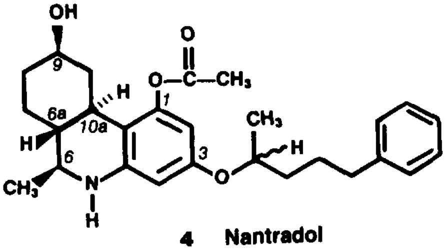
1 Δ^9-THC



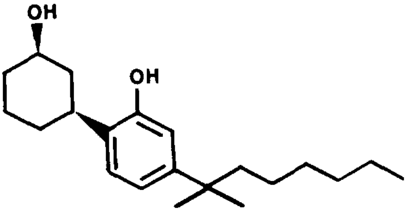
2 HHC

Based on the hypothesis that analgetic activity is a dissociable feature of the cannabinoid molecule, we examined modifications of the side chain, the phenolic moiety, and, most significantly, structures that lack the benzopyran ring present in THC and HHC. In our initial studies, we found that a new grouping, the 1-methyl-4-phenylbutyloxy C-3 side chain (**3**l), elaborates a unique lipophilic region. Replacement of the phenol substituent produced several derivatives which retain analgetic activity in the codeine potency range. Introduction of a weakly basic nitrogen at C-5 and deletion of the axial methyl group in the B ring, two structural changes forbidden by traditional cannabinoid SAR, resulted in a unique family of benzoquinolines with potent analgetic activity. The prototype of this series, nantradol (**4**), exhibits potent and enantiospecific analgetic and antiemetic activity. These early studies supported our three-point receptor model and led to the synthesis of nonclassical cannabinoids.

Synthesis of the first AC-bicyclic cannabinoid followed from our observation that the pyran ring of HHC was not a requirement in this structural class for expression of biological activity. Together, this observation and speculation about the necessity of the lipophilic side chain, phenol and alcohol for biological activity was confirmed with synthesis of 3-Z-[4-(1,1-dimethylheptyl)-2-hydroxyphenyl]cyclohexanol, CP-47,497 (**5**). This compound was shown to possess a biological profile and potency similar to HHC. Such activity and potency was highly dependent on the side chain but was not as influenced by substitution in the cyclohexanol ring. Further structural elaboration and development of SAR around CP-47,497 led to a more potent AC-bicyclic derivative, (-)-3-Z-[4-(1,1-dimethylheptyl)-2-hydroxyphenyl]-4-E-(3-hydroxypropyl)cyclohexanol. The degree of importance of the three hydroxyl groups in this structure was explored and only the phenolic hydroxyl was found to be an absolute necessity. The two alcohol groups were seen to be modifiers of activity and potency. Structural optimization of this derivative of CP-47,497 was achieved by incorporation of the hydroxypropyl chain as a new fused ring and thus the synthesis of the first ACD-tricyclic cannabinoid, (-)-1,2,3,4,5,5aβ,6,7,8,8aα-decahydro-4β-[4-(1,1-dimethylheptyl)-2-hydroxyphenyl]-6β-hydroxymethylnaphthalen-2β-ol. This rigid molecule again exhibits an increase in potency and significantly shows total enantiospecificity in favor of the levorotatory enantiomer. The combination of potency, stereospecificity, enantiospecificity, and well-defined SAR leads us to conclude that the cannabinoidsexertat least their analgetic effect by interaction with a discreet, and as yet unknown, receptor site.



4 **Nantradol**



5 **(CP-47,497)**

ABC-TRICYCLICS

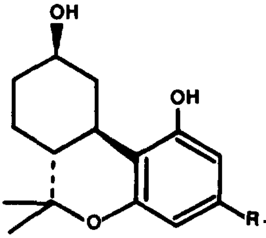
Analgesia and the Side Chain

Adams and his co-workers (1949) had demonstrated quite early that structural alterations of the C-3 side chain of synthetic $\Delta^{6a,10a}$-tetrahydrocannabinols markedly altered biologic potency (Mechoulam et al. 1976). However, since many of the biologic effects measured in these early studies were nonanalgetic, we set out to carefully determine specific structure-analgetic activity relationships of side chains in the HHC series. Our search led us ultimately to examine four distinct classes of side chains (alkyl, aralkyl, alkoxy, and aralkoxy) that are summarized in table 1 along with their analgetic activity (Johnson et al. 1981A, 1981 B).

Initially, we examined the effect of the branched and extended alkyl derivatives 36 and 3*c*, side chains previously used in the $\Delta^{6a,10a}$-, Δ^{8}-, and Δ^{9}-THC (Loev et al. 1973) and in the nabilone (Starkand Archer 1975) and nabitan (Pars et al. 1976) series. The use of a side chain branched at the benzylic position, such as 3*b* and 3*c*, considerably increase biologic activity relative to the *n*-amyl side chain. This increase in potency may be due, in part, to a lessening of metabolic inactivation through benzylic oxidation. Side chain c, with dimethyl substitution at the benzylic position, has the

TABLE 1

Analgetic Activity of HHC Side Chain Derivatives



R=	Compound	Analgetic MPE_{50} (mg/kg)*
(-)-Δ^{9}--THC	-	9.1
$-CH_2CH_2CH_2CH_2CH_3$	3*a*	0.63
$-CH-CH-CH_2CH_2CH_2CH_2CH_3$ CH_3 CH_3	3*b*	0.08
$-C-CH_2CH_2CH_2CH_2CH_2CH_3$ CH_3 CH_3	3*c*	0.06
$-CH_2CH_2C_6H_5$	3*d*	>10**
$-CH-CH_2CH_2C_6H_5$ CH_3	3*e*	4.66
$-CH-CH_2CH_2CH_2H_5$ CH_3	3*f*	0.06
$-CH-CH_2CH_2CH_2$-4-C_6H_5N CH_3	3*g*	0.22
$-CH-CH_2CH_2CH_2C_6H_5$ CH_3	3*h*	0.35
-O-$\underline{c}$-C_6H_{11}	3*i*	<10†
$-O-CH-CH_2CH_2CH_2CH_2CH_3$ CH_3	3*j*	0.17
$-O-CH-CH_2CH_2C_6H_5$ CH_3	3*k*	0.23
$-O-CH-CH_2CH_2CH_2C_6H_5$ CH_3	3*l*	0.07
$-CH-CH_2CH_2OC_6H_5$ CH_3	3*m*	0.23
$-CH-CH_2CH_2CH_2-O-C_6H_5$ CH_3	3*n*	0.38
$-CH-CH_2-O-CH_2CH_2C_6H_5$ CH_3	3*o*	1.28
Morphine	-	0.9

*Phenylbenzoquinone writhing in mice (PBQ), 0.3 hr after dose, s.c. **27% protection at 10 mg/kg, s.c. †75% protection at 10 mg/kg, s.c.

inside chain *b.* As can be seen in table 1, both 3*b* and 3*c* exhibit analgetic activity considerably greater than that of HHC and Δ^{9}-THC.

Having demonstrated that it was possible to increase the analgetic potency of HHC by simple alkyl substitution in the side chain, we next turned our attention to aralkyl-substituted derivatives in order to determine the effect of a pendant phenyl ring on analgetic activity (previously studied in the nabitan series, cf. Winn et al. 1976). The unbranched phenethyl side chain (3*d*). which has approximately the same extended chain length as HHC, was analgetically less active. However, the branched three-, four-, and five-carbon homologs exhibited potent analgetic activity with a maximum effect at four linear carbon atoms (3*f*). The heteroaromatic 4-pyridyl derivative 3*g*, while somewhat less potent than 3*f*, retained analgetic activity. Molecular models show that 3*f* and 3*g* have approximately the same extended side chain length as 3*b* and 3*c.*

Previous studies in the $\Delta^{6a,10a}$-THC series have shown that an oxygen atom directly attached to the phenolic ring has a variable effect on biologic activity. Thus, while unbranched ether side chains were reported to decrease activity (Edery et al. 1972), branched ether side chains exhibited a small increase in activity relative to their carbon isomers (Loev et al. 1973). Furthermore, simultaneous changes in the C-ring and side chain have been shown to produce nonpredictive SAR (Loev et al. 1973). Therefore, we reinvestigated this parameter to specifically assess the effect of a C-3 oxygen atom on analgetic activity in the HHC series. While the cyclohexyl derivative 3*i* retains some analgetic activity, the 2-heptyloxy derivative 3*j* possesses potent analgetic activity (two to three times that of morphine).

TABLE 2

Comparative Analgetic Effect of HHC Side Chains

Compound	MPE_{50} (mg/kg, s.c.)[c]				
	Mouse PBQ Writhing[a]	Mouse Tail Flick[b]	Mouse Hot Plate[b]	Rat Tail Pinch[c]	Rat Flinch Jump[c]
Morphine	0.9	5.72	4.23	4.77	10.3
3*a*	0.63	9.1	34.4	70.5	36.4
3*b*	0.06	3.0	0.32-0.56	N.T.	0.39
3*f*	0.06	0.25	0.55	0.49	0.47
3*j*	0.17	N.T.	0.56-1.0	0.65	N.T.
3*l*	0.07	0.33	0.46	0.38	0.38
3*n*	0.38	0.8	10	N.T.	2.7

[a] Values at 0.3 hr postdose. [b] Values at 1 hr postdose. [c] Values at 2 hr postdose.

Introduction of oxygen in the aralkyl derivative 3*e* yielded *3k.* which is 20 times more potent than 3*e.* Increasing the chain length by one carbon yielded 3*l,* which is fully as potent as 3*f* and 3*c.* Since the extended chain length of 31 is greater than that of 3*c* and 3*f,* this finding indicates that the oxygen atom itself and not just the chain length affects analgetic activity. However, when the oxygen atom is not directly attached to the phenolic ring of HHC, it generally behaves as a carbon unit. Thus, 3*n* and 3*o* more closely resemble 3*h* than 3*l.* However, 3*m* does resemble its isomer 3*k* rather than 3*f.*

We selected a potent member of each side chain class to more fully evaluate analgesia across a range of stringent assays. Our results, reported in table 2, with 3*a*, 3*b*, 3*f*, 3*j*, 3*l*, and 3*n* confirm their ability to exhibit antinociceptive activity in tests traditionally responsive only to the opiates.

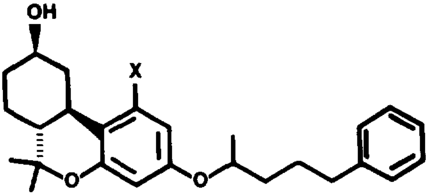
These studies demonstrated that the C-3 side chain of HHC can be modified in a structure-dependent fashion to yield derivatives that produce potent analgesia in animals, previously characteristic only of the opiate analgetic class. In addition, we have shown a unique effect for the placement of an oxygen atom directly attached to the aromatic ring of HHC.

The Importance of the Phenol

Previous studies dealing with modification of only the phenolic portion of cannabinoids have been limited to derivatives of Δ^{8}--THC,Δ^{9}--THC, and$\Delta^{6a,10a}$-THC (Loev et al. 1973; Edery et al. 1972; Kurth et al. 1976; Matsumoto et al. 1977). These studies have suggested, with some ambiguities, that optimal activity is associated with only the phenol. With the potent prototype 31 in hand, we began a study of the aromatic ring of HHC to determine the optimum structural requirements for analgesia. initially, we studied modifications of 31 which replaced the phenol with potential bioisosteric equivalents (Johnson et al. 19818; Melvin et al. 1980).

TABLE 3

Analgetic Activity of Phenol Replacements



X	Analqetic MPE_{50} (mg/kg)[a] Subcutaneous[b]	Oral[c]
OH	0.07	0.11
H	30.5	30.2
CH_3	≥ 56	38.2
CH_2OH	≥ 56	24.6
COOH	≥ 56	-
NH_2	11.3	32.5
$NHSO_2CH_3$	>56	-
$NHCOCH_3$	>56	-
Cl	>56	-
SH	>56	-
Morphine	0.91	-
Codeine	6.7	21.3
Δ^{9}-THC	5.93	7.98

[a]Phenylbenzoquinone writhing in mice (PBQ). [b]0.3 hr postdose. [c]1 hr postdose.

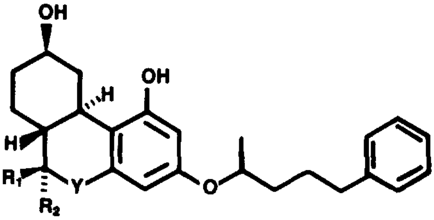
Retaining the phenolic functionality (X = OH) appears to be a prerequisite for optimum analgetic activity (table 3). Further, although analgesia in the range of codeine is obtained for the amino substituent (X= NH_2), substituents possessing acidic hydrogens (X=COOH, $NHSO_2CH_3$, SH) do not mimic the phenol. Complete removal of the phenol (X = H) yields a weakly active compound, as does replacement by methyl and hydroxymethyl groups. The oral test data suggest that these latter two groups are metabolically interconvertible, and it is equally likely that some hydroxylation occurs at C-1 of the X = H analog yielding the active phenol derivative 3*l*.

C-5 and C-6 Positions

Since nuclear modification of the cannabinoids has been largely unsuccessful to date (Mechoulam et al. 1976), we were impressed by the finding of potent analgesia in animals for both the phenanthrene (Y = CH_2) and the phenanthridine (Y = NH) (table 4) (Althuis et al. 1980; Milne et al. 1978).

TABLE 4

Analgetic Activity of C-5 and C-6 Modifications



			MPE$_{50}$ (mg/kg, s.c.)		
R_1	R_2	Y	Mouse PBQ[a] Writhing	Mouse Tail[b] Flick	Rat Tail[c] Pinch
CH_3	CH_3	CH_2	0.78	1.3	1.1
CH_3	CH_3	O	0.07	0.33	0.38
CH_3	H	NH	0.4	0.7	1.0

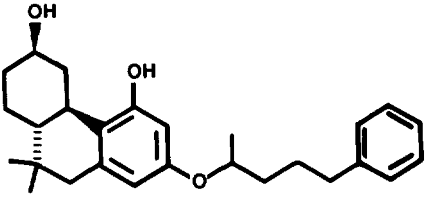
[a]Values at 0.3 hr postdose. [b]Values at 1 hr postdose. [c]Values at 2 hr postdose.

The phenanthrene prototype is one to four times more potent than morphine in the three tests shown in table 4. Although peak activity is generally observed 1 to 2 hours after dibenzo(*b,d*)pyrans, such as THC, this is not true for the phenanthrenes. As shown in table 5, the peak activity of this compound occurs about 4 hours after administration, and this activity remains near the maximum during the period of 2 to 8 hours after administration and drops off at 24 hours. It is further striking to note that even 24 hours after dose, the phenanthrene has a tail-flick MPE$_{50}$ less than that of morphine 1 hour after administration.

The phenanthridine, nantradol (**4**), culminated our initial efforts to design a potent, nonopiate analgetic. This is a significant finding since previous attempts utilizing a cannabinoid-based phenanthridine nucleus resulted in compounds lacking biologic

TABLE 5

Duration of Phenanthrene Analgetic Activity



	MPE$_{50}$ (mg/kg, s.c.)	
Mouse Tail Flick	Rat Tail Pinch	Time (hr)
3.3	-	0.5
1.3	1.1	2.0
1.5	0.74	4.0
-	0.66	6.0
3.5	0.88	8.0
2.2	-	24.0

activity (Hoops et al. 1968; Biel 1970). Nantradol has a number of structural and pharmacologic features which distinguish it from both the opiates and the cannabinoids (Mast et al. 1979; Johnson and Milne 1980A). The most notable structural differences are the presence of a weakly basic nitrogen in place of a pyran oxygen, the deletion of an axial methyl group, and the introduction of an oxygen-containing C-3 side chain. Nantradol is considerably more potent (20- to 100-fold) than Δ^9-THC as an analgetic and possesses two to seven times greater potency than morphine across a full battery of analgetic tests (table 4). Like the opiates, nantradol also possesses antidiarrheal and antitussive activity; however, unlike the opiates, nantradol does not bind to the opiate receptor and its analgetic activity is not blocked by naloxone *in vivo* (Milne et al. 1980A).

Phenanthridine Stereospecificity

Nantradol's substantial potency suggested to us that it might be acting specifically to produce its analgetic effects. Therefore, we sought to determine if nantradol's actions were stereospecific (Milne et al. 1980B; Johnson and Milne 1980B).

Nantradol has a total of five asymmetric centers; however, owing to its defined stereochemistry at positions 6, 6a, 9, and 10a, it is an approximately equal mixture of only 4 of the possible 32 isomers and has been studied in both animals and man as a 50: 50 mixture of two pairs of racemic diastereomers, both of which possess the *trans*-6a,10a stereochemistry and have β-oriented substituents at positions 6 and 9 (figure 1). The first step toward identifying the major active isomer was the separation of nantradol into its component diastereomers, herein designated A and B. Diastereomer A was found to be at least 10 times more potent than its C-3 side chain counterpart diastereomer B in analgetic tests (table 6). These results clearly demonstrate that isomer A contains the majority of nantradol's analgetic activity. Resolution of isomer A into its two optically active enantiomers demonstrated that it

TABLE 6

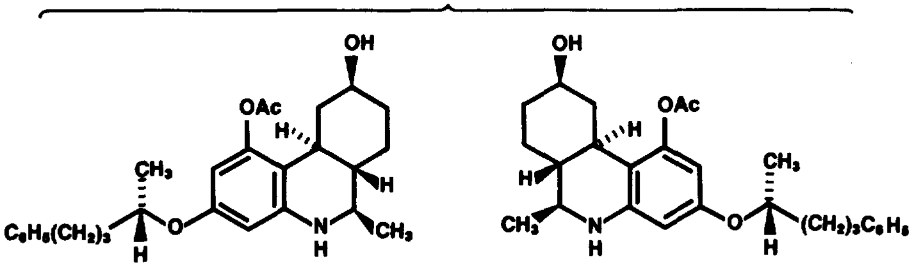
Analgetic Activity of Nantradol Stereoisomers

	MPE$_{50}$ (mg/kg, s.c.)			
Compound	Mouse PBQ Writhing*	Mouse Tail Flick*	Rat tail Pinch†	Rat Flinch Jump†
Nantradol	0.4	0.7	1.0	1.4
Diastereoisomer A	0.2	0.2	0.7	0.3
Diastereoisomer B	1.7	2.4	14.2	2.4
Levonantradol	0.07	0.2	0.2	0.3
Dextronantradol	6.5	>10.0	> 10.0	> 10.0

* 1 Hr after dose. †2 Hr after dose.

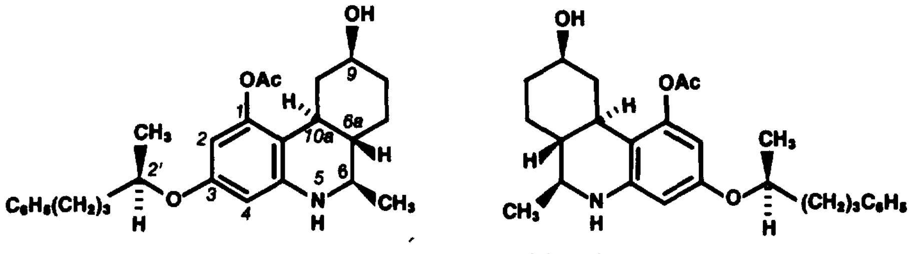
is the levorotatory isomer, levonantradol, that possesses the largest portion of the analgetic activity of nantradol, being four and two times more potent than nantradol and diastereomer A, respectively. Importantly, the enantiomer dextronantradol is at least 100 times less active than levonantradol.

DIASTEREOISOMER A



(+)-2′(S),6(R),6a(S),9(S),10a(S)
Dextronantradol

(–)-2′(R),6(S),6a(R),9(R),10a(R)
Levonantradol



(+)-2′(R),6(R),6a(S),9(S),10a(S)
2′-Epi-dextronantradol

(–)-2′(S),6(S),6a(R),9(R),10a(R)
2′-Epi-levonantradol

DIASTEREOISOMER B

Figure 1

NANTRADOL ISOMERS

AC-BICYCLICS

Previously reported work and studies in our laboratories suggested that the dimethyldihydropyran ring portion of HHC was not required for potent biological activity. Unexpectedly, substitution of carbon (phenanthrenes) or nitrogen (phenanthridines) for the oxygen of the pyran ring in HHC yielded compounds that were of similar analgesic potency to HHC. Additionally, we showed in the work leading to levonantradol that the geminal dimethyl group on the pyran ring of HHC was not necessary for analgesic activity.

Thus, the dihydropyran moiety appeared to serve no other purpose than to firmly anchor the phenolic ring and cyclohexyl ring in an active conformation. This hypothesis led us to further postulate that HHC interacts at a receptor site by a three-point contact (Milne and Johnson 1981). the three binding sites being the equatorial alcohol, phenol, and C-3 side chain.

As a result of these considerations, we prepared (Melvin et al. 1982, 1984A) a novel AC-bicyclic cannabinoid, 5 (CP-47,497), which is lacking the dihydropyran ring of HHC. As table 7 shows, prototype 5 retains all the analgetic activity and potency of HHC and is equipotent with morphine (Johnson et al. 1982; Milne et al. 1983). In addition, 5 still exhibits many of the motor disruption effects in common with HHC and the cannabinoids (Weissman et al. 1982). We believe these striking results can be rationalized by considering the minimum energy conformation of 5. For the calculated minimum energy conformation of 5 and HHC, the dihedral angle between the C-OH (phenol) and C-OH (alcohol) bond is + 54.0° and + 41.3°, respectively. The distance between the oxygen atoms in these calculated conformations was found to be 5.0Å for 5 and 4.8Å for HHC. Thus, we propose that 5 readily assumes a conformation quite similar to the rigid HHC molecule and is recognized by a biological system as being equivalent to a tricyclic cannabinoid.

TABLE 7

Analgetic Activity Test Results of CP-47,497 (5)

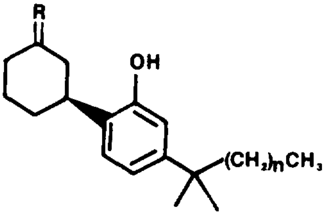
	MPE$_{50}$ (mg/kg)[a]				
Compound	PBQ[b]	RTC[c]	HP[d]	TF[e]	FJ[f]
5	1.0	4.7	8.1	4.4	4.2
HHC	0.63[g]	7.0	34.4	9.1	36.4
Ag-THC	5.9	29.1	>178[h]	55[g,i]	83.1
Morphine	1.8	4.8	4.2	5.7	10.3

[a]The dose to produce 50% of the maximum possible effect (subcutaneous). [b]Mouse phenylbenzoquinone-induced writhing, 1 hr postdose. [c]Rat tail clamp, 2 hr postdose. [d]Mouse hot plate, 1 hr postdose. [e]Mouse tail flick, 1 hr postdose. [f]Rat flinch jump, 2 hr postdose. g20 min postdose. [h]Less than 50% response at this dose. [i]>178 at 1 hr postdose.

We developed side chain SAR for 5 as compiled in tables 8 and 9. These tables illustrate the optimum carbon side chains are 1,1-dimethylheptyl and 1,1-dimethyloctyl, while the optimum alkoxy substituents are 2-octyloxy and 2-nonyloxy. Table 10 compares carbocyclic ring size with analgetic activity, showing the optimum sizes to be cyclohexyl and cycloheptyl (Melvin et al. 1984B). In addition, tables 8 through 10 record the effect on side chain and ring size of varying the stereochemistry of the alcohol in 5 as well as the oxidation state (ketone vs. alcohol) at the same carbon atom. Thus, analgetic potency varies with the alcohol position 5 as equatorial OH>axial OH ≈ ketone.

TABLE 8

PBQ[a] Writhing Results for Alkyl Side Chains



n/R	MPE_{50} (mg/kg)[b] Equatorial OH	Axial OH	=O
0	IA[c]	IA	IA
1	IA	IA	IA
2	IA	IA	IA
3	IA	IA	IA
4	11.9	> 56[d]	34.8
5	1.00	3.8	4.5
6	1.20	3.93	1.06
7	15.2	IA	IA
8	IA	IA	IA
9	IA	IA	IA
–e	IA	IA	IA

[a] Mouse phenylbenzoquinone-induced writhing, 1 hr postdose. [b] The dose to produce 50% of the maximum possible effect (subcutaneous). [c] IA, less than 20% inhibition at 56 mg/kg. [d] 35% inhibition at 56 mg/kg. [e] These compounds have the entire alkyl side chain repiaced by H.

Substitution at the C-4 position of the cyclohexane ring of 5 maximized analgetic potency in this series. The hydroxy propyl analog (-)-6 (CP-55,940) is shown in table 11 to be significantly more potent than morphine and to have at least a 200-fold ratio of stereospecificity (Melvin et al. 1983A). Table 12 compares the effect of simple *n*-alkyl versus *n*-hydroxyalkyl substitution at the C-4 position of 5. Analgetic potency with alkyl substitution was maximized for *n*-propyl, while n-hydroxypropyl or *n*-hydroxybutyl were the optimum hydroxyalkyl substitution.

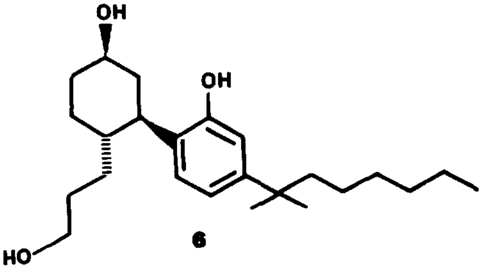


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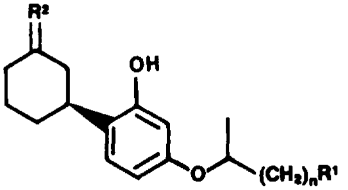
TABLE 9

PBQ[a] Writing Results for Alkoxy Side Chains

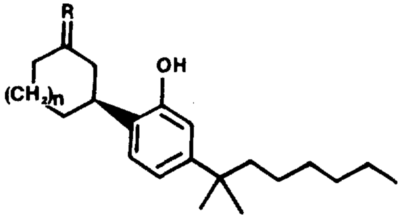


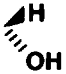
		MPE$_{50}$ (mg/kg)b		
n	R^1/R^2	Equatorial OH	Axial OH	=O
4	CH_3	22.3	>56[c]	>56
5	CH_3	5.04	>56	>56
6	CH_3	9.3	IA[d]	>56
2	C_6H_5	~56[e]	IA	IA
3	C_6H_5	38.0	>56	>56
4	C_6H_5	IA	IA	IA
3[f]	C_6H_S	IA	IA	IA

[a] Mouse phenylbenzoquinone-induced writhing, 1 hr postdose. [b] The dose to produce 50% of the maximum possible effect (subcutaneous). [c] Less than 50% inhibition at 56 mg/kg. [d] IA, less than 20% inhibition at 56 mg/kg. [e] 57% inhibition at 56 mg/kg. [f] These compounds lack the $CHCH_3$ in the side chain of the general structure.

TABLE 10

Analgetic Test Results with Varying Ring Size



		MPE_{50}(mg/kg)[a]				
n	R	PBQ[b]	RTC[c]	HP[d]	TF[e]	FJ[f]
0		~56g	NT[h]	NT	NT	NT
1		1.00	4.7	8.1	44	4.9
2		1.44	7.66	39.4	12.2	3.5
3		4.61	NT	>56[i]	~32j	NT
0		>56[i]	NT	NT	NT	NT
1		3.8	12.3	>56[i]	55.7	~17.8[k]
2		1.53	5.64	<32[l]	7.54	5.36
3		39.2	NT	NT	NT	NT
0	=O	IA[m]	NT	NT	NT	NT
1		4.5	26.1	>32[n]	15.3	11.5
2		1.23	4.36	>32[n]	11.7	10.8
3		~56°	NT	NT	NT	NT

[a] The dose to produce 50% of the maximum possible effect (subcutaneous). [b] Mouse phenylbenzoquinone-induced writhing, 1 hr postdose. [c] Rat tail clamp, 2 hr postdose. [d] Mouse hot plate, 1 hr postdose. [e] Mouse tail flick, 1 hr postdose. [f] Rat flinch jump, 2 hr postdose. [g] 51% inhibition at 56 mg/kg. [h] NT = Not tested. [i] Less than 50% inhibition at 56 mg/kg. [j] 54% inhibition at 32 mg/kg. [k] 58.7% inhibition at 17.8 mg/kg. [l] 65.6% inhibition at 32 mg/kg. [m] IA, less than 20% inhibition at 56 mg/kg. [n] Less than 50% inhibition at 32 mg/kg. °57.5% inhibition at 56 mg/kg.

TABLE 11

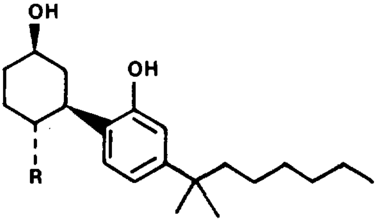
Analgetic Activity and Stereospecificity of 6

	MPE_{50} (mg/kg)				
Compound	PBQ[a]	TF[b]	HP[c]	RTC[d]	FJ[e]
(-)-6	0.07	0.4	0.7	0.13	0.50
(+)-6	14.6	–	–	–	–
(±)-6	0.29	0.55	1.11	0.46	0.63
(±)-5	1.0	4.4	8.1	4.7	4.9
HHC	0.63[f]	9.1	34.4	7.0	36.4
Morphine	1.8	5.7	4.2	4.8	10.3

[a] Mouse phenylbenzoquinone-induced writhing, 1 hr postdose. [b] Mouse tail flick, 1 hr postdose. [c] Mouse hot plate, 1 hr postdose. [d] Rat tail clamp, 2 hr postdose. [e] Rat flinch jump, 2 hr postdose. [f] 0.3 hr postdose.

TABLE 12

Analgetic Activity for Analogs of (±)-5



R	PBQ MPE_{50} (mg/kg)*
H	1.00
CH_3	1.02
CH_2CH_3	1.75
$CH_2CH_2CH_3$	0.54
$CH_2CH_2CH_2CH_3$	0.83
$CH_2CH_2CH_2CH_2CH_3$	50
CH_2OH	1.77
CH_2CH_2OH	2.58
$CH_2CH_2CH_2OH$	0.29
$CH_2CH_2CH_2CH_2OH$	0.23
$CH_2CH_2CH_2CH_2CH_2OH$	0.55

*Phenylbenzoquinone writhing in mice, 1 hr post subcutaneous dose.

ACD-TRICYCLICS

The successful modification of HHC and subsequent derivatization of 5 giving the more potent hydroxypropyl analog 6 encouraged us to further pursue this new lead. Assuming we had found the optimum substitution at C-4 from our studies with 5, we next chose to explore potential stereochemical requirements of this new primary alcohol group. To this end, we prepared a more complex ring system, 7 (CP-55,244), that incorporates a rigidly positioned hydroxypropyl moiety. From table 13, which displays the analgetic test results obtained with rigid decalin 7, it is seen that this compound is 2 to 10 times more potent than less rigid structure 6. A suggestion that the axial hydroxymethyl group of 7 is optimally oriented comes from the lessened (52x) potency observed for the equatorial isomer (±)-8. Even more striking, (-)-7 now exhibits virtually complete stereospecificty of action versus (+)-7. This fact supports our hypothesis that compounds such as 6 and 7 interact with a specific receptor system.

TABLE 13

Analgetic Effect of (-)-7 vs. (-)-6 and Stereospecificity of (-)-7

	MPE_{50} (mg/kg)[a]				
Compound	PBQ[b]	RTC[c]	Hp[d]	TF[e]	FJ[f]
(-)-7	0.02	0.06	0.07	0.03	0.11
(+)-7	>100	-	-	-	-
(±)-7	0.054	0.22	0.24	0.15	-
(±)-8	2.86	6.83	-	-	-
(±)-5	1.0	4.7	8.1	4.4	4.9
(-)-6	0.07	0.13	0.7	0.4	0.5
HHC	0.63[g]	7.0	34.4	9.1	36.4
Morphine	1.8	4.8	4.2	5.7	10.3

[a] The dose to produce 50% of the maximum possible effect. [b] Mouse phenylbenroquinone-induced writhing, 1 hr postdose. [c] Rat tail clamp, 2 hr postdose. [d] Mouse hot plate, 1 hr postdose. [e] Mouse tail flick, 1 hr postdose. [f] Rat flinch jump, 2 hr postdose. g0.3 hr postdose.

DISCUSSION

We have detailed the SAR for side chains of ABC-tricyclic cannabinoids showing optimum effects from both alkyl and alkoxy substitution. The necessity for phenolic functionality at C-1 of HHC was confirmed. Nitrogen and carbon were successfully substituted for the dihydropyran oxygen of HHC. Structural dissection of HHC led to the synthesis of AC-bicyclic cannabinoids and demonstration that the rigid B-ring (dihydropyran ring) is not required for biological activity. The biological activity of AC-bicyclic cannabinoids supports our hypothesis that cannabinoids have three sites of receptor recognition (side chain, phenol, and alcohol). The synthesis of ACD- and ABCE-multicyclic cannabinoids demonstrates that there is structurally unexplored space proximate to the HHC molecule.

The extreme potency and stereospecificity of these synthetic, nonclassical cannabinoids, and their retained activity at the extremes of structural elaboration, lead us to believe that these compounds are exerting their effects by acting at an as yet unknown receptor site.

The current studies demonstrate the ability to achieve extreme biological potency in structures that diverge significantly from the classical cannabinoid ring system. This and the clear relationship between the stereochemistry and biological activity of these compounds provides support for the premise that they interact in very specific and important ways with biological systems. However, it seems likely that further improvements in selectivity between the desired biological activity and the typical cannabinoid side effects will be necessary before the full therapeutic potential of these compounds is fully realized. It is possible that the next round of discovery in this area, possibly leading to greater selectivity, will be sparked by the identification of a novel site of cannabinoid action involving a distinct neurotransmitter system. Toward this end, the potent and stereochemically defined compounds described herein could provide important tools to facilitate this effort.

REFERENCES

Adams, R.; Harfenst, M.; and Lowe, S. New analogs of tetrahydrocannabinol. XIX. J Am Chem Soc 71:1624-1628, 1949.

Althuis. T.H.; Harbert, CA.; Johnson, M.R.; Melvin, L.S.; and Milne, G.M. Analgetic 1,9-dihydroxyoctahydrophenanthrenes. Abstracts of Papers, 180th National Meeting of the American Chemical Society, Las Vegas, NV, August 1980, American Chemical Society, Washington, D.C., 1980. Abstr. MEDI, 66.

Biel, J.H. General discussion. In: Psychotomimetic Drugs. Efron, D.H., ed. NY: Raven, 1970. p. 336.

Edery, H.; Grunfeld, Y.; Porath, G.; BenZvi, Z.; Shani, A.; and Mechoulam, R. Structure activity relationships in the tetrahydrocannabinol series. Arzneimittel-Forsch 22:1995-2003, 1972.

Hoops, J.F.; Bader, H.; and Biel, J.H. A 5-aza analog of $\Delta^{6a(10a)}$-tetrahydrocannbinol. J Org Chem 33:2995-2996,1968.

Johnson, M.R., and Milne, G.M. Recent discoveries in the search for non-opiate analgetics. J Heterocycl Chem 17:1817-1820, 1980A.

Johnson, M.R., and Milne, G.M. The synthesis and stereospecific analgesia of nantadol - A potent, non-opiate analgetic. Abstracts of Papers, 179th National Meeting of the American Chemical Society, Houston, TX, March 1980, American Chemical Society, Washington, D.C., 1980B. Abstr. MEDI 44.

Johnson, M.R.; Althuis, T.H.; Bindra, J.S.; Harbert, C.A.; Melvin, L.S.; and Milne, G.M. Potent analgetics derived from 9-nor-9β-hydroxyhexahydrocannabinol. In: Harris, L.S., ed. Problems of Drug Dependence, 1980. National Institute on Drug Abuse

Research Monograph No. 34. DHHS Pub. No. (ADM) 81-1058. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1981A. pp. 68-74.

Johnson, M.R.; Melvin, L.S.; Althuis,T.H.; Bindra, J.S.; Harbert, CA.; Milne, G.M.; and Weissman, A. Selective and potent analgetics derived from cannabinoids. J. Clin Pharmacol 21:2715-2825, 1981B.

Johnson, M.R.; Melvin, L.S.; and Milne, G.M. Prototype cannabinoid analgetics, prostaglandins and opiates- A search for points of mechanistic interaction. Life Sci 31:1703-1706, 1982.

Johnson, M.R.; Eggler, J.F.; Melvin, L.S.; and Milne, G.M. Cannabinoid-derived therapeutants: Further studies in the search for non-addicting drugs to relieve pain and discomfort. Abstracts of Papers, 186th Meeting of the American Chemical Society, Washington, D.C., Aug. 1983, American Chemical Society, Washington, D.C., 1983. Abstr. MEDI 1.

Johnson, M.R.; Eggler, J.F.; Melvin, L.S.; and Milne, G.M., Cannabinoid-derived therapeutants: Further structural studies in the search for non-addicting drugs to relieve pain and discomfort. Abstracts of Papers, 188th Meeting of the American Chemical Society, Philadelphia, PA, Aug. 1984, American Chemical Society, Washington, D.C., 1984. Abstr. MEDI 45.

Kurth, H.-J.; Kraatz, U.; and Korte, F. Synthese von 1-Mercaptocannabinoiden. Ann Chem 1313-1318:1976.

Lemberger, L.S. Potential therapeutic usefulness of marijuana. Annu Rev Pharmacol Toxicol 20:151-172, 1980.

Li, H.C. An archeological and historical account of cannabis in China. J Econ Botany 28:1437-488, 1974.

Loev, B.; Bender, P.E.; Dowalo, F.; Macko, E.; and Fowler, P.J. Cannabinoids. Structure-activity studies related to 1,2-dimethylheptyl derivatives. J Med Chem 16: 1200-1206, 1973.

Mast, R.W.; Mcllhenny, H.M.; Milne, G.M.; and Johnson, M.R. Behavioral effects and pharmacokinetics of nantradol hydrochloride, a potent cannabinoid-related analgetic. Pharmacologist 21:270, 1979.

Matsumoto, K.; Stark, P.; and Meister, R.G. Cannabinoids. 1. 1-Amino- and 1-mercapto-7,8,9,10-tetrahydro-6H-dibenzo(b,d)pyrans. J Med Chem 20:17-30, 1977.

Mechoulam, R.; McCallum, N.K.; and Burstein, S. Recent advances in the chemistry and biochemistry of cannabis. Chem Rev 76:75-112, 1976.

Melvin, L.S.; Johnson, M.R.; and Milne, G.M. Synthesis and analgetic activity of derivatives of 9-nor-9β-hydroxyhexahydrocannabinol. Abstracts of Papers, 180th National Meeting of the American Chemical Society, Las Vegas, NV, August 1980, American Chemical Society, Washington, D.C., 1980. Abstr. MEDI 58.

Melvin, L.S.; Johnson, M.R.; Harbert, C.A.; and Milne. G.M. A prototypical analgetic derived from cannabinoids. Abstracts of Papers, 183rd National Meeting of the American Chemical Society, Las Vegas, NV, March 1982, American Chemical Society, Washington, D.C., 1982. Abstr. MEDI 45.

Melvin, L.S.; Johnson, M.R.; and Milne, G.M. A cannabinoid derived analgetic (CP-55,940). Abstracts of Papers, 186th National Meeting of the American Chemical Society, Washington, DC, Aug. 1983, American Chemical Society, Washington, DC, 1983A, Abstr. MEDI 2.

Melvin, L.S.; Johnson, M.R.; and Milne, G.M. CP-55,244: A cannabinoid derived analgetic. Abstracts of Papers, 186th Meeting of the American Chemical Society, Washington, D.C., Aug. 1983B, American Chemical Society, Washington, D.C., 1983A. Abstr. MEDI 22.

Melvin, L.S.; Johnson, M.R.; Harbert, CA.; Milne, G.M.; and Weissman, A. A cannabinoid derived prototypical analgetic. J Med Chem 27:67-71, 1984A.

Melvin, L.S.; Johnson, M.R.; and Milne, G.M. Cannabinoid receptor site models, Paper presented at 14th Northeast Regional Meeting of the American Chemical Society, Fairfield, June 1984B.

Milne, G.M., and Johnson, M.R. Levonantradol: A role for central prostanoid mechanisms. J Clin Pharmacol 21:367S-374S, 1981.

Milne, G.M.; Weissman, A.; Koe, B.K.; and Johnson, M.R. CP-44,001, a novel benzo(c)quinoline analgesic. Pharmacologist 20:243, 1978.

Milne, G.M.; Mcllhenny, H.M.; Johnson, M.R.; Koe, B.K.; and Weissman, A. One way cross tolerance from morphine to nantradol -A potent, non-opioid analgetic. In: Way, E.L., ed. Endogenous and Exogenous Opiate Agonists and Antagonists. New York:Permagon, 1980A. pp. 367-370.

Milne, G.M.; Koe, B.K.; and Johnson, M.R. Stereospecific and potent analgetic activity for nantradol- -a structurally novel, cannabinoid related analgetic. In: Harris, L.S., ed. Problems of Drug Dependence, 1979. National Institute on Drug Abuse Research Monograph No. 27. DHHS Pub. No. (ADM) 80-901. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1980B. pp. 84-92.

Milne, G.M.; Johnson, M.R.; Koe, B.K.; Weissman, A.; and Melvin, L.S. Mechanistic insights in the design of novel cannabinoid derived therapeutants. Actua Chim Ther 10:227-242, 1983.

Pars, H.G.; Granchelli, F.E.; Razdan, R.K.; Teiger, D.G.; Rosenberg, F.J.; and Harris, L.S. Drugs derived from cannabinoids. 1. Nitrogen analogs, benzopyranopyridines and benzopyranopyrroles. J Med Chem 19:445-454, 1976.

Stark, P. and Archer, R.A. Preclinical pharmacologic profile of a psychoactive cannabinoid. Pharmacologist 17:210, 1975.

Weissman, A.; Milne, G.M.; and Melvin, L.S. Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. J Pharmacol Exp Ther 223:516-523, 1982.

Wilson, R.S.; May, E.L.; Martin, B.R.; and Dewey, W.K., 9-Nor-9β-hydroxyhexahydrocannabinols. Synthesis, some behavioral and analgesic properties, and comparison with tetrahydrocannabinols. J Med Chem 19:1165-1167, 1976.

Winn, M.; Arendsen, D.; Dodge, P.; Dren, A.; Dunnigan, D.; Hallas. R.; Hwang, K.; Kyncl, J.; Lee, Y.-H.; Plotnikoff, N.; Young, P.; and Zaugg, H. Drugs derived from cannabinoids. 5. $\Delta^{6a,10a}$-tetrahydrocannabinol and heterocyclic analogs containing aromaticside chains. J Med Chem 19:461-471, 1976.

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Structure-Anticonvulsant Activity Relationships of Cannabidiol Analogs

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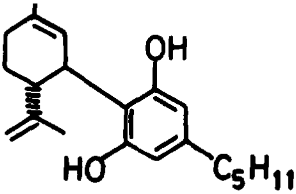
ABSTRACT

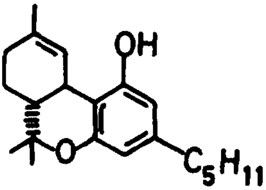
Cannabidiol (CBD) exhibits anticonvulsant activity in experimental animals and in man. As part of a structure-activity study, analogs were prepared wherein the terpene unit, the aryl unit, and/or the side chain were modified. Thus, several pinenyl and carenyl derivatives, aryl ethers and acetates, and a variety of 1",1"-dialkylhexyl and 1",1"-dialkylheptyl analogs were synthesized. The compounds were evaluated for anti-convulsant activity in seizure susceptible (AGS) rats and for neurotoxicity in the rat rotorod (ROT) test. Comparisons of stereoisomers of CBD and several analogs revealed a general lack of stereoselectivity for anticonvulsant and other CNS properties of this class of compounds.

INTRODUCTION

(-)-Cannabidiol [CBD, (-)-1a] is a neutral major cannabinoid of cannabis with potential therapeutic utility. In humans, the drug has antiepileptic efficacy (Cunha et al. 1980) and possibly antidystonic activity (Consroe and Snider 1986) and exhibits no significant side effects. It also has no cannabimimetic activity, i.e., subjective effects in common with cannabis or its neutral major psychoactive ingredient, (-)-delta-9-tetrahydrocannabinol [(-)-delta-9-THC (2), also known as delta-1-THC (Razdan 1986)]. In laboratory animals, (-)-CBD has selectivity of anticonvulsant action relative to neurotoxic effects (Consroe and Snider 1986) and is inactive in models that predict cannabimimetic activity (Razdan 1986). This dichotomy of action between (-)-CBD and (-)-delta-1-THC has led to many investigations of the structure-activity relationships (SAR) in cannabinoids (Razdan 1986). However, most of these studies have been carried out with THC analogs and with laboratory animal models of cannabimimetic activity; thus, virtually all knowledge of cannabinoid SAR is based on the psychoactive component of cannabis-like effect (Razdan 1986). On the other hand, some studies evaluating the anticonvulsant effects of CBD stereoisomers (Leite et al. 1982) and putative metabolites (Carlini et al. 1975) in mice have been reported. We have been carrying out an interdisciplinary research project to evaluate the antiepileptic potential and SAR of new synthetic CBD analogs in rats. Some of our results have been published (Consroe et al. 1981, 1985), and the present report provides an overview of these findings and of new data on the anticonvulsant and differential neurotoxic activities of additional CBD analogs.



(-)-delta-1-CBD (1_a)



(-)-delta-1-THC (2)

MATERIALS AND METHODS

The audiogenic seizure (AGS-susceptible) rat model of epilepsy (Dailey and Jobe 1985) was used to assess anticonvulsant activity, and the rat rotorod (ROT) paradigm (Dunham and Miya 1957) was used to assess differential neurotoxicity (i.e., reflecting mainly sedation and/or incoordination), of CBD analogs. For AGS tests, male and female, 150 to 225-gm, genetically epilepsy prone rats (from the Uaz:AGS-SD colony of the University of Arizona, or from the GEPR-3 colony at the University of Illinois) were used (Consroe et al. 1981, 1985; Dailey and Jobe 1985). For ROT tests, male and female, 150 to 225-gm rats (from the Uaz:AGS-SD colony or the Hsd:(SD)BR colony of the Harlan Sprague Dawley Company, Indianapolis, IN) were used. As detailed previously (Consroe et al. 1981, 1985) CBD analogs (suspended in a vehicle of 10% polysorbate 80 and 90% distilled water) were injected intravenously (iv) and rats were tested 15 minutes later (a peak-effect time for both tests). In the AGS test, responses to sound were recorded as the presence or absence of seizure (i.e., generalized clonus or clonus and generalized flexion). In the ROT test, effects were measured as the ability or inability (i.e., neurotoxicity) of trained rats to remain on a revolving drum (rotorod) for 60 seconds.

Separate groups of ten to fifteen rats were used for each dose of drug tested and, typically, three to six doses of each analog were used for each AGS and ROT test. For appropriate dose-response data, median effective anticonvulsant (ED50) and neurotoxic (TD50) doses and regression line parameters were calculated by computer-assisted probit analysis (Dixon 1977). Subsequent calculations of 95% confidence limits (CL) and comparisons between slopes and median doses (i.e., ED50 and TD50 potency comparisons) were carried out using the method of Litchfield and Wilcoxon (1949). which we have incorporated into a BASIC program for use on a microcomputer. Protective indexes (PI = ROT-TD50/AGS-ED50) also were calculated where possible.

Cannabidiol analogs were prepared by condensation of the appropriate terpene alcohol (+)-p-mentha-2,8-dien-1-ol (-)-P-mentha-2,8-dien-3-ol, (-)-2-caren-4-ol, (+)-verbenol, (-)-verbenol: -(+)-8-acetoxyverbanol, (-)-8-acetoxyverbanol, (+)-8-pivaloxyverbanol, or (-)-8-pivaloxyverbanol with a 5-substituted resorcinol according to the methods of Petrazilka et al. (1969) and Razdan et al. (1974). The synthesis of 5-(1',1',1'-trialkyl)resorcinols have been described previously (Singh et al. 1981). Olivetol (5-n-amylresorcinol), (-)-limonene, (+)- and (-)-α-(-pinene, (-)-myrtenol, and (+)-trans-p-mentha-2,8-dien-1-Ol were obtained from commercial sources and were used as received.

(-)-cis-p-Mentha-2,8-dien-1-ol was prepared from (-)-limonene by the method employed by Rickards and Watson (1980) for the synthesis of (+)-cis-p-mentha-2,8-dien-1-ol from (+)-limonene. (-)-p-Mentha-1,8-dien-3-ol (Leite et al. 1982) was obtained as a 1:3 mixture of cis- and trans-isomers from the sodium borohydride-cerium trichloride reduction (Luchie et al. 1982) of p-mentha-1,8-dien-3-ol (Dauben et al. 1969). (-)-trans-4-Caren-3-ol was obtained by the reaction of sodium phenylselenide on (-)-3β,4β-epoxycarane (Cocker and Grayson 1969), followed by oxidation of the phenylselenide and elimination of the selenoxide (Uzarewicz and Zientek 1977). (+)- and (-)-Verbenol were obtained as mixtures of cis- and trans-isomers from (+)- and (-)-α-pinene (Whitham 1961)) respectively. (+)-Myrtenol was prepared by selenium dioxide oxidation of (-)-α-pinene followed by reduction with sodium borohydride. (+)- and (-)-Myrtenols were acylated with acetic anhydride or pivaloyl chloride in pyridine to obtain the corresponding acetate or pivalate, and the latter were subjected to allylic oxidation with chromium trioxide/pyridine (Dauben et al. 1969) and then reduction with dibutyllithfum aluminium hydride (DiBAH) to give the corresponding 8-acetoxy- and 8-pivaloxy-verbenols.

The optical purity of (+)-CBD obtained from either (-)-p-mentha-1,8-dien-3-ol or (-)-p-mentha-2,8-dien-1-ol was calculated to be 83% based on observed αD values of + 80± 1^0 for the two chemically pure samples. Optical purities for the cannabidiol analogs derived from (+)-3-carene and (+)- and (-)-α-pinene were not determined, but were estimated to be in the range of 80% to 95% based on the optical purities of the starting materials. Structures of the final compounds were confirmed by proton nmr and elemental analysis, which agreed within ± 0.4% of the theoretical values. The cannabidiols prepared along with data for their anticonvulsant and neurotoxic activities are provided in table 1.

RESULTS AND DISCUSSION

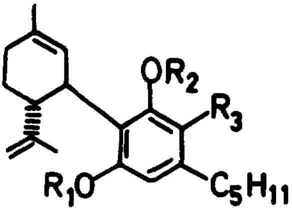
Dose-response data for standard compounds phenytoin (PHT),

TABLE 1

Preliminary and Completed Dose-Response Data of Standard Anticonvulsants and CBD Analogs in ROT and AGS Tests[a]

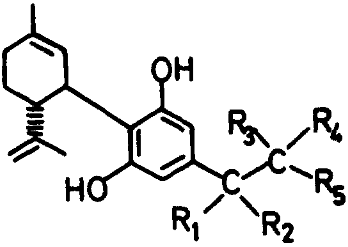
Compd. No.	ROT	AGS	PI
Phenobarbital	28.9 (26.0-32.2)	10.8 (8.5-13.8)	2.7
Carbamazepine	33.9 (32.6-35.4)	12.8 (9.7-16.9)	2.7
Phenytoin	23.9 (22.3-25.6)	14.7 (11.1-19.6)	1.6

(-)-CBD: Aryl Ring Modifications[b]



Compd. No.	R_1	R_2	R_3	ROT	AGS	PI
(-)-1_a	H	H	H	30.7 (26.1-36.1)	14.9 (10.9-20.1)	2.1
1b	Ac	Ac	H	47.9 (43.2-53.2)	11.5 (9.5-13.9)	4.2
1c	H	Ac	Ac	0/10 at 31 mg/Kg	0/12 at 15 mg/Kg	---
1_d	CH_3	CH_3	H	0/10 at 31 mg/Kg	0/10 at 15 mg/Kg	---
1_e	CH_3	H	H	0/10 at 31 mg/Kg	2/10 at 15 mg/Kg	---

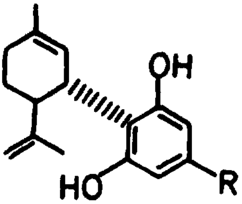
(-)-CBD: Side Chain Modifications[c]



Compd. No.	R_1	R_2	R_3	R_4	R_5	ROT	AGS	PI
(-)-3_a	CH_3	CH_3	H	H	C_5H_{11}	2.1	(2.3-6.3)	0.6
3_b	CH_3	C_2H_5	H	H	C_5H_{11}	31 mg/Kg	6/10 at 15 mg/Kg	---

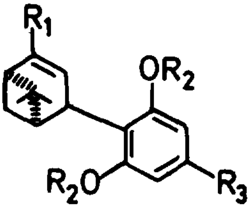
3_c	CH_3	$CH_2CH=CH_2$	H	H	C_5H_{11}	0/10 at 31 mg/Kg	0/10 at 15 mg/Kg	---
(-)-3_d	CH_3	CH_3	H	H	C_4H_9	6/10 at 31 mg/kg	6/10 at 15 mg/Kg	---
3_e	CH_3	C_2H_5	H	H	C_4H_9	9/10 at 31 mg/Kg	4/10 at 15 mg/KG	---
3_f	CH_3	$CH_2CH=CH_2$	H	H	C_4H_9	0/10 at 31 mg/Kg	1/10 at 15 mg/Kg	---
3_g	H	H	CH_3	CH_3	C_5H_{11}	10/10 at 31 mg/Kg	4/10 at 15 mg/Kg	---

(+)-CBDs[c]



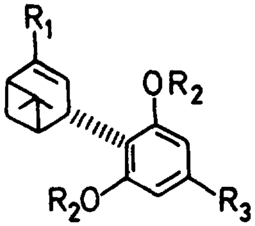
Compd. No.	R	ROT	AGS	PI
(+)-1a	C_5H_{11}	39.7 (36.6-42.9)	16.2 (14.6-18.0)	2.5
(+)-3a	1,1-DMH	10/10 at 31 mg/Kg	9/10 at 4 mg/Kg	---

(-)-Pinenyl CBDs



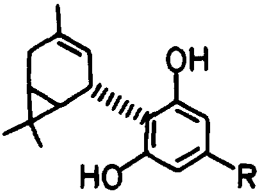
Compd. No.	R_1	R_2	R_3	ROT	AGS	PI
(-)-4_a	CH_3	H	C_5H_{11}	8/10 at 31 mg/Kg	7/10 at 15 mg/Kg	---
(-)-4_b	CH_3	H	1,1-DMH	9.2 (6.8-12.3)	5.2 (3.6-7.4)	1.8
(-)-4_c	CH_2OAC	H	1,1-DMH	2.6 (1.8-3.7)	2.5 (1.4-4.3)	1.0
(-)-4_d	CH_2OAC	Ac	1,2-DMH	4/4 at 31 mg/Kg	4/10 at 15 mg/Kg	---
(-)-4_e	CH_2OPv	H	1,1-DMH	0/6 at 31 mg/Kg	5/10 at 15 mg/Kg	---

(+)-Pinenyl CBDs



Cmpd.	No.	R_1	R_2	R_3	ROT	AGS	PI
(+)-4_a		CH_3	H	C_5H_{11}	5/10 at 31 mg/Kg	1/10 at 15 mg/Kg	---
(+)-4_b		CH_3	H	1,1-DMH	1.9 (1.6-2.2)	0.7 (0.5-1.0)	2.7
(+)-4_c		CH_2OAc	Ac	1,2-DMH	9/10 at 4 mg/Kg	4/10 at 4 mg/Kg	---
(+)-4_e		CH_2OPv	H	1,1-DMH	4/10 at 4 mg/Kg	8/10 at 4 mg/KG	---
(+)-4_f		CH_2OAc	Ac	1,1-DMH	10/10 at 4 mg/Kg	9/9 at 4 mg/Kg	---

(+)-Carenyl CBDs



Compd. No.	R	ROT	AGS	PI
(+)-5_a	C_5H_{11}	99.8 (90.4-110.1)	18.1 (16.2-20.3)	5.5
(+)-5_b	1,1-DMH	3.7 (3.3-4.1)	3.4 (3.0-3.9)	1.1

[a] Median toxic (TD50) and effective (ED50) doses and 95% confidence limits (in parentheses) are in mg/Kg, iv PI = protective index = ROT-TD50/AGS-ED50. Ratios refer to the number of rats protected against AGS or number of rats toxic/ number of rats tested (at the iv dose listed). Chemical abbreviations are: AC = acetyl; DMH = dimethylheptyl; and Pv = pivaloyl.

[b] Data taken, in part, from Consroe et al. (1981).

[c] Data taken, in part, from Consroe et al. (1985).

phenobarbital (PB), and carbamazepine (CBZ); (-)-CBD [(-)-1_a] and a few selected analogs; plus single dose comparisons [for most compounds equivalent to the calculated AGS-ED50 and ROT-TD50 of (-)-1_a] are presented in table 1. In the AGS test, peak-effect times were observed 15 minutes after (-)-1_a, CBD analogs, and PHT; 30 minutes after PB; and 45 minutes after CBZ injections. Vehicle administration neither protected against AGS nor caused ROT toxicity.

In an earlier study (Consroe et al. 1981), it was observed that certain aryl modifications in (-)-1_a markedly reduced both anti-AGS activity and ROT neurotoxicity. For example, 1_d, the dimethylether of (-)-1_a, is inactive in the AGS and ROT tests at 15 and 31 mg/Kg, respectively, and the monomethyl ether (1_e) is marginally active in the AGS test and inactive in the ROT test at the same doses. These earlier data are repeated in table 1. The monoacetate of 3'-acetyl CBD (1_c), obtained by photo-Fries rearrangement of CBD diacetate (1_b), was similarly inactive in both tests. It was therefore concluded (Consroe et al. 1981) that both phenolic hydroxyl groups of CBD must be free for anti-AGS and ROT activities to occur. Weak anti-AGS activity of 1_e could be due to inefficient demethylation to 1_a. Intramolecular hydrogen bonding between the free phenolic group and the 3'-acetyl group of 1_c may be responsible for its lack of activity. The lack of anti-AGS activity in abn-CBD analogs (Consroe et al. 1981) indicates that the positions of the hydroxyl groups on the aromatic unit are important.

CBD diacetate (1_b), in contrast to the ethers, was found to be equivalent to 1_a in anti-AGS potency in a single dose comparison (Consroe et al. 1981). It was tentatively concluded that 1_b is efficiently hydrolyzed by esterases <u>in</u> <u>vivo</u> to la which is responsible for the anti-AGS activity. The neurotoxicity of 1_b, on the other hand, was significantly lower than that associated with 1_a. These preliminary findings prompted us to perform complete dose-response and time course studies on 1_b. Dose-response data (table 1) reveal an ED50 of 11.5 mg/Kg and a TD50 of 47.9 mg/Kg for 1_b, as compared with 30.7 mg/Kg and 14.9 mg/Kg, respectively, for 1_a. Thus, the protective index (PI) of 1_b is two times greater than the PI of 1_a. Comparative time course studies of la and lb at equiactive doses indicate that the anti-AGS effects of 1_a have a more rapid onset and a longer duration than those of 1_b. The ROT neurotoxic effects of the two agents, on the other hand, follow a similar time course. The slightly greater anti-AGS potency of 1_b and its significantly reduced ROT neurotoxicity as compared with 1_a, together with the time course differences, suggest that the sites of action of the two effects may be different. If, indeed, 1_b must be converted to 1_a <u>in</u> <u>vivo</u> in order to be active, then this conversion must- be very efficient. Acetylation of the phenolic groups of 1_a could serve to increase the lipophilicity of the compound, thereby facilitating its penetration into the central nervous system. At the same time, the phenolic groups are protected from inactivation by conjugating enzymes.

We have recently investigated the effects of structural modification of the side chain of CBD on anti-AGS and ROT potencies (Consroe et al. 1985). The compounds investigated were the branched chain compounds 3_a-3_g. For anti-AGS activity, the order of potency (-)-3_a 7 3_b = 1_a >3_c was observed in the 1",1"-dialkylheptyl series, while it was $\underline{1_a \geq 3_e}$ > 3_d = 3_f in the 1",1"-dialkylhexyl series. In the ROT neurotoxicfty test, the rank order of potencies was (-)-3_a > (-)-1_a 7 3_b 7 3_c in the 1",1"-dialkylheptyl series and 3_e > 3_d = 1_a in the 1",1"-dialkylhexyl series. Thus, optimal anti-AGS potency, together with maximal neurotoxicity, was observed with the 1", 1"-dimethylheptyl analog (-)-3_a. The 1" ,1"-dimethylhexyl analog (-)-3_d, on the other hand, is significantly less potent than (-)-3_a. Leite et al. (1982) observed similar enhancemeent in anticonvulsant and CNS depressant potencies of 1" ,1"-dimethylheptyl analogs of CBD in mice. In contrast, the 1"-methyl-1"-ethylheptyl analog 3_b exhibited anti-AGS activity comparable to that of 1_a, but was considerably less neurotoxic. Additional studies on this compound (which, like side chain analogs 3_c-, 3_e, and 3_f, is a diastereoisomeric mixture due to asymmetry at the 1"-carbon) appear to be warranted. The 1"-methyl-1"-ethylhexyl analog 3_e, on the other hand, is significantly more neurotoxic than (-)-1_a, but significantly less potent in the AGS test. The basis for the remarkable differences in the side chain analogs differing by a single carbon atom remain to be explained. Significant neurotoxicity and some anti-AGS activity are retained in the 1",2"-dimethylheptyl analog 3_g.

Early comparisons of CBD analogs derived from (+)- and (-)-α-pinene suggested that the anti-AGS effects of CBD and its derivatives might be stereoselective. Thus, (-)-4_a (which is stereochemically related to (-)-1_a) was significantly more potent than its enantiomer (+)-4_a in the AGS test when each was administered in a dose of 15 mg/Kg (Consroe et al. 1981). However, Leite et al. (1982) found that (-)-1_a and (+)-1_a were essentially equiactive as anticonvulsants (protection against pentylenetetrazole seizures) and as CNS depressants (prolongation of phenobarbital sleeping time) in mice. A parallel lack of stereoselectivity was observed for the highly potent 1" ,1"-dimethylheptyl enantiomers (-)-3_a and (+)-3_a. A general lack of stereoselectivity for the anti-AGS and ROT neurotoxic effects of CBD and its derivatives was recently confirmed (Consroe et al. 1985). Thus, (-)-1_a is marginally more potent than (+)-1_a in both the AGS and ROT tests, while (+)-4_b is somewhat more active than (-)-4_b in the two tests (see table 1). . There are insufficient data with which to quantitatively compare (-)-3_a and (+)-3_a, but it is evident that both are highly active in the AGS and ROT tests. Similarly, the enantiomeric pair (-)-4_d and (+)-4_d appear to exhibit comparable anti-AGS and ROT neurotoxic potencies. On the other hand, (+)-4_e is apparently more potent than (-)-4_e. These two enantiomers seem to be worthy of further study since single dose data suggest that each will exhibit a favorable protective index.

The general lack of stereoselectivity for the anti-AGS and ROT neurotoxic activity of la and its analogs stands in stark contrast to the situation with THC-like compounds (see Mechoulam et al., this volume). Thus, (-)-delta-6-THC analogs that are stereochemically related to (-)-delta-1-THC (2) are markedly more potent than their (+)-enantiomers in both AGS and ROT tests. These findings suggest that the mechanisms of anticonvulsant and central nervous system depressant actions of CBDs and THCs may be different.

The CBD analog derived from (+)-delta-3-carene, i.e., (+)-5_a, is of interest because of its high protective index. Thus, although (+)-5_a has an ED50 of 18.1 mg/Kg in the AGS test and is therefore comparable to (+)-1_a (to which it is stereochemically related) in potency, it has a TD50 of approximately 100 mg/Kg. This separation of activities is, unfortunately, not maintained in the 1",1"-dimethylheptyl derivative (+)-5_b, which has a PI of 1.1.

Solid state (X-ray crystallographic, Jones et al. 1977). solution (Tamir et al. 1980; Kane et al. 1984), and theoretical (Kane et al. 1984) studies all indicate that the 3-olivetyl and 4-isopropenyl groups of CBD prefer a dfequatorfal conformation. These functionalities in the highly ri gfd pinenyl derivatives 4, on the other hand, are held in a diaxial conformation. The less rigid carenyl derivatives 5 no doubt also prefer a diaxial conformation. Since the energy difference between the diequatorial and diaxial conformations of CBD may be slight, it is possible that the "active form" of the molecule may be its energetically less favored diaxial conformer.

The following structure-activity generalizations have emerged from these studies: The aryl unit and the side chain of CBD are highly sensitive to structural modification. Most changes in the aryl unit, e.g., blocking or changing the positions of the phenolic groups, greatly decreased anti-AGS activity. Optimal anti-AGS and ROT neurotoxic effects were observed in 1"-1"-dimethylheptyl derivatives. On the other hand, the terpene unit appears to be relatively insensitive to structural changes. Derivatives of α-pinene and delta-3-carene are as potent as (-)-1_a and there is a general lack of stereoselectivity associated with the terpene unit.

REFERENCES

Carlini, E.A.; Mechoulam, R.; and Lander, N. Anticonvulsant activity of four oxygenated cannabidiol derivatives. Res Commun Chem Pathol Pharmacol 12:1-12, 1975.

Cocker, W., and Grayson, D.H. A convenient preparation of (-)-3,4-epoxycarane. Tetrahedron Lett 4451-4452, 1969.

Consroe, P.; Martin, A.; and Mecholam, R. Anticonvulsant effects of cannabidiol stereoisomers and analoos in rats. In: Harvey, D.J., ed. Marijuana '84. Proceedings of the Oxford Symposium on Cannabis. Oxford: IRL Press, 1985. pp. 705-712.

Consroe. P.; Martin, A.; and Singh. V. Antiepileptic potential cannabidiol analogs. J Clin Pharmacol 21:428s-436s, 1981.

Consroe, P., and Snider, S.R. Therapeutic potential of cannabinoids in neurological disorders. In: Mechoulam, R., ed. Cannabinoids as Therapeutic Agents. Boca Raton: CRC Press, 1986. pp. 21-49.

Cunha, J.M.; Carlini, E.A.; Periera, A.E.; Ramos, O.L.; Pimentel, C.; Gagliardi, R.; Sanvito, W.L.; Lander, N.; and Mechoulam. R. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. Pharmacology 21:175-185, 1980.

Dailey, J.W., and Jobe, P.C. Anticonvulsant drugs and the genetically epilepsy-prone rat. Fed Proc 44:2640-2644, 1985.

Dauben, W.G.; Lorber, M.; and Fullerton, D.S. Alylic oxidation of oleffns with chromium trioxide•pyridine complex. J Org Chem 34:3587-3592, 1969.

Dixon, W.J. BMD Biomedical Computer Programs. Berkeley: University of California Press, 1977. pp. 439-450.

Dominiani, S.J.; Ryan, C.W.; and De Armitt, C.N. Synthesis of 5-(tert-alkyl)resorcinols. J Org Chem 42:344-346, 1977.

Dunham. N.W.. and Miva. T.S. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc 46:208-209, 1957.

Jones, P.G.: Falvello. L.; Kennard, O.; Sheldrick, G.M.; and Mechoulam, R. Cannabidiol. Acta Cryst B33:3211-3214, 1977.

Kane, V.V.; Martin, A.R.; Jaime, C.; and Osawa, E. Dynamic nuclear magnetic resonance and empirical force field studies of cannabidiol. Tetrahedron 40:2919-2927, 1984.

Leite, J.R.; Carlini, E.A.; Lander, N.; and Mecholam, R. Anticonvulsant effects of the (-)- and (+)-isomers of cannabidiol and their dimethylheptyl homologs. Pharmacology 24:141-146, 1982.

Litchfield J.T., and Wilcoxon, F. A Simplified method of calculating dose-effect experiments. J Pharmacol Exp Ther 96:99-133, 1949.

Luchie, J.-L.; Rodriguez-Hahn, L.; and Crabbe, P. Reduction of natural enones in the presence of cerium trichloride. J Chem Soc Chem Comm 601-602, 1982.

Petrizilka, T.; Haeflinger, W.; and Sikemeier, C. Synthese von Haschisch-Inhaltstoffen. Helv Chim Acta, 52:1102-1134, 1969.

Razdan, R.K. Structure-activity relationships in cannabinoids. Pharmacol Rev 38:75-149, 1986.

Razdan, R.K.; Dalzell, H.C.; and Handrick, G.R. Hashish. A simple one-step synthesis of (-)-delta-1-tetrahydro-cannabinol (THC) from p-mentha-2,8-dien-1-ol and olivetol. J Am Chem Soc 96:5860-5865, 1974.

Rickards, R.W., and Watson, W.P. Conversion of (+)-R-limonene into (+)-(IS,4R)-p-mentha-2,8-dien-1-ol, an intermediate in the synthesis of tetrahydrocannabinoids. Aust J Chem 33:451-454, 1980.

Singh, V.; Kane, V.V.; and Martin, A.R. A new synthesis of 5-(1',1',1'-trialkylmethyl)resorcinols. Synth Comm 11:429-437, 1981.

Tamir, I.; Mechoulam, R.; and Meyer, A.Y. Cannabidiol and phenytoin: A structural comparison. J Med Chem 23:220-223, 1980.

Uzarewicz, A., and Zientek, E. Action of diphenyl diselenide on - and + 3,4-epoxycarane and 2β,3β-epoxypinane. Rocz Chem 51:181-184, 1977.

Whitham, G. The reaction of α-pinene with lead tetraacetate. J Chem Soc 2232-2236, 1961.

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Anticonvulsant and Neurotoxic Effects of Tetrahydrocannabinol Stereoisomers

Paul Consroe, Ph.D., and Raphael Mechoulam, Ph.D.

ABSTRACT

Enantiomers of delta-6-tetrahydrocannabinol (THC), delta-6-THC-1", 1"-dimethylheptyl (DMHP), and 7-OH-delta-6-THC-l", 1"-DMHP were assessed for their ability to block audiogenic seizures in genetically epilepsy-prone rats. The stereoisomers were evaluated also for their ability to produce differential neurotoxicity in the rat rotorod (ROT) paradigm. Potency comparisons among the compounds revealed modest to profound stereoselectivity for anticonvulsant and neurotoxic activities, a general increase in both activities with the DMHP and 7-OH modifications of delta-6-THC, and some favorable separation between anticonvulsant and neurotoxic activities with selected THC analogs.

INTRODUCTION

Two important generalizations of tetrahydrocannabinol activity are that DMHP side chain analogs are much more potent than are their n-pentyl side chain homologs (Razdan 1986), and that (+)-optical isomers are substantially less active than are their (-)-enantiomers (Dewey et al. 1984). These structure-activity relationships (SAR) are based largely on studies beginning over four decades ago with analogs of the unnatural racemic delta-3-THC (also known as delta-6-alpha-10-alpha-THC) and on studies beginning over two decades ago with derivatives of (-)-delta-1-THC (also known as (-)-<u>trans</u>-delta-9- THC) and (-)-delta-6-THC (also known as (-)-<u>trans</u>-delta-8-THC), the latter being the major and minor psychoactive principles of cannabis, respectively (Mechoulam and Edery 1973). The activities of the natural THCs (each having an n-pentyl side chain) and a few DMHP analogs of delta-3-THC have been determined in humans --most commonly as a function of their cannabimimetic activity, i.e., cannabis-like subjective effects such as the euphoric "high" (Pradhan

1984; Razdan 1986). The (+)-THC isomers and a wider range of DMHP analogs have been evaluated in laboratory animals -- most commonly in dog, monkey, and/or rodent behavioral paradigms (Dewey et al. 1984; Pradhan 1984). The relative potencies of cannabinoids in these animal tests are thought to be largely reflective of their potential to produce cannabimimetic effects in humans (Razdan 1986).

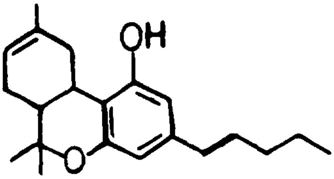
Unfortunately, many investigations carried out to date have used impure optical isomers, such as the often-studied 1", 2" -DMHP homologs of (-)-delta-6-THC (four possible isomers) and racemic delta-3-THC (eight possible isomers). Depending upon the proportion of isomers, the racemic mixtures may show significant variability in their potencies and activities (Pradhan 1984). Interestingly, substantial variability in activity was cited as the reason for the termination (Hardman et al. 1971, p. 296) of an otherwise promising (and the only) clinical trial of DMHP analogs of THC in epileptic patients (Davis and Ramsey 1949).

In light of the above considerations, we sought to evaluate the stereoselectivity relationships of six stereoisomers of THC. The compounds were assessed for anticonvulsant and (rotorod) neurotoxic activities in the rat --activities which pertain to a potential therapeutic application of cannabinoids.

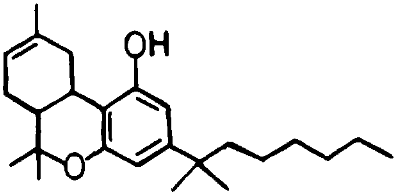
MATERIALS AND METHODS

The cannabinoids employed, along with their structural formulae, are illustrated in figure 1. (+)-Delta-6-THC, and (+)- and (-)- stereoisomers of delta-6-THC-1", 1"-DMHP were circa 97% optically pure, and 7-OH-delta-6-THC-1",1"-DMHP (also known as 11-OH-trans-delta-8-THC-1',1'- DMHP) was 99.9% optically pure. These cannabinoids were synthesized by Mechoulam et al. (this volume). (-)- Delta-6-THC was 96.7% optically pure and was obtained from the National Institute on Drug Abuse.

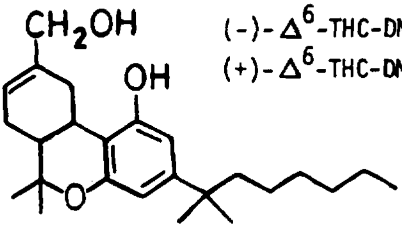
The audiogenic seizure- (AGS-) susceptible rat model of epilepsy (Dailey and Jobe 1985) was used to assess anticonvulsant activity, and the rat ROT paradigm (Dunham and Miya 1957) was used to assess differential neurotoxicity (i.e., reflecting mainly sedation and/or incoordination), of the THC compounds. Male, 150-200 gm, genetically epilepsy-prone rats (Dailey and Jobe 1985) were used for the AGS tests; and male, 150-200 gm rats (Hsd: (SD)BR; Harlan Sprague Dawley Company, Indianapolis, IN) were used for the ROT tests. As detailed previously (Consroe and Man 1973; Consroe et al. 1985), cannabinoids were prepared in a vehicle of 10% polysorbate 80 and 90% distilled water, and were injected intravenously (i.v.); and rats were tested 15 minutes (for DMHP analogs) or 30 minutes (for the parent THC isomers) later, the respective peak-effect times for both tests. In the AGS test, responses to sound were recorded as the presence or absence of seizure (i.e., generalized clonic convulsions). In the ROT test, effects were measured as the ability or inability (i.e., neurotoxicity) of trained rats to remain on a revolving rotorod for 60 seconds.



(-)-Δ^6-THC
(+)-Δ^6-THC



(-)-Δ^6-THC-DMHP
(+)-Δ^6-THC-DMHP



(-)-7-OH-Δ^6-THC-DMHP
(+)-7-OH-Δ^6-THC-DMHP

FIGURE 1

Separate groups of 10 to 15 rats were used for each dose, and 3 to 6 doses of each cannabinoid were used for each AGS and ROT test. Median effective anticonvulsant (ED50) and neurotoxic (TD50) doses and regression line parameters were calculated by computer-assisted probit analysis (Dixon 1977). Subsequent calculations of 95% confidence limits (CL) and comparisons between slopes and median doses (i.e., ED50 and TD50 potency comparisons) were carried out by using the equations of Litchfield and Wilcoxon (1949). We have incorporated these equations into a GW BASIC program for use on a microcomputer operating under MS-DOS. Finally, the protective index (PI) of compounds was calculated by the usual method (i.e., PI = TD50/ED50).

RESULTS AND DISCUSSION

Table 1 presents the dose-effect parameters of the THC stereoisomers. Comparisons among these data reveal several salient findings.

TABLE 1

Dose-Response Parameters of Delta-6-THC Stereoisomers in Audiogenic Seizure (AGS) and Rotorod (ROT) Neurotoxicity Tests in Rats[1]

Drug	ROT-TD50 (95% CL)	AGS-ED50 (95% CL)	PI
(-)-7-OH-THC-1",1"-DMHP	0.007 (0.005-0.009)	0.004 (0.002-0.007)	1.75
(+)-7-OH-THC-l", 1"-DMHP	Not active[2]	Not active[3]	
(-)-THC[4]	1.85 (0.98-3.45)	4.70 (3.11-7.09)	0.39
(+)-THC	21.66 (1g.81-23.68)	15.23 (12.42-18.68)	1.42
(-)-THC-l", 1"-DMHP	0.035 (0.031-0.040)	0.041 (0.037-0.046)	0.85
(+)-THC-1", 1"-DMHP	0.53 (0.37-0.76)	0.89 (0.73-1.1)	0.60

[1] Median toxic (TD50) and effective (ED50) doses and 95% confidence limits (CL) are in mg/kg, i.v. PI = protective index = ROT-TD50/AGS-ED50.

[2] At 1.0, 7.0, and 15.0 mg/kg, i.v.; higher doses were not tested due to insufficient quantity of drug.

[3] At 0.1, 1.0, and 4.0 mg/kg, i.v.; higher doses were not tested due to insufficient quantity of drug.

[4] Data taken from Consroe and Man (1973).

First, the (-)-enantiomers are significantly ($P < .05$) more potent (or substantially more active) than are their respective (+)-enantiomers in both anticonvulsant (AGS) and neurotoxicity (ROT) tests. For anticonvulsant activity, (-)-delta-6-THC is 3 times more potent than (+)-delta-6-THC, and (-)-delta-6-THC- 1",1"-DMHP is 22 times more potent than (+)-delta-6-THC-1",1"- DMHP. Also, (-)-7-OH-delta-6-THC-1",1"-DMHP is extremely potent and its (+)-enantiomer is not active in doses up to 1,000 times higher. For neurotoxic activity, (-)-delta-6-THC is 12 times more potent than (+)-delta-6-THC, and (-)-delta-6-THC-1",1"-DMHP is 15 times more potent than (+)-delta-6-THC-1",1"-DMHP. Again, (-)-7-OH-delta-6-THC-1",1"-DMHP is extremely potent and its (+)- enantiomer is not active in doses up to 2,143 times higher.

Although neither these nor any other pairs of optically pure THC stereoisomers have been evaluated for anticonvulsant or ROT neurotoxicity effects, the comparative activities (or in some cases, dose-response potencies) of the enantiomers of delta-1-THC (Dewey et al. 1984) and of delta-6-THC (Consroe et al. 1982; Dewey et al. 1984) have been ascertained in several other laboratory animal paradigms. In all cases, the (-)-isomers were more active than their respective (+)-isomers; and for the enantiomers of delta-6-THC, differences in activity ranged from about fourfold to fiftyfold depending upon the species and the pharmacological test system used. While it is clear there is a stereoselectivity of action for the THCs, such is not the case for cannabidiol (CBD), a noncannabimimetic cannabinoid of cannabis. In a previous study (Consroe et al. 1985), we found that the natural (-)-CBD was equipotent to the unnatural (+)-CBD in both AGS and ROT tests. These findings suggest that stereoselective receptor mechanisms may be important for many effects of the THCs, including their anticonvulsant and neurotoxic activities. However, it is unlikely that such receptor mechanisms underlie the latter effects of CBD.

Second, the 1", 1"-DMHP and 7-OH modifications of THC greatly increase potencies in both anticonvulsant and neurotoxic paradigms (table 1). (-)-Delta-6-THC-1",1"-DMHP is 115 times more potent in the AGS test and 53 times more potent in the ROT test than is (-)-delta-6-THC. (-)-7-OH-delta-6-THC-1",1''-DMHP is 1,175 times more potent in the AGS test and 264 times more potent in the ROT test than is (-)-delta-6-THC. Additionally, (+)-delta- 6-THC-1", 1"-DMHP is 17 times more potent in the AGS test and 41 times more potent in the ROT test than is (+)-delta-6-THC. However, the "inactivity" of (+)-7-OH-delta-6-THC-1",1"-DMHP is an enigma. Because of limited quantity of the latter drug, we were unable to evaluate doses higher than 4 mg/kg in the AGS test and 15 mg/kg in the ROT test. Nevertheless, these doses are not too different from the AGS-ED50 (15.23 mg/kg) and ROT-TD50 (21.66 mg/kg) of (+)-delta-6-THC.

Aside from this apparent exception, the present results are generally congruent with many previous data showing potency increases with 1",2"-DMHP and 7-OH modifications of the THCs (Mechoulam and Edery 1973; Pradhan 1984; Razdan 1986). Additionally, several 1",2"-DMHP analogs of (+/-)-delta-3-THC (i.e., compounds existing as a mixture of 4 to 8 possible isomers) have been tested for anticonvulsant and ROT neurotoxic activities in rodents and their potencies compared to that of (-)-delta-1-THC (Karler et al. 1974; Plotnikoff et al. 1975). Depending on the analog, species, seizure test, and other experimental conditions, potency increases ranging from about twofold to sixteenfold were reported.

Finally, a perusal of the PIs of cannabinoids evaluated in the present study (table 1) show that only (-)-7-OH-delta-6-THC-1", 1"-DMHP (PI = 1.75) and (+)-delta-6-THC (PI = 1.42) have a favorable separation between anticonvulsant and (ROT) neurotoxic activities.

In a previous study (Consroe et al. 1985), we determined the i.v. potencies of three major antiepileptic drugs in our rat AGS and ROT tests. A collation of these data indicates that the PIs of the above THC analogs are similar to that of phenytoin (PI = 1.6), but are lower than those of phenobarbital (PI = 2.7) and carbamazepine (PI = 2.7). The clinical efficacy of these classic antiepileptic drugs is unquestionably good; but the therapeutic usefulness of each of these agents, especially of phenytoin, is limited in many patients by sedation, incoordination, and/or other neurologic side effects (Livanainen and Savolainen 1983; Keranen and Sivenius 1983). Our comparative, albeit limited, preclinical data suggest that the THC analogs might have this same type of therapeutic limitation. Additionally, (-)-7-OH-delta-6-THC-1", 1"-DMHP has recently been found to be extremely potent (and, in fact, the most potent cannabinoid ever tested) in the drug discrimination learning (DDL) test in rats and pigeons (Mechoulam et al., this volume). As this test (Jarbe et al. 1981; Weissman 1981) is a qualitative and possibly a quantitative predictor of cannabimimetic activity, (-)-7-OH-delta-6-THC-1",1"-DMHP would appear to be a poor candidate for therapeutic application. On the other hand, (+)-delta-6-THC is essentially inactive in the DDL paradigm (Jarbe et al. 1981) and, thus, would be predicted to have minimal, if any, cannabimimetic activity. While the presently determined PI of (+)-delta-6-THC is certainly not optimum, other (+)-THC analogs might have better selectivity of anticonvulsant activity relative to neurotoxic action. Further evaluation of (+)-THC analogs, such as (+)-7-OH-delta-6-

THC-1", 1"-DMHP, would appear to be an important endeavor for future research in this area.

REFERENCES

Consroe, P., and Man, D.P. Effects of delta-8- and delta-9-tetrahydrocannabinol on experimentally induced seizures. Life Sci 13:429-439, 1973.

Consroe, P.; Martin, A.R.; and Fish, B.S. Use of a potential rabbit model for structure-behavioral activity studies of cannabinoids. J Med Chem 25:596-599, 1982.

Consroe, P.; Martin, A.; and Mechoulam, R. Anticonvulsant effects of cannabidiol stereoisomers and analogs in rats. In: Harvey, D.J., ed. Marihuana '84. Proceedings of the Oxford Symposium on Cannabis. Oxford: IRL Press, 1985. pp. 705-712.

Dailey, J.W., and Jobe, P.C. Anticonvulsant drugs and the genetically epilepsy-prone rat. Fed Proc 44:2640-2644, 1985.

Davis, J.P., and Ramsey, H.H. Anti-epileptic action of marijuana-active substances. Fed Proc 8:284, 1949.

Dewey, W.L.; Martin, B.R.; and May, E.L. Cannabinoid stereoisomers: Pharmacological effects. In: Smith D.F., ed. CRC Handbook of Stereoisomers: Drugs in Psychopharmacology. Boca Raton: CRC Press, 1984. pp. 317-326.

Dixon; W.J, BMD Biomedical Computer Programs. Berkelev: University of California Press. 1977. pp. 439-450.

Dunham, N.W., and Miya, T.S. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc 46:208-209, 1957.

Hardman, H.P.; Domino, E.R.; and Seevers, M.H. General pharmacological actions of some synthetic tetrahydrocannabinol derivatives. Pharmacol Rev 23:295-315, 1971.

Jarbe, T.U.C.; Swedberg, M.D.B.; and Mechoulam, R. A repeated tests procedure to assess onset and duration of the cue properties of (-)-delta-9-THC, (-)-delta-8-THC-DMH and (+)- delta-8-THC. Psychopharmacology 75:152-157, 1981.

Karler, R.; Cely, W.; and Turkanis, S.A. Anticonvulsant properties of delta-9-tetrahydrocannabinol and other cannabinoids. Life Sci 15:931-947, 1974.

Keranen, T., and Sivenius, J. Side effects of carbamazepine, valproate and clonazepam during long-term treatment of epilepsy. Acta Neurol Scand (Suppl 97) 68:69-80, 1983.

Litchfield, J.T., and Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96:99-133, 1949.

Livanainen, M., and Savolainen, H. Side effects of phenobarbital and phenytoin during long-term treatment of epilepsy. Acta Neurol Stand (Suppl 97) 68:49-67, 1983.

Mechoulam, R., and Edery, H. Structure-activity relationships in the cannabinoid series. In: Mechoulam, R., ed. Marihuana Chemistry, Metabolism and Clinical Effects. New York: Academic Press, 1973. pp. 101-136.

Plotnikoff, N.P.; Zaugg, H.E.; Petersen, A.C.; Arendsen, D.L; and Anderson, R.F. New benzopyrans: Anticonvulsant activities. Life Sci 17:97-104, 1975.

Pradhan, S.N . Pharmacology of some synthetic tetrahydrocannabinols. Neurosci Biobehav Rev 8:369-385, 1984.

Razdan, R.K.Structure-activity relationships in cannabinnoids. Pharmacol Rev 38:75-149, 1986.

Weissman, J.A. On the definition of cannabinoids: Botanical? Chemical? Pharmacological? J Clin Pharmacol 21:159s-165s, 1981.

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Different Cannabinoids Exhibit Different Electrophysiological Properties

Stuart A. Turkanis, Ph.D., and Ralph Karler, Ph.D.

INTRODUCTION

Delta-9-tetrahydrocannabinol (THC) is a nonselective, centrally active drug that produces a wide variety of pharmacological effects (Truitt and Braude 1974; Paton 1975); for example, in conscious animals, this cannabinoid causes a complex mixture of central depressant and excitatory responses ranging from sedation, anticonvulsant effects, and motor toxicity to overt convulsions (Karler and Turkanis 1981). Fifteen years ago there were few marijuana-related electrophysiological investigations, and those mainly involved marijuana extracts (Boyd et al. 1971, 1974; Pirch et al. 1972; Truitt and Braude 1974; Paton 1975); consequently, we began a series of electrophysiological investigations of the pharmacological properties of THC. Our approach was multifarious: 1) to evaluate the cannabinoid's responses at various levels of organization ranging from the cerebral cortex to the neuromuscular junction, 2) to assess the drug's effects on pools of neurons as well as on single excitable cells, 3) to use conscious animals when possible in order to avoid the confounding problems of anesthesia, and 4) to evaluate the active form of the cannabinoid by using in vitro test preparations. The goals of our research were twofold: to describe the electrophysiological effects of THC, and to uncover possible synaptic mechanisms of action for these effects.

Another important feature of our research has been the electrophysiological evaluation of the properties of cannabidiol (CBD), a drug that was seldom studied prior to our work because it was considered to be pharmacologically inactive (Hollister 1973). The data presented here represent an extensive comparison of the properties of the psychoactive THC with the nonpsychoactive CBD (Perez-Reyes et al. 1973). In addition, it is well documented that THC is metabolized in vivo to various pharmacologically active metabolites (Burstein 1973); therefore, in order to understand the pharmacology of the parent drug, it is necessary to determine the properties of its active metabolites. For this reason, we comparatively evaluated the effects of THC's principal metabolite, 11-hydroxy-THC, and its hydroxylated metabolites. The findings demonstrate that different cannabinoids can produce markedly different neuropharmacological responses.

METHODS

The procedures for the study of the electrophysiological preparations and descriptions of the experimental designs of the various cannabinoid studies have been published in detail elsewhere: electrically produced transcallosal cortical evoked responses in rats (Turkanis and Karler 1981a), photically evoked cortical after discharges in rats (Turkanis et al. 1977), cobalt-caused epilepsy in rats (Chiu et al. 1979), iron-caused epilepsy in rats (Turkanis and Karler 1982), electrically induced limbic afterdischarges in rats (Smiley et al. 1979), cat spinal-cord preparation (Turkanis and Karler 1983, 1984a, 1986), isolated bullfrog ganglia (Turkanis and Karler 1975), and isolated frog sartorius neuromuscular junctions (Turkanis and Karler, in press).

For parenteral preparations, the cannabinoids were dispersed in isotonic sodium-chloride solution with the aid of Tween 80 and ultrasound (Turkanis et al. 1974). For bathing solutions, the cannabinoids were dispersed in Ringer solutions with the nonionic detergent Pluronic F68 and ultrasound (Turkanis and Karler 1975, in press). Pentylenetetrazol (PTZ) was dissolved in isotonic sodium-chloride solution. Vehicle and drug preparations were administered intraperitoneally (i.p.) for the cortical and limbic studies and intravenously (i.v.) for the spinal-cord studies.

RESULTS

The effects of THC, CBD, and PTZ on the amplitude of the initial phase of transcallosal cortical evoked responses in rats were investigated (Turkanis and Karler 1981a). Specifically, THC in the lower dosage range (0. 1-1.0 mg/kg) markedly increased the evoked response amplitude, whereas higher dosages (1.2-6.0 mg/kg) elicited only depression. Our reference convulsant, PTZ, in subconvulsant doses (10-20 mg/kg), like THC, enhanced potential amplitude. Both THC and PTZ also increased electrically caused cortical evoked potentials in the monkey (Boyd et al. 1971). In contrast, CBD over a wide dosage range (0.l-80 mg/kg) exerted only depressant effects. In summary, the findings demonstrate qualitative differences in the pharmacological properties of the two cannabinoids.

We also compared the effects of THC, CBD, and PTZ on photically evoked cortical afterdischarges in rats (Turkanis et al. 1977); THC (5-10 mg/kg), but not CBD, produced a PTZ-like increase of cortical potentials. In contrast to the results with electrically caused responses, high THC doses (50-100 mg/kg) did not affect photic potentials; furthermore, neither THC nor CBD displayed any depressant properties in this test system.

The results of our limbic afterdischarge investigations also demonstrate that THC and CBD exhibit different neuropharmacological properties (Smiley et al. 1979). THC in a dosage range of 0.3 to 3.0 mg/kg elicited only a threshold increase--a depressant response; doses of 5 to 15 mg/kg, however, caused an even greater rise in threshold, but also a prolongation of the afterdischarge. This prolongation of the response is an electrophysiological manifestation of the drug’s excitatory properties. In contrast to THC, CBD (0.3-200 mg/kg) only depressed the afterdischarge; that is, it increased the threshold, shortened the duration, and reduced the amplitude. In summary, low THC doses and all CBD doses yielded depression, whereas relatively high THC doses produced concurrent excitation and depression of the limbic afterdischarge.

The effects of cannabinoid on iron-epileptic rats are complex: THC (1-20 mg/kg) elicited excitation by producing behavioral signs of central nervous system (CNS) stimulation, generalized bursts of epileptiform potentials, and frank convulsions (table 1 and figure 1); in addition, it produced depression by causing motor toxicity and reduction of focal epileptic activity (table 1 and figures 1 and 2).

TABLE 1

Influences of Cannabinoids on Conscious Iron-Epileptic Rats with Chronically Implanted Cortical Electrodes

Treatment	Dosage Range (mg/kg, i.p.)	Focal Epileptic Activity	Precipitation of Convulsions	Bursts of Epileptiform Potentials	CNS Excitation	Motor Toxicity
THC	1-20	-	Yes	Yes	Yes	Yes
11 -Hydroxy-THC	1-20	-	No	Yes	Yes	Yes
8-Beta-hydroxy-THC	0.5-20	0	Yes	Yes	No	No
CBD	1-100	0	No	No	No	No*
PTZ	5-20	0	Yes	Yes	No	No

- = decrease; 0 = no change. Each drug was studied in at least six different experiments. Behavioral signs of CNS excitation were vocalization, hissing, and jumping. For additional details, refer to figures 1 and 2 and to Turkanis and Karler 1982.

*The median-effective motor-toxic dose of CBD in nonepileptic control rats is 173 mg/kg, i.p. (Smiley et al. 1979).

11-Hydroxy-THC (1-20 mg/kg) exerted THC-like effects with one major exception; that is, it did not precipitate convulsions (table 1 and figure 2). The properties of 8-beta-hydroxy-THC, another THC metabolite, were different compared with those of either the parent compound or the 11-hydroxy metabolite. In doses of 0.5 to 20 mg/kg, it exerted only excitation, as evidenced by bursts of epileptiform potentials and convulsions (table 1 and figures 1 and 2). In contrast, CBD, even in doses up to 100 mg/kg, was devoid of all of THC's effects. In addition, pretreatment with CBD markedly altered the responses to THC in the following ways: Focal depression was partially blocked, generalized epileptiform activity was enhanced, and convulsions were abolished. In general, the cannabinoids exhibited a wide spectrum of CNS effects in iron-epileptic rats, functional significance of the cannabinoid-caused generalized but the pharmacological profile of each agent was demonstrably different. The bursts of functional significance of the cannabinoid-caused generalized epileptiform

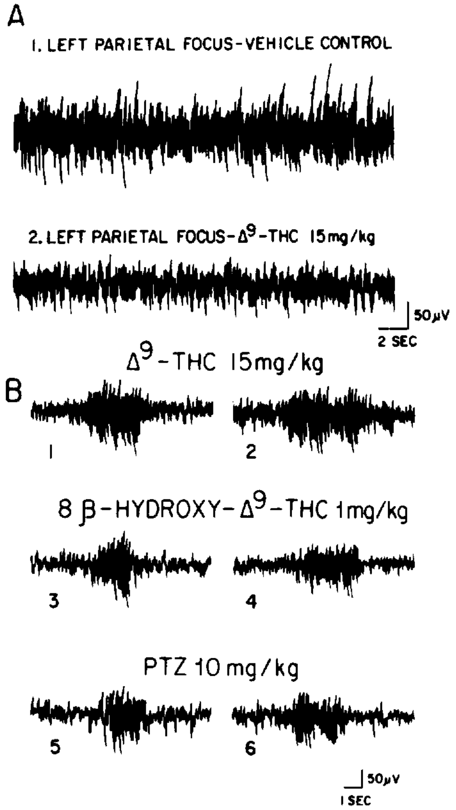

FIGURE 1. Influences of cannabinoids on electrocorticogram of conscious iron-epileptic rats. The iron-caused epileptic focus was located in the parietal cortex; recordings were obtained from screw electrodes chronically implanted on the focus and other cortical sites. The position of the iron focus was identical to that of the cobalt focus (figure 3 and table 2). A1 depicts recording 15 min after vehicle; A2, recording 15 min after THC. B illustrates generalized bursts of epileptiform potentials from three different rats: B1 and B2 represent potentials observed 45-60 min after THC: B3 and B4, potentials 15-20 min after 8-beta-hydroxy-THC; B5 and B6, potentials 10-15 min after PTZ (adapted from Turkanis and Karler 1982).

potentials is unknown, but these potentials look like the electrical manifestation of a seizure. In support of such a possibility, the convulsant control PTZ produced seizurelike discharges that are indistinguishable from those caused by THC and its metabolites (table 1 and figure 1). In addition, THC and its metabolites also evoked these generalized seizurelike discharges in nonepileptic control rats.

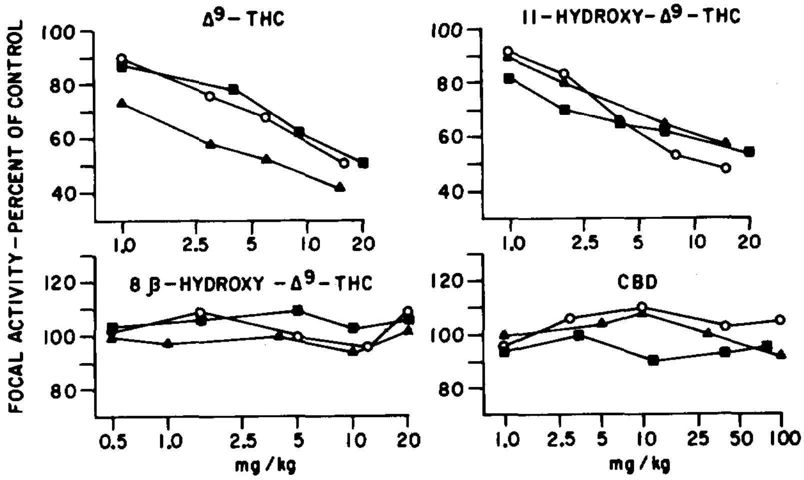

FIGURE 2. Influences of cannabinoids on iron-induced focal epileptic potentials in conscious rats. Throughout each experiment, focal electrocorticograms and integrals of the electrocorticogram voltage were recorded. The data shown are maximum changes in the integrals of the electrocorticographic voltage over a 5-min period, which are expressed as a percentage of the internal vehicle control values obtained in each experiment. Percentage oj control focal activity is plotted against the cumulative dose. Each dose-effect curve represents the results obtained from an experiment with one rat. For additional details, refer to figure 1 and to Turkanis and Karler 1982. Copyright 1982 by Pergamon Press (New York).

The effects of THC, its 11-hydroxy metabolite, and CBD were assessed in cobalt-epileptic rats. As shown in table 2 and in figure 3, THC (0.5-100 mg/kg) increased the frequency of focal epileptic potentials, elicited generalized bursts of epileptiform potentials, and precipitated or exacerbated convulsions.

11-Hydroxy-THC (1.0-20 mg/kg) exerted only one of the three THC excitatory responses, namely, bursts of epileptiform potentials. Again, CBD (0.5-200 mg/kg) only produced depression-suppression of the spontaneous convulsions. Each of the drugs, therefore, produced characteristic effects in this test system.

The five investigations with conscious animals described above involved pools of cortical and limbic neurons, and the results demonstrated that the cannabinoids can exert a complex mixture of excitatory and depressant electrophysiological effects. The next step in our research program was to uncover possible synaptic sites and mechanisms of cannabinoid actions. Initial studies with cat spinal monosynaptic reflexes indicated that cannabinoid effects at this level were comparable to those in higher centers. Specifically, THC in low doses (0.05-0.15 mg/kg, i.v.) increased the reflex itself and posttetanic potentiation (PTP), whereas higher doses of THC (0.2-0.75 mg/kg, i.v.) and all doses of CBD

TABLE 2

Influences of Cannabinoids on Conscious Cobalt-Epileptic Rats with Chronically Implanted Cortical Electrodes

Treatment	Dosage Range (mg/kg, i.p.)	Frequency of Focal Epileptic Potentials	Effect on Convulsions	Bursts of Epileptiform Potentials
THC	0.5-100	+	+	Yes
11-Hydroxy-THC	1 0-20	0	0	Yes
CBD	0.5-200	0	-	No

+ = increase; - = decrease; 0 = no change. THC and CBD were studied in eight epileptic rats, and 11-hydroxy-THC was studied in three animals due to a limited drug supply. The production of generalized bursts of epileptiform cortical potentials by THC and its metabolite also occurred in control rats. For additional details, refer to figure 3 and to Chiu et al. 1979.

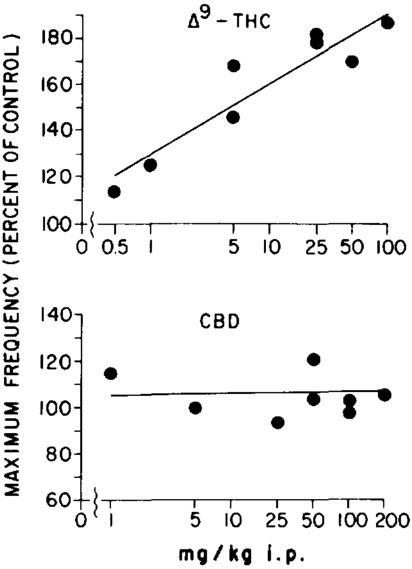

FIGURE 3. Influence of THC and CBD on frequency of spontaneously firing focal epileptic potentials recorded from a cobalt-induced focus in the left parietal cortex. Each point represents the results from a different conscious rat and is the maximum frequency expressed as a percentage of predrug vehicle control. For additional details, refer to table 2 and to Turkanis and Karler 1981b. Copyright 1981 by The American College of Clinical Pharmacology (New York).

(0.05-14 mg/kg, i.v.) were only depressant (Tramposch et al. 1981; Turkanis and Karler 1984a). In this context, intracellular recording techniques were used on single spinal motoneurons in order to define the cannabinoids' synaptic pharmacology.

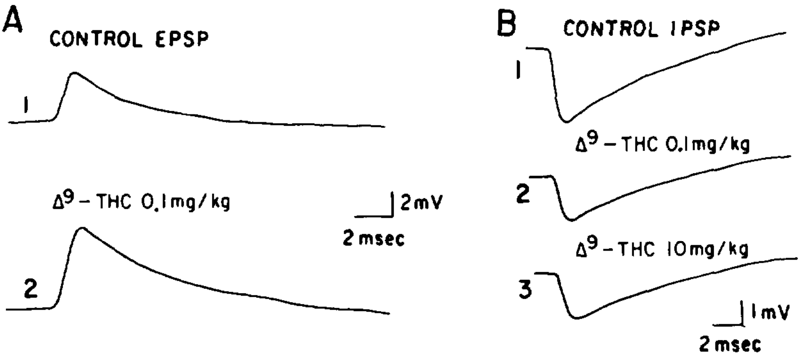

FIGURE 4. Excitatory effects of THC on electrophysiological responses of single spinal motoneurons in spinal cats. Each trace represents the electronically obtained average of 16 consecutive potentials. A and B are from two different cats. A depicts THC-caused increase of EPSP amplitude: AI, control response 20 min after vehicle; A2, response 20 min after drug. The increase in amplitude is at least partially attributable to an increase in membrane resistance. EPSPs were recorded jrom a triceps surae motoneuron. B depicts THC-caused limited depression of IPSP amplitude: B2, control response 20 min after vehicle: Bs, response 20 min after drug; B3, response 30 min after drug. The figure illustrates the limited efficacy of THC in reducing the amplitude of lPSPs because the effect of 10 mg/kg (B3) is no greater than that of 0.1 mg/kg (B2). For additional details, refer to table 3. (Adapted from Turkanis and Karler 1983.)

THC effects on the parameters of single spinal motoneurons are complicated: The drug (0.01-0.1 mg/kg) produced excitation by increasing excitatory postsynaptic potentials (EPSPs) and by decreasing inhibitory postsynaptic potentials (IPSPs) (table 3 and figure 4).

THC's responses on the two synaptic potentials appear to be unrelated to alterations of the afferent input because up to 10 mg/kg, i.v., of the drug had no measurable effects on compound-nerve action potentials recorded from the dorsal roots (Turkanis and Karler 1984b). EPSP enhancement, however, is at least partially attributable to an increase in membrane resistance (table 3). In addition to excitation, THC produced depression by raising the firing threshold and reducing soma action-potential amplitude (table 3); thus, in single neurons, as in conscious animals and pools of neurons, the cannabinoid exerted dual effects. 11-Hydroxy-THC's effects were similar to those of the parent compound, except

TABLE 3

Influences of Cannabinoids on the Afferent Input and on Single Spinal-Motoneuron Parameters in Spinal Cats

Treatment	EPSP Amplitude	Membrane Resistance	IPSP Amplitude	Firing Threshold	Nerve Action Potential	Soma Action Potential
THC	+	+	-	+	0	-
11-Hydroxy-THC	+	+	0	+	0	-
CBD	-	0	0	+	0	-

+ = increase; - = decrease; 0 = no change. All of the effects occurred between 0.01 and 0.1 mg/kg, i.v., except for the reduction of the soma action potentials; the latter was affected by 0.2-0.5 mg/kg 11-hydroxy-THC and 1-5 mg/kg THC and CBD. Compound nerve-action potentials (afferent input) were recorded extracellularly from dorsal roots; the other measurements were made with intracellular microelectrodes from motoneuron somas. Measurement of excitatory EPSPs, IPSPs, membrane resistance, and firing threshold were made from single spinal motoneurons with intracellular glass microelectrodes filled with 3M potassium-acetate solution. EPSPs were evoked by stimulating the triceps surae or posterior-biceps semitendinosus nerves, and IPSPs were evoked by stimulating the quadriceps nerve. Membrane resistance was determined by passing sufficient current to produce small depolarizations. The threshold for the production of the soma potential was the voltage required to fire the cell consistently. For additional details, refer to figures 4 and 5 and to Turkanis and Karler 1983, 1984a, 1984b, 1986.

that the metabolite did not depress IPSPs (table 3). Again, CBD (0.1-5 mg/kg) was only a depressant; that is, it raised the firing threshold, depressed the amplitude of soma action potentials, and reduced the EPSP amplitude (table 3 and figure 5). In relation to mechanisms and sites of drug action, the cannabinoids' effects on the firing threshold and amplitude of soma action potentials and on membrane resistance indicate that changes in postsynaptic ionic conductances are involved.

Subsequent cannabinoid investigations were carried out with isolated synaptic preparations. The goals of these studies were twofold: to determine the active form of the cannabinoid by avoiding in vivo drug metabolism, and to uncover possible synaptic mechanisms and sites of drug action. To this end, the effects of several cannabinoids were evaluated on PTP of extracellularly recorded action potentials in isolated bullfrog ganglia: Two THC metabolites, 11-hydroxy-THC

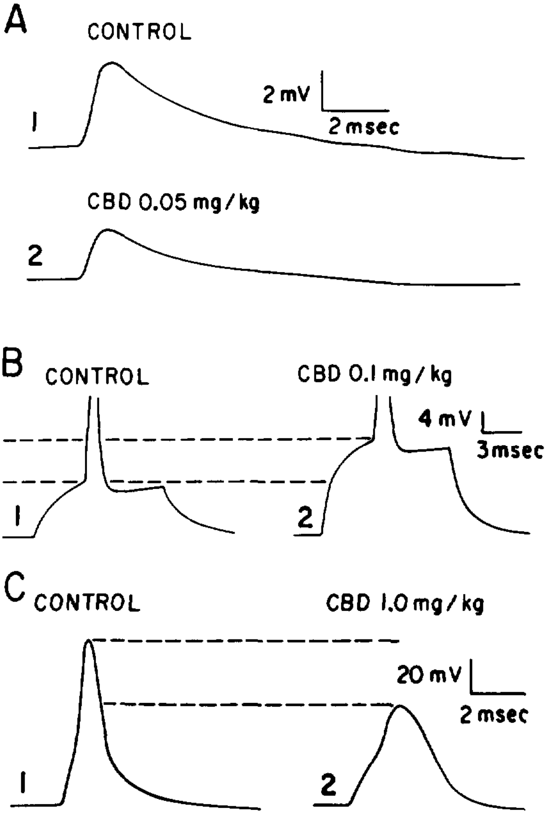

FIGURE 5. Depressant effects of CBD on electrophysiological responses of single spinal motoneurons in spinal cats. Traces in A are electronically obtained averages of 16 consecutive potentials: traces in B and C are of single potentials. A, B, and C are from three different cats. A depicts CBD-caused depression of EPSP amplitude; responses measured 30 min after vehicle and 30 min after drug. B illustrates CBD-elicited increase in firing threshold; responses measured 20 min after vehicle and 20 min after drug. C shows the CBD-caused depression of the soma action potential; responses measured 30 min after vehicle and 30 min after drug. The effects shown in A, B, and C are not the result of changes in membrane resistance or resting membrane potential. For additional details, refer to table 3. (Adapted from Turkanis and Karler 1986.)

and 8-alpha, 11-dihydroxy-THC, in low concentrations (01.-0.33 μM) reduced PTP, whereas THC, in concentrations as high as 25μM, exerted no effect (figure 6).

Drug-induced changes in PTP may also relate to THC's anticonvulsant as well as to its convulsant properties, since a reduction in PTP has been reported to be an electrophysiological mechanism of action for such antiepileptic agents as

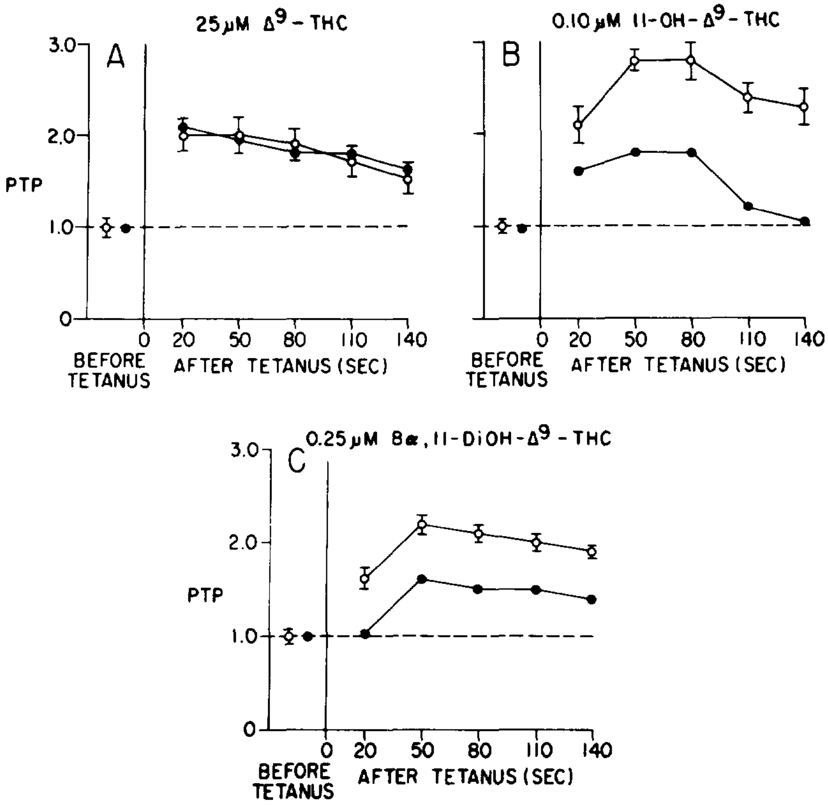

FIGURE 6. Influences of cannabinoids on PTP at isolated bullfrog paravertebral ganglia X. PTP was defined as an increase in the amplitude of the extracellucellularly recorded ganglionic potentials following a period of tetanic stimulation and is expressed as the ratio of posttetanic to pretetanic potential amplitudes. Bracketed o = the mean and standard deviation of four control values; ● = values 60 min after drug treatment. Raising the THC concentration above 25 μM reduced the amplitude of the pretetanic potential, thereby invalidating calculations of PTP (Turkanis and Karler 1975). Copyright 1975 by Pergamon Press (New York).

phenytoin (Esplin 1957). Our research demonstrates that THC in vivo, depending upon the dose, can either increase or decrease PTP of cat spinal monosynaptic reflexes; therefore, similar effects in higher centers may contribute to the drug's convulsant and anticonvulsant properties. Our CBD results with spinal reflexes substantiate a role for PTP because the drug only depresses PTP and is only anticonvulsant.

The cannabinoids' effects on IPSPs of spinal motoneurons may also provide a possible explanation for THC's convulsant properties. In this instance, THC, like the convulsant strychnine (Kuno and Weakly 1972), reduces the amplitude of spinal motoneuron IPSPs. The 11-hydroxy-THC data are consistent with involvement of inhibitory potentials because the metabolite, unlike THC, does not affect IPSPs and is not a convulsant. On the other hand, THC, unlike strychnine, precipitates convulsions only in special circumstances; that is, in cobalt- and iron-epileptic rats (tables 1 and 2), in epileptic beagles (Feeney 1979), and in a special strain of rabbits (Martin and Consroe 1976). The reason for the restricted convulsant activity of THC may be due to its limited efficacy in reducing inhibitory potentials. In short, the cannabinoids' anticonvulsant and convulsant activities are probably associated with several pharmacological properties of the drugs as well as related to the susceptibility of the subjects.

Intracellular recordings from such single cells as spinal motoneurons and neuromuscular junctions may provide information about possible electrophysiological mechanisms and sites of the cannabinoids' central actions. First, the synapse is likely to be the major site of drug action: As demonstrated in in vivo experiments, synapses are affected by relatively low THC doses (0.01-0.1 mg/kg, i.v.), whereas doses up to 10 mg/kg, i.v., failed to modify compound action potentials of axons (table 3). Second, cannabinoids can exert both pre- and post-synaptic effects; for example, THC caused excitation presynaptically by enhancing neurotransmitter release and postsynaptically by increasing membrane resistance. In addition, THC produced depression presynaptically by reducing neurotransmitter release and postsynaptically by decreasing neurotransmitter response. Lastly, cannabinoid-induced effects on the motoneuron soma, that is, changes in membrane resistance, firing threshold, and action-potential amplitude, indicate that the drugs alter postsynaptic ionic conductances. Depression of neurotransmitter responses may be due to drug-receptor interactions or to changes in postsynaptic ionic conductances. Nevertheless, if these drug responses occur in the CNS, they suggest possible mechanisms and synaptic sites of action for the cannabinoids' central excitatory and depressant properties, and the consistent pattern of the drugs' effects at every level of organization studied--from the cerebral cortex to the neuromuscular junction--validate these conclusions.

It is possible that a higher THC concentration might reduce PTP, but raising the concentration above 25 μM markedly reduced the amplitude of the pretetanic potential and invalidated the calculation of PTP. These findings support those of Brady and Carbone (1973), who found that 11-hydroxy-THC, but not the parent compound, depressed the action potentials of squid giant axons. Like THC's metabolites, CBD (33-100 μM) was pharmacologically active; that is, it reduced PTP. It is noteworthy that the isolated ganglion, like the squid axon, only displayed depression in response to the cannabinoids.

The purpose of the next study was to determine whether THC affects neurotransmitter release in an electrophysiologically functional system; specifically, a preparation in which nerve-action potentials release neurotransmitter. To accomplish this aim, we evaluated the influences of THC on neurotransmitter release by determining electrophysiologically the mean quantum content of the endplate potential (m) (Katz 1969). A neuromuscular preparation was selected because it is one of the limited number of preparations in which m can be measured, while the rationale for using a cholinergic system was that cannabinoids have been reported to affect cholinergic pathways (Drew and Miller 1974); therefore, intracellular recording techniques were used with isolated frog sartorius neuromuscular junctions to describe the effects on transmitter release (Turkanis and Karler, in press). The results were again complex: The frequency of miniature endplate potentials (m.e.p.ps) was increased by THC, decreased by CBD, and unaffected by 11-hydroxy-THC, whereas m.e.p.ps amplitude was depressed by all three cannabinoids. In addition, m was first increased and then decreased by THC and its 11-hydroxy metabolite, but CBD produced only depression. Changes in m and m.e.p.ps frequency indicate presynaptic sites of drug action, and reduction of m.e.p.ps amplitude suggests a postsynaptic site. The pre- and post-synaptic effects described here are possible mechanisms of action for the drug-caused amplitude changes of evoked and synaptic potentials in the CNS.

DISCUSSION

At every level of organization studied--from pools of cortical neurons to individual spinal motoneurons and neuromuscular junctions--the cannabinoids produce excitation and/or depression. This pattern of drug response suggests that the observed effects on single neurons may account for the mechanisms of action of the cannabinoids in conscious animals; for example, if the effects on single spinal motoneurons are applicable to higher centers, then they may contribute to the cannabinoids' anticonvulsant activity. In fact, THC and CBD raise thresholds for electrically induced minimal convulsions in conscious animals (Karler and Turkanis 1981b), and the drugs raise the threshold for electrically induced afterdischarge (seizure discharges) in pools of limbic neurons (table 3). Both effects reflect the drugs' anticonvulsant activity, and both effects may be the direct result of an increased firing threshold in individual neurons.

The results of comparative electrophysiological investigations of THC with its 11-hydroxy metabolite can be classified, as shown in table 4, into three categories: In the first, both drugs produced similar responses; that is, the two drugs were either equipotent or 11-hydroxy-THC was more potent than the parent cannabinoid; for example, dose-effect relations for depression of cobalt-induced focal epileptic activity were identical for both drugs; in contrast, the 11-hydroxy metabolite was 10 times more potent than THC in the production of generalized cortical bursts of epileptiform potentials in cobalt-epileptic rats. In the second category, only THC has pharmacological activity (table 4). Finally, in two in vitro test systems, only 11-hydroxy-THC has activity (table 4). In this case, the interpretation of the findings is not complicated by drug metabolism, and the data suggest that the 11-hydroxy derivative may account for some of the in vivo responses to the parent drug. In brief, the results indicate that there are both quantitative and qualitative differences between THC and its principal metabolite and that, depending upon the effect, the metabolite may contribute to, be dissociated from, or account for THC's activity.

The data also demonstrate marked differences in the neuropharmacological properties of two major marijuana constituents, that is, THC and CBD. These findings confirm our previously drawn conclusions (Turkanis and Karler 1981b): First, THC is a nonselective, centrally active drug producing both excitation and depression; second, CBD is a relatively selective central depressant. In fact, CBD appears to be devoid of all of THC's central stimulatory properties, including psychotoxicity. In general, the results of the electrophysiological studies of the cannabinoids repeatedly illustrate that different cannabinoids possess different properties.

TABLE 4

Comparison of the Electrophysiological Effects of THC and its 11-Hydroxy Metabolite

I. Responses produced by both cannabinoids:

  A. Cortical bursts of epileptiform potentials in cobalt- and iron-epileptic rats,
  B. Depression of cortical focal epileptic activity in iron-epileptic rats,
  C. Enhancement of spinal motoneuron EPSPs,
  D. Increase in motoneuron membrane resistance,
  E. Depression of motoneuron action potential,
  F. Depression of m.e.p.ps amplitude, and
  G. Increase and subsequent decrease of mean quantum content of endplate potentials.

II. Responses produced only by THC:

  A. Depression of spinal motoneuron IPSP, and
  B. Enhancement of m.e.p.ps frequency.

III. Responses produced only by 11-hydroxy-THC:

  A. Depression of PTP at isolated bullfrog ganglia, and
  B. Depression of action potentials of isolated squid giant axons (Brady and Carbone 1973).

REFERENCES

Boyd, E.S.; Boyd, E.H.; Muchmore, J.S.; and Brown, L.E. Effects of two tetrahydrocannabinols and of pentobarbital on cortico-cortical evoked responses in the squirrel monkey. J Pharmacol Exp Ther 176:480-488, 1971.

Boyd, E.S.; Boyd, E.H.; and Brown, L.E. The effects of some drugs on an evoked response sensitive to tetrahydrocannabinols. J Pharmacol Exp Ther 189:748-758, 1974.

Brady, R.O., and Carbone, E. Comparison of the effects of delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol and ethanol on electrophysioiogical activity of the giant axon of the squid. Neuropharmacology 12:601-605, 1973.

Burstein, S.H. Labeling and metabolism of the tetrahydrocannabinols. In: Mechoulam, R., ed. Marijuana--Chemistry. Pharmacology, Metabolism and Clinical Effects. New York: Academic Press, 1973. pp. 167-190.

Chiu, P.; Olsen, D.M.; Borys, H.K.; Karler, R.; and Turkanis, S.A. The influence of cannabidiol and delta-9-tetrahydrocannabinol on cobalt epilepsy in rats. Epilepsia 20:365-375, 1979.

Drew, W.G., and Miller, L.L. Cannabis: Neural mechanisms and behavior--A theoretical review. Pharmacology 11:12-21, 1974.

Esplin, D.W. Effects of diphenylhydantoin on synaptic transmission in cat spinal cord and stellate ganglion. J Pharmacol Exp Ther 120:301-323, 1957.

Feeney, D.M. Marihuana and epilepsy: Paradoxical anticonvulsant and convulsant effects. In: Nahas, G.G., and Paton, W.D.M., eds. Marihuana Biological Effects. Oxford: Pergamon Press, 1979. pp. 643-657.

Hollister, L.E. Cannabidiol and cannabinol in man. Experientia 29:825-826, 1973.

Karler, R., and Turkanis, S.A. The cannabinoids as potential antiepileptics. J Clin Pharmacol 21:437S-448S, 1981.

Katz, B. The Release of Neural Transmitter Substances. Springfield, IL: Charles C Thomas, 1969. p. 12.

Kuno, M., and Weakly, J.N. Quantal components of the inhibitory synaptic potential in spinal motoneurones of the cat. J Physiol (Long) 224:287-303, 1972.

Martin, P., and Consroe, P. Cannabinoid induced behavioral convulsions in rabbits. Science 194:965-967, 1976.

Paton, W.D.M. Pharmacology of marijuana. Annu Rev Pharmacol 15:191-220, 1975.

Perez-Reyes, M.; Timmons, M.C.; Davis, K.H.; and Wall, E.M. A comparison of the pharmacological activity in man of intravenously administered delta-9-tetrahydrocannabinol, cannabinol and cannabidiol. Experientia 29:1368- 1369, 1973.

Pirch, J.H.; Cohn, R.A.; Barnes, P.R.; and Barratt, E.S. Effects of acute and chronic administration of marijuana extract on the rat electrocorticogram. Neuropharmacology 11:231-240, 1972.

Smiley, K.A.; Turkanis, S.A.; Borys, H.K.; Olsen, D.M.; and Karler, R. An electrophysiological analysis of the anticonvulsant action of cannabidiol on limbic seizures in conscious rats. Epilepsia 20:351-363, 1979.

Tramposch, A.; Sangdee, C.; Franz, D.N.; Karler, R.; and Turkanis, S.A. Cannabinoid-induced enhancement and depression of cat monosynaptic reflexes. Neuropharmacology 20:617-621, 1981.

Truitt, E.B., Jr., and Braude, M.C. Preclinical pharmacology of marihuana. In: Gibbins, R.J.; Israel, V.; Kalant, H.; Popham, R.E.; Schmidt, W.; and Smart, R.G., eds. Research Advances in Alcohol and Drug Problems. Vol. 1. New York: Wiley, 1974. pp. 199-242.

Turkanis, S.A., and Karler, R. Influence of anticonvulsant cannabinoids on posttetanic potentiation at isolated bullfrog ganglia. Life Sci 17:569-578, 1975.

Turkanis, S.A., and Karler, R. Excitatory and depressant effects of delta-9-tetrahydrocannabinol and cannabidiol on cortical evoked responses in the conscious rat. Psvchopharmacology 75:294-298, 1981a.

Turkanis, S.A., and Karler, R. Electrophysiologic properties of the cannabinoids. J Clin Pharmacol 21:449S-463S, 1981b.

Turkanis, S.A., and Karler R. Central excitatory properties of delta-9-tetrahydrocannabinol and its metabolites in iron-induced epileptic rats. Neuropharmacology 21:7-13, 1982.

Turkanis, S.A, and Karler, R. Effects of delta-9-tetrahydrocannabinol on cat spinal motoneurons. Brain Res 288:283-287, 1983.

Turkanis, S.A, and Karler, R. Influence of 11-hydroxy-delta-9-tetrahydrocannabinol on spinal motoneurons in cat. Neuropharmacology 23:1435-1439, 1984a.

Turkanis, S.A., and Karler, R. Mechanisms of delta-9-tetrahydrocannabinol's convulsant actions. In: Agurell, S.; Dewey, W.L.; and Willette, R.E., eds. The Cannabinoids; Chemical, Pharmacologic and Therapeutic Aspects. Orlando: Academic Press, 1984b.

Turkanis, S.A., and Karler, R. Cannabidiol-caused depression of spinal motoneuron responses in cats. Pharmacol Biochem Behav 25:85-94, 1986.

Turkanis, S.A., and Karler, R. Effects of delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol and cannabidiol on neuromuscular transmission in frog. Neuropharmacology, in press.

Turkanis, S.A.; Cely. W.; Olsen, D.M.; and Karler, R. Anticonvulsant properties of cannabidiol. Res Commun Chem Pathol Pharmacol 8:231-246, 1974.

Turkanis, S.A.; Chiu, P.; Borys, H.K.; and Karler, R. Influence of delta-9-tetrahydrocannabinol and cannabidiol on photically evoked after-discharge potentials. Psychopharmacology 52:207-212, 1977.

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Molecular Determinants for Cannabinoid Activity: Refinement of a Molecular Reactivity Template

Patricia H. Reggio, Ph.D.

INTRODUCTION

Numerous studies of structure-activity relationships (SARs) in the cannabinoid series have been published; e.g., see review by Sofia (1978) and some more recent work by Martin et al. (1984a, 1984b) and Narimatsu et al. (1985). An observation which emerges from a review of all of these studies is that some cannabinoids can be structurally dissimilar as in delta-9-tetrahydrocannabinol (delta-9-THC) and Abbott 40656 (Rosell et al. 1979) and yet can have similar activities; whereas others, which show only slight structural differences as in 10-alpha-OH-delta-8-THC and 10-beta-OH-delta-8-THC (Edery et al. 1971). can exhibit dramatic activity differences (figure 1). This observation emphasizes that a common basis for the activity of these compounds must be sought in more than their mere three-dimensional structures.

Traditionally, cannabinoid SARs have been focused almost entirely on the independent contribution of certain structural groups ("functional groups") of the molecules. These SARs have been compiled into extensive "lists of requirements" (Razdan 1984). Certain regions or conformations of the molecules have been proposed to be important. These regions or conformations include a methyl group in the plane of the aromatic ring, a free phenol, and a free C-4(C-5') aromatic position (Edery et al. 1971); the phenolic hydroxyl group (Uliss et al. 1975); the side chain (Osgood et al. 1978); nonplanarity (Binder et al. 1979); and planarity of the molecule (Burstein et al. 1983). Stereoselectivity in the activity of the tetrahydrocannabinols has been reported (Dewey et al. 1984). This type of approach in SAR studies often assumes the following: that functional groups must react directly with specific sites in the receptor, that modification of one group does not affect the reactivity of another, and that geometric and stereometric factors, such as distance and spatial relationships between functional groups, are all important. Such focus on isolated aspects of the cannabinoids ignores the fact that the molecular properties that are directly responsible for the molecular interactions (that lead to the pharmacological effect) are encoded in the entire molecular structure. To date, no characterization of the entire molecule and correlation of this characterization with activity has been attempted in the cannabinoid field.

The importance of quantum chemical methods in describing the physical and chemical properties of molecules is evident in every facet of modern chemical

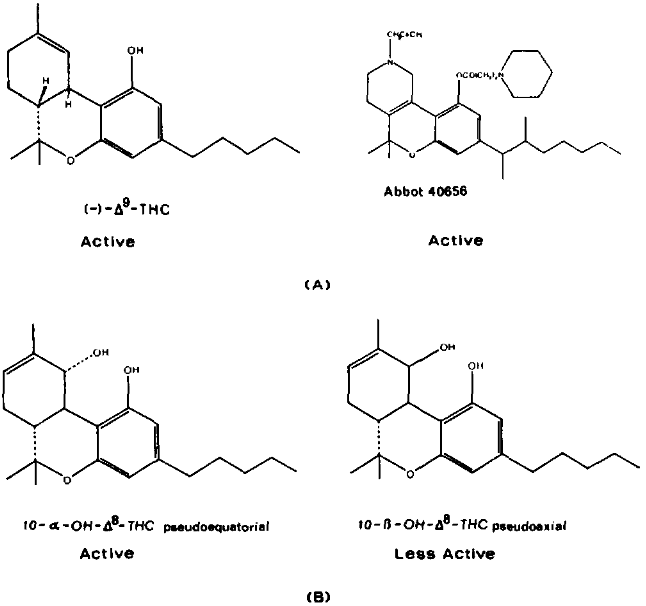
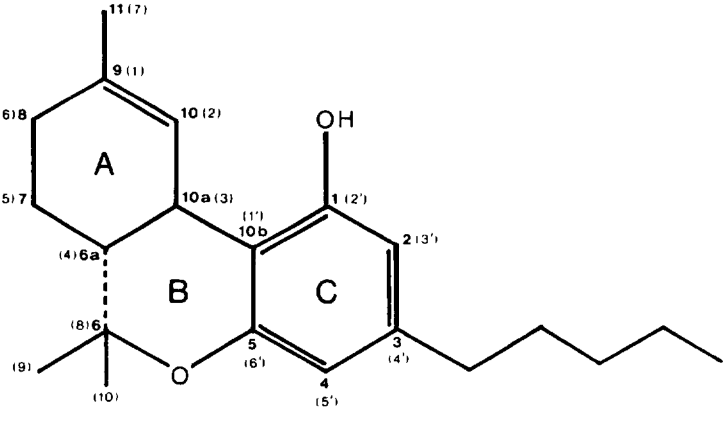


FIGURE 1. Cannabinoids (a) which are structurally dissimilar yet exhibit similar activities, and (b) which are structurally similar yet exhibit different activities. From Reggio and Mazurek 1987. Copyright 1986 by Elsevier Science Publishers B.V. (Amsterdam).

research and is very well documented. In the study of biological systems, experimental data must be obtained in a variety of forms and from many different sources. The value of quantum chemical approaches to the study of biological systems is in an ability to analyze, interpret, and rationalize all experimental observations on molecular processes in a unified language based on the primary principles of physics. Considerable evidence exists proving the relative success of theoretical approaches. These approaches have elucidated specific mechanisms of drug action, have provided a basis for rational drug design, and have led to conclusions about the nature of drug-receptor interactions (Dearden 1983; Vida and Gordon 1984). We have used theoretical approaches to successfully describe the molecular determinants for binding of methylenedioxytryptamines at SHT/LSD receptors (Reggio et al. 1981) and have also used theoretical approaches to explain the role that tautormerism plays in the activation of the histamine-H_2 receptor by 2-methyl and 4-methylhistamine (Reggio and Mazurek 1987). The basic assumption in any of these theoretical studies of drug action is that the interaction between drugs and their biological targets is dependent on the same molecular parameters that determine chemical interactions and reactions. Molecular Reactivity Characteristics (MRCs) such as molecular electrostatic potentials (MEP), polarizabilities, and proton affinities are molecular properties that determine specific chemical reactivity. In theoretical studies of structure-activity relationships, such MRCs are used to establish how the information necessary for receptor recognition and activation is encoded in the molecular structure of the drug.

Despite the extensive application of theoretical methods in the study of many pharmacological systems, very little attention has been focused on the cannabinoids to date. Those theoretical studies on cannabinoids which have been published have not focused on the calculation of molecular reactivity characteristics. Most of these studies have concentrated on the calculation of conformation in an attempt to predict the most probable geometry (lowest energy conformer) and its relation to activity. The structures and energies of delta-9-THC and three other cannabinoids obtained by Westheimer calculations and extended Huckel molecular orbital calculations have been reported (Archer et al. 1970). Theoretical studies of cannabinoids with analgetic and anticonvulsant activity have also been reported (Johnson et al. 1982; Tamir et al. 1980). The work presented here extends beyond the considerations which have prevailed in theoretical studies of the cannabinoids. The aim of this work is to reveal elements of molecular reactivity that are related to psychopharmacological activity.

A major step in identifying the MRCs necessary for cannabinoid activity was to select a template molecule (a molecule with demonstrated activity whose MRCs can be used as a basis for comparison with those of other cannabinoids). Since (-)trans-delta-9-THC (delta-9-THC) has been reported to be the major psychoactive component of cannabis (Gaoni and Mechoulam 1964, 1971) and since studies indicate that it can exert behavioral effects without metabolic activation (Carney et al. 1979), delta-9-THC was chosen as the template molecule for this study. Figure 2 illustrates the numbering systems which are commonly employed in the literature for delta-9-THC.



Δ^9-THC ≡ Δ^1-THC

FIGURE 2. An illustration of the two numbering systems which are commonly employed for the cannabinoids. From Reggio and Mazurek 1987. Copyright 1986 by Elsevier Science Publishers B.V. (Amsterdam).

Our first working hypothesis deals with two components of the delta-9-THC structure which confer reactivating characteristics upon the molecule that are crucial to activity. These components are the lone pairs of electrons of the phenol oxygen (these lone pairs generate reactivity properties dependent on the orientation of the OH bond relative to the carbocyclic ring, Ring A), and the orientation of the carbocyclic ring, Ring A (this ring and its orientation generate hydrophobic properties). The spatial arrangements of these reactivity characteristics are the components of the template to which the characteristics of other cannabinoids can subsequently be compared.

Template for Cannabinoid Activity

Recently, we reported the characterization of the template molecule delta-9-THC (Reggio and Mazurek, 1987). Since the molecular reactivity characteristics of delta-9-THC are to be used as a basis of comparison with other cannabinoids, the essential features of the template are presented here. The reader should refer to the original paper (Reggio and Mazurek 1987), however, for all calculational details.

The lowest energy conformer of delta-9-THC as obtained by conformational analysis is shown in figure 3. It is clear from this figure that delta-9-THC is a nonplanar molecule. The carbocyclic ring (Ring A) of delta-9-THC exists in a half-chair conformation. Ring B assumes a conformation such that the axial C_6 methyl group is on the same side of the molecule as H_{10a} and is much closer to H_{10a} than is the other methyl group. In this optimized structure, the phenolic hydrogen points away from the carbocyclic ring (dihedral angle, C_2-C_1-0-H = -1^0). Figure 4 shows a different perspective of the molecule. Here, delta-9-THC is shown in the direction parallel to the vector from C_2 to C_{10b}.From this perspective, the conformation of the carbocyclic ring (Ring A) causes the top part of the ring to move to the left, thus permitting no protrusion of Ring A into the bottom face of the molecule. It is part of our working hypothesis that this orientation is necessary for cannabinoid activity. The conformation of the carbocyclic ring may allow for nondirectional hydrophobic interactions.

The lone pairs of electrons of the phenol oxygen generate directional reactivity properties dependent on the orientation of the OH bond relative to the carbocyclic ring. Studies of molecular energy as a function of rotation about the C_1-O axis reveal that two minimum energy conformations of the phenolic OH exist. In Conformation I, the phenolic OH is slightly bent out of the plane of the aromatic ring with the hydrogen pointing away from Ring A (C_2-C_1-O-H dihedral angle, τ = -1°). In Conformation II, the phenolic hydrogen is below the plane of the aromatic ring and is pointing toward Ring A (τ = 155°). The conformation of the rest of the molecule remains essentially unchanged.

The molecular electrostatic potential (MEP) is a molecular reactivity characteristic that determines specific chemical reactivity. It is an important determinant of drug action. The electrostatic potential field extends into space and influences nearby molecules. Unless the fields of a drug and its receptor are complementary, they will not attract or bind to one another. Indeed, electrostatic forces appear to guide an incoming drug into its receptor site and align so that shorter range binding forces can take effect (Tucker 1984). Therefore, a very direct indication of the nature and extent of electrostatic drug-receptor interactions can be obtained by mapping the potential generated by a molecule.

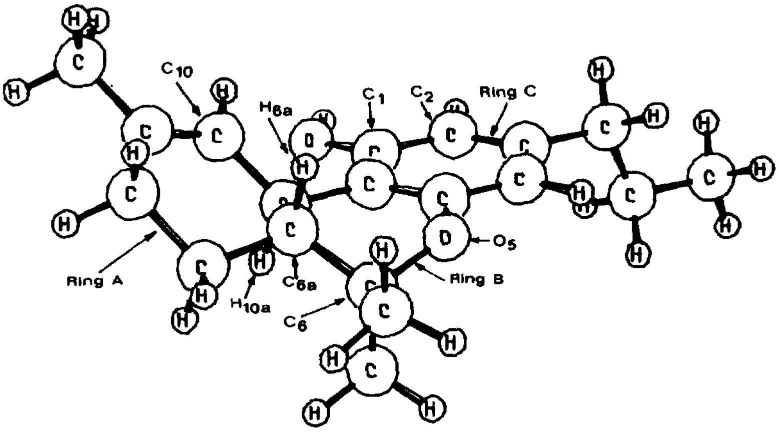


FIGURE 3. Conformation of delta-9-THC (with propyl side chain) as determined by MM2. From Reggio and Mazurek 1987. Copyright 1986 by Elsevier Science Publishers B.V. (Amsterdam).

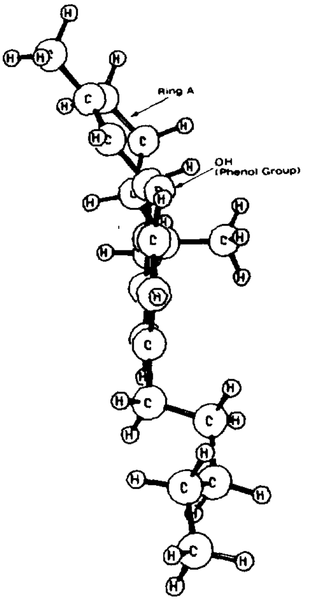


FIGURE 4. Conformation of delta-9-THC as determined by MM2: here the perspective of Ring A is viewed in the direction parallel to the vector from C_2 to C_{10b} . From Reggio and Mazurek 1987. Copyright 1986 by Elsevier Science Publishers B.V. (Amsterdam).

The calculated electrostatic potential patterns generated by the bottom face of delta-9-THC in each of its minimum energy conformers are shown in figures 5 and 6. These patterns are composed of lines of equipotential energy in kcal/mole that would be felt by a test positive charge placed at any specified point on the map. Figures 5 and 6 illustrate the regions of negative potential in planes parallel to the benzene ring at distances of 1.5 Ås below the plane of the ring. Because delta-9-THC is a nonplanar molecule. the MEPs differ depending upon whether the molecule is viewed from above or below. We found that the change in the position of the phenol group from Conformation I to Conformation II caused a distinguishable difference in the MEPs of the two conformers when viewed from the bottom face.

At this stage of the investigation, it was impossible to identify which conformation of the phenolic OH of delta-9-THC (I or II) was more relevant at the site of action. Hence, the MEPs shown in figures 5 and 6 along with the conformation of Ring A, as illustrated in figure 4, formed the preliminary set of molecular reactivity characteristics for cannabinoid activity.

Refinement of the Template

In order to refine our template, a comparison of the MRCs of delta-9-THC with those of another cannabinoid was undertaken. Edery et al. (1971) reported that in rhesus monkey behavioral tests the replacement of the phenolic hydrogen of delta-9-THC with a methyl group renders the molecule inactive. This inactive cannabinoid, O-methyl-delta-9-THC (figure 7), was selected for comparison in order to assess the importance of the position of the lone pairs of electrons of the phenol oxygen. We report here the results of our comparison of the properties of O-methyl-delta-9-THC with those of the delta-9-THC reactivity template.

METHODS OF PROCEDURE

Conformational Study of O-Methyl-Delta-9-THC

The first step in the characterization was the conformational analysis of O-methyl-delta-9-THC. The force field or molecular mechanics method as encoded in the program MM2 (Allinger and Yuh 1980) was used in this analysis. The force field available in MM2 has been parameterized for oxygen containing molecules and has proven satisfactory for the calculation of structures and energies of oxygen containing compounds (Allinger et al. 1980). The X-ray structure of delta-9-THC Acid B (Rosenquist and Ottersen 1975) was used to obtain an input geometry (i.e., bond lengths, bond angles, and dihedral angles for the fused ring skeleton of delta-9-THC). Necessary atoms were added to this skeleton at standard bond lengths and bond angles (Mitchell and Cross 1965) to produce an input geometry for O-methyl-delta-9-THC in the MM2 calculation. In the calculational scheme for the template molecule, delta-9-THC, molecular size constraints necessitated reducing the pentyl side chain to propyl (Reggio and Mazurek 1987). In order to maintain consistency, the side chain in O-methyl-delta-9-THC was also reduced to propyl. Such a modification was acceptable since the focus of this study is on the fused ring structure and the phenol group and not on the side chain.

Because the position of the methoxy group was very important to our working hypothesis, two studies of the molecular energy as a function of rotation about the C_1-O axis were conducted. In the first study, the dihedral driver option in

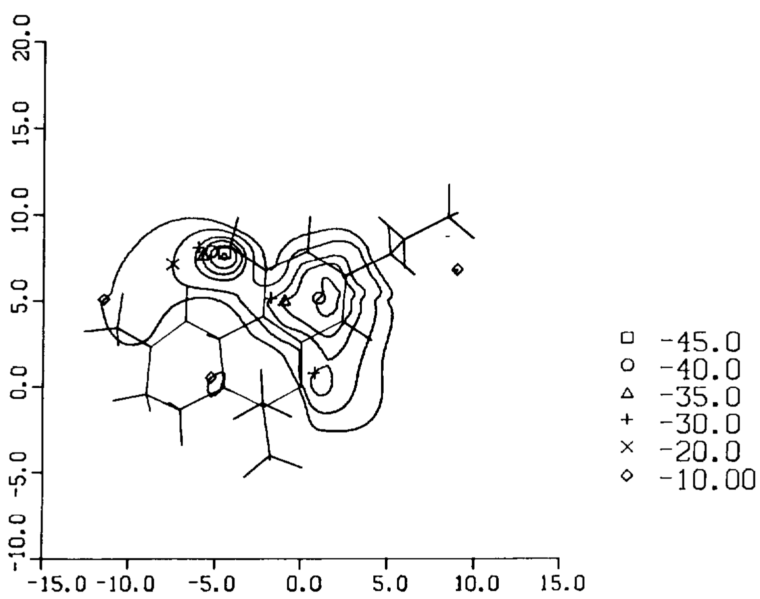


FIGURE 5. Molecular electrostatic potential of the bottom face of delta-9-THC (Conformational I). Contours represent the MEP in a plane 1.5Å below the aromatic ring. Values are in Kcal/mole. From Reggio and Mazurek 1987. Copyright 1986 by Elsevier Science Publishers B.V. (Amsterdam).

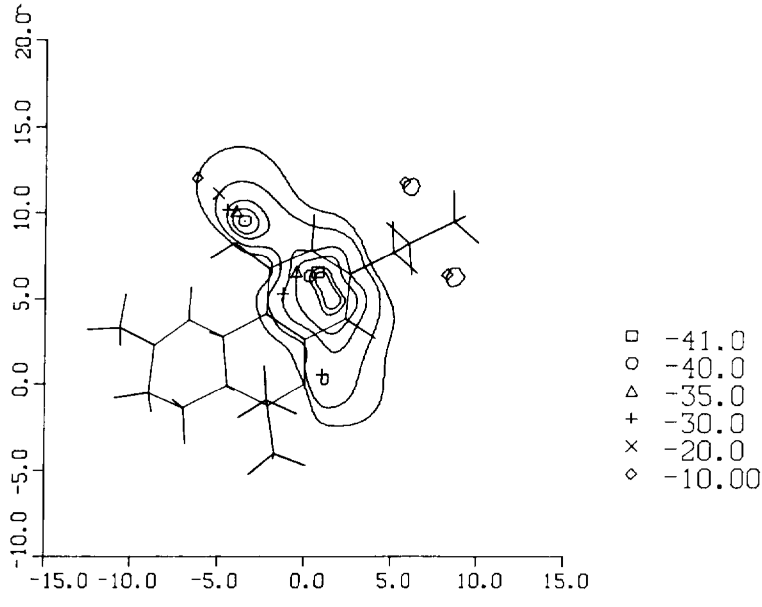


FIGURE 6. Molecular electrostatic potential of the bottom face of delta-9-THC (Conformational II). See figure 5 for details. From Reggio and Mazurek 1987. Copyright 1986 by Elsevier Science Publishers B.V. (Amsterdam).

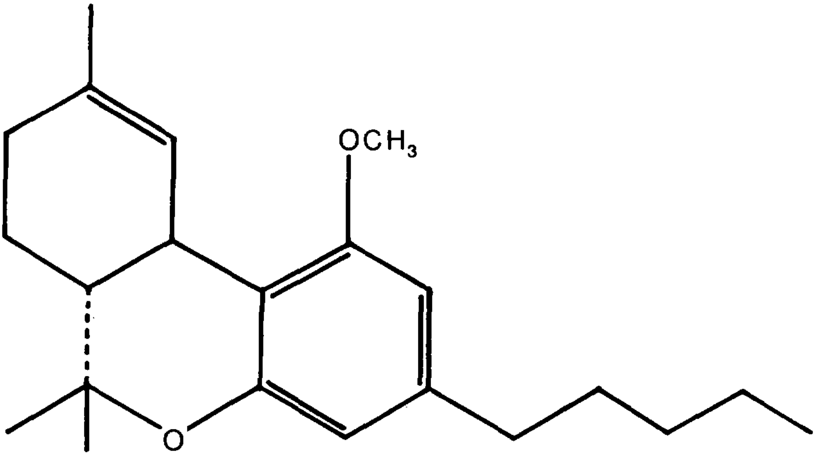


FIGURE 7. O-methyl-delta-9-THC

the MM2 program (Allinger and Yuh 1980) was used. Rotations in 36 steps were made about the C_1-O axis.

In the second study, an SCF calculation was performed for a model fragment of O-methyl-delta-9-THC (see figure 8). The Gaussian 80 system of programs and the STO-3G basis set were employed here (Fluder et al. 1980). Initially, all atoms in the fragment were frozen in their optimized positions. These positions were determined by MM2 calculations for the full molecule. Rotations of the methoxy group in 18 steps were made about the C_1-O axis.

RESULTS AND DISCUSSION

Conformational Analysis

Figure 9 shows the minimum energy conformer of O-methyl-delta-9-THC as obtained from the MM2 calculation. Previous studies have predicted that the cyclohexene ring (Ring A) of delta-9-THC should exist predominantly in a half-chair-like conformation (Archer et al. 1970). The present calculations indicate that Rng A retains this conformation in O-methyl-delta-9-THC. As a result of the interaction between the C_6 methyl groups and H_{6a} , Ring B assumes a conformation such that the axial C_6 methyl group is on the same side of the molecule as H_{10a} and is much closer to H_{10a} than is the other methyl group. The substituents on C_6 and C_{6a} are staggered with respect to one another (optimized C_{10a}-C_{6a} -C_6 -O_5 dihedral angle is 63°). Here, the numbering system employed is the same as that for delta-9-THC (see figure 2).

The C_1 methoxy group is subject to steric interaction with the C_{10} proton. This interaction causes the methoxy oxygen to bend slightly out of the plane of the benzene ring. The methoxy group optimizes at a position away from Ring A (optimized C_2-C_1-O-C dihedral angle is -5°).

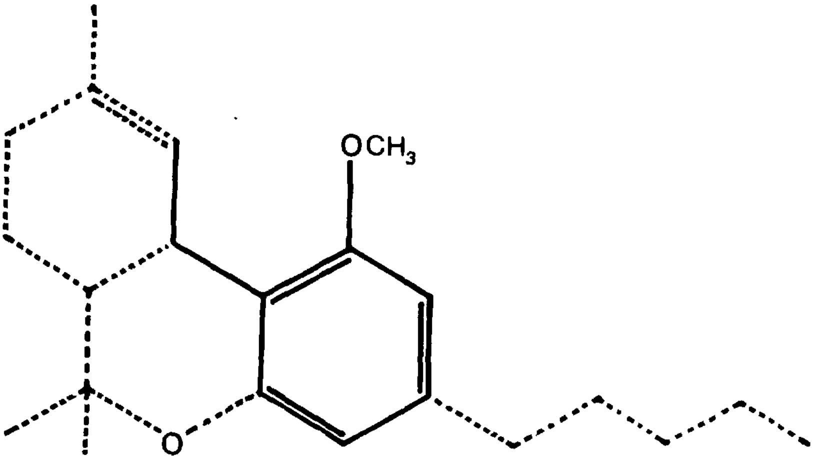


FIGURE 8. Model fragment for O-methyl-delta-9-THC.

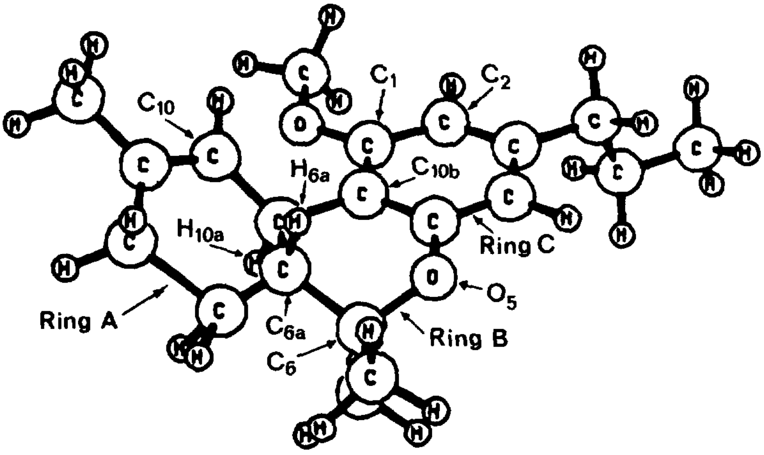


FIGURE 9. Conformation of O-methyl-delta-9-THC (with propyl side chain) as determined by MM2.

Optimized bond lengths are within .01 Å of the following values: C_{sp} P2-C_{sp}2 1.40 Å, C_{sp} 2-C_{sp}3 1.51 Å, C_{sp} 3-C_{sp} 3 1.54 Å, C_{sp}2-O 1.37 Å, C_{sp}3-O 1.41 Å, C_{sp}2-H 1.10 Å, and C_{sp}3-H 1.11 Å.

Figure 10 shows a perspective of the molecule viewed in the direction parallel to the vector from C_2 to C_{10b} From this perspective, the conformation of Ring A causes the top part of the ring to move to the left, thus permitting no protrusion of Ring A into the bottom face of the molecule. A comparison of figure 10 with figure 4 reveals that the position of Ring A in O-methyl-delta-9-THC mimics that of Ring A in delta-9-THC.

The results of the MM2 study of molecular energy as a function of rotation about the C_1-O axis are summarized in figure 11. There are local minima at τ = -50^0 and 85° separated by a 0.8 kcal barrier. The SCF results for the model fragment of O-methyl-delta-9-THC agreed very well with those above. The SCF method pinpointed two minimum energy conformers τ = -5° and τ = 85° separated by a 1.0 kcal barrier.

It is clear from figure 11 that a τ of 155° (which corresponds to Conformation II of delta-9-THC) does not represent an energy minimum for the molecule. In fact, in the MM2 study, the conformation with τ = 155° is 7.3 kcal higher in energy than the minimum energy conformer of O-methyl-delta-9-THC.

In its minimum energy conformation, the methoxy group of O-methyl-delta-9-THC essentially adopts Conformation I of the template. In this conformation, the lone pairs of electrons of the phenol oxygen are directed toward Ring A. However, the methoxy group cannot mimic Conformation II of the template in which the lone pairs of electrons of the phenol oxygen are directed away from Ring A. On the basis of these results, the inactivity of O-methyl-delta-9-THC can be explained by the fact that the molecule cannot mimic Conformation II of delta-9-THC. These results seem to indicate, then, that it is Conformation II which is the more relevant conformation at the site of action.

In an early SAR study, Edery et al. (1971) postulated the need for a free phenol for cannabinoid activity. Recently, Binder and Franke (1982) have proposed a picture of delta-9-THC interacting with its hypothetical receptor. In this picture, the phenolic hydrogen is shown to form a hydrogen bond with the receptor. Such a hypothetical drawing implies the necessity of a phenolic hydrogen for activity. It is possible to argue from this viewpoint that O-methyl-delta-9-THC is inactive due to its lack of a phenolic hydrogen. Although the demonstrated activity of some esterified THC would argue against the necessity of a phenolic hydrogen for activity, there appears to be no definitive compound yet synthesized which can answer this question. In the next stage of our investigation, we hope to propose new cannabinoids which could be useful in delineating an answer.

CONCLUSIONS

The finding that O-methyl-delta-9-THC cannot mimic Conformation II of delta-9-THC in which the lone pairs of electrons of the phenol oxygen are directed away from Ring A has led to a refinement of our template for cannabinoid activity. This inability of O-methyl-delta-9-THC to mimic Conformation II may cause the inactivity of the molecule. As a result of this finding, we have tentatively refined our template for cannabinoid activity to include two aspects.

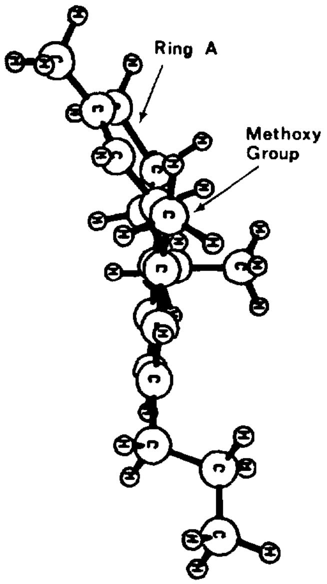


FIGURE 10. Conformation of O-methyl-delta-9-THC (with propyl side chain) as determined by MM2.

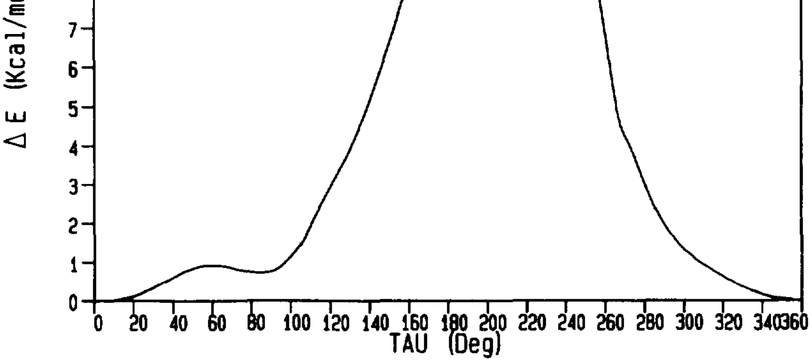


FIGURE 11. Conformation of O-methyl-delta-9-THC as determined by MM2: here the perspective of Ring A is viewed in the direction parallel to the vector from C_2 to C_{10b}.

1. The first aspect is the orientation of the lone pairs of electrons of the phenol oxygen such that these lone pairs point away from Ring A (Conformation II) (these lone pairs generate directional reactivity properties).

2. The second aspect is the orientation of Ring A such that the ring moves out of the bottom face of the molecule (this conformation is likely to allow for nondirectional hydrophobic interactions).

Further refinements of this template will be made in subsequent comparisons of the template MRCs with those of other cannabinoids.

REFERENCES

Allinger, N.L., and Yuh, Y.H. MM2. Ouantum Chemistry Program Exchange 13:395, 1980.

Allinger, N.L.; Chang, S.-H.; Glaser, D.H.; and Honig, H. An improved molecular mechanics force field for alcohols, and ethers. Isr J Chem 20:51-56, 1980.

Archer, R.A.; Boyd, D.B.; Demarco, P.V.; Tyminski, I.J.; and Allinger, N.L. Structural studies of cannabinoids. A theoretical and proton magnetic resonance analysis. J Am Chem Soc 92(17):5200-5206, 1970.

Binder, M., and Franke, I. Is there a THC-receptor? In: Hucho, F., ed. Neuroreceotors. Berlin and New York: Walter de Gruyter, 1982. pp. 151-161.

Binder, M.; Edery, H.; and Porath, G. Delta-7-tetrahydrocannabinol, a non-psychotropic cannabinoid: Structure-activity considerations in the cannabinoid series. In: Nahas, G.G., and Paton, W.D.M., eds. Marihuana Biological Effects: Analysis, Metabolism, Cellular Responses, Reproduction, and Brain. Oxford: Pergammon Press, 1979. pp. 71-80.

Burstein, S.; Hunter, S.A.; and Ozman, K. XII. The effect of cannabinoid structure on the synthesis of prostaglandins by human lung fibroblasts. Mol Pharm 23:121-126, 1983.

Carney, J.M.; Balster, R.C.; Martin, B.R.; and Harris, L.S. Effects of systemic and intraventricular administration of cannabinoids on schedule-controlled responding in the squirrel monkey. J Pharmacol Exp Ther 210:399-404, 1979.

Dearden, J.C., ed. Ouantitative Approaches to Drug Design. Amsterdam: Elsevier, 1983. 296 pp.

Dewey, W.; Martin, B.; and May, E. Cannabinoid stereoisomers: Pharmacological effects. In: Smith, D.E., ed. CRC Handbook of Stereoisomers: Drugs in Psychopharmacology. Boca Raton: CRC Press, 1984. pp. 317-321.

Edery, H.; Grunfeld, Y.; Ben-Zvi, Z.; and Mechoulam, R. Structural requirements for cannabinoid activity. Ann NY Acad Sci 191:40-53, 1971.

Fluder, E.J.; Schlegel, H.B.; and Topiol, S.W. Gaussian 80 (IBM Version), unpublished, 1980.

Gaoni, Y., and Mechoulam, R. Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 86:1646-1647, 1964.

Gaoni, Y., and Mechoulam, R. The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. J Am Chem Soc 93:217-224, 1971.

Johnson, M.R.; Melvin, L.S.; and Milme, G.M. Prototype cannabinoid analgetics, prostaglandins and opiates - a search for points of mechanistic interaction. Life Sci 31:1703-1706, 1982.

Martin, B.R.; Harris, L.S.; and Dewey, W.L. Pharmacological activity of delta-g-THC metabolites and analogs of CBD, delta-8-THC and delta-9-THC. In: Agurell, S.; Dewey, W.L.; and Willette, R.E., eds. The Cannabinoids; Chemical, Pharmacologic, and Therapeutic Aspects. New York: Academic Press, 1984a. pp. 523-544.

Martin, B.R.; Kallman, M.J.; Kaempf, G.F.; Harris, L.S.; and Dewey, W.L. Pharmacological potency of R- and S-3'-hydroxy-delta-9-tetrahydrocannabinok Additional structural requirement for cannabinoid activity. Pharmacol Biochem Behav 21:61-65, 1984b.

Mitchell, A.D., and Cross, L.C., eds. Tables of Interatomic Distances and Configurations in Molecules and Ions, Special Publication No. 11, 1958; Special Publication No. 18, 1965.

Narimatsu, S.; Matsubara, K.; Shimoniski, T.; Watanabe, K.; Yamamoto, I.; and Yoshimura, H. Pharmacological activities in the mouse of delta-9-tetrahydrocannabinol metabolites oxidized at the 8-position. Chem Pharm Bull 33(1):392-395, 1985.

Osgood, P.R.; Howes, J.F.; Razdan, R.K.; and Pars, H.G. Drugs derived from cannabinoids. 7. Tachycardia and analgesia structure-activity relationships in delta-9-tetrahydrocannabinol and some synthetic analogues. J Med Chem 2(8):809-811, 1978.

Razdan, R.K. Chemistry and structure-activity relationships of cannabinoids: An overview. In: Agurell, S.; Dewey, W.L.; and Willette, R.E., eds. The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects. New York: Academic Press, 1984.

Reggio, P.H. and Mazurek, A.P. A molecular reactivity template for cannabinoid activity. J Mol Struc (Theochem) 149:331-343, 1987.

Reggio, P.; Weinstein, H.; Osman, R.; and Topiol, S. Molecular determinants for binding of methylenedioxytryptamines at 5HT/LSD receptors. Int J Quantum Chem QBS8:373-384, 1981.

Reggio, P.; Topiol, S.; and Weinstein, H. Molecular determinants for the agonist activity of 2-methylhistamine and 4-methylhistamine at H2-receptors. J Med Chem 29:2412-2415, 1986.

Rosell, S.; Bjorkroth, V.; Agurell, S.; Leander, K.; Ohlsson, A.; Martin, B.; and Mechoulam, R. Relation between effects of cannabinoid derivatives on the twitch response of the isolated guinea pig ileum and their psychotropic properties. In: Nahas, G.G., and Paton, W.D.M., eds. Marihuana Biological Effects: Analysis, Metabolism, Cellular Responses, Reproduction, and Brain. Oxford: Pergammon Press, 1979. pp. 63-70.

Rosenquist, E., and Ottersen, T. The crystal and molecular structure of delta-9-tetrahydrocannabinolic acid B. Acta Chem Scand 1329:379-384, 1975.

Sofia, R.D. Cannabis: Structure-activity relationships. In: Iverson, L.L.; Iverson, S.D.; and Snyder, S.H., eds. Handbook of Psychopharmacology. Vol. 12. Drugs of Abuse. New York: Plenum Press, 1978. pp. 319-371.

Tamir, I.; Mechoulam, R.; and Meyer, A. Cannabidiol and phenytoin: A structural comparison. J Med Chem 23:220-223, 1980.

Tucker, J.B. Designing molecules by computer. High Technology JAN:52-59, 1984.

Uliss, D.B.; Calzell, H.D.; Handrick, G.R.; Howes, J.F.; and Razdan, R.K. Hashish. Importance of the phenolic hydroxyl group in tetrahydrocannabinols. J Med Chem 18:213-215, 1975.

Vida, J.A., and Gordon, M. Conformationally Directed Drug Design. American Chemical Society Symposium Series 251. Washington: American Chemical Society, 1984. 271 pp.

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Different Cannabinoids Exhibit Different Pharmacological and Toxicological Properties

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INTRODUCTION

By the mid-1970s. the anticonvulsant properties of the cannabinoids were well documented (Loewe and Goodman 1947; Karler et al. 1973, 1974; Carlini et al. 1975; Consroe et al. 1976). but an analysis of their therapeutic potential as antiepileptics had not yet been carried out. For this reason, a major goal originally of our marijuana-related research was to determine the cannabinoids' antiepileptic potential by the use of conventional laboratory tests for anticonvulsant and toxic properties (Turkanis and Karler 1981). The aim was to define the relative selectivity of desired-to-undesired effects of these agents. In the course of these studies, which involved many different cannabinoids, we observed that there exist among these drugs, not only quantitative differences, but also qualitative differences. The accretion of these differences ultimately provides a profile of the pharmacological characteristics of the cannabinoids as a class of drugs. The data presented below represent examples from our investigations of some properties of the cannabinoids, specifically related to their antiepileptic potential.

METHODS

The anticonvulsant characteristics were measured in a variety of tests in order to obtain a spectrum of properties for comparative purposes; the tests included both electro- and chemo-shock tests. Because a major toxic effect of the clinical antiepileptics is central depression, neurotoxicity was determined in terms of motor toxicity, a classical measure of central nervous system (CNS) depression. The details of the anticonvulsant and motor-toxicity tests have been previously described (Karler et al. 1974; Turkanis et al. 1974).

The cannabinoids were dispersed in isotonic sodium chloride solution with the aid of Tween 80 and ultrasound (Turkanis et al. 1974), and all drug and vehicle preparations were administered intraperitoneally (i.p.). All determinations of potency were made from dose-effect curves, which were obtained at peak-effect times; and anticonvulsant potencies were expressed as either the effective-dose 50 (ED50) or the anticonvulsant-dose 50 (AD50), and motor toxic potency as the toxic-dose 50 (TD50).

RESULTS

Figure 1 illustrates the dose-effect curves for anticonvulsant activity in a maximal electroshock (MES) test and motor toxicity in a bar-walk test.

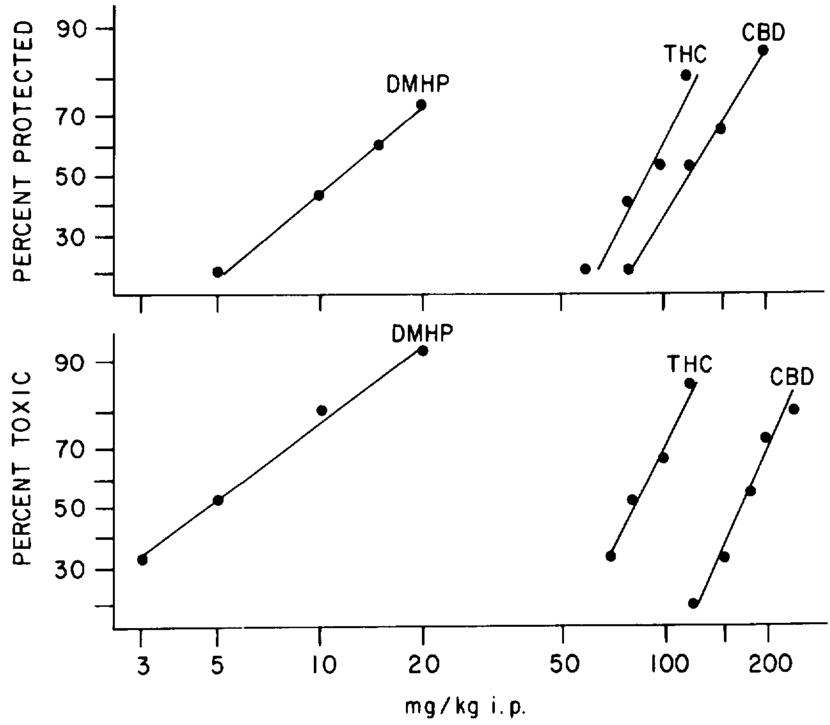

FIGURE 1. Dose-effect relationships for cannabinoids in MES and bar-walk tests. The bar-walk test was carried out just prior to the MES test at the peak-effect times: THC and DMHP, 2 hr; CBD, 1 hr. The AD50s and their 95% confidence limits (mg/kg, i.p.) are 12 (8-19) for DMHP, 92 (74-115) for THC, and 122 (94-159) for CBD. The TD50s and their 95% confidence limits (mg/kg, i.p.) are 4.7 (3-7) for DMHP, 82 (66-103) for THC, and 170 (144-200) for CBD. Each point represents the results obtained jrom about 15 male Charles River mice (ICR) weighing 15-26 gm. AD50s and TD50s and their 95% confidence limits were calculated by methods described by Litchfield and Wilcoxon (1949). THC = delta-9-tetrahydrocannabinol; CBD = cannabidiol; DMHP = dimethylheptylpyran.

In this study, we compared DMHP, THC, and CBD. At the time, these three compounds were selected for our initial studies for a variety of reasons: THC was included because we intended to use it as the prototype cannabinoid; CBD was included because it lacks the psychotoxicity of THC; and, finally, DMHP was studied because it was thought to be the compound that had been tried with apparently some success in epileptic children (Davis and Ramsey 1949). DMHP was later found, like CBD, to be nonpsychoactive in humans (Lemberger et al. 1974). In the upper half of this figure, we compared the dose-response curves for these three drugs against MES, which is one measure of anticonvulsant activity. Some points are clear from these data: first, that all three compounds were anticonvulsant in the MES test; second, that the psychotoxicity of THC is separable from the anticonvulsant activity because the nonpsychoactive CBD and DMHP are both anticonvulsant; and, finally, in terms of anticonvulsant potency, the dose-response curves illustrate that there is a big difference in potency between DMHP and the other two drugs.

The separation between psychoactivity and anticonvulsant activity encouraged us to continue this study and to pursue other aspects of the potential toxicity problem. One property that especially interested us was motor toxicity because all antiepileptic drugs are motor toxic. The lower half of the figure illustrates that the cannabinoids are also motor toxic, as measured in a bar-walk test; in these instances, potency in terms of motor toxicity appeared to be directly proportional to the anticonvulsant potency; that is, the greater the anticonvulsant potency, the greater the motor-toxic potency.

The data shown in table 1 represent the continuation of our study of relative potencies with respect to the anticonvulsant and toxic effects of a large number of cannabinoids. The drugs included in table I were not selected for their structural characteristics; rather, they were included in the study because they were the drugs that were available to us at the time. Nevertheless, the data shown illustrate that most of these compounds displayed both anticonvulsant and motor-toxic activity and that the potency relative to THC varied. For example, in the MES test, the 11-hydroxy derivative is about seven times as potent as is THC; and, similarly, it is about three or four times as potent in terms of motor toxicity. The remaining compounds listed in the table illustrate the quantitative differences in both anticonvulsant activity and motor toxicity, even in such a limited sample of cannabinoids. The anticonvulsant activity ranged from a fraction of that of THC, as illustrated by THC Acid B, to many times that of THC, as in the case of d,1,threo-DMHP. Similar ranges were observed for the motor-toxic activity in the bar-walk test.

At this point in our studies, one of the frequent criticisms that we received was that the anticonvulsant dose of THC in the mouse, about 100 mg/kg, is very high--approximately 100 times that of the psychoactive dose in humans; therefore, the meaning of the anticonvulsant effects vis-a-vis humans was questioned. To address this question, we examined the potency of some of the cannabinoids in three different species: the mouse, the rat, and the frog. The data are shown in table 2.

Here, we can see a very wide range of potency for these drugs. At the extremes, the AD50 for both THC and CBD in the mouse is about 100 mg/kg; in the frog, it is about 0.1 mg/kg. This species variability is not seen for either phenytoin or phenobarbital. The point of this table is that there are large species differences in the potency of the cannabinoids, which means that it is not possible to extrapolate meaningful potency data from animals to humans; therefore, the anticonvulsant dose in the mouse (or the frog) cannot be equated with a psychoactive dose in humans. An anticonvulsant dose in humans can only be determined in humans.

TABLE 1

Relative Potencies of Cannabinoids in Anticonvulsant and Motor Performance Tests in Mice

Drug	MES Test	Bar-walk Test
THC	1.0	1.0
11-Hydroxy-THC	7.1	3.6
8 Alpha-hydroxy-THC	*	0.7
8 Beta-hydroxy-THC	1.0	*
THC Acid A	0.5	0.2
THC Acid B	0.3	0.2
Delta-8-THC	1.0	1.7
9-Nor-delta-8-THC	0.8	1.3
CBN	0.4	0.4
9-Nor-9 alpha-hydroxy-hexahydro CBN	0.8	0.8
9-Nor-9 beta-hydroxy-hexahydro CBN	2.2	1.7
CBD	0.8	0.5
DMHP	7.7	15
d,l,Threo-DMHP	14	–
d,l,Erythro-DMHP	4.0	–
Cannabichromene	*	0.4

The relative potency is the ratio of the median-effective THC dose to that of another cannabinoid. The average median-effective THC dose was 100 mg/kg, i.p., for anticonvulsant effects in the MES test and 80 mg/kg, i.p., for motor toxicity measured in a bar-walk test.

*Compound lacks activity within the dosage range tested.

–No data available.

The data shown in figure 2 represent another example of species differences in response to the cannabinoids. The figure depicts dose-effect curves for the motor toxicity of THC and CBD in the rat. These two drugs are of similar motor-toxic potency in the mouse (about 100 mg/kg); but in the rat, the TD50 for THC is about 7 mg/kg, compared with 175 mg/kg of CBD. In the rat, therefore, THC is about 25 times as toxic as is CBD and about 10 times as toxic as it is in the mouse. Again, the species differences in response to these two cannabinoids are striking; and they serve to re-emphasize the inappropriateness of extrapolating data from one species to another, including humans.

TABLE 2

Species Differences in Drug Potencies in the Maximal Electroshock Test

	AD50 (mg/kg)*		
Drug	Mouse	Rat	Frog
THC	100	5	0.1
CBD	120	50	0.1
Phenytoin	9	5	10
Phenobarbital	12	12	8

*Values calculated from dose-response data. From Karler and Turkanis 1981. Copyright 1981 by The American College of Clinical Pharmacology, Inc. (New York).

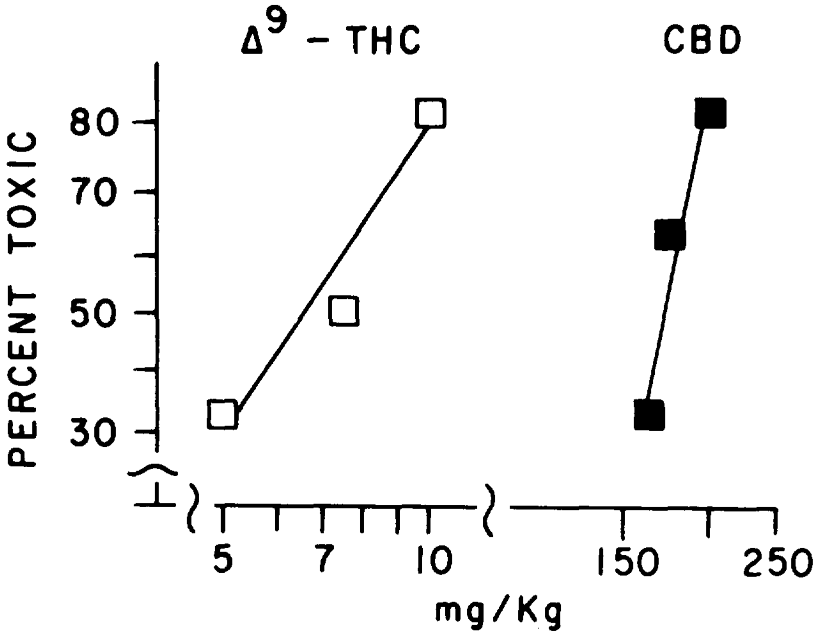

FIGURE 2. Dose-effect relationships for THC and CBD in a bar-walk test with rats. The TD50s and their 95% confidence limits (mg/kg, i.p.) are 6.6 (4.8-9.0) for THC and 173 (156-192) for CBD. The values were calculated by methods described by Litchjield and Wilcoxon (1949). Each point represents results jrom 6-8 male Sprague-Dawley rats weighing 250-280 gm. The relative potency for CBD compared to THC was 0.04 (Smiley et al. 1979).

The data in table 3 illustrate the comparative selectivity of the cannabinoids, as described conventionally in terms of the protective index, that is the ratio of the TD50 to the ED50. The point of the experiment was to examine the quantitative relationship between anticonvulsant activity and motor toxicity in order to determine if these two effects are separable. Motor toxicity is a major side effect of all antiepileptic drugs. In pharmacological terms, we were raising the issue here of the selectivity of these compounds as anticonvulsants relative to their general CNS depressant activity. Clinically, the usefulness of any drug, in part, depends upon its selectivity for a desired effect.

TABLE 3

Effects of a Single Dose of Cannabinoid in Mice in a Maximal Electroshock and a Bar-walk Test

Drug	Peak-effect Time (hr)*	ED50 (mg/kg)	TD50 (mg/kg)	Protective Index (TD50/ED50)
DMHP	2	13	5	0.4
Delta-9-THC	2	100	80	0.8
CBN	1	230	230	1.0
CBD	1	120	180	1.5
11-Hydroxy-THC	1	14	22	1.6
Delta-8-THC	2	83	152	1.8

All experiments were performed at an ambient temperature of approximately 22°C; ED50 and TD50 values were calculated by the method of Litchfield and Wilcoxon (1949) from conventional dose-response curves based on 3-6 doses with 15-20 animals for each dose; drugs were administered i.p.

*The peak-effect times for the anticonvulsant and toxic effects were similar.

From Karler and Turkanis 1976. Copyright 1976 by Plenum Publishing Corp. (New York).

In this table are listed the protective index data for DMHP, delta-9-THC, cannabinol (CBN), CBD, the 11-hydroxy metabolite, and delta-8-THC. For DMHP, the ED50 against the maximal electroshock is 11.5 mg/kg and the TD50 is 4.6 mg/kg, yielding a protective index of 0.4. Similarly, the protective index for delta-9-THC is 0.8; for CBN, 1.0; for CBD, 1.5; for 11-hydroxy-THC, 1.6; and for delta-8-THC, 1.8. For comparative purposes, the protective index for phenobarbital is 1.5 (Karler and Turkanis 1981). These data clearly illustrate that the anticonvulsant and motor-toxic activities are at least partially separable, ranging from DMHP and THC, which are more motor toxic than anticonvulsant, to CBD, 11-hydroxy-THC, and delta-8-THC, which exhibit more anticonvulsant than toxic activity.

The data in table 4 represent a summary of the results obtained from five different convulsive threshold tests. All of the anticonvulsant data described above have been limited to only one convulsive test, that is, the MES test. There are, however, many such tests, and table 4 contains the comparative results of THC and CBD from a variety of electro- and chemo-shock tests.

TABLE 4

Anticonvulsant Properties of the Cannabinoids

Convulsive Threshold Test	THC	CBD
Minimal		
60-Hz-EST	-	+
6-Hz-EST	+	+
Pentylenetetrazol	-	0
Maximal		
MEST	+	+
Pentylenetetrazol	+	+

+ = anticonvulsant activity; - = proconvulsant activity; 0 = no activity.

CBD is anticonvulsant in four of the five tests; THC, however, has a very different spectrum of activity because it is anticonvulsant in three tests, but is proconvulsant in the 60-Hz-EST and the pentylenetetrazol minimal threshold test. This was the first evidence that we had to indicate that THC exerts not only depressant effects but also excitatory effects. Subsequently, we have observed THC's excitatory properties in many other experimental situations. For example, THC can precipitate frank convulsions in cobalt- and iron-epileptic rats (Chiu et al. 1979; Turkanis and Karler 1982); THC enhances electrically induced limbic seizure activity in the rat (Smiley et al. 1979); and THC increases the rate of kindling to both electrical and chemical stimuli (Karler et al. 1984). In striking contrast, in all of these test situations, CBD displays only anticonvulsant or depressant properties. We have also observed THC excitatory effects at every level of organization of the nervous system, from the intact animal to the synapse (Turkanis and Karler 1981). In none of these tests has CBD shown any excitatory activity. On these grounds, we have concluded that the excitatory properties of the cannabinoids are completely separable from the depressant properties.

The data shown in figures 3 and 4 represent the influence of chronic THC or CBD treatment on their anticonvulsant properties. All of the anticonvulsant data described above were derived from acute experiments. In contrast, the data in figure 3 were obtained from animals treated for 22 days with a single daily dose of THC. The top of the figure shows the results from the 6-Hz-EST test. On day 1, THC raised this convulsive threshold about 20 V, which reflects its anticonvulsant activity in this test. During the course of the 22 days, tolerance developed because the anticonvulsant activity decreased. In the next test, the 60-Hz-EST, THC is proconvulsant because on day 1 it decreased seizure threshold by 2 mA, but again tolerance appeared to develop with repeated daily treatment. Finally, in the third test, the maximal EST, THC acutely is anticonvulsant because it elevated the threshold on day 1, but again tolerance developed to this effect. The two curves at the bottom of the figure illustrate that the tolerance in these instances is a true tolerance because there were no significant changes in either the brain- or the plasma-THC concentrations with chronic treatment.

Figure 4 presents the results of chronic CBD treatment in the identical experimental paradigm as that used for the previous THC study. But the results are very different. In the two seizure-threshold tests shown at the top of the figure, that is, the 6-Hz- and 60-Hz-EST tests, the anticonvulsant activity of CBD progressively increased with chronic treatment, just the opposite response from the tolerance observed with THC.

An enhanced response to a given dose of drug administered chronically has been termed by some investigators as "reverse tolerance," and it is a phenomenon that has been repeatedly described for CNS locomotor stimulants, such as cocaine, amphetamine, and morphine (Post and Kopanda 1975; Segal and Mandell 1974; Shuster et al. 1975). This may be the first report of "reverse tolerance" developing to depressant effects, in this case, anticonvulsant effects. The instances of "reverse tolerance" again clearly distinguish CBD from THC. The results of the maximal EST indicate that tolerance develops to the other two effects. With respect to this test, CBD resembles THC because tolerance developed to both of the drugs.

Although antiepileptics are taken chronically, studies of chronic effects of these drugs are conspicuously absent from the literature. For this reason, we included phenytoin in our cannabinoid study and found that in one of the tests, tolerance developed; in another test, there was no change in pharmacodynamics; and in the third test, "reverse tolerance" developed (Karler et al. 1982). The "reverse tolerance" in this case is of special interest because it is clinically recognized that there exists a time lag of a few weeks before the antiepileptic activity of phenytoin completely manifests itself. Currently, there is no explanation for the time lag, but our animal data suggest that the lag may be due to the time required to develop "reverse tolerance." In any event, the phenomenon of "reverse tolerance" may add a new dimension to the study of antiepileptic drugs, as well as to the study of cannabinoids.

DISCUSSION

The data presented were selected to provide an overview of what we consider to be some of the more important pharmacological characteristics of the cannabinoids that have emerged from our whole-animal studies of their anticonvulsant properties. We have seen, for example, as have others, that the anticonvulsant activity is separable from the psychoactivity. Without such a separation, these drugs would have no clinical potential as antiepileptics. We also

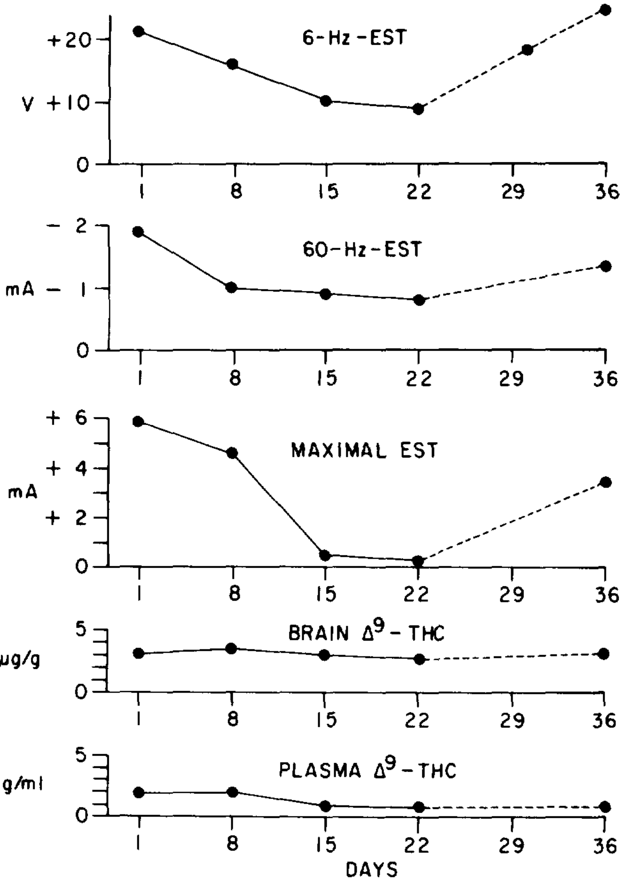

FIGURE 3. Influence of repeated THC treatment on ESTs. In each test, 60 mice were given 10 mg/kg THC i.p. once daily, and another 60 mice, vehicle. The points indicate the differences in EST between drug- and vehicle-treated groups. —— = period of daily treatment: ----- = period oj withdrawal. Brain and plasma values represent data from 6-8 mice. On day 1 the vehicle-control values were 23 (21-24) V for the 6-Hz-EST, 5.9 (5.7-6.1) mA Jor the 60-Hz-EST and 7.5 (7.0-8.0) mA for the MEST and, as determined by a relative potency test of Litchfield and Wilcoxon (1949) (P < 0.05), were significantly different from the drug-treated groups. From Karler et al. 1982. Copyright 1982 by Springer-Verlag (Heidelberg).

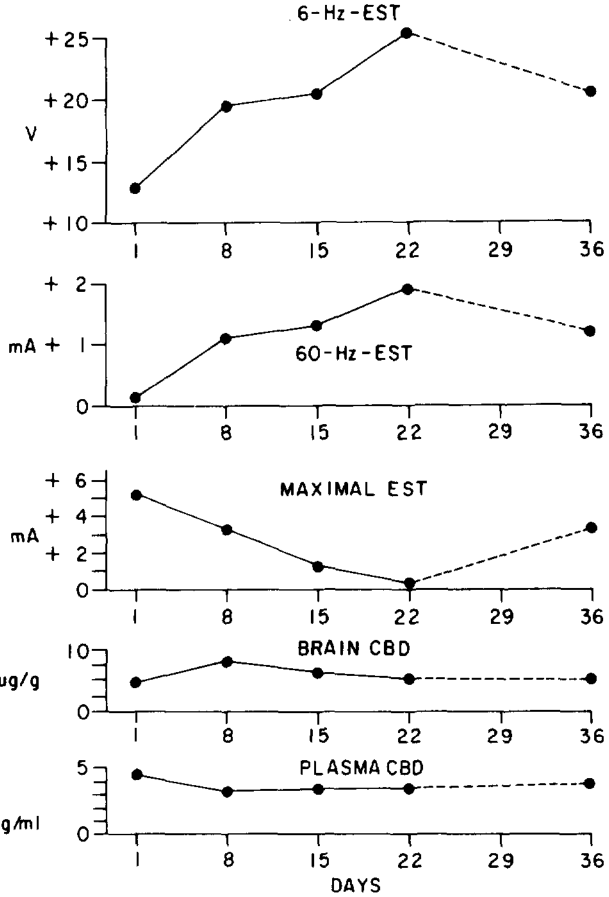

FIGURE 4. Influence of repeated CBD treatment on the ESTs and on brain and plasma-drug concentrations. On day 1 vehicle-control values were 21.5 (20-23) V for the 6-Hz-EST, 5.4 (5.1-5.8) mA for the 60-Hz-EST and 7.9 (7.6-8.3) mA for the MEST, and on day 1 only the 6-Hz-EST and MEST vehicle-control values were significantly different from those obtained from CBD-treated groups as determined by a relative potency test. See figure 3 for additional information. From Karler et al. 1982. Copyright 1982 by Springer-Verlag (Heidelberg).

observed that among the different cannabinoids, there are differences in potency, there are differences in the protective index, and there are species differences in response to them. None of these observations is too surprising because such differences are general characteristics of drugs. Detailed structure-activity studies would probably yield cannabinoids with more selectivity as anticonvulsants than those that we have studied. The "reverse tolerance" to CBD was a surprise. What this means vis-a-vis an antiepileptic drug is not clear, but the observation that a similar response to chronic phenytoin treatment raises the possibility that the clinical efficacy of phenytoin may, in part, be due to "reverse tolerance." Although "reverse tolerance" was not observed for THC in the anticonvulsant study, the CBD data illustrate that "reverse tolerance" can occur to cannabinoid effects; therefore, the toxicological or pharmacological evaluation of any cannabinoid should involve the assessment of effects in chronically treated, as well as acutely treated, animals.

In the course of our studies, we have tried to emphasize that anticonvulsant activity is not a single entity; rather, it is a complex group of effects. That point is clearly seen in the case of THC which is anticonvulsant in some tests and proconvulsant in others. This resembles the clinical response generally to antiepileptic drugs. Phenytoin, for example, is effective in the treatment of grand mal epilepsy; however, it can exacerbate absence seizures. The reverse is true for ethosuximide. In animal studies, these drugs resemble THC because they all exhibit both anticonvulsant and proconvulsant properties. In retrospect, what is interesting about CBD as an anticonvulsant is that we have not yet seen any indication of an excitatory effect on CNS function. In this sense, it may be unique as an anticonvulsant. Despite a number of favorable properties of CBD, its future as an antiepileptic--at least in this country--is not very promising. Although the anticonvulsant drug-screening program sponsored by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) screened CBD and found it to have anticonvulsant activity, it has not been recommended for clinical trial, and it is the NINCDS recommendation that determines which new antiepileptics will be clinically tested in this country. The drug industry in the past has been reluctant to develop new antiepileptics because the potential market is too small to justify the drug developmental expense. Even now that the NINCDS is providing much of the developmental costs, what drug company would be willing to bear the marketing cost of a nonpatentable, marginally profitable drug such as CBD? It is for this reason that we are pessimistic about the future of CBD as an antiepileptic. It may remain just as we found it: a very interesting laboratory drug.

REFERENCES

Carlini E.A.; Mechoulam, R.; and Lander, N. Anticonvulsant activity of four oxygenated cannabidiol derivatives. Res Commun Chem Pathol Pharmacol 12:1-15, 1975.

Chiu, P.; Olsen, D.M.; Borys, H.K.; Karler, R.; and Turkanis, S.A. The influence of cannabidiol and delta-9-tetrahydrocannabinol on cobalt epilepsy in rats. Epilepsia 20:365-375, 1979.

Consroe, P.; Jones, B.; Laird, H., II; and Reinking, J. Anticonvulsant-convulsant effects of delta-9-tetrahydrocannabinol. In: Cohen, S., and Stillman, R.C., eds. The Therapeutic Potential of Marihuana. New York: Plenum Publishing Corp., 1976. pp. 343-362.

Davis, J.P., and Ramsey, H.H. Antiepileptic action of marihuana-active substances. Fed Proc 8:284-285, 1949.

Karler, R., and Turkanis, S.A. The antiepileptic potential of the cannabinoids. In: Cohen, S., and Stillman, R.C., eds. The Therapeutic Pontential of Marihuana. New York: Plenum Publishing Corp., 1976. pp. 383-397.

Karler, R., and Turkanis, S.A. The cannabinoids as potential antiepileptics. J Clin Pharmacol 21:437S-448S, 1981.

Karler, R.; Cely, W.; and Turkanis, S.A. The anticonvulsant activity of cannabidiol and cannabinol. Life Sci 13: 1527- 1531, 1973.

Karler, R.; Cely, W.; and Turkanis, S.A. Anticonvulsant properties of delta-9-tetrahydrocannabinol and other cannabinoids. Life Sci 15:931-947, 1974.

Karler, R.; Borys, H.K.; and Turkanis, S.A. Influence of 22-day treatment on the anticonvulsant properties of cannabinoids. Naunym Schmiedebergs Arch Pharmacol 320:105-109, 1982.

Karler, R.; Calder, L.D.; and Turkanis, S.A. Changes in CNS sensitivity to cannabinoids with repeated treatment: Tolerance and auxoesthesia. In: Sharp, C., ed. Mechanisms of Tolerance and Dependence: 1984. National Institute on Drug Abuse Research Monograph 54. DHHS Pub. No. (ADM) 84-1330. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1984. pp. 212-222.

Lemberger, L.; McMahon, R.; Archer, R.; Matsumoto, K.; and Rowe, H. Pharmacologic effects and physiologic disposition of delta-6-alpha, 10-alpha-dimethylheptyltetrahydrocannabinol (DMHP) in man. Clin Pharmacol Ther 15:380-386, 1974.

Litchfield, J.T., Jr., and Wilcoxon, F.A. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 95:99-113, 1949.

Loewe, S., and Goodman, L.S. Anticonvulsant action of marihuana-active substances. Fed Proc 6:352, 1947.

Post, R.M., and Kopanda, R.T. Cocaine, kindling and reverse tolerance. Lancet 1:409-410, 1975.

Segal, D.S., and Mandell, A.J. Long-term administration of d-amphetamine: Progressive augmentation of motor activity and stereotypy. Pharmacol Biochem Behav 2:249-255, 1974.

Shuster, L.; Webster, G.W.; and Yu, G. Increased running response to morphine in morphine-treated mice. J Pharmacol Exp Ther 192:64-72, 1975.

Smiley, K.A.; Turkanis, S.A.; Borys, H.K.; Olsen, D.M.; and Karler, R. An electrophysiological analysis of the anticonvulsant action of cannabidiol on limbic seizures in the conscious rat. Epilespsia 20:351-363, 1979.

Turkanis, S.A., and Karler, R. Electrophysiologic properties of the cannabinoids. J Clin Pharmacol 21:449S-463S, 1981.

Turkanis, S.A., and Karler, R. Central excitatory properties of delta-9-tetrahydrocannabinol and its metabolites in iron-induced epileptic rats. Neuropharmacology 21:7-13, 1982.

Turkanis, S.A.; Cely, W.; Olsen, D.M.; and Karler, R. Anticonvulsant properties of cannabidiol. Res Commun Chem Pathol Pharmacol 8:231-246, 1974.

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Pharmacological Evaluation of Agonistic and Antagonistic Activity of Cannabinoids

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INTRODUCTION

The currently proposed mechanisms by which the cannabinoids could be producing their central effects involve either general membrane perturbation or direct interaction with a specific cannabinoid receptor. The difficulty in defining the mechanism by which Δ^9-THC alters central function could be because the cannabinoid behavioral syndrome represents a composite of several independent effects, rather than a single event. Therefore, it is possible that both mechanisms play an important role in the total behavioral effect of the cannabinoids. The observed behavioral syndrome may be a composite of multiple independent effects, each of which could be a function of either a nonspecific cannabinoid action or, alternatively, of a specific cannabinoid effect which might be modified (at higher drug doses) by nonspecific interactions. It is our objective to determine whether either of these mechanisms is responsible for Δ^9-THC's effects. If cannabinoids are interacting with specific binding sites, then there should be a strict structure-activity relationship (SAR) as well as a specific antagonist. Alternately, if the cannabinoids are producing their effects solely by perturbation of membranes, there should not be a specific antagonist, but there may be a correlation between activity and membrane (lipid) solubility as measured by partition coefficients. The partial development of a SAR for several cannabinoid behavioral effects has been established by evaluating the agonistic and antagonistic activity of newly synthesized analogs in a variety of behavioral test systems. These tests include spontaneous activity, rectal temperature and analgesia (mouse), blood pressure, heart rate, and respiratory rate (rat), static ataxia (dog), and conditioned operant behavior (monkey) (Martin et al. 1975, 1984a, 1984b; Beardsley et al., in press). Additionally, attempts have been made to distinguish those cannabinoid structural features associated with only a portion of the behavioral syndrome normally observed with THC as an effort to determine if a unique SAR exists for each behavioral measure, which would support the notion that the cannabinoid syndrome is composed of multiple behavioral effects (Martin 1986). We propose that the pharmacological properties unique to the cannabinoid class of drugs are produced by the interaction of Δ^9-THC with specific receptors, while cannabinoid-induced central nervous system (CNS) depression is a general phenomenon produced by nonspecific membrane interactions.

METHODS

Cannabinoids

The analogs **1-24, 32,** and **33** were synthesized by Dr. Raj Razdan and **25, 29,** and **31** by Dr. Everette May. Compounds **26-28** were obtained from the National Institute on Drug Abuse and compound **30** was provided by the National Cancer Institute. The procedure of Olson et al. (1973) was used to prepare micellar suspensions of cannabinoids suitable for injection. The cannabinoids (10 mg) were dissolved in 100 µl of a 1:1 mixture of 95% ethanol and emulphor (a polyoxyethylated vegetable oil manufactured by GAF) by sonification. This solution was then diluted with ethanol:emulphor:saline (1:1:18) to provide the desired concentration, and injected intravenously (i.v.), unless stated otherwise.

Spontaneous Activity, Rectal Temperature, and Antinociceptive Activity in Mice

Analogs are evaluated initially in these three tests in order to assess their general pharmacological activity. To conserve limited supplies of drugs, these measurements are recorded sequentially in the same animal as described previously (Martin et al. 1984a, 1984b; Jorapur et al. 1985). Male ICR mice (22-30 g) are housed in the laboratory for 24 hr before treatment. The ambient temperature of the laboratory, which varies from 22 to 24 °C from day to day, is recorded at the beginning and end of each experiment. Rectal temperature is determined by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) just prior to vehicle or drug administration. Following the initial temperature determinations, control tail-flick reaction times are determined for each animal as described below. The mice are then injected i.v. with either vehicle or drug (0.1 ml/10 g of body weight) and immediately placed in individual photocell activity chambers. After the animals have acclimated for 5 min, interruptions of the photocell beams are recorded for a 10-min session. The mice are removed from the activity chambers, and tail-flick response is determined 5 min later. Rectal temperature is measured 60 min after drug administration. The spontaneous activity of the treated animals is expressed as percent of control, and the ED_{50}s(and confidence limits) are determined by the method of Litchfield and Wilcoxon (1949). The difference between rectal temperature before and 60 min after treatment is calculated for each mouse.

The antinociceptive activity procedure is essentially the same as that described by D'Amour and Smith (1941) and Dewey et al. (1970). A mouse's tail is placed on a notched block so as to occlude a slit over a photocell detector. Heat is applied by a 100-watt lamp mounted in a reflector. The apparatus is arranged so that when the operator turns on the lamp a timer is activated. When the mouse flicks its tail, the light falls on the photocell and automatically stops the timer. The intensity of the lamp is adjusted to give a normal reaction time between 2 and 4 sec. A control reaction time is taken for each animal prior to treatment. The mice then receive an i.v. injection of the drug and are tested at the appropriate time. A maximum 10-sec cut-off (latency) time is employed in order to prevent damage to the animal's tail. The antinociceptive response is expressed as a percent of the maximum possible increase in latency. Percent response is calculated from the equation: $\% R = (T - Tc/10 - Tc) \times 100$ where T is the mean test reaction time, and Tc is the control reaction time. The ED_{50} (and confidence limits) are determined by the method of Litchfield and Wilcoxon (1949).

Static Ataxia in Dogs

The ability of cannabinoids to produce static ataxia (an effect unique to psychoactive cannabiioids) and other characteristic behavioral effects is examined in mongrel dogs of either sex (10-18 kg). On the test day, the animals are moved from their home cages to the observation room, and behavior is evaluated for 5 to 10 min prior to drug administration. The normal behavior of each animal (which includes spontaneous activity, gait, tail tuck, hyperreflexia, etc.) is recorded. Each animal is then injected i.v. with either the cannabiioid or the vehicle (1 ml/kg of body weight), and their behavior is rated at 5-min intervals according to the static ataxia scale as described previously (Walton et al. 1938; Martin et al. 1975) and as depicted in table 1. Typical test sessions consist of four animals that receive either vehicle, Δ^9-THC (0.2 mg/kg which typically produces a rating of 3-4), or one of two other cannabinoid treatments. Only one of the experimenters is knowledgeable to the treatment. Behavior is scored by three observers who are "blind" with regard to treatment.

TABLE 1

Quantitation of the Behavioral Effects Produced by Cannabinoids in Dogs

Score	Behavioral Effects
0	No effect
1	Slight depression of activity, slight static ataxia seen only after the dog has been standing in one position for 3 to 5 min.
2	Walks with a prance-like placement of feet, exaggerated reflex to a swinging hand, and static ataxia after standing in one position for 2 to 3 min.
3	Tail is often tucked, some loss of tone in hind legs as evidenced by a semisquatting position, static ataxia more pronounced and seen after dog stands in one position for 1 to 2 min, and nodding may be observed 30 to 60 min after injection.
4	Marked static ataxia, sways forward and backward and/or side to side, and almost falls after standing in one position for 1 min.
5	Cannot stand for longer than 30 sec without almost falling and often plunges about.
6	Lies prostrate on the floor.

Conditioned Operant Responding in Monkeys

Rhesus monkeys are trained on a multiple timeout 3-min fixed-ratio x schedule to lever press for food pellets as described by Beardsley et al. (in press). During drug testing, the animals receive cumulative dosing of cannabinoids, as described in table 5, and the dose at which the behavioral sign appears is recorded. The vehicle did not produce any of the indicated behavioral signs nor did it suppress conditioned responding.

Protocol for Determining Antagonistic Activity

Analogs with little or no agonistic activity in these tests are evaluated for antagonistic activity. Antagonism in spontaneous activity and rectal temperature measures is studied in one group of mice, whereas antinociceptive activity is evaluated in a separate group of animals. A subthreshold dose of the analog (or vehicle) is administered i.v. 10 min prior to an i.v. administration of Δ^9-THC (ED_{50} dose for producing hypoactivity) or vehicle. Spontaneous activity and rectal temperature are measured (described above) in the following treatment groups: analog plus Δ^9-THC (A), analog (B), Δ^9-THC (C), and vehicle (D). Antagonism of hypothermia is calculated from the following equation:
$\%AD_{hypothermia} = \{1 - [(A - B) \div (C - D)]\}$ x 100 where A through D represent the differences between preinjection and postinjection (60 min) rectal temperatures. The antagonism of hypoactivity is calculated from the following equation:
$\%AD_{hypoactivity} = \{1 - [1 - (A \div B)] \div [1 - (C \div D)]\}$ x 100 where A through D represent the spontaneous activity (counts per 10-min period). To measure antagonism in the tail-flick procedure, the analog is administered i.v. 10 min prior to an i.v. dose of Δ^9-THC (ED_{80} dose for producing antinociception). The mice are tested 5 min after the second injection. Two injections of vehicle into the tail vein have no effect on the tail-flick response. The results are expressed as percent antagonism of the THC antinociceptive effect. The AD_{50} values (and confidence limits) are determined by the probit analysis method of Litchfield and Wilcoxon (1949).

Antagonistic activity of analogs is evaluated in dogs by pretreating i.v., with either vehicle or analog, 10 min prior to the i.v. administration of either vehicle or Δ^9-THC (0.2 mg/kg). In this way, each test session consists of the following treatments: vehicle, analog, Δ^9-THC, and analog +Δ^9-THC. Behavioral activity is evaluated for 60 min after the Δ^9-THC injection. The maximum effect of Δ^9-THC is usually observed between 15 and 30 min after administration. Analogs are evaluated at the highest possible dose in duplicate or triplicate. Analogs with antagonistic activity are evaluated at two other doses (in duplicate) so that AD_{50} values can be calculated. Dogs are retested at weekly intervals, but never more than four times total. Development of tolerance has not been observed when low doses of cannabinoids are given as infrequently as once a week.

RESULTS

Antinociceptive Studies

Cannabinoids have previously been considered to be weak antinociceptive agents (Buxbaum 1972; Dewey et al. 1972; Chesher et al. 1973; Sofia et al. 1973; Noyes et al. 1975; Wilson et al. 1976; Bloom et al. 1977; Gilbert 1981; Sofia and Barry

1983). However, i.v. administration of Δ^9-THC demonstrates that it has antinociceptive properties comparable to those of morphine sulfate (Martin 1985a). SAR studies demonstrate that the structural requirements for cannabinoid-induced antinociceptive activity, as measured in the tail-flick procedure, are quite similar to those necessary for inducing hypoactivity and hypothermia (Martin 1985b). These findings, coupled with the observation that i.v. administration of Δ^9-THC (ED_{50} = 1.9 mg/kg) results in consistent and potent antinociceptive activity, suggest that the tail-flick procedure is a reasonable test for assessing agonistic and antagonistic activity of cannabinoids.

Evaluation of Agonistic Activity of CBD Analogs

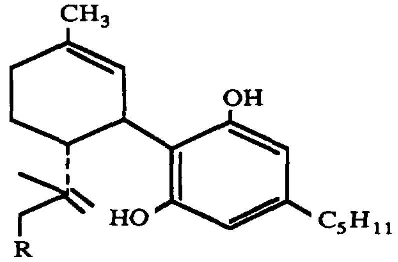
The behavioral profiles and structures of CBD analogs evaluated for pharmacological activity in mice are presented in table 2. Interest in these 10-N-substituted analogs is derived from early suggestions that CBD itself exhibited some antagonistic properties (see Martin 1986 for a recent review). The ethylamino (**1**) and propylamino (**2**) analogs are as potent as Δ^9-THC in decreasing spontaneous activity and rectal temperature, but exhibit only slight antinociceptive activity (Jorapur et al. 1985). Additionally, **1** lacks specific cannabinoid effects in the dog static-ataxia test, although it does produce CNS depresson at i.v. doses of 1 to 2 mg/kg. An interesting aspect of these two analogs' activity profile is their high toxicity (LD_{50} = 5 mg/kg), whereas the LD_{50} of CBD is >100 mg/kg. However, other lo-substituted CBD analogs (**3-10**) have been prepared, and they are relatively inactive in all mouse tests. The N-methyl- N-propargyl (**7**) and morpholino (**10**) CBD analogs are diacetyl derivatives which are weakly active (≈ Δ^8-THC) in producing hypoactivity, but inactive in the other two tests. Due to concerns regarding the penetrability of N-mustards into the CNS, analog 3 was also evaluated following intraventricular (i.v.t.) administration, and doses up to 150 µg/mouse (≈ 5 mg/kg) do not alter spontaneous activity or tail-flick latency in mice, but they do produce a 3.8 °C drop in rectal temperature. An identical i.v.t. dose of Δ^9-THC produces a 5.5 °C decrease in temperature, as well as a 68% decrease in spontaneous activity and a 55% increase latency in the tail-flick response. Only the ethylamino analog **1** has been evaluated in the dog static-ataxia test, and it failed to produce the classical cannabinoid syndrome. However, it did produce CNS depression at i.v. doses of 1 to 2 mg/kg, which was consistent with its effects in mice. Therefore, it appears that this analog may be prototypic of a cannabinoid with potent CNS depressant properties, but without antinociceptive or static-ataxia properties. The lack of agonistic properties in the other CBD analogs make them suitable for testing as antagonists.

Evaluation of Agonistic Activity of Δ^8-THC Analogs

The importance of the C-11 position has been well documented. The general consensus is that hydroxylation at this position increases biological activity, whereas removal of the C-11 position decreases potency (Martin et al. 1975). Numerous substitutions have been made at C-11 of Δ^8-THC (**11-21**) and the results are summarized in table 3. Alkylamino (**11-14**) or thioacetyl (**16**) substitutions have more or less resulted in loss of agonistic activity in the mouse tests with the exception of the N-methyl analog (**11**) which was as potent as Δ^8-THC in depressing spontaneous activity. The N-mustard (**13**, acetate of **14**) was administered i.v.t. as described for analog **3** and found to be inactive up to doses of 150 µg/mouse. The alkyl analogs (**17-21**) are primarily additions of a bulky substituent at C-11 which dramatically attenuate behavioral activity.

TABLE 2

Phamacological Activity of CBD and Structurally Related Analogs in Mice[a]



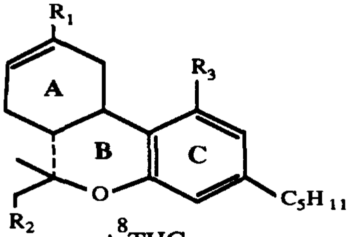
R		Pharmacological Profile S.A. [b]	R.T. [c]	T.F. [d]
CBD		0.08	0.03	<0.03
1	$-NHC_2H_5$	1.15	0.44	~0.30
2	$-NHC_3H_7 \cdot HCl$	1.10	1.00	~0.40
3	$-N(CH_2CH_2Cl)_2$	N.T.[e]	N.T.	N.T.
4	$-N_3$	<0.20	<0.05	<0.20
5	-SH	<0.02	0.05	<0.20
6	-Br [f]	< 0.08	0.15	<0.20
7	$-N(CH_3)CH_2C{=}CH$[f]	0.25	0.03	<0.06
8	$-N[CH(CH_3)_2]_2$[f]	<0.05	0.03	<0.06
9	$-NHCO(CH_2)_3NH_2$	0.08	0.11	<0.08
10	$-cN(CH_2CH_2)_2O$[f]	0.2	0.02	<0.06

---

[a] Pharmacological potency of i.v. analogs relative to that of i.v. Δ9-THC. [b] Spontaneous activity — the ED_{50} of Δ9-THC (2.3 mg/kg) was divided by that of the analog. Less than (<) indicates the analog did not produce a 50% effect at the highest dose tested. Therefore, the ED50 was divided by the highest dose tested. [c]Rectal temperature — the dose of Δ9-THC (3.0 mg/kg) that produced a 1.6 °C decrease in rectal temperature divided by the dose of the analog that produced a comparable effect. [d] Tail-flick response — the ED50 of Δ9-THC (1.9 mg/kg) was divided by that of the analog. Less than (<) indicates the analog did not produce a 50% effect at the highest dose tested. [e] Not tested. [f]Diacetyl derivatives previously reported (Jorapur et al. 1985).

TABLE 3

Pharmacological Activity of Δ^8-THC and Structurally Related Analogs in Mice[a]



Δ^8-THC

	R[b]	Pharmacological Profile S.A.	 R.T.	 T.F.
Δ^8-THC		0.23	0.98	0.28
	R_1			
11	$-CH_2NHCH_3$[c]	0.21	0.04	N.T.
12	$-CH_2NHCH_2CH_3$	<0.20	< 0.05	~ 0.20
13	$-CH_2N(CH_2CH_2Cl)_2$ acetate	<0.20	0.08	< 0.20
14	$-CH_2N(CH_2CH_2Cl)_2$	< 0.07	0.03	0.06
15	-SH	N.T.	N.T	N.T
16	$-SCOCH_3$	< 0.20	0.06	< 0.20
17	$-C_6H_5$[c]	0.03	0.08	N.T.
18	$-CH_2C_6H_5$[c]	0.07	0.04	"
19	$-CH(CH_3,)_2$[c]	0.09	0.02	"
20	$-CH_2CH_2CH_2CH_3$[C]	0.07	0.27	"
21	$-CH(OH)CH_2CH_3$[c]	0.06	0.03	"
	R_2			
22	$-NH_2$	0.82	0.94	< 0.30
23	$-NHCH_2CH_3$	0.92	0.82	< 0.60
24	$-SCOCH_3$	<0.20	0.06	< 0.20
	R_3 and/or Side Chain			
25	$-O_2C(CH_2)_3N(CH_2CH_2)_2O$	0.22	0.34	0.11
26	$-O^-$ Side chain = $-(CH_2)_5N^+(CH_3)_3$	1.1	1.13	N.T.
27	Side chain = $-(CH_2)_5Br$	1.1	2.44	"
28	Side chain = $-(CH_2)_5OAc$	0.79	~1.0	"

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[a] Potency of analogs relative to Δ^9-THC as described in legend of table 2.
[b] R_1 and R_2 = $-CH_3$, R_3 = -OH unless indicated. [c] Martin et al. 1984a.

Substitutions have also been made at position 12 of Δ^8-THC, which is comparable to position 10 of CBD. Both 12ß-amino-Δ^8-THC (**22**) and the corresponding ethylamino derivative (**23**) effectively reduce spontaneous activity and rectal temperature as potently as Δ^9-THC, but have little or no effect on tail-flick response. In contrast, introduction of a thioacetyl group (**24**) reduces activity compared to Δ^8-THC. Analogs **22** and **23** were similar to the CBD analogs **1** and **2** in that they too were quite toxic (lethal at 6-10 mg/kg). The pharmacological effects of 1-morpholinobutyryl-Δ^8-THC (MB-Δ^8-THC, 25) were compared to the values obtained for Δ^9-THC tested during the same session, and the data suggest MB-Δ^8-THC is somewhat less potent (0.3-0.1) than Δ^9-THC. The values obtained with Δ^9-THC were similar to those previously reported, and the potency range for the effects of MB-Δ^8-THC is similar to that obtained for Δ^8-THC relative to Δ^9-THC (Martin et al. 1984a). These data would seem to support the notion that MB-Δ^8-THC is metabolized rapidly by liver enzymes *in vivo* to Δ^8-THC, which is probably responsible for the observed behavioral effects (Zitko et al. 1972).

The possibility that Δ^9-THC exerts some of its effects by interacting with a specific receptor has recently gained partial support from the studies of Nye et al. (1985) who defined a stereoselective, saturable, high affinity site for tritiated 5'-trimethylammonium-Δ^8-THC phenolate (5'-TMA, 26) binding. Therefore, in order to determine the relevance of this binding site to the behavioral effects of the cannabinoids, 5'-TMA has been evaluated and compared to Δ^8-THC in our cannabinoid assays. Pharmacological comparison reveals that both Δ^8-THC and 5'-TMA induce hypothermia (2.6° and 3.0°, respectively) at 5 mg/kg, and decrease spontaneous activity (ED_{50} = 10 mg/kg and 2.1 mg/kg, respectively) in the mouse after i.v. administration. Δ^8-THC and 5'-TMA also induce hypothermia (4.4° and 5.0°, respectively at 100 μg), as well as hypoactivity (ED_{50} = 46 μg and 133 μg, respectively) after i.v.t. administration in the mouse. In the do both drugs, as well as Δ^9-THC, produce mild sedation. However, only Δ^8- and Δ^9-THC produce static ataxia (at 0.4 mg/kg and 0.2 mg/kg i.v., respectively), while 5'-TMA is inactive at doses up to 1.0 mg/kg (i.v.). Although 5'-TMA exhibits CNS depressant properties, it appears to lack the specific behavioral effects that are typically produced by psychoactive cannabiioids.

The 5'-bromo- (**27**) and 5'-acetyl- (**28**) Δ^8-THC analogs are similar in potency to to both 5'-TMA and Δ^9-THC in producing hypoactivity. However, **27** is more potent in decreasing rectal temperature (2.8 °C at 1 mg/kg). Analog **28** does produce hypothermia (maximum effect of 2.8 °C at 30 mg/kg), but not in a dose-related fashion (a 1.5 °C drop at doses of 1,3, and 10 mg/kg).

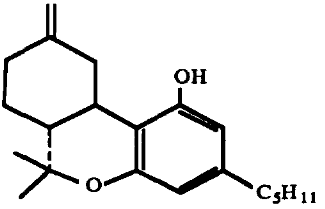
Evaluation of Agonistic Activity of Δ^9-THC Analogs

The behavioral data demonstrate that 1-O-succinyl-Δ^9-THC (**29**) possesses a pharmacological profile similar to that of Δ^9-THC. However, the analog is less potent than Δ^9-THC in depressing spontaneous activity and in producing hypothermia and antinociception. While **29** appears to retain cannabinoid activity, one cannot rule out the possibility that the pharmacological activity is due to *in vivo* hydrolysis to Δ^9-THC. These data support the contention that substitutions at the phenolic hydroxyl of Δ^9-THC reduce pharmacological activity.

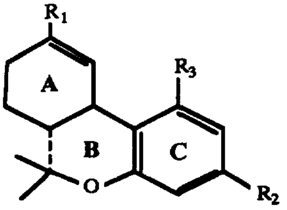
Alkylating agents of Δ^8- and Δ^9-THC have been prepared for the purpose of acting as receptor probes. The N-mustard Δ^9-THC-1-bis(2-chloroethyl)carbamate (**30**) was found to be inactive if administered intraperitoneally (i.p.) or orally (p.o.), which suggests that this ester is not rapidly hydrolyzed *in vivo* to Δ^9-THC. However, **30** is active after either i.v. or i.v.t. administration. Following i.v. administration (table 4), **30** is at most only 0.06 times as potent as Δ^9-THC. In contrast, i.v.t. administration of the **30** produces effects on rectal temperature equivalent to that of Δ^9-THC and is 0.2 times as potent as Δ^9-THC in producing hypoactivity (the tail-flick latency was not tested). These data suggest that the ability of **30**, and possibly other N-mustards, to enter the CNS may be limited.

TABLE 4

Pharmacological Activity of Δ^9-THC and Structurally Related Analogs in Mice[a]



$\Delta^{9\text{-}11}$-THC



Δ^9-THC

Rb	Pharmacological Profile S.A.	R.T.	T.F.
Δ^9-THC[c]	1.00	1.00	1.00
29 R_3 = succinyl	0.15	0.30	0.34
30 $R_3 = -O_2CN(CH_2CH_2Cl)_2$	0.06	0.01	<0.01
31 $R_2 = -(CH_2)_5Br$	1.21	2.80	19.0
32 $R_2 = -(CH_2)_2CHOH-C_2H_3$[d]			
S-3'-OH	6.20	1.00	N.T.
R-3'-OH	0.90	0.92	N.T.
R/S-3'-OH	2.50	0.89	N.T.
33 $\Delta^{9\text{-}11}$ -THC	0.28	0.07	0.09

[a] Potency of i.v. analogs relative to i.v. Δ^9-THC on the spontaneous activity (S.A.), rectal temperature (R.T.), and tail-flick (T.F.) response measures; calculated as described in the legend of table 2. [b] $R_1 = -CH_3$, $R_2 = -C_5H_{11}$, and $R_3 = -OH$ unless indicated otherwise. [c] The ED50 is 2.3 and 1.9 mg/kg for S.A and T.F., respectively, and a 1.6 °C drop occurs at 3 mg/kg. [d] Based on previously reported values (Martin et al. 1984a).

The 5'-bromo-Δ^9-THC analog (**31**) is similar to the 5'-bromo-Δ^8-THC analog (**27**) in potency and profile. Analog **31** (table 4) is equipotent to Δ^9-THC in reducing spontaneous activity and is roughly 3-fold more potent in producing hypothermia. Interestingly, **31** is 19-fold more potent than Δ^9-THC in the mouse antinociception test. Tests are currently under way to determine if **27**, the 5'-bromo-Δ^8-THC analog, also shares this characteristic.

Introduction of a hydroxyl group at the 3' position of the side chain (**32**) results in an R/S 3'-OH racemic mixture that retains cannabinoid-like activity (Martin et al. 1984a). Final resolution of each of the compounds results in a S-3'-OH mixture contaminated by 9% of the R isomer, and in an R-3'-OH mixture contaminated by 15% of the S isomer. Therefore, it is possible that the pharmacological effects observed by one of the "pure" isomers is partially (or totally) due to the opposite isomer. However, the pharmacological profiles of the racemate and each of the isomers suggest that the S isomer is 6-fold more potent at reducing spontaneous activity than Δ^9-THC. The R isomer also produces hypoactivity, but the result could be because 15% of the administered compound is actually the S isomer. In contrast, the ability of both the R and the S isomer, as well as the racemate, to produce hypothermia is nearly identical, and in fact is equipotent with Δ^9-THC. These data suggest that there is a specific side chain stereospecificity required for one effect (hypoactivity) that is not required for another (hypothermia). In fact, it is possible that the S, but not the R, isomer produces hypoactivity, while both isomers are equipotent in producing hypothermia.

Evaluation of Cannabinoids for Antagonistic Properties

Since 10-N-ethylamino-CBD (**1**) is a partial antagonist of Δ^9-THC-induced antinociception (Jorapur et al. 1985), this compound has been tested further for antagonistic activity. Although **1** lacks specific cannabinoid effects in the dog static-ataxia test, pretreatment with 0.5 mg/kg (i.v.) of **1** does not alter the behavioral effects of Δ^9-THC in dogs. Compound **1** also does not produce an antagonism of Δ^9-THC's effects on spontaneous activity and rectal temperature. Therefore, the attenuation of Δ^9-THC's effects by **1** appears to be limited to mouse antinociception. Additionally, **2** also produces a partial antagonism (38% at 3 mg/kg) of Δ^9-THC-induced antinociception, similar to **1**. However, compound **2** also does not block Δ^9-THC's effects on spontaneous activity and rectal temperature, and therefore is not being tested further. The evaluation of the 10-bromo-CBD diacetate analog (**6**) for antagonistic activity indicates no alteration of Δ^9-THC-induced antinociception at doses up to 30 mg/kg.

The 12ß-amino-Δ^8-THC analog (**22**) does not alter Δ^9-THC-induced hypoactivity and hypothermia as expected due to its agonistic activity. However, **22** does produce a slight attenuation in Δ^9-THC's antinociceptive activity. Doses of **22** of 0.3 to 3.0 mg/kg antagonized Δ^9-THC's effects by 25%.

Assessment of the antagonistic activity of the N-mustard Δ^9-THC-1-bis (2-chloroethyl) carbamate (**30**) following i.v. administration reveals that this compound is not an antagonist. High doses of **30** (100 mg/kg) attenuate the effects of Δ^9-THC administered 24 hr later, but this blockade appears to be due to desensitization, rather than antagonism, since Δ^9-THC itself produces a similar effect. However, it is possible that lower doses of **30**, or a different experimental protocol, might reveal antagonistic properties.

TABLE 5

Δ^{9}-- and Δ^{9-11}-THC's Effects on Conditioned Operant Responding in Monkeys

		Cumulative Dose (mg/kg)[a]			
Test	Monkey	Ptosis	Ataxia	Sedation	Operant Suppression
Veh[b]	639	—[c]	—	—	—
	M661	—	—	—	—
	M572	—	—	—	—
	824	—	—	—	—
Δ^{9}-THc[d]	639	0.250	0.25	0.50	0.050
	M661	0.125	0.50	1.00	0.250
	M572	0.125	0.50	1.00	0.125
	824	0.250	1.00	0.25	0.005
Δ^{9-11}-THC[e]	639	—	—	—	1.000
	M661	—	—	—	—
Δ^{9-11}-THC[f]	639	—	—	—	1.000
	M661	—	—	—	—
	M572	—	—	—	—
	824	—	—	—	0.500
INT[g]	639	1.00	—	1.50	0.500
	M661	1.00	1.00	1.50	—
	M575	1.00	—	1.00	0.250
	824	1.00	—	1.00	0.125
Δ^{9}-THC[d]	639	0.25	1.00	0.25	0.125
	M661	0.25	1.00	0.50	0.500
	M572	0.25	1.00	0.50	0.500
	824	0.25	0.50	0.25	0.005

[a] Cumulative dose equals the summation of all previous doses. [b] Vehicle consists of emulphor: ethanol: saline in a ratio of 1:1:18, respectively. [c] The effect was not observed. [d-f] Total cumulative dose of 1.0 mg/kg of Δ^{9}-THC[d], 1.0 mg/kg of Δ^{9-11}-THC[e] and 5.0 mg/kg of Δ^{9-11}-THC[f]. [g] A cumulative dose of 1.0 mg/kg Δ^{9-11}-THC preceded infusions of Δ^{9}-THC. All monkeys received a maximum cumulative dose of 10 mg/kg of Δ^{9}-THC within the normal 1-hr experimental session. Monkeys 639 and M661 received additional 0.5 mg/kg infusions of Δ^{9}-THC after the session to provide a total cumulative dose of Δ^{9}-THC of 1.5 and 2.0 mg/kg, respectively.

TABLE 6

Selective Pharmacological Profiles of Cannabinoids Analogs

Class 1 - All Cannabinoid Effects

Δ^{9}-THC
Δ^{8}-THC
11-OH-Δ^{9}-THC
S-3'-OH-Δ^{9}-THC

Class 2 - Weak CNS Depressant Devoid of other Cannabinoid Effects

10-Azido-CBD (**4**)
10-Sulfhydryl-CBD (**5**)
10-Bromo-CBD diacetate(**6**)
10-Morpholino-CBD diacetate (**10**)
10-(N-Methyl-N-propargylamino)CBD (**7**)
10-(N,N-Diisopropylamino)CBD (**8**)
10-[(4-Aminobutyryl)amino]CBD (**9**)
CBD
$\Delta^{9\text{-}11}$-THC (**33**)
11-N-Ethylamino- Δ^{8}-THC (**12**)
11-N-Bis(2-chloroethyl)- Δ^{8}-THC (**13 & 14**)
11-Thiol-Δ^{8}-THC (**15**)
11-Thioacetyl- Δ^{8}-THC (**16**)
9-*nor*-9-Phenyl- Δ^{8}-THC (**17**)
9-*nor*-9-Benzyl- Δ^{8}-THC (**18**)
9-*nor*-9-Isopropyld Δ^{8}-THC (**19**)
9-*nor*-9-nButyl- Δ^{8}-THC (**20**)

Class 3 -Potent CNS Depressant Devoid of other Cannabinoid Effects

10-N-Ethylamino-CBD (**1**)
10-N-Propylamino-CBD (**2**)
12ß-amino- Δ^{8}-THC (**22**)
12ß-ethylamino- Δ^{8}-THC (**23**)
$\Delta^{9\text{-}11}$-THC (**33**) (dogs only)
5'-trimethylammonium- Δ^{8}-THC phenolate (**26**)

Class 4- Selective CNS Depressant Devoid of other Cannabinoid Effects

10-N,N-Bis-chloroethyl-CBD (**3**) - hypothermia, i.v.t.
11-Methylamino- Δ^{8}-THC (**11**) - hypoactivity
9-*nor*-9-nButyl- Δ^{8}-THC (**20**) - hypothermia

Agonistic Effects of Δ^{9-11}-THC in the Mouse, Dog and Monkey

The exocyclic analog (**33**), Δ^{9-11}-THC, was investigated extensively as a result of the suggestions of Binder and his colleagues (Binder et al. 1979; Binder and Franke 1982; Semjonow and Binder 1985) that this analog should lack psychoactive properties and would be useful for delineating nonspecific and specific cannabinoid binding in *in vitro* assays. The pharmacolocal profile of Δ^{9-11}-THC in mice is similar to that of Δ^{9}-THC, even though Δ^{9-11}-THC is less potent in all tests (table 4). It appears to be approximately 4, 15, and 11 times less active than Δ^{9}-THC in depressing spontaneous activity, reducing body temperature, and producing antinociception in mice, respectively (Beardsley et al., in press).

Antagonistic Effects of Δ^{9-11}-THC

The effects of Δ^{9-11}-THC pretreatment on the actions of Δ^{9}-THC in mice, dogs, and monkeys have also been studied (Beardsley et al., in press). Δ^{9-11}-THC pretreatment did not attenuate any of the effects of Δ^{9}-THC in mice. Evaluation of Δ^{9-11}-THC for cannabinoid antagonistic properties in the dog is hampered by the fact that it has some depressant properties in this behavioral test. However, pretreatment with either 0.5 or 1.0 mg/kg of Δ^{9-11}-THC 15 min before Δ^{9}-THC does not result in an attenuation of the effects of the latter. One of the most intriuing observations in the monkey tests is the finding that pretreatment with Δ^{9-11}-THC attenuates Δ^{9}-THC-induced ptosis, sedation, ataxia. and operant behavior in monkeys (table 5). The importance of this observation is that Δ^{9-11}-THC produces more than a modest reduction in the effects of Δ^{9}-THC in most cases. Δ^{9}-THC-induced operant suppression is decreased by as much as 25 times. Although it may be premature to describe this interaction in monkeys as antagonism, two exciting leads emerge. This exocyclic compound suggests further possible alterations in the cannabinoid structure for developing an antagonist, and implies that the monkey may be better suited for characterizing cannabinoid antagonistic activity than other behavioral tests.

SUMMARY

The behavioral effects of cannabinoids are dependent upon numerous variables which include species differences, dose, experimental conditions, etc. The complexity of the cannabinoid behavioral syndrome certainly complicates attempts to establish biochemical correlates. It is for this reason that attempts are being made to develop analogs that exhibit selective behavioral effects. The pharmacological profile of the analogs summarized in table 6 suggests a tentative classification for the cannabinoids. While there are numerous analogs which produce the entire spectrum of cannabinoid effects, only those discussed within this article are grouped in class 1. CBD and many of its analogs appear to be devoid of cannabinoid effects, but they are capable of producing CNS depression at large doses (class 2). It is our working hypothesis that the compounds in class 2 may be altering membrane peturbation in a nonspecific fashion. This latter effect may or may not be related to the psychoactive cannabinoids. Class 3 compounds are potent CNS depressants that appear to lack other effects that are unique to cannabinoids. These analogs are particularly interesting in that they may be altering membrane function in a specific fashion in contrast to nonspecific effects exerted by analogs in class 2. Analogs in class 4 appear to exert selective CNS depression. While the mechanism by which these analogs are producing their effects may be proven to be different from those postulated, it does appear that a classification of cannabinoid effects is possible through structural alterations in the cannabinoid molecule.

REFERENCES

D'Amour, F.E., and Smith, D.L. A method for determining loss of pain sensation. J Pharmacol Exd Ther 72:74-79, 1941.

Beardsley, P.M.; Scimeca, J.A.; and Martin, B.R. Studies on the agonistic activity of Δ^{9-11}-tetrahydrocannabinol in mice, dogs and rhesus monkeys and its interactions with Δ^{9}-tetrahydrocannabinol. J Pharmaco Exp Ther, in press.

Binder, M., and Franke, I. Is there a THC receptor? Current perspectives and approaches to the elucidation of the molecular mechanism of action of the psychotropic constituents of *Cannabis sativa L.* In: Hucho, F., ed. Neuroreceptors. Berlin: Walter de Gruyter and Co., 1982. pp. 71-80.

Binder, M.; Edery, H.; and Porath, G. Delta-7-tetrahydrocannabinol, a nonpsychotropic cannabinoid: Structure-activity considerations in the cannabinoid series. In: Nahas, G.G., and Paton, W.D.M., eds. Marihuana: Biological Effects. London: Pergamon Press, 1979. pp. 71-80.

Bloom, A.S.; Dewey, W.L.; Harris, L.S.; and Brosium, K.K. 9-*nor*-9ß-OH-Hexahydrocannabinol, a cannabinoid with potent antinociceptive activity: Comparisons with morphine. J Pharmacol Ex Ther 200:263-270, 1977.

Buxbaum, D.M. Analgesic activity of Δ^{9}-tetrahydrocannabinol in the rat and mouse. Psychopharmacology 25:275-280, 1972.

Chesher, G.B.; Dahl, C.J.; Everingham, M.; Jackson, D.M.; Marchant-Williams, H.; and Starmer , G.A. The effect of cannabinoids on intestinal motility and their antinociceptive effect in mice. Br J Pharmacol 49:588-594, 1973.

Dewey, W.L.; Harris, L.S.; Howes, J.F.; Kennedy, J.S.; Granchelli, F.E.; Pars, H.G.; and Razdan, R.K. Pharmacology of some marijuana constituents and two heterocyclic analogues. Nature 226: 1265-1267, 1970.

Dewey, W.L.; Harris, L.S.; and Kennedy, J.S. Some pharmacological and toxicological effects of *1-trans*-Δ^{8}- and *1-trans*-Δ^{9}-tetrahydrocannabinol in laboratory rodents. Arch Int Pharmacodyn 196:133-145, 1972.

Gilbert, P.E. A comparison of THC, nantradol, nabilone, and morphine in the chronic spinal dog. J Clin Pharmacol 21:311S-319S, 1981.

Jorapur, V.S.; Khalil, Z.H.; Duffley, R.P.; Razdan, R.K.; Martin, B.R.; Harris, L.S.; and Dewey, W.L. Hashish: Synthesis and pharmacological activity of some novel analogs of cannabidiol and oxepin derivatives of Δ^{9}-tetrahydrocannabinol. J Med Chem 28:783-787, 1985.

Litchfield, L.T., and Wilcoxon, F.A. A simplified method of evaluating dose effect experiments. J Pharmacol Exp Ther 96:99-113,1949.

Martin, B.R. Characterization of the antinociceptive activity of intravenously administered delta-9-tetrahydrocannabinol in mice. In: Harvey, D.J., ed. Marihuana '84, Proceedings of the Oxford Symposium on Cannabis. Oxford: IRL Press, 1985a. pp. 685-691

Martin, B.R. Structural requiremdnts for cannabinoid-induced antinociceptive activity in mice. Life Sci 36:1523-1530, 1985b.

Martin, B.R. Cellular effects of cannabinoids. Pharmacol Rev 38:45-74, 1986.

Martin, B.R.; Dewey, W.L.; Harris, L.S.; Beckner, J.; Wilson, R.S.; and May, E.L. Marihuana-like activity of new synthetic tetrahydrocannabinols. Pharmacol Biochem Behav 3:849-853, 1975.

Martin, B.R.; Dewey, W.L.; and Harris, L.S. Pharmacological activity of Δ^9-THC metabolites and analogs of CBD, Δ^8-THC and Δ^9-THC. In: Agurell, S.; Dewey, W.L.; and Willette, R.E., eds. The Cannabinoids: Chemical, Pharmacologic and Therapeutic Aspects. New York: Academic Press, 1984a. pp. 523-544.

Martin, B.R.; Kallman, M.J.; Kaempf, G.F.; Harris, L.S.; Dewey, W.L.; and Razdan, R.K. Pharmacological potency of R- and S-3'-hydroxy- Δ^9-tetrahydro- cannabinol: Additional structural requirement for cannabinoid activity. Pharmacol Biochem Behav 21:61-65, 1984b.

Noyes, J.R.; Brunk, S.F.; Avery, D.H.; and Canter, A. The analgesic properties of Δ^9-tetrahydrocannabinol and codeine. Clin Pharmacol Ther 18:84-89, 1975.

Nye, J.S.; Seltzman, H.H.; Pitt, C.G.; and Snyder, S.H. High-affinity cannabinoid binding sites in brain membranes labeled with [^{3}H]5'-trimethyl ammonium Δ^8-tetrahydrocannabinol. J Pharmacol Exp Ther 234:784-791. 1985.

Olson, J.L.; Makhani, M.; Davis, K.H.; and Wall, M.E. Preparation of Δ^9-teuahydrocannabinol for intravenous injection. J Pharm Pharmacol 25:344, 1973.

Semjonow, A., and Binder, M. Generalization of the discriminative stimulus properties of Δ^9-THC to $\Delta^{9(11)}$-THC in rats. Psychopharmacology 85:178-183, 1985.

Sofia, R.D., and Barry, H. The effects of SKF 525-A on the analgesic and barbiturate-potentiating activity of Δ^9-tetrahydrocannabinol in mice and rats. Pharmacology 27:223-236, 1983.

Sofia, R.D.; Nalepa, S.D.; Harakal, J.J.; and Vassar, H.B. Antiedema and analgesic properties of Δ^9-tetrahydrocannabinol (THC). J Pharmacol Exp Ther 186:646-655, 1973.

Walton, R.P.; Martin, L.F.; and Keller, J.H. The relative activity of various purified products obtained from American grown hashish. J Pharmacol Exp Ther 62:239-251, 1938.

Wilson, R.S.; May, E.L.; Martin, B.R.; and Dewey, W.L. 9-*nor*-9-Hydroxyhexahydrocannabinols. Synthesis, some behavioral and analgesic properties, and comparison with the tetrahydrocannabinols. J Med Chem 19:1165-1167, 1976

Zitko, B.A.; Howes, J.F.; Razdan, R.K.; Dalzell, B.C.; Dalzell, H.C.; Sheehan, J.C.; Pars, H.G.; Dewey, W.L.; and Harris, L.S. Water-soluble derivatives of Δ^1-tetrahydrocannabinol. Science 177:442-444, 1972.

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Interactions of Cannabinoids With Membranes. The Role of Cannabinoid Stereochemistry and Absolute Configuration and the Orientation of Delta-9-THC in the Membrane Bilayer

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Cornelis Van Der Schyf, Ph.D.; and Harold Jarrell, Ph.D.

INTRODUCTION

Cannabinoid Membrane Effects

Many pharmacological actions of the cannabinoids, such as psychotropic effects, bronchodilatation, increased heart rate, reduced intraocular pressure, analgesia, alteration in body temperature, and anticonvulsant activity, can be related to their known effects on a variety of cellular membranes. Indeed, cannabinoids are known to affect a variety of membrane functions (Martin 1986; Mechoulam et al. 1985; Hillard et al. 1985, 1986). Among these we can list their effects on numerous membrane enzymes (e.g., adenylate cyclase; Na^+, K^+-, Mg^{2+}-, Ca^{2+}-ATPases; NADH dehydrogenase; LPC acyltransferase; phospholipase A_2; monoamine oxidase; cholesterol esterase; 5'-nucleotidase), brain synaptosomes, blood platelets, human fibroblasts, neuroblastoma cells, rabbit neutrophils (Naccache et al. 1982), excitable membranes (Martin 1986), and membrane receptors (e.g., beta-adrenergic, cholinergic, opioid, dopaminergic) (Bloom 1984; Bloom and Hillard 1985). The membrane functions affected by the cannabinoids can be related to a wide variety of membrane-bound proteins. A common feature with all of these effects is that the cannabinoids do not seem to interact directly with the protein catalytic sites and do not compete with those ligands known to interact with these sites.

Cannabiniod Activity Through Interactions with Phospholipids

It is generally accepted that the lipid microenvironment surrounding membrane-associated enzymes (Sandermann 1978), receptors, or other functional proteins (Loh and Law 1980) can influence protein function. On this basis, the view was advanced that cannabinoids may exert their effects by interacting with the lipid component of the membrane. The following evidence is offered in support of this view: (I) Cannabinoids are highly lipophilic molecules and partition favorably in membrane lipids. (2) Cannabinoids have been shown to affect the thermotropic properties of phospholipids. They thus decrease the phase transition temperature of pure (Bruggemann and Melchior 1983; Makriyannis et al. 1987) and brain membrane (Bach and Sela 1981) phospholipids. Also, they eliminate the discontinuity in the Arrhenious plot for microsomal O-demethylase (Bach et al. 1977), an effect attributed to a change in the phase transition properties of the lipids associated with the enzyme. (3) Cannabinoids affect the "microviscosity" of model and biological membranes as determined by electron spin resonance (ESR) (Lawrence and Gill 1975), fluorescence (Hillard et al. 1986), and NMR methods (Makriyannis et al. 1987; Kriwacki 1986). The lipid hypothesis is bolstered by the fact that some of these studies were carried out using cannabinoid concentrations considered to be "physiological" (Hillard et al. 1985).

The Role of Drug Geometry in Drug: Phospholipid Interactions

Traditionally, the potency of drugs that are believed to act on the lipid component of the membrane has been correlated with their lipid solubility and no consideration was made for variations in their conformational properties or their absolute configurations. This assumption was shown to be true with the low molecular weight general anesthetics (Kendig et al. 1973), but does not seem to hold with the larger membrane-active drugs. It was thus reported that enantiomeric pairs of barbiturates showed marked differences in potency (Wahlstrom et al. 1970). This has also been well documented in the case of anesthetic sterioids where small structural variations in the steroid molecule, having no effect on its lipid solubility, produce dramatic changes in anesthetic activity (Atkinson et al. 1965). When tested for their effectiveness in inhibiting the function of a membrane-bound protein (Makriyannis and Fesik 1980) and for their abilities to perturb model phospholipid membranes (Makriyannis and Fesik 1983; Makriyannis et al. 1986), the anesthetic steroids showed variations that paralleled those of their respective potencies. We explained the observed differences in steroid membrane interactions on the basis of observed conformational differences between the drug molecules (Fesik and Makriyannis 1985).

Interestingly, an analogous effect was demonstrated by Bloch and coworkers, who found that small variations in sterol structure influence in a dramatic manner their effects on model membranes, including solute permeation, microviscosity, and steroid mobility in the membrane (Lala et al. 1978; Dahl et al. 1980), as well as the growth rates of microorganisms (Lala et al. 1978).

Analogous evidence is also available for cannabinoids, pointing to the role of structure and geometry in their interactions with phospholipids. Earlier work using ESR (Lawrence and Gill 1975) showed that cannabinoid analogs with only slight structural differences, or having the same structure but differing in absolute configuration, exhibited relatively large differences in their effects on model membrane preparations. As reported in a recent publication, fluorescence polarization was used to study the effects of cannabinoids on synaptosomal plasma membranes (Hillard et al. 1985). The study found significant changes in membrane "fluidity" when "physiological" concentrations of cannabinoids were used. Another study compared the thermotropic properties of model membrane preparations containing each of the two delta-9-THC enantiomers and found differences between them (Burstein et al. 1986). We would like here to report on more detailed work from our laboratories using solid-state ^{2}H-NMR spectroscopy.

RESULTS AND DISCUSSION

Model Membranes and the Use of Solid-State NMR Techniques

Because of their demonstrable applicability to the natural systems, studies with model membranes have contributed a great deal toward understanding the properties of biological membranes. Model membranes have also been used successfully to obtain information on drug:membrane interactions. Many of these studies have made use of fluorescence, E.S.R., and high resolution NMR techniques. However, the availability of solid-state NMR has opened new horizons in this field of research. During the past few years, these methods have led to previously unavailable molecular information on the conformational and dynamical properties of model and biological membranes (semisolids) (Griffin 1981; Davis 1983). The use of solid-state NMR has also been extended to study drug:membrane interactions and showed it to be the method of choice (Boulanger et al. 1981; Makriyannis et al. 1986). The model membranes used for the solid-state NMR experiments are multilamellar bilayer phospholipid dispersions that have proven to be excellent models, sharing to a surprisingly large degree many of the properties of the lipid component in biological membranes (Davis 1983).

The Role of Cannabinoid Conformation and Absolute Configuration During its Interaction with Membranes as Studied by Solid-State ^{2}H-NMR

In systems undergoing anisotropic motions, as is the case with phospholipid multilamellar bilayers, the deuterium nucleus gives rise to a "powder spectrum" in which the doublet with the maximum intensity is the ^{2}H quadrupolar splitting ($\Delta\nu_Q$]) and equal to:

$$\Delta\nu_Q = (3/4)\ (e^2qQ/h)\ S_{CD} \qquad \text{(Davis 1983)}$$

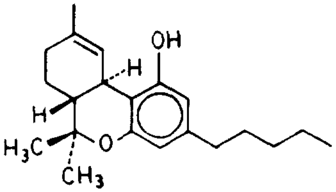
where e^2qQ/h) is the static quadrupole coupling constant which for, paraffinic C-D bonds, is approximately 170 KHz. S_{CD} is the order parameter which can be used to describe the amount of motional averaging of the C-D bond vector with respect to a fixed symmetry axis and has been used as a measure of membrane "fluidity."

In its liquid crystalline phase, the ^{2}H spectrum of DPPC labeled in a single site has the powder pattern features observed in the ^{2}H spectra of solids. However, the quadrupolar splitting is reduced due to motional averaging, including axial diffusion of the chains and gauche:trans isomerization in the methylene chain segments.

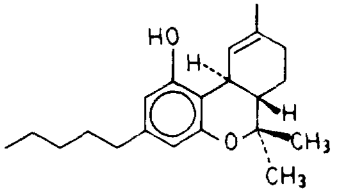
To compare the effects of cannabinoid analogs on membranes, we used DPPC model membranes containing 0.15M cholesterol into which 0.15M cannabinoid was incorporated. From earlier studies with anesthetic steroids (Makriyannis, unpublished), we have found that model membranes of such composition are very suitable for discriminating between membrane-acting drug analogs and that the relative effects of these molecules on the model membrane parallel their effects on biological membranes.

We compared the effects of (-)-delta-9-THC to those of its inactive (-)-delta-9,11-THC isomer on the model membrane by obtaining solid-state ^{2}H-NMR spectra as a function of temperature for each of the two preparations as well as spectra for the membrane preparation without cannabinoid. We also compared in a similar manner the effects of the two delta-9-THC enantiomers.

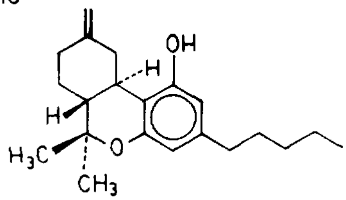
Figure 1 shows representative spectra from the model membrane preparations with (-)-delta-9-THC, (-)-delta-9,11-THC, and no drug, obtained at two different temperatures. The spectra are of the liquid crystalline type and allow us to extract directly values for the residual ^{2}H quadrupolar splittings ($\Delta\nu_Q$). As



(-)-Δ^9-THC



(+)-Δ^9-THC



(-)-$\Delta^{9,11}$-THC

expected, the splittings decrease as the temperature is increased, reflecting the higher ratio of gauche:trans chain conformers in the chain at higher temperatures.

In figure 2, we compare the $\Delta\nu_Q$ values for the above three preparations as a function of temperature, while figure 3 compares the effects of the two delta-9-THC enantiomers on the same membrane preparation. As can be seen, the presence of each of the three cannabinoids in the membrane results in a decrease in the respective ^{2}H quadrupolar splittings. Also, membrane preparations containing the pharmacologically active (-)-delta-9-THC analog give ^{2}H spectra with consistently smaller splittings than those from its inactive or less active isomers, (-)-delta-9,11-THC or (+)-delta-9-THC. This is evidence that, although all three cannabinoids increase the ratio of gauche:trans conformers in the membrane chain, the more active analog produces the larger increase in gauche:trans methylene ratio and the smaller order parameter (S_{CD}). This, in turn, may be interpreted to mean that the active (-)-delta-9-THC analog "perturbs" the model membrane more effectively than its two less active isomers.

The above data show that the interaction of cannabinoids with the phospholipid component of the membrane is governed by specific stereoelectronic requirements. Our results also provide support for the view that at least some of the cannabinoid effects result from their interactions with the lipid component of the membrane. The above observations on the stereoselectivity of cannabinoid: phospholipid interactions are understandable if the defined geometric parameters of the phospholipid bilayer are considered. These include the natural asymmetry of the lecithin and cholesterol molecules as well as the conformational preferences and dynamic properties of the lipid hydrocarbon chains and/or polar head groups.

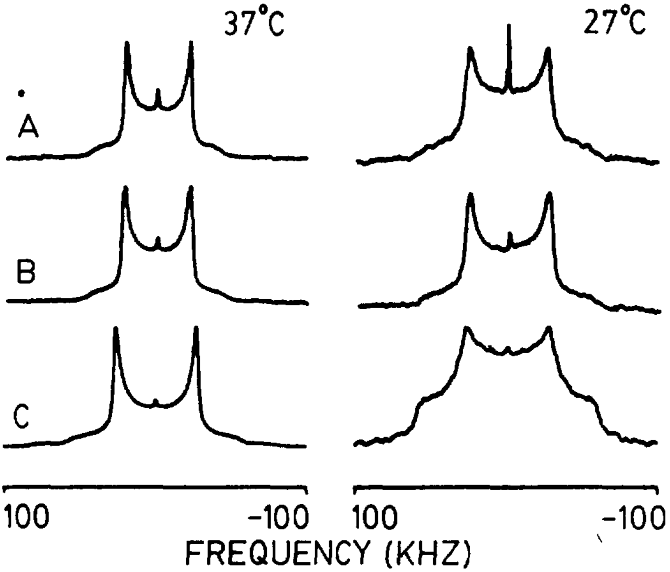


FIGURE 1. Solid-state ^{2}H-NMR spectra due to the 2(7',7'-C^{2}H) segment of DPPC obtained using a 90°$_x$ - τ -90° quadrupolar echo sequence at 45.3 MHz with a 90° pulse width of 2.1-2.4 μs. To obtain each spectrum, 8,000-16,000 ethos were signal averaged. (A) DPPC + 0.15m cholesterol + 0.15M (-)-delta-9-THC; (B) DPPC + 0.15M cholesterol + 0.15M (-)- delta-9,11-THC; (C) DPPC + 0.15M cholesterol.

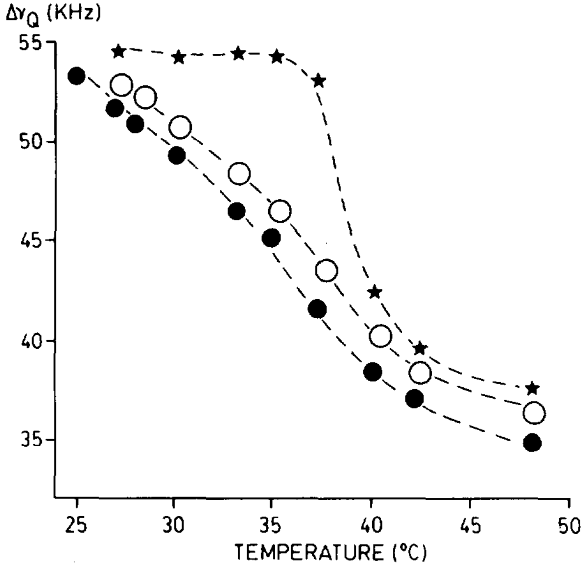


FIGURE 2. Temperature dependence of the ^{2}H quadrupolar splittings in the ^{2}H-NMR spectra of the 2(7',7'-C^{2}H$_2$) segment DPPC. (●)DPPC + 0.15M cholesterol + 0.15M (-)-delta-9-THC: (○)DPPC + 0.15M cholesterol + 0.15M (-)-delta-9'11-THC; (★) DPPC + 0.15M cholesterol.

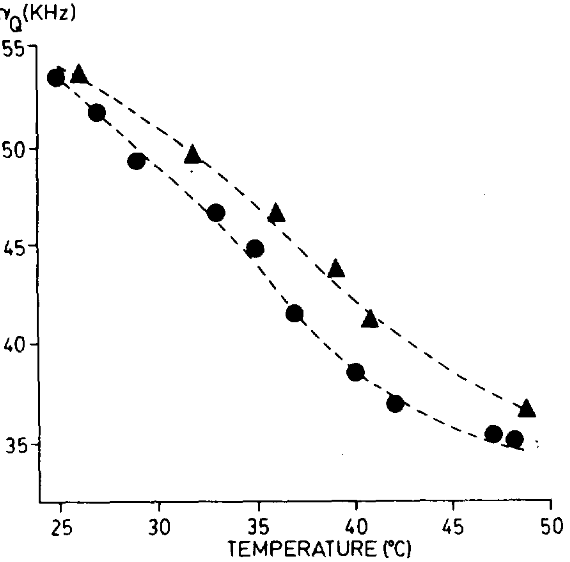


FIGURE 3. Temperature dependence of the ^{2}H quadrupolar splittings in the ^{2}H-NMR spectra of the 2(7', 7'-C^2H_2) segment of DPPC. (●) DPPC + 0.15M cholesterol + 0.15M (-)-delta-9-THC; (▲) DPPC + 0.15M cholesterol + 0.15M (+)-delta-9-THC.

Since the drug concentrations used in our experiments are much higher than "pharmacological," the results presented here cannot be viewed as a quantitative description of the membrane perturbation produced by cannabinoids in vivo. However, the data give us a qualitative description of the type of conformational and dynamical changes produced by cannabinoids on the membrane bilayer. Moreoever, such experiments provide us with a method for comparing the effects of structurally related cannabinoid analogs on membranes.

The Orientation of (-)-delta-9-THC in DPPC Bilayers

In our effort to understand the geometric parameters involved in the interaction of cannabinoids with membrane bilayers. we have made use of solid-state ^{2}H-NMR to study the orientation of (-)-delta-9-THC in the membrane bilayer. This experiment required the strategic introduction of ^{2}H labels in different positions of the tricyclic cannabinoid ring system. To that effect, we synthesized (-)delta-9-THC with specific ^{2}H labels in each of the 2, 4, 8 alpha, 8 beta, 11-CH_3, 10, and 10a positions (Banijamali et al. 1987). The labeled drug molecule was then introduced in DPPC bilayers. ^{2}H spectra were generally obtained for membrane preparations containing (-)-delta-9-THC with one ^{2}H label or with up to three ^{2}H labels if these could be unambiguosly assigned in the solid-state spectra. Spectra from the ^{2}H-labeled THC in the membrane in the liquid crystalline phase were axially symmetrical or nearly so and had different quadrupolar splittings ($\Delta\nu_Q$) depending on the position of the ^{2}H label in the molecule (figure 4). The value of these splittings was then used to calculate the orientation of (-)-delta-9-THC in DPPC bilayers.

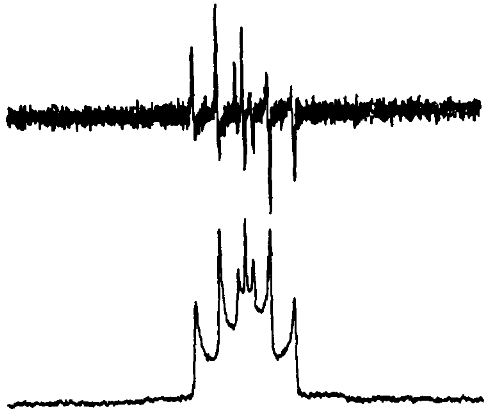


FIGURE 4. Below: Representative solid-state ^{2}H-NMR spectrum of 2,4-d_2-(-)-delta-9-THC (.3M) in hydrated DPPC bilayers at 42°C; $\Delta\nu_Q$ ($^2H_4$) = 43.0 KHz, $\Delta\nu_Q$ ($^2H_2$) = 20.8 KHZ. The central doublet ($\Delta\nu_Q$ =6.6 KHz) is due to the phenolic deuteron. Above: Firs& derivative of the same spectrum from which ^{2}H splittings could be obtained more accurately.

Briefly, the principle of this determination is as follows (Taylor 1981): Deuterons attached to a rigid structure will give quadrupolar splittings described by the equation:

$$\Delta\nu_Q = 3/4\ \frac{e^2qQ}{h}\ \left(\frac{3\cos^2 a - 1}{2}\right)\ \left[\frac{3\cos^2\gamma - 1}{2}\right]$$

where is the angle between the individual C-^{2}H bond and the rotation axis. The last term, which is known as the molecular order parameter (S_{mol}), defines the anisotropic motion of the entire molecule. The angles between the rotation axis and the C-^{2}H bonds cannot be calculated directly because S_{mol} is not known. However, S_{mol} is expected to be identical for all ^{2}H atoms attached to the cannabinoid ring system in which overall tumbling is expected to dominate the dynamics of this part of the molecule. Thus, the ratios (R) between two quadrupolar splittings for two different deuterium atoms (^2H_i and ^2H_j) are equal to:

$$R_a\ \frac{\Delta\nu_i}{\Delta\nu_j} = \frac{3\cos^2\alpha_{i-1}}{3\cos^2\alpha_{j-1}}$$

Our calculations showed that (-)-delta-9-THC orients with its axis of rotation almost in the plane of the aromatic ring. However, the molecule does not orient with its long axis parallel to the bilayer chains, as is the case with cholesterol, but almost orthogonal to the chains. Details of the above calculations will be given elsewhere.

The above-described orientation allows the cannabinoid phenolic hydroxy group to direct itself toward the polar side of the bilayer, indicating that this group serves as the orienting anchor in the molecule. We refer to this cannabinoid: bilayer interaction as "amphipathic interaction" in which its primary incentive is to direct the polar and hydrophobic components of the cannabinoid molecule toward the respective components in the amphipathic bilayer. This anchoring effect of the THC phenolic OH on the membrane is intriguing and deserves more extensive investigation.

Indeed, the requirement of a free OH for activity in THC is well established. Also, differences in activities have been reported between cannabinoid isomers, differing only in the presence and/or orientation of hydroxy and keto groups in the C ring. Since it is reasonable to assume that these hydrogen bonding groups may also be involved in the orientation of the cannabinoid molecule, one may speculate that cannabinoid orientation in the membrane may have an important effect on its biological activity. The drug orientation in the amphipathic membrane may, thus, play an important (although not unique) role in determining the nature of the cannabinoid:membrane interaction and its ability to produce "productive membrane perturbations." We are currently investigating the above hypothesis.

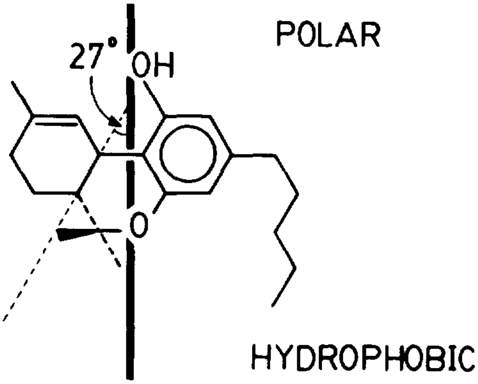


FIGURE 5. The orientation of (-)-delta-9-THC in DPPC bilayers as determined from solid-state ^{2}H-NMR data.

We combined our results on the orientation of delta-9-THC in the bilayer with previously obtained data on the conformation of delta-9-THC (Kriwacki 1986) to model its interaction with a DPPC bilayer using computer graphics. The parameters for modeling the phospholipid bilayer were obtained from available crystallographic data. As can be seen, the THC molecule inserts between the two fatty acid chains of the bilayer while maintaining its phenolic hydroxy group at the level of the bilayer interphase. Also, it can be noted that the plane of the C ring in delta-9-THC deviates from those of the A and B rings. This conformational property of delta-9-THC may explain its ability to perturb membranes.

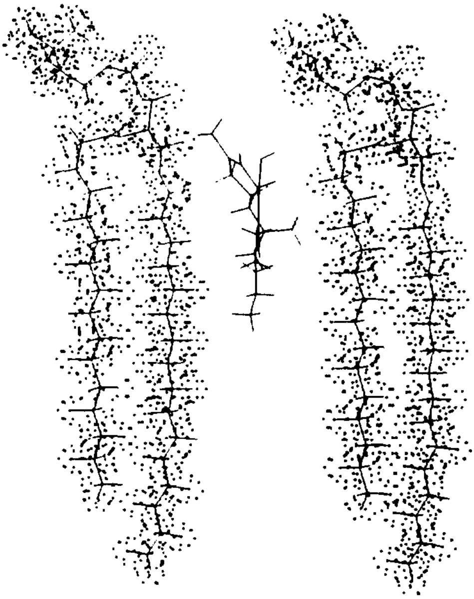


FIGURE 6. Modeling of (-)-delta-9-THC in a DPPC bilayer from experimental data using a E&S PS 300 system and ChemX graphics software (developed and distributed by Chemical Design Ltd., Oxford, England).

REFERENCES

Atkinson, R.M.; Davis, B.; Pratt, M.A.; Sharpe, H.M.; and Tomich, E.G. Action of some steroids on the central nervous system of the mouse. II Pharmacology. J Med Chem 8:426-432, 1965.

Bach, D.; Bursuker, L.; and Goldman, R. Differential scanning calorimetry and enzymic activity of rat liver microsomes in the presence and absence of delta-1-tetrahydrocannabinol. Biochim Biophys Acta 469:171-179, 1977.

Bach, D., and Sela, B. Thermotropic properties of calf brain lipids interacting with drugs. Biochem Pharmacol 30:1777-1780, 1981.

Banijamali, A.; Abou Taleb, N.; Charalambous, A.; Van der Schyf, C.J.; and Makriyannis, A. Synthesis of deuterium labeled cannabinoids. Submitted for publication, J Label Comp, 1987.

Bloom, A.S. Effects of cannabinoids on neurotransmitter receptors in the brain. In: Agurell, S.; Dewey, W.L.; and Willette, R.E., eds. The Cannabinoids; Chemical, Pharmacologic, and Therapeutic Aspects. New York: Academic Press, 1984. pp. 575-590.

Bloom, A.S., and Hillard, C.J. Cannabinoids, neurotransmitter receptors and brain membranes. In: Harvey, D.J., ed. Marihuana '84. Proceedings of the Oxford Symposium on Cannabis. Oxford: IRL Press, 1985. pp. 217-231.

Boulanger, Y.; Schreier, S.; and Smith, I.C.P. Molecular details of anesthetic-lipid interactions as seen by deuterium and phosphorus-31 nuclear magnetic resonance. Biochemistry 20:6824-6830, 1981.

Bruggemann, E.P., and Melchior, D.L. Alterations in the organization of phosphatidylcholine/cholesterol by tetrahydrocannabinol. J Biol Chem 258:8293-8303, 1983.

Burstein, S.; Hunter, S.A.; Latham, V.; Mechoulam, R.; Melchoir, D.; and Renzuli, L. Prostaglandins and cannabis. XV. Comparison of enantiomeric cannabinoids in stimulating prostaglandin synthesis on fibroblasts. Life Sci 39:1813-1823, 1986.

Dahl, C.E.; Dahl, J.S.; and Bloch, K. Effect of alkyl-substituted precursors of cholesterol on artificial and natural membranes and on the viability of Mycoplasma capricolum Biomchemistry 19:1462-1467, 1980.

Davis, J.H. The description of membrane lipid conformation, order and dynamics using ^{2}H NMR. Biochim Biophys Acta 737:117-171, 1983.

Fesik, S.W., and Makriyannis, A. Geometric requirements for membrane perturbation and anesthetic activity. Conformational analysis of alphaxalone and delta-16-alphaxalone and ^{2}H NMR studies on their interactions with model membranes. Mol Pharmacol 27:624-629, 1985.

Griffin, R.G. Solid state nuclear magnetic resonance in lipid bilayers. Methods Enzymol 72:108-174, 1981.

Hillard, C.J.; Harris, R.A.; and Bloom, A.S. Effects of cannabinoids on physical properties of brain membranes and phospholipid vesicles: Fluorescence studies. J Pharmacol Exp Ther 232:579-588, 1985.

Hillard, C.J.; Bloom, A.S.; and Houslay, M.D. Effects of delta-9-tetrahydrocannabinol on glucagon receptor coupling to adenylate cyclase in rat liver plasma membranes. Biochem Pharmacol 35:2797-2803, 1986.

Kendig, J.J.; Trudell, J.R.; and Cohen, E.N. Halothane stereoisomers: Lack of stereospecificity in two model systems. Anesthesiology 39:518-524, 1973.

Kriwacki, R. Studies on the conformations of two cannabinoids and their interactions with model membranes using high resolution NMR techniques. Dissertation Abstr 1986.

Lala, A.K.; Lin, H.; and Bloch, K. The effect of some alkyl derivatives of cholesterol on the permeability properties and microviscosities of model membranes. Biorg Chem 7:437-445, 1978.

Lawrence, D.K., and Gill, E.W. The effects of delta-1-tetrahydrocannabinol and other cannabinoids on spin-labeled liposomes and their relationship to mechanisms of general anesthesia. Mol Pharmacol 11:595-602, 1975.

Loh, H.H., and Law, P.Y. The role of membrane lipids in receptor mechanisms. Annu Rev Pharmacol Toxicol 20:201-234, 1980.

Makriyannis, A., and Fesik, S.W. Effects of anesthetics on sulfate transport in the red cell. J Neurosci Res 5:25-33, 1980.

Makriyannis, A., and Fesik, S.W. Mechanism of steroid anesthetic action. Interactions of alphaxalone and delta- 16-alphaxalone with bilayer vesicles. J Med Chem 26:463-465, 1983.

Makriyannis, A.; Siminovitch, D.; Das Gupta, SK.; and Griffin, R.G. Studies on the interaction of anesthetic steroids with phosphatidylcholine using ^{2}H and ^{13}C solid state NMR. Biochim Biophys Acta 859:49-55, 1986.

Makriyannis, A.; Siminovitch, D.; Das Gupta, S.K.; and Griffin, R.G. The perturbation of model membranes by (-)-delta-9-tetrahydrocannabinol. Studies using ^{2}H and ^{13}C-solid state NMR. In preparation, 1987.

Martin, B.R. Cellular effects of cannabinoids. Pharmacol Rev 38:45-75, 1986.

Mechoulam, R.; Srebnik, M.; and Burstein, S. Cannabis chemistry, biochemistry and therapeutic applications - An overview. In: Harvey, D.J., ed. Marihuana '84. Proceedings of the Oxford Svmposium on Cannabis. Oxford: IRL Press, 1985. pp. 1-12.

Naccache, P.H.; Volpi, M.; Becker, E.L.; Makriyannis, A.; and Sha'afi, R.I. Cannabinoid induced degranulation of rabbit neutrophils. Biochem Biophys Res Commun 106:1286-1290, 1982.

Sandermann, H., Jr. Regulation of membrane enzymes in lipids. Biochim Biophys Acta 515:209-237, 1978.

Taylor, M.G.; Alkiyama, T.; and Smith, I.C.P. The molecular dynamics of cholesterol in bilayer membranes: A deuterium NMR study. Chem Phys Lipids 29:327-338, 1981.

Wahlstrom, G.; Buch, H.; and Buzello, W. Unequal anaesthetic activity despite equal brain concentration of hexobarbital antipodes. Acta Pharmacol Toxicol 28:493-498, 1970.

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The High Affinity Cannabinoid Binding Site in Brain: Regulation by Guanine Nucleotides and Isolation of an Endogenous Inhibitor

Jeffrey S. Nye and Solomon H. Snyder, M.D.

INTRODUCTION

The molecular mechanism by which delta-9-tetrahydrocannabinol (delta-9-THC) produces its characteristic psychotropic effect in man and animals remains a mystery. The very high potency of cannabinoids and their stereochemical and structural requirements for eliciting characteristic psychotropic effects suggest that a neuronal receptor exists for delta-9-THC. Neuronal receptors have been identified for a wide range of psychotropic agents; these sites have been characterized by studying the binding of radiolabeled analogues of high potency drugs.

The biochemical labeling of a drug receptor helps illuminate the drug's mechanism of action. The identification of receptor subtypes in different organs or regions of the brain has helped clarify the pharmacological effects of certain drugs. Sometimes the site of action of a drug may in fact be the receptor for a well known neurotransmitter, as was the case for the antipsychotic drugs which act at dopamine and serotonin receptors. Alternatively, a drug receptor may physiologically interact with a previously unknown neurotransmitter, as in the case of opiate receptors and the enkephalins.

DEVELOPMENT AND CHARACTERIZATION OF THE RADIOLIGAND [^{3}H]5'-TRIMETHYLAMMONIUM-DELTA-8-THC ([^{3}H]TMA)

To identify a receptor for cannabinoids, we developed a cationic analogue of THC which was water-soluble and biologically active. Because of the extensive membrane partioning of cannabinoids, studies with ^{3}H-delta-9-THC have not revealed saturable binding sites. TMA (figure 1) (Seltzman et al. 1985) possesses a quaternary ammonium moiety at the 5' position. Pharmacological studies reveal that modification of the 5' position does not result in loss of psychotropic activity (Ohlsson et al. 1979). A tritiated version of TMA was prepared from 5'dimethylammonium delta-8-THC and [^{3}H]CH_3I.

Cannabimimetic Activity of TMA

To evaluate the biological activity of TMA, we studied its effects on the field-stimulated contractions of the guinea pig ileum (Nye et al. 1985, table 1). The ileum serves as an excellent model of cannabinoid action since its contractions

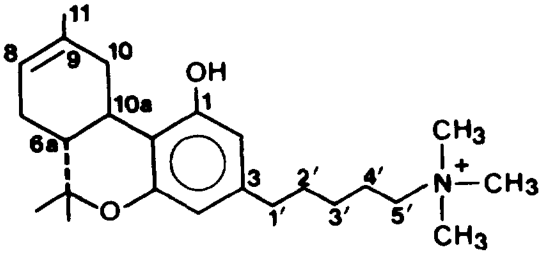

FIGURE 1. 5'-Trimethylammonium delta-8-tetrahydrocannabinol (TMA)

are inhibited by cannabinoids in a stereospecific manner which accurately parallels their psychotropic effects (Rosell et al. 1979). Active cannabinoids suppress the contractions of the ileum when stimulated by low voltage fields or serotonin application, but not those elicited by acetylcholine. Contractions are suppressed by an inhibition of release of acetylcholine from the presynaptic nerves.

TMA and 5'-dimethylammonium delta-8-THC inhibit contractions of the ileum with IC_{50}s of 1000 and 900 nM, respectively. TMA also inhibits serotonin-induced contractions, but not those elicited by acetylcholine. These studies suggest that TMA is an active cannabinoid. Because of its permanent positive charge, TMA may not cross the blood-brain barrier and, thus, be inactive when administered i.v.

Binding of [^{3}H]TMA to Neuronal Membranes

Binding of [^{3}H]TMA to homogenates of membranes from whole rat brain is saturable (Nye et al. 1985). Typically, we use 75 pM [^{3}H]TMA with 5 to 10 ug protein of brain membranes. Inhibition of binding by unlabeled TMA reveals a half-maximal inhibition at 100 nM TMA (figure 2). The dissociation constant (K_D) is 89 nM, calculated from Scatchard analysis of the data. The maximal number of binding sites is calculated as 1.1 nmol mg protein. The Hill coefficient is 1.1, indicating a single class of binding sites and the absence of significant cooperative interactions.

Detailed kinetic analysis of the binding of [^{3}H]TMA to brain membranes indicates very slow association and dissociation rates (figure 3). The association rate constant (k_1) is 4.5 x 10^9 $M^{-1}h^{-1}$. Dissociation of radioligand was initiated with 10 uM unlabeled TMA yielding a dissociation rate constant (k_{-1}) of 0.24 h^{-1}. The dissociation constant (K_D) calculated as the quotient of the rate constants (k_{-1}/k_1) is 5.7 x 10^{-11} M, which is more than 1000-fold lower than the K_D determined above, calculated from inhibition of equilibrium binding. This discrepancy may reflect a nonspecific interaction of [^{3}H] TMA with membranes, resulting in a low free concentraction of TMA.

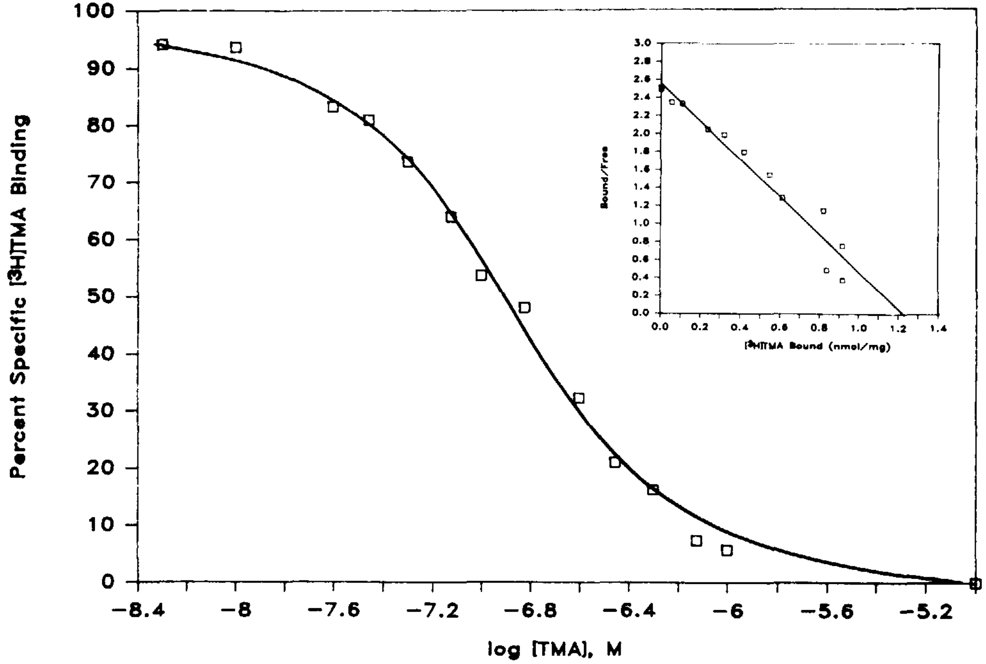

FIGURE 2. $[^3H]$TMA binding to rat cortical membrane homogenates. Triplicate tubes containing $[^3H]$TMA (75 pM) were incubated with crude homogenates (20 ug protein) at 37°C for 3 hr with varying concentrations of unlabelled TMA. Nonspecific binding in the presence of 10 uM TMA is subtracted and the result is compared to binding in the absence of unlabelled TMA. Data presented are from a representative experiment which was repeated four times with results varying less than 15%. Inset: Scatchard analysis of $[^3H]$TMA binding. B_{max}, maximum binding. From Nye et al. 1985. Copyright 1985 by American Society for Pharmacology and Experimental Therapeutics (Baltimore).

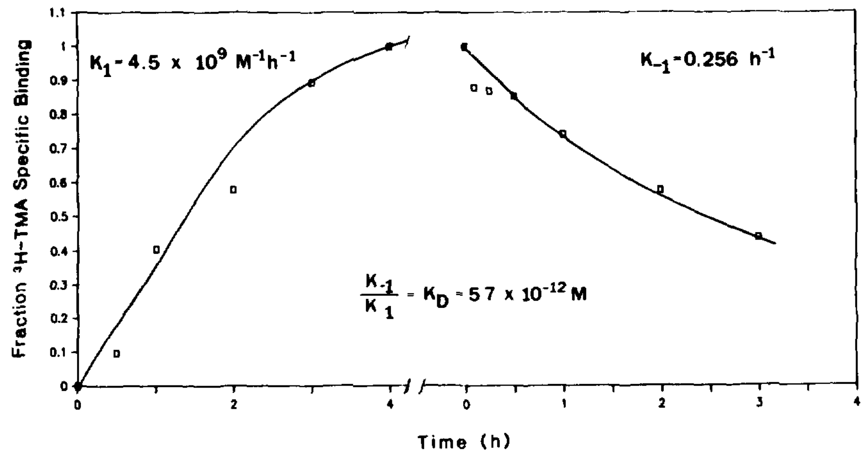

FIGURE 3. Kinetic analysis of [^{3}H]TMA binding. Left: [^{3}H]TMA (100 pM) was incubated with crude rat brain membrane homogenates at 37°C for varying periods of time before rapid filtration and washing. Right: [^{3}H]TMA (100 pM) and crude membrane homogenates were preincubated for 4 hr. Ten micromolar TMA was added to aliquots of the mixture to initiate dissociation for varying times before rapid filtration and washing. Fraction specific binding is calculated as the total binding minus the binding in the presence of 10 uM TMA divided by the maximal specific binding (at 4 hr). The rate constants are calculated assuming first-order dissociation (r=.99) and pseudo first-order association kinetics (r=.96).

Cannabinoid Specificity of [^{3}H]TMA Binding

The binding of [^{3}H]TMA is not inhibited at concentrations less than 1 uM by a wide range of drugs and compounds spanning numerous drug-receptor classes, active peptides, and highly hydrophobic compounds (legend to table 1). However, cannabinoids show high affinity for the [^{3}H]TMA site. Delta-9-THC, the major naturally occurring psychotropic cannabinoid, inhibits [^{3}H]TMA binding with an inhibitory constant (K_i) of 27.3±5.0 nM, and delta-8-THC has a K_i of 32.9±0.8 nM. These concentrations represent the calculated concentration of compound in the assay volume. Because of extensive nonspecific membrane partitioning, the actual free concentrations are substantially lower. Similar concentrations of delta-9-THC are found in the blood of human subjects and rhesus monkeys after threshold doses (Agurell et al. 1985).

Binding studies of a series of cannabinoid analogues reveal a well-defined structure-potency relationship for the [^{3}H]TMA site in brain (Nye et al. 1985, table 1). Taken together, the potencies for all of the cannabinoids at the [^{3}H]TMA site do not correlate with their potencies observed in the psychotropic test or in any other known behavioral assay. However, among groups of

TABLE 1

Comparison of potencies for cannabinoids

Compound	$[^3H]$TMA Binding[a] K_i(S.E.M.)	Inhibiting Ileal Contraction[b] (IC_{50})	Rhesus Behavioral Potency[c]
	nM	*nM*	
Natural cannabinoids and stereoisomers			
l-Δ^9THC	27.3 (5.0)	100	H
d-Δ^9THC	40.4 (2.1)[d]	>2,000	I
l-Δ^8THC	32.9 (0.8)	100	M
d-Δ^8THC	40.3 (6.3)[e]	nt[f]	I
Cannabinol	34.4 (10.9)	>2,000	I
Cannabidiol	73.1 (2.2)	>2,000	I
Cannabigerol	8.3	>2,000	I
Cannabicyclol	73.1	nt	I
Side-chain metabolites			
1′-OH Δ^9THC (epimer A)	417	1,100	I[g]
1′-OH Δ^9THC (epimer B)	430	>2,300	I
2′-OH Δ^9THC	121	500	L
3′-OH Δ^9THC	174	20	M
4′-OH Δ^9THC	120	80	M
5′-OH Δ^9THC	58	110	M
1′OH cannabinol	84	nt	nt
1′-oxo cannabinol	342	nt	nt
3-COOH, penta-nor-Δ^9THC	>10,000	nt	nt
Terpenoid metabolites			
11-OH Δ^9THC	74	15	H
11-OH Δ^8THC	69	nt	M
11-OH cannabinol	56	<1,000[h]	nt
10-OH cannabidiol	94	nt	nt
8 α-OH Δ^9THC	70	3,500	L
8 β-OH Δ^9THC	122	1,000	L
8 α, 11-diOH Δ^9THC	136	nt	nt
8 β, 11-diOH Δ^9THC	181	nt	nt
9-COOH-11-nor-Δ^9THC	452	>10,000	nt
9-COOH-11-nor-Δ^8THC	395	nt	I
9-COOH-11-nor-cannabinol	219	nt	nt
Syntnetic cannabinoids			
*threo*1′,2′-dimethylheptylΔ^8THC	21	nt	H[i]
β-Hexahydrocannabinol	19	nt	M
Nabilone	351	100	nt
Levonantradol	297	10	nt
A42236 (Ia, *erythro*)[j]	6.4	nt	nt
A42235 (Ia, *threo*)	6.8	nt	nt
A47632 (7a)	4.6	nt	nt
A47972 (7b)	9.7	nt	nt
A48131 (11)	7.6	nt	nt
5′Methylamino-Δ^8THC	117	nt	nt
DMA	99	900	nt
TMA	109	1,000	nt
Cannabinol acetate	264	nt	nt
$\Delta^{9(11)}$THC	13.6	~1,000[k]	I
Olivetol	952	>10,000	I

[a] Inhibitory constant (K_i) for $[^3H]$TMA site. Compounds with negligible effects at 1 uM are: amphetamine, atropine, capsaicin, captopril, carbamazepine, chloral hydrate, cholesterol, choline, cocaine, cortisone, cyclohexyladenosine, diazepam, dihydro-ergotamine, doxepin, 17-beta-estradiol, GABA, haloperidol, imipramine, 2-isobutyl-3-methoxypyrazine, lysergic acid diethylamide, mazindol, meprobamate, mianserin, muscimol, naloxone, norepinephrine, nitrendipine, nortriptyline, orcinol, ouabain, paraldehyde, pargyline, phencyclidine, phenobarbitol, phentolamine, phenytoin, phorbol dibutyrate, picrotoxin, progesterone, resorcinol, SKF525a. strychnine, testosterone, thiothixene, thyroxine, tyrosine, valproic acid, verapamil, and zopiclone. [b] Potency of cannabinoids on guinea-pig ileum. [c] Threshold for rhesus psychotropic effects from Mechoulam and Edery (1973) and references therein. Codes are: H, high, <.1 mg/kg; M, medium, .1-.5 mg/kg; L, low, .5-2 mg/kg; I,inactive, >2 mg/kg. [d] Different from levo isomer (P<.05). [e] Different from levo isomer (P<.1). [f] nt-Not tested. [j] See Lee et al. 1983. From Nye et al. 1985. Copyright 1985 by American Society for Pharmacology and Experimental Therapeutics (Baltimore).

cannabinoids which are active in eliciting a psychotropic response, the order of affinity for the [^{3}H]TMA site parallels the order of potency in behavioral studies and in inhibiting contractions of the ileum. The features of [^{3}H]TMA pharmacology which parallel behavioral potencies are as follows:

1. The [^{3}H]TMA site shows a stereochemical preference for the natural isomer of THC. Thus, for both delta-8- and delta-9-THC, the levo stereoisomer is significantly more potent than the dextro isomer.

2. The pentyl side chain is essential for [^{3}H]TMA binding. Substitution of the side chain with a carboxylic acid abolishes potency, while the addition of 1' and 2' methyl groups enhances affinity for the [^{3}H]TMA site. The order of inhibitory potencies for side-chain hydroxylated analogues of delta-9-THC accurately reflects their order of potency in both psychotropic tests and physiological assays.

3. The phenolic hydroxyl (position 1) must be unobstructed for significant [^{3}H]TMA binding potency. Thus, desacetyl-levonantradol is 10-fold more potent than levonantradol (figure 4). This deacetylation occurs in vivo and is essential for psychotropic activity (McIlhenny et al. 1981). Cannabinol acetate is also significantly weaker than cannabinol.

4. The addition of a carboxylic acid moiety inactivates delta-9-THC in inhibiting [^{3}H]TMA binding and in behavioral assays. The addition of a hydrox to the 11 position of delta-9- or delta-8-THC does not impair affinity for the[^{3}H]TMA site, whereas 8-hydroxyl derivatives have significantly reduced affinity.

A number of natural cannabinoids and synthetic analogues display significant affinity for the [^{3}H]TMA site despite their apparent inactivity in numerous tests for psychotropic efficacy. Thus cannabinol, cannabidiol, cannabigerol, cannabicyclol and delta-9,11-THC lack psychoactivity but show affinities for the [^{3}H]TMA site of 34.4, 73.1 8.3, 73.1, and 13.6 nM, respectively. These discrepancies suggest that [^{3}H]TMA may not be labeling the site of action of delta-9-THC for producing psychotropic effects.

An alternative possibility suggested by analogy to other drug-receptor systems is that structural isomers that lack bioactivity may be antagonists. Although the pharmacological data are incomplete for many of the inactive cannabinoids, a growing body of evidence indicates that antagonists do exist. The best studied is cannabidiol, which antagonizes the effects of delta-9-THC in humans, rodents (Karniol and Carlini 1973; Karniol et al. 1974) and seizure-sensitive animals (Consroe et al. 1982, this volume). More recently, Martin et al. (this volume) have demonstrated that delta-9, 11-THC suppresses the psychoactivity of delta-9-THC in rhesus monkeys. Burstein et al. (this volume) have shown that 9-catboxy,11-nor-delta-9-THC reduces the catalepsy in mice produced by THC.

Since most measures of the psychotropic effects of cannabinoids in man and monkey are not quantitative, it is difficult to observe the shifts in dose-response relationships that result from antagonism. Moreover, several of these compounds may be weak agonists or partial agonists. This is supported by the observation that cannabinol in high doses elicits psychotropic effects indistinguishable from delta-9-THC in humans (Perez-Reyes et al. 1973).

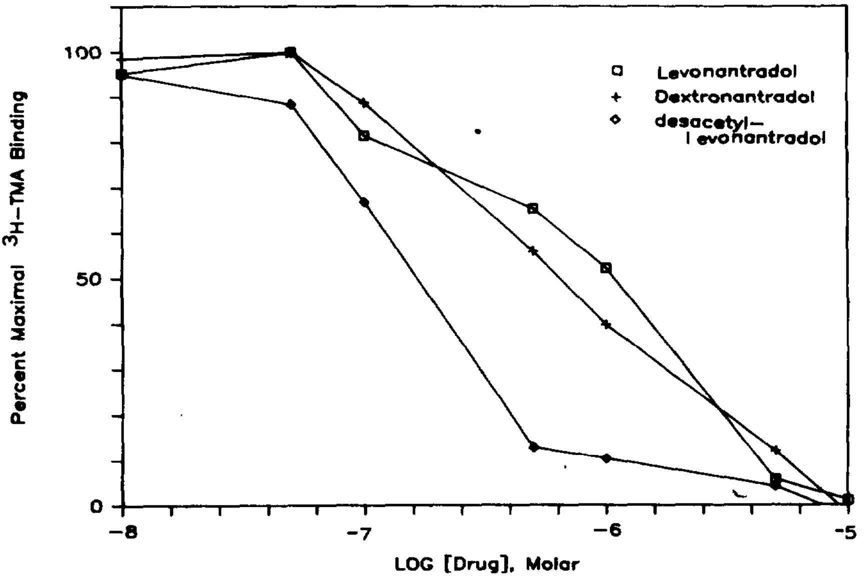

FIGURE 4. Inhibition of [^{3}H]TMA binding by nantradol isomers. [^{3}H]TMA (100 pM) was incubated with crude rat brain membranes in the presence of varying concentrations of nantradol isomers under standard assay conditions. Nonspecific binding is determined in incubations with 10 uM TMA and is subtracted from all data.

Localization and Physical Properties of the High Affinity Cannabinoid Site

[^{3}H]TMA binding is found in brain as well as a number of peripheral organs. The density of binding sites is greatest in brain compared to other organs, and the highest binding levels occur in the hippocampus. The relative potencies for several cannabinoids at the [^{3}H]TMA site are similar in all brain regions. Of peripheral organs, the kidney shows the highest density of sites, while lung and muscle show the lowest densities.

The [^{3}H]TMA site is an integral membrane component which is fully extracted by detergents, such as CHAPS and Triton X-100. Molecular sieve chromatography of the [^{3}H]TMA binding site in CHAPS-extracted membranes reveals a bimodal peak of activity with an M_r of 60,000 daltons. This molecular size probably includes an undetermined number of bound detergent molecules.

REGULATION OF THE HIGH AFFINITY CANNABINOID SITE BY PURINE NUCLEOTIDES

We evaluated the effect on [^{3}H]TMA binding of guanine and adenine nucleotides (figure 5). The specific binding of [^{3}H]TMA is enhanced about 3-fold by the nonhydrolyzable analogue GTP-gamma-S. This enhancement occurs between 0.1 and 10 micromolar; at higher concentrations the enhancement is abolished, with small inhibition observed at >200 uM. GTP does not produce an increase in [^{3}H]TMA binding; instead, it shows a similar inhibition at high concentrations. GTP is probably hydrolyzed to GDP and GMP by nonspecific hydrolases during

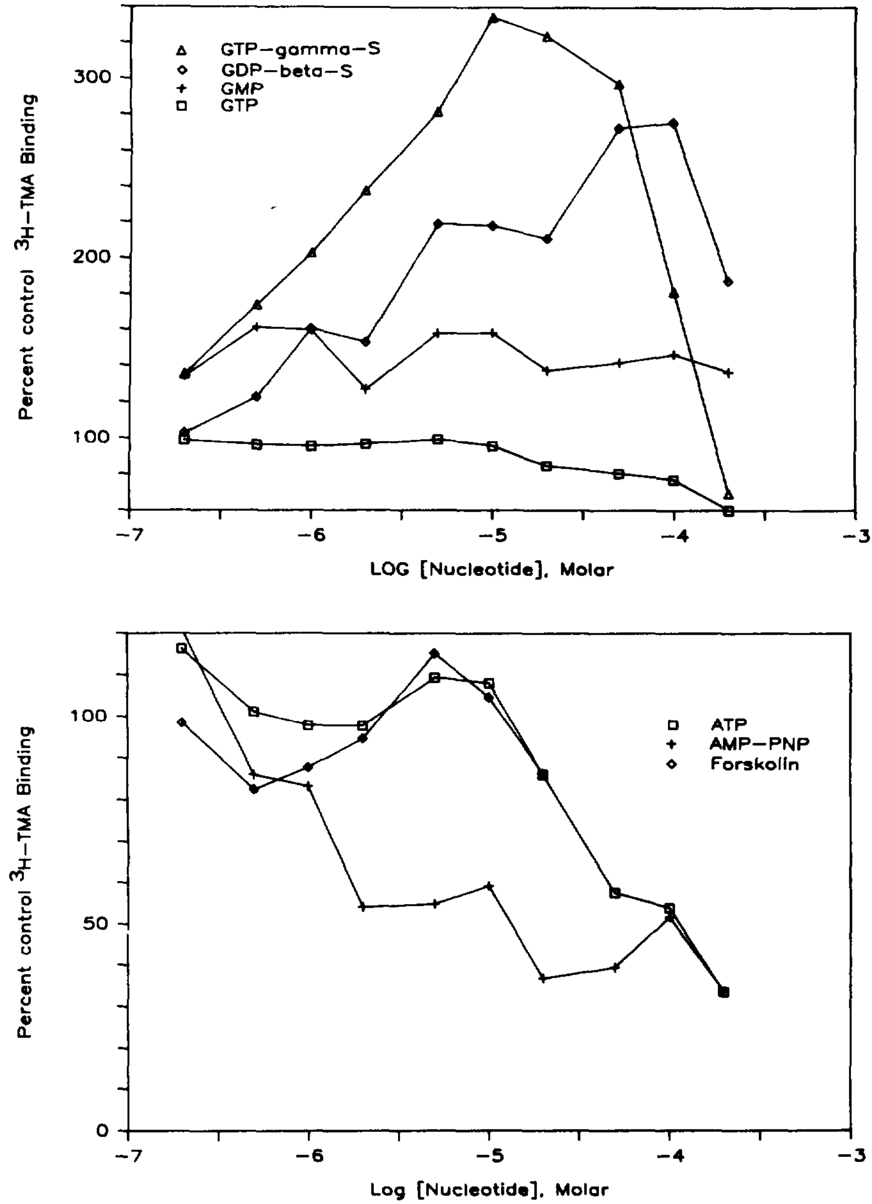

FIGURE 5. Regulation of [^{3}H]TMA binding by purine nucleotides. [^{3}H]TMA was incubated with washed crude brain membranes under standard assay conditions in the presence of added nucleotides. Control binding is defined as the specific [^{3}H]TMA binding measured in the absence of added nucleotides minus nonspecific binding (10 uM TMA). Each data point is the average of duplicate assays and is representative of three experiments.

the long incubations required for measuring [^{3}H]TMA binding. The nonhydrolyzable analogue GDP-beta-S produces a similar enhancement of binding, but at somewhat higher concentrations. The ED_{200} (concentration that doubles binding) is about 1 uM for GTP-gamma-S and about 10 uM for GDP-beta-S. GMP is without either stimulatory or inhibitory effect. These concentrations are in the relevant range for an effect which is mediated by a GTP binding protein (Lefkowitz et al. 1983).

Adenine nucleotides fail to display high potency stimulation of specific [^{3}H]TMA binding. ATP inhibits binding, but only at high concentrations (IC_{50} = 100 uM) (figure 5). The nonhydrolyzable analogue of ATP, ADP-imidophosphate (AMP-PNP), inhibits [^{3}H]TMA binding with an IC_{50} between 5 and 10 uM. These concentrations of AMP-PNP are sufficient to inhibit ATP-dependent processes, such as adenylate cyclase or GDP-phosphorylation. Forskolin, a stimulator of adenylate cyclase, has no effect on [^{3}H]TMA binding at the concentrations tested.

The enhancement of [^{3}H]TMA binding by nonhydrolyzable GTP and GDP analogues suggests that the site may be regulated by a guanine nucleotide binding protein or G-protein. G-proteins are coupled to a large number of neurotransmitter and hormone receptors, and they regulate a variety of intracellular processes, including adenylate cyclase and phospholipase C (Stryer 1986). Paradoxically, both GTP and GDP analogues produce an apparent increase in [^{3}H]TMA binding. In the case of another receptor linked to a G-protein, the beta-adrenergic receptor, the affinity of agonists is decreased by GTP analogues leading to a reduction in binding. Antagonists, on the other hand, are unaffected by GTP analogues (Lefkowitz et al. 1976).

Recent work by Howlett et al. (1986) suggests that cannabinoids act via a G-protein in the neuroblastoma cell line N18tg2. Psychotropically active annabinoids inhibit the adenylate cyclase of these cells at submicromolar concentrations (Howlett and Fleming 1984; Howlett, this volume). Interestingly, stimulation of adenyl cyclase via a number of different receptors is inhibited by cannabinoids. Moreover, Howlett et al. (1986) have demonstrated that the inhibition is reversed by ADP-ribosylation with pertussis toxin, suggesting that the cannabinoids are acting via the inhibitory G-protein or G_i.

ISOLATION OF AN ENDOGENOUS INHIBITOR OF CANNABINOID BINDING

The presence of a neuronal receptor for a psychoactive drug implies that some endogenous neural mechanism is perturbed by that drug. One possible neurochemical mechanism is the interaction of a neurotransmitter or neuromodulator and its receptor. Accordingly, we decided to search in brain extracts for an endogenous inhibitor of [^{3}H]TMA binding.

Extracts of rat brain were prepared according to the method of Bennett et al. (1978). Such extracts preserve peptides from proteolysis and precipitate most proteins. These preparations inhibit [^{3}H]TMA binding in a dose-dependent manner when added to standard assays. Because inhibitory activity was stable to acid and acetonitrile, we partially purified the active component using a combination of conventional chromatography and HPLC. The extracts were loaded on a C18 reverse-phase Sep Pak (Waters), washed with 0.1% trifluoracetic acid, and eluted with 80% acetonitrile 0.1% aqueous TFA. This material was chromatographed on a Biogel P10 column (Biorad) and eluted with 10% formic acid 0.1% 2-mercaptoethanol. The fractions were evaporated and assayed for inhibition of [^{3}H]TMA binding (figure 6). A single peak of inhibitory activity is observed in fractions corresponding to the void volume of the column. HPLC gel filtration chromatography of the pooled fractions on a TSK column (Biorad) reveals a single peak of [^{3}H]TMA inhibitory activity corresponding to a calculated molecular size of 15-20,000 daltons (figure 7).

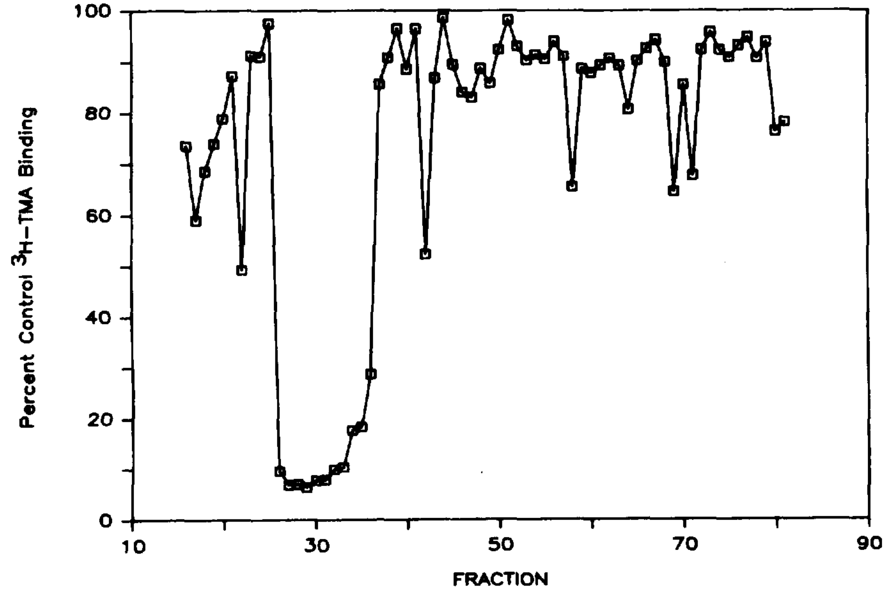

FIGURE 6. Gel filtration chromatography of the endogenous inhibitor of [^{3}H]TMA binding. Rat brain extracts. prepared as described in the text, were loaded onto a Biogel P10 (Biorad) column (1x25 cm) equilibrated in 10% formic acid/0.1% 2-mercapto-ethanol running at 5 ml/hr. Fractions (0.35 mL) were evaporated and resuspended in 25 mM TES buffer (pH 6.8) before being added to standard [^{3}H]TMA binding assays in the presence of 75 pM [^{3}H]TMA. Fractions 26-32 correspond to the void volume of the column. Assays were performed in singlicate and represent two similar experiments.

Partially purified fractions of the endogenous inhibitor reduce [^{3}H]TMA binding (figure 8) in a concentration-dependent manner. Although the preparation tested is only partially purified, it shows an inhibitory potency (IC$_{50}$) of 1.5-2.0 x 10^{-4} g protein/L. Assuming that a 15-20,000 dalton protein accounts for the inhibitory activity, the maximal molar concentration of the preparation at the IC$_{50}$ is 10 nM. When fully purified, the actual IC$_{50}$ of the endogenous inhibitor of [^{3}H]TMA binding may therefore be substantially lower than 10 nM. Many possibilities exist for the role of the endogenous inhibitor of [^{3}H]TMA binding. It is enticing to speculate that this protein is a neurotransmitter or neuromodulator whose normal functioning is perturbed by cannabinoids. Thus, further characterization of the endogenous inhibitor may lead to additional insights into the effects of cannabinoids upon the brain. Alternatively, the page endogenous inhibitor may be an intracellular regulatory protein which interacts with the cannabinoid site to alter certain cellular processes.

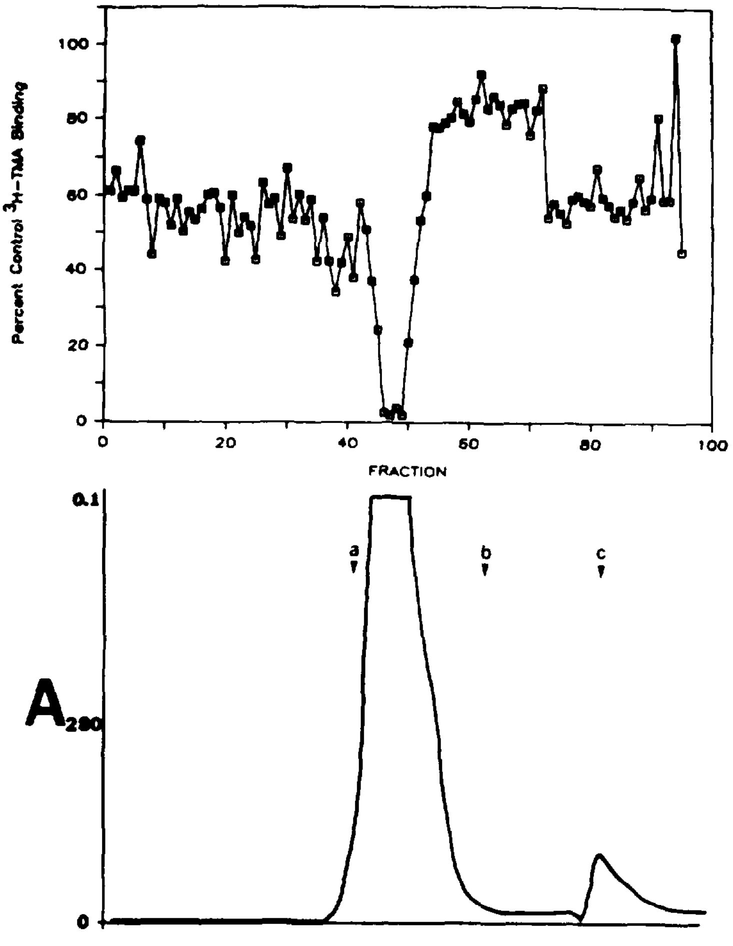

FIGURE 7. Molecular sizing of the endogenous inhibitor of [^{3}H]TMA binding by high pressure liquid chromatography. Partially purified rat brain extracts (see text) were loaded onto a Biosil TSK column triplet (TSK-400, TSK 250, and TSK-125) connected in series. The columns were eluted with 32% acetonitrile 0.1% aqueous trifluoroacetic acid at a flow rate of mL/min. Fractions (0.5 mL) were evaporated, resuspended in 0.1 N HCl, and added to standard [^{3}H]TMA binding assays. The data presented (upper tracing) are from single determinations on each fraction. Equivalent quantities of HCl reduced binding to 60% to 70% of maximal levels. The eluate was monitored for absorbance at 280 nm (lower tracing). Bovine serum albumin (a, M_r=66,000), alpha-MSH (b, M_r=1572), and 2-mercaptoethanol (c, M_r=78) were used as standards to calibrate the column.

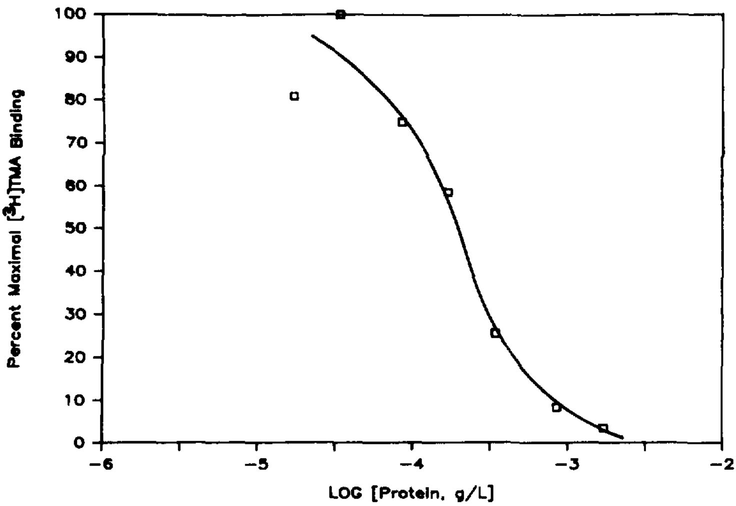

FIGURE 8. Inhibitory potency of a partially purified preparation of the endogenous inhibitor of [^{3}H]TMA binding. Dilutions of partially purified extracts obtained from gel filtration chromatography were added to triplicate standard [^{3}H]TMA binding assays with crude bruin membranes. Maximal binding is defined as the total binding minus the binding in the presence of 10 uM TMA. The abscissa represents the logarithm of the concentration of added protein in the final volume of the [^{3}H]TMA binding assay. Protein values were determined from protein assay (BCA, Pierce) of the partially purified endogenous inhibitor preparation.

MODEL OF CANNABINOID ACTIONS

One of the most perplexing phenomena in studying the cellular actions of cannabinoids is their profound effects on a host of enzymes, transport mechanisms, and neurochemical processes (Martin 1986). One possible explanation for these diverse effects is that cannabinoids modulate the metabolism of intracellular second messengers, such as cyclic AMP or the phosphoinositide phospholipids (PIP,). These intracellular mediators in turn regulate the activity of multiple cellular processes by stimulating the phosphorylation of proteins, by the release of calcium ion stores, and by other mechanisms.

A mechanism for the regulation of intracellular second messengers by cannabinoids is suggested by the finding that the high affinity cannabinoid site as labelled by [^{3}H]TMA appears to be coupled to a G-protein. Moreover, the effect of cannabinoids on adenylate cyclase is mediated via G_i (Howlett, this volume). Since G-proteins are the regulatory proteins which modulate the activities of adenylate cyclase, phospholipase, and other second messenger-metabolizing enzymes, an effect by cannabinoids upon these critical proteins would in turn affect multiple cellular processes. Although the evidence is incomplete, the hypothesis that all of the cellular actions of cannabinoids are mediated via G-proteins offers a unified explanation for the diverse and multiple effects of cannabinoids in Vitro and in vivo.

REFERENCES

Agurell, S; Halldin, M.; Lindgren, J.-E.; Ohlsson, A.; Widman, M. Hollister, L.E.; and Gillespie, H.K. Recent studies on the pharmacokinetics and metabolism of d^1 tetrahydrocannabinol, cannabidiol and cannabinol in man. In: Harvey, D.J., ed. Marijuana '84: Proceedings of the Oxford Svmposium on Cannabis. Oxford: IRL Press, 1985. pp. 49-62.

Bennett, H.P.J.; Hudson, A.M.; Kelly, L.; McMartin, C.; and Purdon, G.E. A rapid method using octadecasilyl silica for the extraction of certain peptides from tissue. Biochem J 175:1139-1141, 1978.

Consroe, P.; Martin, A.R.; and Fish, B.S. Use of a potential rabbit model for structure-behavioral activity studies of cannabinoids. J Med Chem 25:596-599, 1982.

Hewlett, A.C., and Fleming, R.M. Cannabinoid inhibition of adenylate cyclase: Pharmacology of the response in neuroblastoma cell membranes. Mol Pharm Acol 23:532-538, 1984.

Howlett, A.C.; Qualy, J.M.; and Khachatrian, L.L. Involvement of G_i in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol Pharm Acol 29:307-313, 1986.

Karniol, I.G. and Carlini, E.A. Pharmacological interaction between cannabidiol and delta-9-tetrahydrocannabinol. Psychopharmologia 33:53-70, 1973.

Karniol, I.G.; Sirakawa, I.; Kasinski, N.; Pfefferman, A.; and Carlini, E.A. Cannabidiol interferes with the effects of delta-9-tetrahydrocannabinol in man. Eur J Pharmacol 28:172-177, 1974.

Lee, C.-M.; Zuagg, H.E.; Michaels, R.J.; Dren, A.T.; Plotnikoff, N.P.; and Young, P.R. New azacannabinoids highly active in the central nervous system. J Med Chem 26:278-280, 1983.

Lefkowitz, R.J.; Mullikin, D.; and Caron, M.G. Regulation of beta-adrenergic receptors by guanyl-5'-yl imidodiphosphate and other purine nucleotides. J Biol Chem 251:4686-4692, 1976.

Lefkowitz, R.J.; Stadel, J.M.; and Caron, M.G. Adenylate cyclase-coupled beta-adrenergic receptors: Structure and mechansims of activation. Annu Rev Biochem 52:159-186, 1983.

McIlhenny; A.M, Mast; R.W., Johnson, M.R.; and Mime, G.W. Nantradol hydrochloride: Pharmacokinetics and behavioral effects after acute and chronic treatment. J Pharm Acol Exp Ther 219:363-369, 1981.

Martin, B.R. Cellular effects of cannabinoids. Pharm Rev Acol 38:45-74, 1986.

Mechoulam, R., and Edery, H. Structure-activity relationship in the cannabinoid series. In: Mechoulam, R., ed. Marijuana: Chemistry, Pharmacology, Metabolism and Clinical Effects. New York: Academic Press, 1973. pp. 101-136.

Nye, J.S.; Seltzman, H.H.; Pitt, C.G.; and Snyder, S.H. High-affinity cannabinoid binding sites in brain membranes labeled with [^{3}H]-5'-trimethylammonium d^8-tetrahydro-cannabinol. J Pharm Acol Exper Ther 234:784-791, 1985.

Ohlsson, A.; Agurell, S.; Leander, K.; Dahmen, T.; Edery, H.; Porath, G.; Levy, S.; and Mechoulam, R. Synthesis and psychotropic activity of side-chain hydroxylated delta-6-tetrahydrocannabinol metabolites. Acta Pharm Suec 16:21-33, 1979.

Perez-Reyes, M.; Timmons, M.C.; Davis, K.H.; and Wall, E.M. A comparison of the pharmacological activity in man of intravenously administered delta-9-tetrahydrocannabinol, cannabinol and cannabidiol. Experientia 29:1368- 1369, 1973.

Rosell, S.; Bjorkroth, U.; Agurell, S.; Leander, K.; Ohlsson, A.; Martin, B.; and Mechoulam, R. Relation between effects of cannabinoid derivatives on the twitch response of the isolated guinea-pig ileum and their psychotropic properties. In: Nahas, G.G. and Paton, W.D.M., eds. Marijuana Biological Effects: Analysis, Metabolism, Cellular Response, Reproduction and Brain. Oxford: Pergamon Press. 1979. pp. 63-69.

Seltzman, H.H.; Setzer, S.R.; Williams, D.L.; Demian, I.; Wyrick, C.D.; and Pitt, C.G. Synthesis of cannabinoid radioligands and haptens for use in radioimmunoassay and receptor site studies. In: Harvey, D.J., ed. Marijuana '84: Proceedings of the Oxford Symposium on Cannabis. Oxford: IRL Press, 1985. pp. 183-190.

Stryer, L. G-Proteins: A family of signal transducers. Annu Rev Cell Biol 2:391-419, 1986.

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Regulation of Adenylate Cyclase in a Cultured Neuronal Cell Line by Marijuana Constituents, Metabolites of Delta-9-tetrahydrocannabinol, and Synthetic Analogs Having Psychoactivity

Allyn C. Howlett, Ph.D.

The psychoactive properties of cannabinoid drugs have been described in man and investigated in a variety of animal models. However, in vitro studies have failed to elucidate the mechanism of cannabinoid action at the neuronal level. The activities of a number of enzymes have been reported to be modified by cannabinoid compounds (Martin 1986). However, in many studies, problems arise in correlating these cellular events with changes in animal behavior. This may be because the drug concentrations required for alterations in enzyme activity are greater than would be expected at the site of action in the brain. In other studies, cannabinoid drugs having little or no psychoactivity produce a cellular response similar to that of psychoactive cannabinoid drugs. These difficulties in defining a mechanism of action for cannabimimetic drugs have been discussed by Martin (1986) in a recent review on the cellular effects of cannabinoid drugs. Studies in my laboratory have determined that cannabimimetic drugs inhibit cyclic AMP accumulation in neuronal cells (Howlett 1984; Howlett and Fleming 1984). These studies will be described in this chapter. It is proposed that the cannabimimetic inhibition of adenylate cyclase is a receptor-mediated cellular response that may be one mechanism of action for these drugs.

A neuroblastoma cell culture system is used for these studies as a model for neurons. The N18TG2 cell line was cloned from the cultured C1300 tumor (Augusti-Tocco and Sato 1969; Gilman and Minna 1973; Schubert et al. 1969). This tumor arose spontaneously in the region of the spinal cord of the A/J mouse (Augusti-Tocco and Sato 1969) and, therefore, may have been derived from sympathetic ganglial cells. N18TG2 cells have retained a typical neuronal morphology in culture (Augusti-Tocco and Sato 1969; Schubert et al. 1969), and they exhibit electrophysiological responses to neurotransmitters such as acetylcholine (Chalazonitis et al. 1977). These cells also synthesize the neuromodulator, vasoactive intestinal peptide (VIP) (Brick et al. 1985). The advantage of using cultured neuroblastoma cells rather than brain cells for biochemical studies of adenylate cyclase is that plasma membranes may be isolated from large quantities of cells that are genetically and phenotypically identical.

A number of neuromodulators interact with receptors that regulate the synthesis of cyclic AMP in neuronal cells. Cyclic AMP diffuses into the neuron as the "second messenger" that activates cyclic AMP-dependent protein kinase. Protein kinase modifies the activity of key enzymes involved in such functions as neurotransmitter synthesis or membrane excitability (Bloom 1975; Daly 1977; Greengard 1976). The regulation of adenylate cyclase by neuromodulator receptors is complex and has been reviewed by Gilman (1984). According to our current understanding, stimulatory receptors interact with a regulatory protein complex, G_s, and inhibitory receptors interact with a homologous complex, G_i (figure 1). Both regulatory proteins are heterotrimers comprising α, β,, and γ subunits. Although the β and γ subunits of G_s and G_i are thought to be nearly identical, the a subunits differ structurally and functionally. Stimulatory and inhibitory neuromodulator-receptor interactions promote the binding of GTP onto the α_s and α_i subunits, respectively. This causes dissociation of the α_s or α_i from the β and γ proteins. The α_s subunit is then free to activate the catalytic protein of adenylate cyclase. The free a_i subunit may inhibit the catalytic protein directly. However, inhibition of adenylate cyclase may also occur as the free β-γ proteins form a new equilibrium with α_s proteins and thereby prevent the interaction of α_s with the catalytic subunit.

INHIBITION OF ADENYLATE CYCLASE BY CANNABINOID COMPOUNDS

My laboratory provided the first evidence that cannabimimetic drugs decreased cyclic AMP accumulation in neuronal cells (Howlett 1984). Δ^9-Tetrahydrocannabinol (THC) and Δ^8-THC decreased both basal and prostacyclin- (prostaglandin I_2-) stimulated cyclic AMP accumulation in neuroblastoma cells. Prostanoid-stimulated adenylate cyclase in a membrane preparation from these cells was inhibited by cannabimimetic compounds, indicating that this enzyme complex was the target for the effect of Δ^9-THC on cellular cyclic AMP concentrations. The cannabimimetic drugs caused a decrease in V_{max} of the enzyme, with no alteration in the K_m for substrate (Howlett 1985). Adenylate cyclase inhibition was apparent immediately upon addition of Δ^9-THC and the effects of the drug were readily reversible after the membranes were sedimented and resuspended (Howlett 1985). The inhibition was shown to be concentration-dependent over a nM range for both Δ^9-THC and Δ^8-THC. The activity of cyclic nucleotide phosphodiesterase, responsible for the metabolic breakdown of cyclic AMP, was unaltered by these agents (Howlett 1984).

The cannabimimetic inhibition of adenylate cyclase was not observed universally in all cell types. The response was not observed in either the soluble adenylate cyclase from rat sperm or the membrane-bound adenylate cyclase from C6 glioma or S49 lymphoma cells (Howlett et al. 1986). This cellular selectivity provides evidence for the existence of specific receptors for the cannabimimetic drugs. If these drugs were influencing enzyme activity by intercalating into lipid membranes and altering membrane fluidity, then one would have expected universal inhibition of adenylate cyclase in all cell types. This was not the case.

The inhibition of adenylate cyclase by Δ^9-THC in neuroblastoma plasma membranes was not due to an effect on stimulatory receptor regulation of the enzyme (Howlett and Fleming 1984). Inhibition by Δ^9-THC was not competitive with prostaglandin E_1 or prostacyclin. Furthermore, non-competitive inhibition was also observed when the peptide hormones

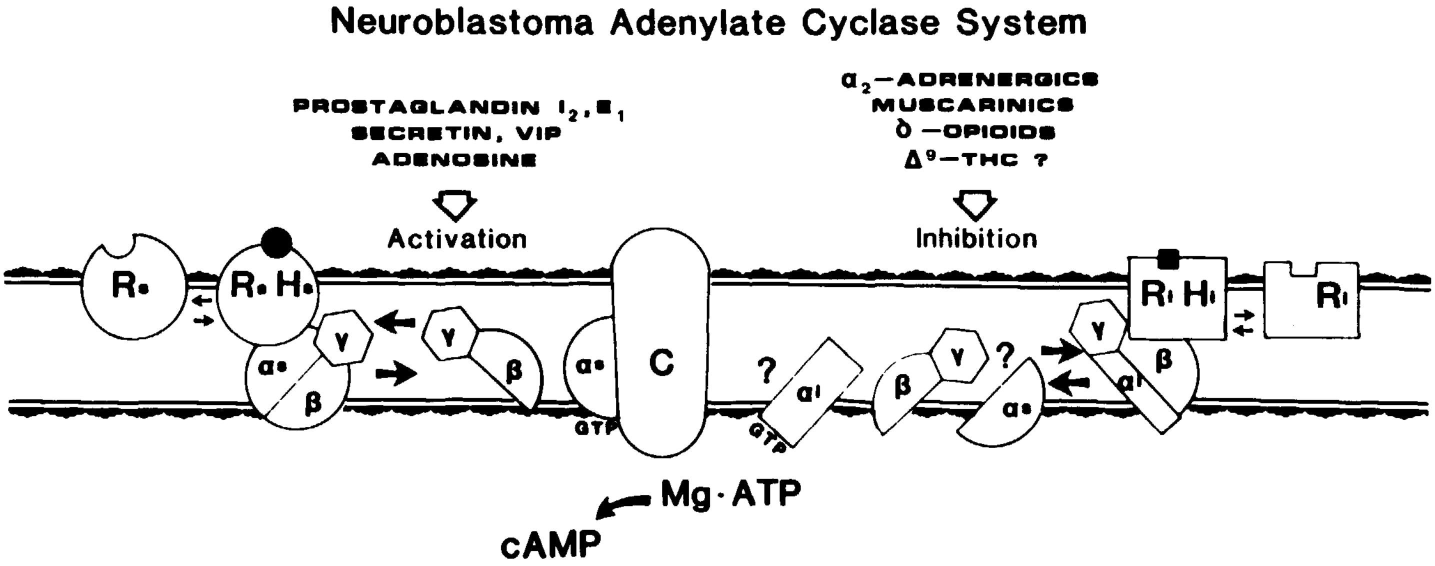

FIGURE 1. Hypothetical model of the regulation of adenylate cyclase by stimulatory and inhibitory neuromodulators. Rs and R_i represent one of several pharmacologically distinct receptors for stimulatoy and inhibitory neuromodulators, respectively, and Hs and H_i indicate occupancy by their respective agonist ligands. The G-protein complexes, G_s and G_i are denoted as their subunit constituents, α_s, β, γ and α_i, β, γ, respectively. The catalytic protein is designated C. Details concerning the interaction of these protein subunits are found in the text.

secretin and VIP stimulated the enzyme. Additional evidence that the Δ^9-THC was not acting at the level of the receptors that stimulate adenylate cyclase was provided by the observation that inhibition also occurred when forskolin was used to activate the enzyme. Forskolin is believed to act directly at the catalytic subunit (Seamon and Daly 1981).

The cannabimimetic inhibition of adenylate cyclase appeared to resemble the regulation resulting from inhibitory neuromodulator receptors that act via G_i (Howlett 1985). The effects of cannabimimetic drugs were related to the ability of the enzyme to be regulated by divalent cations and guanine nucleotides. The inhibition was greatest at micromolar Mg^{2+} or Mn^{2+} concentrations and was attenuated by millimolar concentrations of Mn^{2+}. Half-maximal inhibition of adenylate cyclase was observeo at 800 nM GTP for both cannabimimetic and muscarinic cholinergic agents. This concentration is higher than that required for stimulation of the enzyme by hormones and is typical of the high GTP concentrations required by inhibitory hormones and neuromodulators in other systems (Cooper 1982). These observations illustrate the similarities between the enzyme inhibition by cannabimimetic drugs and by muscarinic cholinergic drugs. It is inferred that the cannabimimetic drugs must act via regulatory mechanisms similar to those operating for hormonal inhibition of adenylate cyclase.

Receptor-mediated inhibition of adenylate cyclase in most eukaryotic systems requires the presence of the guanine nucleotide-binding protein complex, G_i. Further studies confirmed that cannabimimetic inhibition of adenylate cyclase also required the presence of G_i (Howlett et al. 1986). C_i can be functionally inactivated as the result of an ADP-ribosylation modification catalyzed by pertussis toxin. Therefore, pertussis toxin can be used as a tool to identify Gi-mediated events in the cell. Pertussis toxin treatment of intact N18TG2 cells abolished the cannabimimetic regulation of cellular cyclic AMP content. The adenylate cyclase response to Δ^9-THC was also attenuated in membranes isolated from cells that had been treated with pertussis toxin. The action of pertussis toxin for in vitro modification of neuroblastoma G_i requires NAD as substrate for the ADP-ribosylation. The toxin was able to catalyze the labeling of a neuroblastoma membrane protein in vitro using (^{32}P)NAD under conditions similar to those by which attenuation of the cannabimimetic inhibition of adenylate cyclase could be demonstrated. Protein labeling and the loss of the response to Δ^9-THC followed the same dose-response curve for pertussis toxin. This strengthens the argument that cannabimimetic drugs act via receptors that interact with the inhibitory GTP-binding protein complex.

THE PHARMACOLOGY OF CANNABIMIMETIC INHIBITION OF ADENYLATE CYCLASE

Inhibition of adenylate cyclase in N18TG2 cells is known to be regulated by muscarinic cholinergic, α-adrenergic, and δ-opioid receptors. Thus, it was possible that Δ^9-THC may be acting via one of these pharmacological receptor types. When this was tested, it was found that inhibition by Δ^9-THC was neither synergistic nor additive with muscarinic cholinergic or α-adrenergic agents when each was present at maximally inhibitory concentrations (Howlett and Fleming 1984). Furthermore, the response to Δ^9-THC was not blocked by the antagonists to these receptors, atropine and yohim bine, respectively.

The opioid drugs are another pharmacological class of neuromodulatory compounds that inhibit adenylate cyclase in N18TG2 cells. Because of the interaction of cannabimimetic and opioid drugs in animal models of tolerance, the possible involvement of opioid receptors was examined (see Devane et al. 1986 and references contained therein). δ-Opioid receptors were found on neuroblastoma membranes using (^{3}H)-D-ala^2-D-ieu^5-enkephalin. No μ- or κ-opioid receptors were detected. The δ-opioid receptor binding affinity and capacity were unaltered by cannabimimetic drugs. Opioid drugs as well as Δ^9-THC and the nantradol series of analgesic cannabimimetic analogs decreased cyclic AMP accumulation in intact cells and inhibited adenylate cyclase in membrane preparations. Opioid antagonists blocked the response to the opioid agonists but not to the cannabimimetic drugs. The interaction between cannabimimetic drugs and the opioid agonist etorphine showed no antagonistic, synergistic, or additive effects at maximal concentrations. These results suggest that the opioid and cannabimimetic drugs operate via distinct, noninteractive receptors that can be coupled to the same effector, adenylate cyclase.

Other neuronal cell lines having a δ-opioid response were also examined (Devane et al. 1986). The cannabimimetic inhibition of cyclic AMP accumulation in NC108-15 neuroblastoma x glioma hybrid cells was not as great as the response in N18TG2 cells. N4TG1 neuroblastoma cells, which exhibit a prominent inhibition of adenylate cyclase in response to opioid drugs, did not respond to cannabimimetic drugs under any conditions tested. Thus, the cannabimimetic response does not correlate with the efficacy of the δ-opioid response.

STRUCTURE-ACTIVITY RELATIONSHIPS AMONG THE CANNABINOID DRUGS

Given that the cannabimimetic response in neuroblastoma cells is mediated by G_i and does not result from drug interaction with δ-opioid, muscarinic cholinergic, or α-adrenergic receptors that inhibit adenylate cyclase, the hypothesis can be proposed that these drugs act via a "cannabinoid" receptor. The classical means to describe a receptor is to define its pharmacological spectrum of activity. This was performed using constituents of marijuana extracts and metabolites of these compounds (Howlett 1987). The concentration of Δ^9-THC exhibiting half-maximal inhibition was less than 500 nM (figure 2A). Δ^8-THC was less active, cannabinol was only partially active, and cannabidiol was inactive. Other constituents of marijuana that have no psychoactivity (cannabigerol, cannabichromene, olivetol) and compounds having a reduced length of the C3 alkyl side chain were not active as inhibitors of adenylate cyclase (table 1). These inactive compounds did not behave as antagonists to the effects of Δ^9-THC.

The nantradol class of cannabinoid analogs were potent inhibitors of adenylate cyclase (figure 2B). Desacetyllevonantradol was more potent than nantradol. The nonpsychoactive isomer dextronantradol was a poor inhibitor of adenylate cyclase.

The metabolities of Δ^8-THC and Δ^9-THC hydroxylated at the C11-position were more potent than the parent drugs (table 1). However, hydroxylation at the C8-position of the terpenoid ring resulted in loss of activity of Δ^9-

THC. Compounds hydroxylated along the C3 alkyl side chain were equally efficacious but less potent than Δ^9-THC.

These structure-activity relationships are consistent with cannabinoid pharmacology reported for psychological effects in humans and for behavioral effects reported in a variety of animal models. Potency estimates have suggested that the psychological high experience in humans is greater for Δ^9-THC than for Δ^8-THC (Hollister 1974; Hollister and Gillespie 1973). Neither cannabinol nor cannabidiol produced responses in human subjects at the oral dosages tested (Hollister 1973, 1974). However, when infused intravenously, cannabinol (but not cannabidiol) produced a psychological high and cardioacceleration at ten-fold the dose of Δ^9-THC (Hollister 1973; Perez-Reyes et al. 1973a). 11-OH-metabolites of Δ^9-THC and Δ^8-THC were up to twice as active as the parent compounds in producing a psychological high (Hollister 1974; Lemberger et al. 1973). The ability of these compounds to inhibit adenylate cyclase parallel these responses.

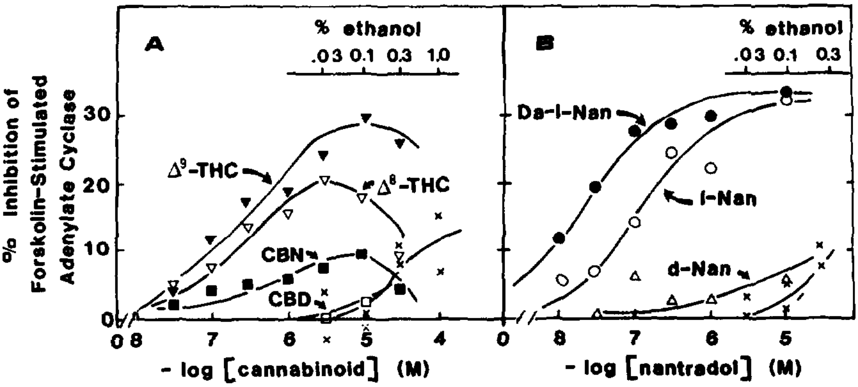

FIGURE 2. ***Inhibition of adenylate cyclase by cannabinoid and nantradol compounds. Forskolin was present at 1.0 µM to activate adenylate cyclase. Other compounds present at the indicated concentmtfons were cannabinol (CBN), cannabidiol (CBD), levonantradol (ℓ-Nan), dextronantradol (d-Nan), and desacetyllevonantradol (Da-ℓ-Nan).***

In animal studies, Δ^8-THC was less potent than Δ^9-THC in such tests as dog ataxia (Martin et al. 1981; Wilson et al. 1976), monkey behavior (Edery and Crunfeld 1971), mouse hypothermia (Martin et al. 1981), and hot-plate analgesia (Uliss et al. 1975; Wilson and May 1975) and in genetically THC-seizure prone rabbits (Consroe and Fish 1981). Cannabidiol (Consroe and Fish 1981; Edery and Crunfeld 1971; Pertwee 1972; Uliss et al. 1975), cannabinol (Edery and Grunfeld 1971), and cannabichromene (Edery and Grunfeld 1971) were inactive in a number of these models. The THC-seizure prone rabbits responded to cannabinol at high doses (Consroe and Fish 1981). The 11-OH metabolites of Δ^8-THC and Δ^9-THC were more potent than the parent compounds in the dog ataxia (Wilson et al. 1976), mouse immobility (Gill et al. 1973), hot-plate analgesia (Wilson and May 1975), and THC-seizure prone rabbit models (Consroe and Fish 1981) and in the drug discrimination studies using rats or pigeons (Jarbe and McMillan 1980). Animal studies of the hydroxylated metabolites at the 8-position are inconsistent; although in each study, 8-OH metabolites are either inactive or less potent than Δ^9-THC (Ben-Zvi et al. 1971; Wilson and May 1975; Perez-Reyes et al. 1973b; Jarbe and McMillan 1980). The data reported here for inhibition of adenylate cyclase are consistent with this pharmacological pattern.

TABLE 1

Pharmacological Parameters of Marijuana Constituents and Metabolites

Compound	Efficacy[a]	Potency K_{inh}
Δ^9-THC	1.0	430
Δ^8-THC	0.9	560
Cannabinol	0.5	1400
Cannabidiol	0[b]	--
Cannabichromene	0	--
Cannabigerol	0	--
Olivetol	0	--
$\Delta^{9,11}$-THC	0.6	290
11-OH-Δ^9-THC	1.0	100
11-OH-Δ^8-THC	1.0	260
11-OH-cannabinol	1.0	320
8α-OH-Δ^9-THC	0	--
8β-OH-Δ^9-THC	0	--
8α,11-diOH-Δ^9-THC	0	--
2′-OH-Δ^9-THC	1.0	2800
3′-OH-Δ^9-THC	1.0	840
4′-OH-Δ^9-THC	1.0	1800
5′-OH-Δ^9-THC		1580
Tri Nor-Δ^9-THC-carboxylic acid	0	--
Penta Nor-Δ^9-THC-carboxylic acid	0	--

[a] *Relative to Δ^9-THC equal to 1.*

[b] *An efficacy of 0 was assigned to all compounds exhibiting a lack of inhibition at concentrations up to 50 µM.*

The parallels in cannabinoid structure-activity relationships exhibited by the neuronal cells compared to the human and animal behaviors is intriguing. These findings suggest that certain behavioral effects of cannabimimetic drugs may be the result of an interaction of these drugs with a pharmacologically distinct receptor associated with neuronal adenylate cyclase. At least one possible mechanism of action for the cannabimimetic compounds may involve regulation of cyclic AMP synthesis in populations of neurons associated with modification of such behaviors.

REFERENCES

Augusti-Tocco, G., and Sato, G. Establishment of functional donal lines of neurons from mouse neuroblastoma. Proc Natl Acad Sci USA 64:311-315, 1969.

Ben-Zvi, Z.; Mechoulam, R.; Edery, H.; and Porath, G. 6β-Hydroxy-Δ^{1}-tetrahydrocannabinol synthesis and biological activity. Science 174:951-952, 1971.

Bloom, F. The role of cyclic nucleotides in central synaptic function. Rev Physiol Biochem Pharmacol 74:1-104, 1975.

Brick, P.L.; Howlett, A.C.; and Beinfeld, M.C. Synthesis and release of vasoactive intestinal polypeptide (VIP) by mouse neuroblastoma cells: Modulation by cyclic nucleotides and ascorbic acid. Peptides 6:1075-1078, 1985.

Chalazonitis, A.; Minna, J.D.; and Nirenberg, M. Expression and properties of acetylcholine receptors in several clones of mouse neuroblastoma x L cell somatic hybrids. Exp Cell Res 105:269-280, 1977.

Consroe, P., and Fish, B.S. Rabbit behavioral model of marijuana psychoactivity in humans. Med Hypothesis 7:1079-1090, 1981.

Cooper, D.M.F. Bimodal regulation of adenylate cyclase. FEBS Lett 138:157-163, 1982.

Daly, J. Cyclic Nucleotides in the Nervous System. New York: Plenum Press, 1977.

Devane, W.A.; Spain, J.W.; Coscia, C.J.; and Howlett, A.C. An assessment of the role of opioid receptors in the response to cannabimimetic drugs. J Neurochem 46:1929-1935, 1986.

Edery, H., and Grunfeld, Y. Structural requirements for cannabinoid activity. Ann NY Acad Sci 191:40-53, 1971.

Gill, E.W.; Jones, G.; and Lawrence, D.K. Contribution of the metabolite 7-hydroxy-Δ^{1}-tetrahydrocannabinol toward the pharmacological activity of Δ^{1}-tetrahydrocannabinol in mice. Biochem Pharmacol 22:175-184, 1973.

Gilman, A.G. G proteins and dual control of adenylate cyclase. Cell 36:577-579, 1984.

Gilman, A.G., and Minna, J.D. Expression of genes for metabolism of cyclic adenosine 3':5'-monophosphate in somatic cells I. responses to catecholamines in parental and hybrid cells. J Biol Chem 248:6610-6617, 1973.

Greengard, P. Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters. Nature 260:101-108, 1976.

Hollister, L.E. Cannabidiol and cannabinol in man. Experientia 29:825-826, 1973.

Hollister, L.E. Structure-activity relationships in man of cannabis constituents, and homologs and metabolites of Δ^{9}-tetrahydrocannabinol. Pharmacology 11:3-11, 1974.

Hollister, L.E., and Gillespie, H.K. Delta-8- and delta-9-tetrahydrocannabinol. Comparison in man by oral and intravenous administration. Clin Pharmacol Ther 14:353-357, 1973.

Howlett, A.C. Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. Life Sci 35:1803-1810, 1984.

Howlett, A.C. Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. Mol Pharmacol 27:429-436, 1985.

Howlett, A.C. Cannabinoid inhibition of adenylate cyclase: Relative activities of marihuana constituents and metabolites of marihuana. Neuropharmacology 26:507-512, 1987.

Howlett, A.C., and Fleming, R.M. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol Pharmacol 26:532-538, 1984.

Howlett, A.C.; Qualy, J.M.; and Khachatrian, L.L. Involvement of G; in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol Pharmacol 29:307-313, 1986.

Jarbe, T.U.C., and McMillan, D.E. Δ^9-THC as a discriminative stimulus in rats and pigeons: Generalization to THC metabolites and SP-111. Psychopharmacology 71:281-289, 1980.

Lemberger, L.; Martz, R.; Rodda, B.; Forney, R.; and Rowe, H. Comparative pharmacology of Δ^9-tetrahydrocannabinol and its metabolite, 11-OH-Δ^9-tetrahydrocannabinol. J Clin Invest 52:2411-2417, 1973.

Martin, B.R. Cellular effects of cannabinoids. Pharmacol Rev 38:45-74, 1986.

Martin, B.R.; Balster, R.L.; Razdan, R.K.; Harris, L.S.; and Dewey, W.L. Behavioral comparisons of the stereoisomers of tetrahydrocannabinols. Life Sci 29:565-574, 1981.

Perez-Reyes, M.; Timmons, M.C.; Davis, K.H.; and Wall, M.E. A comparison of the pharmacological activity in man of intravenously administered Δ^9 -tetrahydrocannabinol, cannabinol, and cannabidiol. Experientia 29:1368-1369, 1973a.

Perez-Reyes, M.; Timmons, M.C.; Lipton, M.A.; Christensen, H.D.; Davis, K.H.; and Wall, M.E. A comparison of the pharmacological activity of Δ^9 -tetrahydrocannabinol and its monohydroxylated metabolites in man. Experientia 29:1009-1010, 1973b.

Pertwee, R.G. The ring test: A quantitative method for assessing the "cataleptic" effect of cannabis in mice. Br J Pharmacol 46:753-763, 1972.

Schubert, D.; Humphreys, S.; Baroni, C.; and Cohn, M. In vitro differentiation of a mouse neuroblastoma. Proc Natl Acad Sci USA 64:316-323, 1969.

Seamon, K.B., and Daly, J.W. Forskolin: A unique diterpene activator of cyclic AMP-generating systems. J Cyclic Nucleotide Res 7:201-224, 1981.

Uliss, D.B.; Dalzell, H.C.; Handrick, C.R.; Howes, J.F.; and Razdan, R.K. Hashish. Importance of the phenolic hydroxyl group in tetrahydrocannabinols. J Med Chem 18:213-215, 1975.

Wilson, R.S., and May, E.L. Analgesic properties of the tetrahydrocannabinols, their metabolites, and analogs. J Med Chem 18:700-703, 1975.

Wilson, R.S.;’ May, E.L.; Martin, B.R.; and Dewey, W.L. 9-Nor-9-hydroxyhexahydrocannabinols. Synthesis, some behavioral and analgesic properties, and comparison with the tetrahydrocannabinols. J Med Chem 19:1165-1167, 1976.

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Inhibitory and Stimulatory Effects of Cannabinoids on Eicosanoid Synthesis

Sumner H. Burstein, Ph.D.

BACKGROUND

Introduction

More than a decade ago, we suggested that some of the actions of tetrahydrocannabinol (THC) were mediated by changes in prostaglandin synthesis. This was based on a comparison of the literature from both fields and the results of pilot experiments in our laboratory (Burstein and Raz 1972). Much evidence in support of this hypothesis has since been reported both by us and by others. At this point, it appears that such a mechanism is still quite viable and can provide a rational framework for new experiments aimed at developing a mechanism of action for THC (for a review, see Martin 1986).

Role of Prostaglandins in the Brain

Arachidonic acid, the precursor of all eicosanoids, is a major lipid component of brain, and the ability of this organ to synthesize eicosanoids is well established (Chiu and Richardson 1985). In addition, peripherally administered prostaglandins are known to produce potent effects on central nervous system (CNS) function (Curro et al. 1982) and can cross the blood-brain barrier quite readily (Bito et al. 1976). Moreover, evidence for the existence of receptors in the brain for several of the prostaglandins has been reported, although their functional significance is not clear at present (Malet et al. 1982; Shimizu et al. 1982).

Of particular interest to this discussion are reports that both centrally and peripherally injected prostaglandins can produce behavioral changes in rodents (Poddubiuk 1976; Hollingsworth and Patrick 1984; Laychock et al. 1980). Sedation and the inhibition of locomotor activities are prostaglandin-induced responses which have been known for some time (Gilmore and Shaikh 1972). The molecular mechanisms accompanying these effects are not well understood. However, there is some evidence suggesting that prostaglandins regulate neurotransmitter levels and/or calcium transport in neuronal tissue (Bergstrom et al. 1973).

In Vitro Evidence for the Hypothesis

The data reported thus far from our laboratory using a cell culture model are strongly supportive for a role for prostaglandin E_2 (PGE_2) as a mediator of THC action. The structure-activity relationships (SAR) (Burstein et al. 1983a, 1984a), the development of tolerance (Burstein et al. 1985), and the existence of stereospecificity (Burstein et al. 1986a) give a profile of activity similar to that for the psychotropic effects of THC in *vivo*. The properties of the other primary cannabinoids (CBD,CBN, etc.) in our model do not show a good correlation with psychoactivity (Burstein et al. 1983). However, these substances do have other activities which may be mediated by eicosanoids (Martin 1986). Clearly, any model has its limitations; however, this does not prevent its utilization as part of a broader approach to establishing a mechanism.

The experiments we have reported thus far using subcellular preparations from mouse brain demonstrate that THC can stimulate lipid turnover by increasing phospholipase activities (Hunter et al. 1986). We studied phospholipase A_2 (PLA_2) and phospholipase C (PLC) activity in synaptosomes, myelin and mitochondrial fractions using both exogenous and endogenous substrates containing radiolabelled arachidonate. Submicromolar levels of THC produced significant increases in PLA_2 activity in synaptosomes. Diacylglyceride (DAG) levels were also increased by THC, probably due to its inhibitory action on diglyceride lipase combined with its stimulatory action on phosphatidyl inositol (PI) hydrolysis by PLC.

Hillard and Bloom (1983) have suggested that there may be a role for prostaglandins in the effects of cannabinoids on adenylate cyclase activity, which they have observed. They reported that aspirin, indomethacin, and mepacrine all inhibited THC-induced stimulation of adenylate cyclase in homogenates of mouse cerebral cortex. It is well known that PGE_2 is an effective stimulator of cyclase in a variety of systems and that cAMP has an essential role in catecholamine-mediated neurotransmission.

Using an experimental design very similar to ours, White and Tansik (1980) reported stimulation of human platelet PLA_2 activity by THC and cannabidiol (CBD). The released arachidonate was converted primarily into lipoxygenase products. A similar finding was reported by Laychock et al. (1986) in which rat pancreatic islet preparations were exposed to THC. Again, when arachidonic acid levels were increased, it resulted in increased synthesis of 12-HETE which enhanced insulin secretion.

The synthesis of F-type prostaglandins by rat brain synaptosomes was reported to be inhibited by THC (ID_{50}, 750 μM) (Raffel et al. 1976). The high doses needed for this effect cast some doubt on its relevance to any *in vivo* action of THC. In a symposium report, Howes and Osgood (1976) described a THC-induced inhibition of PGE_1 synthesis in rat striatal synaptosomes with an ID_{50} of approximately 0.5 uM.

In Vivo Evidence for the Hypothesis

Some time ago, Jordan and Castracane (1976) reported that THC produced a 50% rise in PGE levels in rat uterine venous blood. The animals were given a dose of 40 mg/kg following estradiol stimulation, and samples of blood were taken by means of a uterine vein cannula. They raised the possibility that some of the actions of THC were due to its ability to increase PGE levels.

Dalterio et al. (1981) found that a complex response was produced by cannabinoid exposure in mice when pituitary and testicular levels of PGE and PGF were monitored. Significant increases or decreases could be observed depending on subtle experimental conditions. They concluded that, "prostaglandins may mediate some effects of cannabinoids on male reproductive function in mice."

We have provided evidence for a role for prostaglandins in the hypotensive action of THC in the dog (Burstein et al. 1982b). A THC-induced decrease in mean arterial blood pressure could be profoundly inhibited by the prior administration of aspirin. We postulated that the hypotensive effect of THC could be mediated by the release of prostacyclin in the cardiovascular system.

Fairbairn and Pickens (1979, 1980) presented convincing evidence that the cataleptic response to THC in mice is mediated by PGE_2. Animals treated with any of several prostaglandin synthetase inhibitors showed a reduced response to THC which could be restored by intraperitoneal injection of PGE_2. Moreover, mice placed on an essential fatty acid deficient diet showed little response to THC. However, the administration of arachidonic acid resulted in a rapid restoration of the cataleptic effect. These findings raise the possibility that the CNS effects of THC in humans may be mediated by eicosanoids.

A study in humans was recently reported (Laviolette and Belanger 1986) in which cannabis-induced bronchodilation was measured in a group of 11 volunteers. A second exposure to marijuana smoke was monitored in which the subjects were heavily dosed with aspirin over a 30-hour pretreatment period. No inhibition of bronchodilation was observed, prompting the authors to conclude that eicosanoids were not mediators of this effect of marijuana.

In the next section, we describe recent findings on the *in vitro* antagonism of THC by its major metabolite, THC-7-oic acid-(Burstein et al. 1986b). In subsequent experiments, mice were given oral doses of this acid (40 mg/kg), and a 50% reduction in the cataleptic effect of THC (50 mg/kg) was observed by us (see table 7). The most likely explanation for this result is that the metabolite blocks the conversion of arachidonic acid into PGE_2 based on our *in vitro* findings. Thus, THC is able to both stimulate and inhibit prostaglandin production via its metabolite depending on experimental parameters.

This possibility for dual effects could explain reports where there is an apparent reduction of prostaglandin synthesis following THC exposure. Ayalon et al. (1977) reported a decrease in rat ovarian PGE content after THC; Green and Podos (1974) similarly found that the arachidonic-induced rise in rabbit intraocular pressure was inhibited by intravenous THC. Hypothalamic "PGE_2-like material" in THC-treated rats was reduced in experiments reported by Coupar and Taylor (1982). The tissue extracts were monitored by a bioassay procedure using the rat gastric fundus strip which is not a particular specific assay for PGE_2. The same authors have subsequently reported (1983) that intestinal "PGE_2" is also reduced by THC.

In summary, a number of *in vitro* studies have demonstrated that cannabinoids are potent stimulators of eicosanoid synthesis. The data obtained from our cell culture model suggest that this effect is due to the action of the cannabinoids on the phospholipases which control the levels of free arachidonic acid needed for prostaglandin production. In certain instances, some cannabinoids may inhibit prostaglandin synthesis, indicating the possibility of complex interactions. The *in vivo* support for the "prostaglandin hypothesis" is less complete at this time. Most convincing are the reports by Fairbairn and Pickens (1979, 1980) which strongly suggest that PGE_2 is an important mediator in THC-induced catalepsy in mice. While this may or may not be related to THC effects on the human psyche, it does demonstrate a role for prostaglandins in a CNS action of THC.

RECENT FINDINGS

In Vivo Studies

Encouraging data in support of a role for prostaglandins in the actions of THC were obtained by us some time ago (Burstein et al. 1982b). In collaboration with members of the Pharmacology Department, we were able to demonstrate inhibition of THC-induced hypotension in dogs by the prior administration of aspirin. Such an effect is consistent with a mechanism in which THC stimulates prostacyclin synthesis in the cardiovascular system. Prostacyclin is a potent vasodilator and could well mediate the observed hypotensive effect of THC.

Evidence for a role for prostaglandins in the CNS actions of THC has been more difficult to obtain. Recently (Hunter et al., unpublished data), we have shown a 29% rise in mouse brain prostaglandins (table 1) following oral administration of Δ^1-THC (50 mg/kg). Prior administration of probenecid (120 mg/kg) was necessary, presumably to slow the rapid clearance of PGE_2 from the brain; however, this had no effect on arachidonate metabolism (table 2). We also utilized a double isotope labelling procedure to reduce the difficulties in the analysis of the brain extracts (table 3). This approach reduced the variability of results caused by losses during the extraction and chromatographic separations.

TABLE 1

Effect of Δ^1-Tetrahydrocannabinol on [^{14}C]-Arachidonate Metabolism in the Mouse Brain[a]

Metabolite	Control[b]	Treated[b]	Δ(%)	P(n=4)
Phospholipids	47.2 ± 5.8	39.3 ± 9.1	- 17	> .05
Prostaglandins	23.7 ± 13	30.5 ± 3.8	+ 29	< .01
Arachidonate	10.3 ± 2.9	10.5 ± 2.1	+ 1.7	> .05
Diglycerides	9.90 ± 3.9	12.8 ± 3.3	+ 29	> .05
Triglycerides	8.00 ± 4.3	6.10 ± 3.6	- 24	> .05

[a] Values are expressed as percentage of total radioactivity recovered from tlc ± S.D. P was determined by the Student's t test.

[b] Δ^1-Tetrahydrocannabinol (50 mg/kg) administered orally in sesame oil to the treated group. Control and treated groups received probenecid (120 mg/kg) i.p.

TABLE 2

Effect of Probenecid (120 mg/kg) on [^{14}C]-Arachidonate Metabolism in the Mouse Brain[a]

Metabolite	Control	Probenecid Treated	P(n=8)
Phospholipids	44.0 ± 12	46.4 ± 6.6	> .05
Prostaglandins	18.9 ± 6.9	18.8 ± 5.5	> .05
Diglycerides	15.8 ± 4.6	14.8 ± 3.0	> .05
Triglycerides	8.5 ± 2.4	8.0 ± 2.9	> .05

[a] As in table 1.

Cell Culture Experiments

Our initial observations on the stimulatory effect of cannabinoids on prostaglandin synthesis were made using cells in culture (Burstein and Hunter 1978). More recently, we have described this effect in some detail and attempted to develop it into an in vitro model for the CNS actions of THC (Burstein et al. 1982a). Using such model systems, we plan to investigate the biochemical basis for the effects of THC on arachidonate turnover and metabolism.

TABLE 3

Effect of Δ^1-Tetrahydrocannabinol on [$^{14}C/^{3}H$]Arachidonate Metabolism in the Mouse Brain

Metabolite	Isotope Ratio[a]		Δ(%)	P(n=4)
	Control[b]	Treated[b]		
"Phospholipids"	0.52 ± 0.15	0.69 ± 0.04	+ 34	< 0.0005
$PGF_{2\alpha}$	1.19 ± 0.22	1.16 ± 0.05	-2.5	> 0.05
TXB_2	1.23 ± 0.33	1.32 ± 0.09	+ 7.5	> 0.05
PGE_2	0.99 ± 0.21	1.24 ± 0.18	+ 25	< 0.05
HETEs	0.35 ± 0.37	0.38 ± 0.18	+ 7.2	> 0.05
PGA_2 and HETEs	1.26 ± 0.20	1.58 ± 0.34	+ 25	> 0.05
Arachidonate	1.24 ± 0.21	1.08 ± 0.23	- 13	> 0.05
Neutral lipids	0.70 ± 0.35	1.02 ± 0.36	+ 46	> 0.05

[a] Values are given as the ratios of the percentage of total radioactivity recovered from tlc for each isotope ± S.D.

[b] As in table 1.

For a variety of reasons, we have primarily utilized the WI-38 human lung fibroblast cell line for our studies. This cell line is the most THC-sensitive of those we have tested, which included mouse neuroblastoma cells, mouse glioma cells, human skin fibroblasts, and HeLa cells. Moreover, it produces predominantly PGE_2 in response to a variety of agonists, including the cannabinoids. We have also demonstrated that mouse peritoneal macrophages are a suitable cell type for studying cannabinoid effects on arachidonate metabolism by lipoxygenases (Burstein et al. 1984a).

Dose-response relationships were reported for both primary cannabinoids and several metabolites of Δ^1-THC in which we measured the release of free arachidonic acid and the levels of PGE_2 in WI-38 cells (Burstein et al. 1983). Responses were obtained starting at around 0.8 μM and a good correlation was found between PGE_2 synthesis and arachidonate release, suggesting that, as expected, the two events are related. Of particular interest was the finding that, within the THC series, a good SAR could be seen when PGE_2 levels were compared with literature reports on the subjective "high" in humans and in the monkey behavioral assay. A similar SAR was demonstrated with mouse peritoneal cells showing that this was not a special feature of WI-38 fibroblasts (Burstein et al. 1984a).

A wide range of agents were shown to be effective in blocking the stimulatory actions of THC in cell culture, including phospholipase inhibitors (such as mepacrine and dexamethasone) and cyclooxygenase inhibitors (such as aspirin, indomethacin, and naproxen). Lysolecithin acyltransferase inhibitors, such as p-hydroxymercuribenzoate, were shown to greatly augment the stimulatory effects of THC, presumably by preventing released arachidonate from being incorporated into phospholipids that would render it unavailable for conversion to eicosanoids (Hunter et al. 1984).

Tolerance to the stimulatory action of THC could also be readily observed in our model system (Burstein et al. 1985). Three successive 30-minute exposures with 24-hour intervals in THC-free media reduced the response to control levels. The effect could also be seen when arachidonate release was monitored, and it was reversed by allowing a 5-day recovery period.

We have recently examined the stereospecificity of these effects again using WI-38 cells as the model Burstein et al. 1986a). Three pairs of enantiomers (Δ^1-THC, Δ^6-THC, and Δ^6-dimethylheptyl-THC) were studied and all three showed statistically significant twofold to threefold greater potency for the (-) natural isomer in stimulating PGE_2 synthesis. Interestingly, we did not observe a significant effect when arachidonate release was measured. A possible explanation is that PGE_2 is derived from a single stereoselective lipid pool; the free arachidonate we measured was released from several pools, some of which were not stereoselective.

As an offshoot of our research, we tested the possibility of using the lipid mobilizing properties of the cannabinoids to provide symptomatic relief for certain of the lipid storage diseases, such as Niemann-Pick. Cells derived from Niemann-Pick patients when treated with cannabinoids showed a favorable decrease in sphingomyelin content (Burstein et al. 1984b). Cannabidiol (which is nonpsychoactive) was particularly effective, thus raising the possibility of its use in managing patients with such problems.

Brain Subcellular Experiments

Synaptosomes, myelin, and mitochondria were prepared from whole mouse brain, and cannabinoid effects on their lipases were studied (Hunter et al. 1986). PLA_2 activity was increased in the presence of THC and its metabolites and the effect exhibited structural specificity (table 4). Synaptosomal PLC-mediated hydrolysis of phosphatidyl inositol (PI) was also stimulated by THC (table 5), giving rise to increased levels of DAG. This is of interest since DAG has a potent effect on C-kinase activity which has been linked to neurotransmitter action. In addition, THC inhibited DAG lipase activity, which could amplify the PLC effect.

Metabolite Studies

As part of our long-standing general interest in the metabolites of THC, we tested several of the available examples for interactions with THC in our model system. The simplicity of the model allowed us to examine a number of metabolites over a range of doses with relative ease (table 6). It became immediately apparent that the carboxyl-containing metabolites, in particular, Δ^1-THC-7-oic acid, were antagonists to the stimulatory action of THC on PGE_2 synthesis (Burstein et al. 1986a).

A kinetic analysis of the inhibition suggested that it was noncompetitive. The structure of Δ^1-THC-7-oic-acid is similar to many of the nonsteroidal antiinflammatory agents, prompting us to test it for cyclooxygenase inhibition. The metabolite showed potent inhibitory action in several different preparations with cyclooxygenase activity. Moreover, it had no effect on the release of arachidonate from labelled WI-38 cells, showing that it has a specific action in antagonizing the effect of THC.

An obvious question was whether this antagonism would manifest itself _in_ _vivo._ The cataleptic response to THC in mice has been suggested to involve prostaglandins as mediators. We established the procedure as described by Pertwee (1972) in our laboratory and treated mice with the 7-oic acid (40 mg/kg) orally followed by Δ^1-THC (50 mg/kg) orally (Burstein et al., unpublished data). The cataleptic effect was inhibited by at least 50% by this procedure, showing that the metabolite can greatly affect this particular CNS action of THC (table 7).

SUMMARY

Experimental support for a role for prostaglandins in the actions of THC has been greatly expanded by data from our laboratory during the last 5 years. The majority of the data has been obtained using a cell culture model. However, important findings have also come from _in_ _vivo_ experiments as well as subcellular preparation studies. We have shown that aspirin can inhibit the hypotensive action of THC in dogs, which is consistent with a mechanism in which THC stimulates prostaglandin synthesis in the cardiovascular system. _In_ _vivo_ treatment of mice with THC was followed by a significant in the level of PGE_2 in whole brain extracts, suggesting a role for this eicosanoid in the CNS actions of THC.

We have also shown that our cell culture model displays several of the characteristics of THC action _in_ _vivo._ These include tolerance, stereospecificity, and an SAR which resembles that for THC and its metabolites in humans and in the rhesus monkey. These findings would seem to justify the use of this model for further experiments aimed at elucidating the biochemical events connected with THC action.

Lipid metabolism in brain preparations such as synaptosomes have been found by us to be responsive to THC, which generates such potent mediators as arachidonate and DAG by stimulating the

TABLE 4

Effects of Cannabihoids on Arachidonic Acid Formation From [1-^{14}C] Arachidonylphosphatidylcholine by Synaptosomes

Cannabinoid	μM	Arachidonic Acid (dpm ± SD)	Change (%)
Vehicle	-	302 ± 14	-
Δ^1-THC	0.32	379 ± 40	25.4
Δ^1-THC	0.8	370 ± 20	22.4
Δ^1-THC	1.6	353 ± 15	16.7
Δ^1-THC	3.2	368 ± 43	21.9
Δ^1-THC	8.0	423 ± 35	40.1
Δ^1-THC	16.0	397 ± 38	31.3
7-oxo-Δ^1-THC	1.6	357 ± 32	18.3
7-oxo-Δ^1-THC	3.2	303 ± 16	0.3[a]
7-oxo-Δ^1-THC	8.0	371 ± 11	22.8
7-oxo-Δ^1-THC	16.0	351 ± 20	16.1
7-OH-Δ^1-THC	8.0	464 ± 16	53.8
7-OH-Δ^1-THC	16.0	503 a 26	66.5
Δ^1-THC-7-oic acid	8.0	279 ± 15	-7.8
$\Delta 1$-THC-7-oic acid	16.0	364 ± 24	20.4
6α-OH-Δ^1-THC	8.0	284 ± 15	-5.9
6α-OH-Δ^1-THC	16.0	360 ± 31	19.0
6β-OH-Δ^1-THC	8.0	298 ± 15	-1.3[a]
6β-OH-Δ^1-THC	16.0	308 ± 17	2. 1[a]

Synaptosomes (0.22 mg protein) were incubated with [1-^{14}C]arachidonylphosphatidylcholine (35,000 dpm) in 1 ml of 5 mM-$CaCl_2$:0.05 M Tris-HCl buffer (pH 7.4) for 45 min. Cannabinoids were added in 10 μl ethanol. Arachidonic acid levels were obtained by hexane extraction of the incubate. Values represent mean (dpm) ± SD of triplicate samples. All values were significantly different from the vehicle, $p < 0.0005$ by Student's t test. [a] Not significant.

TABLE 5

Synaptosomal Hydrolysis of [1-^{14}C]Arachidonylphosphatidylinositol

Cannabinoid	μM	Product Formation	
		Diacylglyceride (%)	Arachidonic acid (%)
Control		10.9 ± 9 (4)	2.5 ± 0.4 (4)
Δ^1-THC	1.6	25.2 ± 2.3 (3)[a]	2.5 ± 0.6 (3)[d]
Δ^1-THC	8	30.4 ± 1.8 (3)[b]	3.2 ± 0.8 (3)[d]
7-COOH-Δ^1-THC	8	18.2 ± 2.4 (3)[c]	2.2 ± 0.1 (3)[c]

Synaptosomes (0.1 mg protein per ml) were suspended in 5 mM $CaCl_2$: S.T.E.M. buffer (pH 7.4) and incubated for 30 min with [1-^{14}C]arachidonylphosphatidylinositol (25,000 dpm) and either cannabinoid or vehicle (10 μl). The values are the percentage mean (dpm) ± SD of the number of determinations given in arentheses. Values were significantly different from control: [a] $p < 0.005$; [b] $p < 0.0005$; [c] $p < 0.01$; [d] Not significant.

appropriate lipases. This showed that the brain responds in a manner analogous to other tissues with regard to lipid mobilization.

A surprising and potentially important discovery was made using the cell culture model we have developed. The major metabolite of Δ^1-THC, Δ^1-THC-7-oic acid, was found to antagonize the stimulatory action of THC by inhibiting a specific step in the synthesis of prostaglandins. We were also able to demonstrate antagonism by this metabolite <u>in vivo</u> using THC-induced catalepsy in the mouse as the biological parameter. We propose, based on these observations, that tissue levels of this metabolite will have a profound influence on THC responses. Future pharmacokinetic studies should consider this possibility since the acid has been shown to be present in amounts comparable and even exceeding those of THC itself. In passing, it is worth noting that THC exhibits potent antiinflammatory activity in animal models (Sophia et al. 1973a, 1973b) which can now be readily explained by a metabolite that inhibits prostaglandin synthesis. Finally, all of these facts are most consistent with a mechanism of action in which THC stimulates the production of prostaglandins and other eicosanoids.

TABLE 6

Interactions of Δ^1-THC with Its Metabolites on PGE_2 Synthesis in Fibroblasts*

Metabolite	Δ^1-THC Concentration (μM)			
	0.32	0.80	1.6	3.2
6 α-OH-Δ^1-THC (3.2 μM)	0.93	0.93	1.76	0.99
6 α-OH-Δ^1-THC (8.0 μM)	1.15	0.93	1.26	0.89
1"-OH-Δ^1-THC (3.2 μM)	1.31	1.44	0.93	1.07
1"-OH-Δ^1-THC (8.0 μM)	2.16	1.89	1.46	1.45
1"-oic Acid (16 μM)	0.96	0.93	1.43	1.56
2"-oic Acid (16 μM)	0.83	0.49	0.51	0.74
3"-oic Acid (16 μM)	1.43	1.14	1.20	0.87
7-oic Acid (8 μM)	-	0.28	0.28	0.27
7-oic Acid (16 μM)	-	< 0.15	0.13	0.11

* The metabolite was added to the cells 30 min prior to the addition of Δ^1-THC at the indicated concentrations. The values shown are the ratios of PGE_2 levels in treated over control cells; the controls consisted of cells to which vehicle (10 μl ethanol) had been added in place of a metabolite. PGE_2 was measured by RIA. Duplicate determinations were done on three individual monolayers.

TABLE 7

The Inhibition of Δ^1-THC Induced Catalepsy in Mice by Δ^1-THC-7-oic Acid

	Control	Treated	Pretreated
Experiment 1	0.05 ± 0.05 (4)	0.28 ± 0.02 (4)	0.11 ± 0.04 (5)
Experiment 2	0.09 ± 0.04 (3)	0.26 ± 0.03 (3)	0.19 ± 0.10 (5)
Experiment 3	0.08 ± 0.03 (4)	0.42 ± 0.01 (4)	0.19 ± 0.10 (4)
Mean	0.07 ± 0.02	0.32 ± 0.01*	0.16 ± 0.05*

Female, CD-1 mice (Charles River) weighing 20 to 25 grams were given Δ^1-THC (50 mg/kg) in sesame oil (10 µl) orally. One hour later the cataleptic effect was measured using the procedure described by Pertwee (1972). The "control" groups received only sesame oil and the "pretreated" groups were given Δ^1-THC-7-oic acid (40 mg/kg) in oil (50 µl) 1 hour prior to the administration of Δ^1-THC. The values are expressed as the means of the fraction of time the mice remained immobile ± S.D. Values in parentheses indicate the number of mice in each group. Asterisks denote a p value of < 0.01 as determined by a one-way analysis of variance (F = 26.4, DF = 2,33) and a post hoc Newman-Keuls test.

REFERENCES

Ayalon, D.; Nir, I.; Cordova, T.; Bauminger, S.; Pudar, M.; Naor, Z.; Kashi, R.; Zor, W.; Harell, A.; and Lindner, H.R. Acute effect of Δ^1-THC on the hypothalamic pituitary-ovarian axis in the rat. Neuroendocrinology 23:31-42, 1977.

Bergstrom, S.; Farnebo, L.; and Fuxe, K. Effect of PGE_2 on central and peripheral catecholamine neurons. Eur J Pharmacacol 21:362-368, 1973.

Bito, L.Z.; Davson, H.; and Hollingsworth, J.R. Facilitated transport of PGs across the blood-brain barriers. J Physiol 256:273-285, 1976.

Burstein, S., and Hunter, S.A. Prostaglandins and cannabis-VI. Release of arachidonic acid from HeLa cells by Δ^1-tetrahydrocannabinol and other cannabinoids. Biochem Pharmacol 27:1275-1280, 1978.

Burstein, S., and Raz, A. Inhibition of prostaglandin E_2 biosynthesis by delta-1-tetrahydrocannabinol. Prostaglandins 2:369-374, 1972.

Burstein, S.; Hunter, S.A.; Sedor, C.S.; and Shulman, S. Prostaglandins and cannabis IX Stimulation of PGE_2 synthesis in human lung fibroblasts by Δ^1-THC. Biochem Pharmacol 31:2361-2365, 1982a.

Burstein, S.; Ozman, K.; Burstein, E.; Palermo, N.; and Smith, E. Prostaglandins and cannabis XI. Inhibition of Δ^1-THC-induced hypotension by aspirin. Biochem Pharmacol 31:591-592, 1982b.

Burstein, S.; Hunter, S.A.; and Ozman, K. Prostaglandins and cannabis XII. The effect of cannabinoid structure on the synthesis of prostaglandins by human lung fibroblasts. Mol Pharmacol 23:121-126, 1983.

Burstein, S.; Hunter, S.A.; Ozman, K.; and Renzulli, L. Prostaglandins and cannabis XIII. Cannabinoid-induced elevation of lipoxygenase products in mouse peritoneal macrophages. Biochem Pharmacol 33:2653-2656, 1984a.

Burstein, S.; Hunter, S.A.; and Renzulli, L. Stimulation of sphingomyelin hydrolysis by CBD in fibroblasts from a Niemann-Pick patient. Biochem Biophys Res Commun 121:168-173, 1984b.

Burstein, S.; Hunter S.A.; and Renzulli, L. Prostaglandins and cannabis XIV. Tolerance to the stimulatory actions of cannabinoids on arachidonate metabolism. J Pharmacol Exp Ther 235: 87-91, 1985.

Burstein, S.; Hunter, S.A.; Latham, V.; Mechoulam, R.; Melchior, D.L.; Renzulli, L.; and Tefft, R.E., Jr. Prostaglandins and cannabis XV. Comparison of enantomeric cannabinoids in stimulating PG synthesis in fibroblasts. Life Sci 39: 1813-1823, 1986a.

Burstein, S.; Hunter, S.A.; Latham, V.; and Renzulli, L. Prostaglandins and cannabis XVI. Antagonism of Δ^1-THC action by its metabolites. Biochem Pharmacol 35:2553-2558, 1986b.

Burstein, S.; Hunter, S.A.; Latham, V.; and Renzulli, L. A major metabolite of Δ^1-THC reduces its cataleptic effect in mice. Unpublished.

Chiu, E.K.Y., and Richardson, J.S. Behavioral and neurochemical aspects of prostaglandins in brain function. Gen Pharmacol 16:163-175, 1985.

Coupar, I.M., and Taylor, D.A. Alteration in the level of endogenous hypothalamic prostaglandins induced by Δ^1-tetrahydrocannabihol in the rat. Br J Pharmacol 76:115-119, 1982.

Coupar, I.M., and Taylor, D.A. Effect of Δ^9-tetrahydrocannabinol on prostaglandin concentrations and fluid absorption rates in the rat small intestine. J Pharm Pharmacol 35:392-394, 1983.

Curro, F.A.; Greenberg, S.; Gardier, R.W. Effect of prostaglandin on central nervous system function. In: Greenberg, S., Kadowitz, P.J. and Burks, T.F., eds. Prostaglandins. New York: Dekker, 367-405, 1982.

Dalterio, S.; Bartke, A.; Harper, M.J.K.; Huffman, R.; and Sweeney, C. Effects of cannabinoids and female exposure on the pituitary-testicular axis in mice: Possible involvement of prostaglandins. Biol Reprod 24:315-322, 1981.

Fairbairn. J.W.. and Pickens, J.T. The oral activity of Δ^1-THC and its-dependence on prostaglandin E_2. Br J Pharmacol 67:374-385, 1979.

Fairbairn, J.W., and Pickens, J.T. The effect of conditions influencing endogenous prostaglandins on the activity of Δ^1-THC in mice. Br J Pharmacol 69:491-493, 1980.

Gilmore, D.P., and Shaikh, A.A. The effect of PGE_2 in inducing sedation in the rat. Prostagladins 2:143-151, 1972.

Green, K.,and Podos, S.M. Antagonism of arachidonic acid-induced ocular effects by delta-1-THC. Invest Ophthalmol 13:422-429, 1974.

Hillard, C.J., and Bloom, A.S. Possible role of PGs in the effects of the cannabinoids on adenylate cyclase activity. *Eur J Pharmacol* 91:21-27, 1983.

Hollingsworth, E.B., and Patrick, G.A. Sedation produced by PGs is not a nonspecific fatty acid effect. *Psychopharmacology* 84:423-425, 1984.

Howes, J.F., and Osgood, P.F. Cannabinoids and the inhibition of prostaglandin synthesis. In: Nahas, G.G., ed. *Marihuana: Chemistry, Biochemistry and Cellular Effects.* New York: Springer-Verlag, 415-424, 1976.

Hunter, S.A.; Burstein, S.; and Sedor, C. Stimulation of PG synthesis in WI-38 human lung fibroblasts following inhibition of phospholipid acylation by p. hydroxymercuribenzoate. *Biochim Biophys Acta* 793:202-212, 1984.

Hunter, S.A.; Burstein, S.; and Renzulli L. Effects of cannabinoids on the activities of mouse brain lipases. *Neurochem Res* 11:1273-1288, 1986.

Hunter, S.A.;Renzulli, L.; and Burstein, S. Elevation of mouse brain PGE_2 levels by Δ^1-THC. Submitted for publication, 1987.

Jordan, V.C., and Castracane, V.D. The effect of reported PG synthetase inhibitors on estradiol-stimulated uterine prostaglandin biosynthesis in vivo in the ovariectomized rat. *Prostaglandins* 12:1073-1081, 1976.

Laviolette, M., and Belanger, J. Role of PGs in marihuana-induced bronchodilation. *Respiration* 49:10-15, 1986.

Laychock, S.G., Johnson, D.N., and Harris, L.S. PGD_2 effects on rodent behavior and EEG patterns in cats. *Pharmacol Biochem Behav* 12:747-754, 1980.

Laychock, S.G.; Hoffman, J.M.; Meisol, E.; and Bilgin, S. Pancreatic islet arachidonic acid turnover and metabolism and insulin release in response to Δ^9-THC. *Biochem Pharmacol* 35:2003-2008, 1986.

Malet, C.; Scherrer, H.; Saavedia, J.M.; and Dray, F. Specific Binding of PGE_2 to rat brain membranes and synaptosomes. *Brain Res* 236:227-233, 1982.

Martin, B.R. Cellular effects of cannabinoids. *Pharmacol Rev* 38:45-68, 1986.

Pertwee, R.G. The ring test: A quantitative method of assessing the "cataleptic" effect of cannabis in mice. *Br J Pharmacol* 46:753-763, 1972.

Poddubiuk, Z.M. A comparison of the central actions of PG A, E, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in the rat. *Psychopharmacology* 50:86-94, 1976.

Raffel, G., Clarenback, P., Peskar, B.A., and Hertting, G. Synthesis and release of prostaglandins by rat brain synaptosomal fractions. *J Neurochem* 26:493, 1976.

Shimizu, T., Namosluta, A.,and Hayaishi, O. Specific binding of PGD_2 to rat brain synaptic membrane. *J Biol Chem* 257:13570-13575, 1982.

Sofia, R.D., Nalepa, S.D., and Harakal, J.J. Anti-edema and analgesic properties of delta-9-THC. *J Pharmacol Exp Ther* 186:646-655, 1973a.

Sofia, R.D., Knobloch, L.C., and Vassar, H.B. The anti-edema activity of various naturally occurring cannabinoids. *Res Commun Chem Pathol Pharmacol* 6:909-918, 1973b.

White, H.L.,and Tansik, R.L. Effects of Δ^9-THC and CBD on phospholipase and other enzymes regulating arachidonate metabolism. *Prostaglandins Med* 4:409-411, 1980.

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Pharmacokinetics and Disposition of Cannabinoids

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INTRODUCTION

Cannabis was used for fiber or medicinal purposes for centuries (Camp 1936). Recently, it has been widely used for recreational purposes, due largely to its euphoric effect. Cannabis contains hundreds of compounds. Among them, a class of compounds unique to cannabis has been identified as cannabinoids, which have been associated with cannabis activities. So far, over 60 naturally occurring cannabinoids have been identified in cannabis (Turner et al. 1980). In addition, a large number of cannabinoids have been synthesized for structure-activity studies and for the development of therapeutic drugs. Due both to concerns about health hazards related to the social use of marijuana and to the potential use of cannabinoids for therapeutic purposes, extensive research on their chemistry, pharmacokinetics and disposition, pharmacology, cellular effects, and therapeutic effects has been carried out and recently reviewed (Razdan 1986; Agurell et al. 1986; Dewey 1986; Martin 1986; Hollister 1986).

As information on the pharmacokinetics and disposition of cannabinoids is important for understanding their use and abuse, this chapter summarizes current understanding of the pharmacokinetics and disposition of these substances. The cannabinoids to be discussed include delta-9-tetrahydrocannabinol (THC), the major psychoactive component In cannabis; cannabinol (CBN) and cannabidiol (CBD), which are usually abundant in plants that are rich in THC; and nabilone, a synthetic cannabinoid approved for therapeutic use. The major focus will be on THC, as it is the most widely used cannabinoid and has been the subject of extensive research. In addition to pharmacokinetics, regulatory requirements for the approval of drugs by the United States Food and Drug Administration (FDA) will also be reviewed briefly.

PHARMACOKINETICS AND DISPOSITION

TETRAHYDROCANNABINOL

Delta-9-tetrahydrocannabinol, the major psychoactive component of marijuana, has been shown to have a broad range of cellular, biological, and behavioral effects (Martin 1986; Dewey 1986). It was recently approved for therapeutic use in the treatment of nausea and emesis caused by cancer chemotherapy (Hollister 1986). The health hazards from the social use of marijuana are a major concern. In particular, marijuana use has been associated with decrements in behavior and cognitive performance. For example, safe operation of motor vehicles or machinery at the workplace when a person is under the influence of THC is a major concern. There is an urgent need for the interpretation of a certain concentration of THC in urine or blood as related to the pharmacological effects, such as performance impairment. So far, data from controlled clinical studies indicate that there is a correlation for both the "high" effect and the performance impairment with plasma THC concentrations (Chiang and Barnett 1984; Barnett et al. 1985) when a pharmacokinetlc and a pharmacodynamic analysis are performed on a series of plasma samples. However, the prediction of the extent of high effect or impairment from a single plasma level of THC cannot be established (Hollister et al. 1981) because of the complex nature of the pharmacokinetics and pharmacodynamics of THC (Agurell et al. 1986; Mason and McBay 1985). Further research is still needed in these areas.

Pharmacokinetic Parameters

Half-Life. Following an intravenous dose of THC, plasma levels of THC decline very rapidly over the first few minutes and then decline at slower rates. A four-exponential equation with a four-compartment model, proposed by Hunt and Jones (1980), indicates that there are at least four different composites of tissues into which THC is distributed with different rates of permeation and different tissue binding strengths. The averages of the associated disposition half-lives are 1 minute, 4 minutes, 1 hour, and 19 hours. The half-lives of 1 minute, 4 minutes, and 1 hour are attributable to the rapid distribution of THC into different tissues as well as to the rapid metabolism of THC. At approximately 6 hours after an intravenous dose, a psuedoequilibrium is reached between plasma and tissues.

After the pseudoequilibrium is reached, THC is then slowly eliminated. This slow elimination is primarily due to the slow return of THC from sequestered tissues to blood. The terminal half-life of THC is approximately 1 day. Hunt and Jones (1980) suggested a terminal half-life of 19 hours: Ohlsson et al. (1982), of greater than 20 hours; and Wall et al. (1983), of 25 to 36 hours. There are no significant differences in plasma profiles for chronic and infrequent users or in half-lives in moderate users before and after a 2-week exposure of THC (Ohlsson et al. 1982; Hunt and Jones 1980). The difference between the earlier reported terminal half-life of 28 hours for chronic marijuana users and 57 hours for naive smokers, in studies of Lemberger et al. (1970, 1971a), could be due to relatively nonspecific analytical methods used for their pioneering studies.

Volume of distribution. The volume of distribution of THC is estimated to be 10 1/kg (Hunt and Jones 1980: Wall et al. 1983). which is over 200 time; the plasma volume. This large volume of distribution is commonly seen for lipophilic drugs, where the majority of the drug in the body is in tissues and only a small fraction is in the blood. In blood, THC is preferentially distributed in plasma and only about 10% in the red blood cells (Garrett and Hunt 1974; Widman et al. 1974).

Clearance. The average plasma clearance is about 605-980 ml/min (Hunt and Jones 1980; Ohlsson et al. 1982) and estimated blood clearance of 1.0 to 1.6 l/min is close to the hepatic blood flow. This suggests that the rate of metabolism of THC is clearly dependent on hepatic blood flow to the liver, and enzyme induction/inhibition appears to have little effect on the metabolic clearance of THC (Hunt and Jones 1980). Because of the high metabolic clearance, part of the drug absorbed from the gastrointestinal tract is metabolized in the liver before it enters the systemic circulation. As a result, the bioavailability of THC is low and is in the range of 10% to 20% (Wall et al. 1983).

Distribution

In blood, THC is extensively bound, approximately 97% to 99%, to plasma proteins and only limited amounts of THC (about 10%) are present in red blood cells (Garrett and Hunt 1974; Widman et al. 1974).

Because THC is a highly lipophilic compound, it is readily distributed into tissues from blood circulation. THC concentrations in brain reach a maximum, about three to six times higher than in plasma, within 30 minutes (the first sampling time after drug administration) after a single intravenous dose of THC in mice (Harvey et al. 1984). THC brain concentrations roughly parallel the plasma concentrations. This suggests that there is no barrier for the partition of THC into brain and that an equilibrium between the brain and plasma is quickly reached.

Earlier studies in animals using labeled THC demonstrated that tissue levels of THC (in most cases, of THC and metabolites) were higher in fat, liver, heart, and lung than in brain and blood (Ryrfeldt et al. 1973; Kreuz and Axelrod 1973; Leighty 1973; Siemens et al. 1979). A recent study in rabbits, using a more specific GC-MS method for the assay of unchanged THC, also suggests that much higher concentrations of THC are found in fat and heart than in brain (Leuschner et al. 1986). THC concentrations in fat are about 1,000 times those in plasma, while in brain they are only about 3 to 10 times those in plasma. This is consistent with the fact that THC is a very lipophilic compound and, therefore, accumulates in fatty tissues. The relatively low THC levels in brain, in contrast to what would be expected from the partition coefficient, are primarily due to the fact that the brain is well perfused and the blood flow moves THC in and out of the brain very rapidly.

Animal studies indicate that THC passes through the placenta and affects fetal development (Lee and Chiang 1985). THC levels in fetal tissues and blood are shown to be lower than in maternal tissues for dogs and sheep (Martin et al. 1977; Abrams et al. 1985). In humans, THC is also found in cord blood, and the cord blood levels are three to six times lower than in maternal blood (Blackard and Tennes 1984). However, its effects on the fetus are not yet fully understood. THC is also secreted in milk at a higher concentration than in plasma, which may be attributed to the high lipid content in milk (Perez-Reyes 1982).

Metabolism

Metabolism is the major route for the elimination of THC from the body; only negligible amounts of this substance are excreted as unchanged THC. So far, approximately 80 metabolites of THC have been reported from studies in humans, animals, and in vitro liver preparations (Agurell et al. 1986). In humans, over 20 metabolites have been identified in urine and feces (Halldin et al. 1982a, 1982b; Widman et al. 1985; Wall and Perez-Reyes 1981). Still, a significant number of the metabolites, in both urine and feces, have not been identified.

The metabolism of THC in humans involves allylic oxidation, epoxidation, aliphatic oxidation, decarboxylation, and conjugation (Burstein 1985; Harvey 1984; Widman et al. 1985). Allylic oxidation at C-8 and C-11 and aliphatic oxidation at the side chain result in the formation of hydroxylated metabolites. The mono- and di-hydroxy metabolites are further oxidized to form acid and hydroxy-acid.

Allylic oxidation at C-11 position is the major metabolic pathway, and the resultant metabolite 11-OH-THC is identified as a predominant metabolite from in vitro studies using human liver preparations. This metabolite is also found in significant amounts in feces (Halldin et al. 1982c; Wall and Perez-reyes 1981). 11-Nor-THC-9-COOH, produced from the oxidation of 11-OH-THC, is a major metabolite identified in plasma, urine, and feces (Widman et al. 1985; Wall and Perez-Reyes 1981; Wall et al. 1983). The side chain is also easily degraded to form decarboxylated metabolites, and 4',5'-bisnor-THC-11,3'-dioic acid is the second most abundant metabolite found in urine.

Conjugation with glucuronide acid may occur to THC and to hydroxylated and carboxylated metabolites. Glucuronic acid conjugates of 11-nor-THC-9-COOH and THC have been identified in human urine (Halldin et al. 1982a, 1982b; Widman et al. 1985; Wall and Perez-Reyes 1981). Epoxide of THC (11-OH-epoxyhexahydrocannabinol) is found in trace amounts in the liver preparation (Halldin et al. 1982c).

Pharmacological activities of metabolites have been studied in humans. The major metabolite 11-nor-THC-9-COOH is found to be inactive, while some hydroxylated metabolites are found to be pharmacologically active (Perez-Reyes et al. 1973; Perez-Reyes 1985). 11-OH-THC, another major metabolite, produces pharmacological activity similar to THC and follows similar disposition (Lemberger et al. 1972; 1973; Perez-Reyes et al. 1972; Perez-Reyes 1985). 8-Beta-OH-THC is less potent and 8-alpha-OH-THC is essentially inactive in producing THC-like activity (Perez-Reyes 1985).

The activity of side-chain hydroxylated metabolites has not been studied well in humans. Pharmacological tests in dogs and mice indicate that 3'-OH-THC has a pharmacological profile similar to that of THC and is about 2 to 3 times more potent than THC, while the dihydroxy metabolite 3',11-dihydroxy-THC is less potent than THC (Martin et al. 1984).

Excretion

About 80% to 90% of the dose of THC is excreted as metabolites within 5 days, 65% in feces and 20% in urine. Only negligible amounts of THC were recovered as unchanged drug (Hunt and Jones 1980; Wall et al. 1983). Since a significant portion of the metabolites is excreted in the feces, enterohepatic recirculation (which may also be a contributing factor for the slow elimination of a drug) of THC metabolites in humans is also possible. Studies in dogs indicate that 10% to 15% of the dose of THC is enterohepatically recirculated (Garrett and Hunt 1977).

Among the metabolites, 11-nor-9-THC-COOH is a major metabolite identified in both urine and feces (Wall and Perez-Reyes 1981; Widman et al. 1985). Metabolites in the urine consist of primary acidic compounds; while the metabolites in feces consist of both acidic and neutral compounds. In urine, a total of 20 metabolites of THC, 2 as glucuronic acid conjugates and 18 as nonconjugated acids, have been identified. All of the other nonconjugated acidic metabolites identified in urine, except for 11-nor-9-THC-COOH, have their side chains further oxidized or degraded to form various hydroxylated or carboxylated metabolites (Widman et al. 1985). In contrast to urine, neutral metabolites appear in significant amounts in feces: the amount of 11-OH-THC is about the same as that of 11-nor-THC-9-COOH; and 8,11-dihydroxy, 8-alpha-OH, and 8-beta-OH metabolites are also found in significant amounts. However, one-third of the metabolites in feces are less well-defined acids (Wall and Perez-Reyes 1981). Glucuronic acid conjugates of THC and 11-nor-THC-9-COOH are found in urine (Widman et al. 1985). In general, the glucuronic acid conjugates are labile in the basic conditions and, hence, it is likely that most of the acidic metabolites identified could also be partly present in the original urine or feces as glucuronic acid conjugates which are later readily hydrolyzed during the experimental procedures.

Absorption

THC from various routes of administration is absorbed quite differently. Smoking marijuana cigarettes is the most common route for the self-administration of THC in the United States. In cancer treatment, THC capsules are ingested orally for the antiemetic effect. Parenteral injectables of THC are used primarily in research. As THC is practically insoluble in water (Garrett and Hunt 1974), organic vehicles or surfactants are generally employed to make parenteral solutions of THC. Commonly used vehicles for the intravenous administration of THC in humans are alcohol and albumin solution. For use in animals, many other vehicles--such as propyleneglycol, polyvinylpyrrolidone, Tween-80, and Emulohor--have been used for the parenteral preparations. The bioavailability and pharmacokinetics of THC from the different preparations are quite variable. As a result, the time profiles and the magnitude of pharmacological effects are also different.

Smoking. During the smoking process, part of the dose is lost by pyrolysis and from side stream smoke. In an in vitro smoking study (Davis et al. 1984) when cigarettes were consumed as a single puff to minimize side stream smoke, about 30% of THC was degraded during smoking. This suggests that the maximum amounts of THC absorbed from smoking will not exceed 70% of the THC content of cigarettes. When puff volume and puff duration of a typical marijuana smoker were simulated, about 16% to 19% of the THC in the cigarettes was recovered in the smoke condensate (Davis et al. 1984).

THC is readily absorbed from the lungs or respiratory tract into the systemic circulation. Peak plasma concentrations are rapidly reached, and the plasma profile is very similar to that of an intravenous dose (Ohlsson et al. 1982; 1985; Chiang and Barnett 1984). The bioavailability of THC from this route of administration is quite variable (Barnett et al. 1982; Lindgren et al. 1981; Ohlsson, et al. 1980; 1982; 1985; Perez-Reyes et al. 1982), as it is affected not only by the burning characteristics of the cigarettes, but also by the depth of inhalation, puff duration, and other factors. Experienced smokers seem to inhale marijuana more efficiently than nonexperienced ones. The THC bioavailablity from actual smoking of a 1% to 2% marijuana cigarette is 16 to 40% for heavy users and 13% to 14% for light users (Ohlsson et al. 1982).

When cigarettes of different potencies are used, smokers may tend to titrate the amount of THC inhaled. In one study, subjects were instructed to smoke cigarettes containing 1.3%, 2.0%, and 2.5% of THC in their usual fashion (Perez-Reyes et al. 1982). Plasma THC levels increased with THC potencies, indicating that higher amounts of THC were absorbed from high potency cigarettes. However, the actual fractions of THC inhaled were relatively less for high potency cigarettes. This self-adjustment of THC intake became more apparent in a study using marijuana cigarettes of 1% and 3.8% THC. The actual amount of THC inhaled from a 3.8% cigarette was about 1.8 times of that from a 1% cigarette. That indicates that the fraction of THC inhaled from a 3.8% cigarette was only about half of that estimated for a 1% cigarette (Perez-Reyes 1985).

Oral Administration. The THC oral dosage form that is currently available in the market is a soft gelatin capsule containing THC in sesame oil. The absorption of this dosage form is slow, the peak THC levels are reached at about 2 hours, and the bioavailability is in the range of 10% to 20% (Wall et al. 1983). This low bioavailability of THC is primarily due to the "first-pass effect." In other words, a large fraction of THC is metabolized to metabolites in the liver before it reaches the systemic circulation, thus resulting in a small fraction of the administered dose of THC getting into the systemic blood. Consequently, the active metabolites appear in significant amounts in the systemic blood and therefore may contribute significantly to the effects of THC (Wall et al. 1983; Hunt and Jones 1980).

Parenteral administration. After an intravenous dose, THC is distributed instantly in the systemic circulation. Since THC is highly bound to plasma protein, a vehicle made from plasma protein (and premixed with THC) may compete with tissue uptake of THC. A study by Fehr and Kalant (1974) suggested that tissue distribution could be partly retarded when THC is administered in a vehicle made from plasma protein as compared with a vehicle made from polyethylene glycol.

Generally, intramuscular injectables of THC are used in animal studies. These injetables are commonly made in Tween-80 and in Emulphor. Studies in monkeys, using radioimmunoassay methods, suggest that the systemic absorption of THC from the Emulphor preparation is greater than from Tween-80 preparation (Perlin et al. 1985). Although peak levels attained for these two preparations are similar, times required to reach peak levels are different: 30 minutes for Tween 80 and about 2.5 hours for Emulphor.

Other routes of administration. THC is absorbed from the ophthalmic route of administration. Studies in rabbits indicate the absorption of THC in light mineral oil is slow, and peak levels are reached in about 1 hour and maintained for approximately 4 to 8 hours. The bioavailability is quite variable, ranging from 6% to 40%. (Chiang et al. 1983).

Rectal route of administration of THC has been evaluated for several suppository formulations using Carbowax, Witepsol, sesame oil, cocoa butter, and Cetomacrogol in monkeys (Perlin et al. 1985). No drug is detected in plasma, possibly due to the high lipophilicity of THC, which might prevent the partitioning of THC out of the vehicles.

Kinetics of the THC Metabolites

THC is rapidly and extensively metabolized. After in intravenous dose of THC. metabolites appear immediately in plasma, and peak levels are reached in about 15 to 30 minutes (Hunt and Jones 1980; Wall et al. 1983). The concentrations of metabolites reach levels equal to that of THC in about 20 minutes and, at 1 hour, the metabolite levels increase to about 5 to 10 times that of THC. A major portion of metabolites in plasma are polar and acidic compounds; neutral metabolites (mostly hydroxy metabolites) account only for about 10% of total metabolites. A significant fraction of metabolites in plasma has yet to be identified.

Consistent with the first pass-effect, ratios of concentrations of metabolites to THC in plasma for an oral dose are much higher than those for an intravenous dose (Wall et al. 1983). For example, the concentration levels of 11-nor-THC-9-COOH (a major metabolite that is devoid of THC activity) in plasma are about 2 to 3 times higher than that of THC after an intravenous dose and about 6 to 10 times after an oral dose. Plasma levels of the active metabolite 11-OH-THC are about 10% to 15% of THC levels for an intravenous dose and about 50% of THC levels for an oral dose. Also found in plasma are 8-alpha-OH, 8-beta-OH, and 8,11-di-OH metabolites. The levels of active metabolites 11-OH-THC and 8-beta-OH-THC from an intravenous dose or smoked dose may not contribute significantly to THC effects, but they could contribute significantly to THC effects from an oral dose.

Metabolites are also highly bound to plasma protein (88% to 99%). The terminal plasma half-life of metabolites from THC administration is approximately 50 hours, which is longer than that of THC. This slower elimination rate of THC metabolites is not due to the slower elimination rate of the metabolites, but rather to their slower rates of formation, which, in turn, depends on the slower return of THC from sequestered tissues to form metabolites (Hunt and Jones 1980).

NABILONE

Nabilone, a synthetic cannabinoid, is currently used as an antiemetic (Herman et al. 1979; Hollister 1986). Following an intravenous dose of nabilone, plasma levels sharply decline due to extensive tissue distribution and metabolism. The plasma half-life is reported to be about 2 hours. Metabolites have not been well identified for nabilone; however, it is suggested that the long duration of its action may be due to the active metabolites. Similarly to THC, 65% of dose of nabilone is excreted in feces and about 20% in urine within 4 days. Urinary metabolites are primarily polar, acidic metabolites. The half-life of total metabolites in plasma is about 35 hours. The oral absorption of nabilone preparation made from coprecipitation with polyvinylpyrrolidone is complete and the peak plasma levels are reached in 1 hour (Rubin et al. 1977).

CANNABIDIOL

Cannabidiol, one of the major cannabinoids found in cannabis, is shown to be devoid of any psychotomimetic activity in humans (Perez-Reyes et al. 1973; Perez-Reyes 1985), but it possesses significant anticonvulsant activity (Martin 1986). Plasma profiles of CBD following an intravenous dose are quite similar to those of THC. Plasma levels decline rapidly shortly after intravenous dose and decline at slower rates later. Similar to THC, plasma clearances of CBD are 960 to 1560 ml/min and terminal plasma half-life averages 24 hours (range 18 to 33 hours). Volume of distribution for CBD is estimated to be 30 l/kg (Ohlsson et al. 1986).

CBD, like THC, is extensively metabolized (Burstein 1985). Hydroxylation is a major metabolic pathway and occurs primarily at 11 position. The 11-OH metabolite is further oxidized to form dihydroxy and carboxy metabolites. 9-carboxy-CBD is a major metabolite identified in plasma. Similar to THC, the majority of the administered dose of CBD is excreted in feces and a relatively small fraction is excreted in urine (about 16% of the dose in urine and 33% in feces over 72 hours). Unlike THC, unchanged CBD is excreted in large portion in the feces (Wall et al. 1976).

After smoking, CBD is quickly absorbed into the systemic circulation. The bioavailabilities of CBD averaged 31% (range, 11% to 45%) (Ohlsson et al. 1986). Coadministration of CBD with THC may alter THC's effects (Hollister and Gillespie 1975; Karniol et al. 1974); it has minimal or no effects on plasma levels of THC, however, suggesting that there are no apparent pharmacokinetic interactions of CBD with THC (Agurell et al. 1981; Hunt et al. 1981).

CANNABINOL

Cannabinol is a major cannabinoid found in cannabis and has about one-tenth the activity of THC in humans (Perez-Reyes et al. 1973; Perez-Reyes 1985). Similar to THC, plasma levels of CBN following an intravenous dose decline rapidly shortly after the dose and then decline at slower rates (Ohlsson et al. 1984). CBN is also extensively metabolized (Burstein 1985). 9-COOH-CBN is a major metabolite identified in plasma. The majority of the dose of CBN is excreted in feces, while a relatively small fraction is found in urine: About 8% of the dose is found in urine and 35% in feces over 72 hours. Major metabolites found in feces for CBN are mono-OH-CBN, 9-COOH-CBN, and di-OH-CBN (Wall et al. 1976).

CBN is also quickly absorbed from smoking. The bioavailabilities of CBN average 41% (range, 8% to 77%) (Ohlsson et al. 1984, 1985). It is likely that a very limited pharmacokinetic interaction exits between CBN and THC, because the coadministration of CBN with THC results in plasma concentrations of THC slightly higher than, but not statistically significant from, THC concentrations obtained from THC administered alone (Agurell et al. 1981).

MEDICINAL ASPECTS OF CANNABIS

Several ancient civilizations used cannabis and related products for the "treatment" of a number of disease conditions, and the therapeutic claims were very extensive. As systematic investigation, isolation, and identification of the active ingredients proceeded and these ingredients were pharmacologically investigated, most of the earlier claims were not substantiated.

During the early sixties, Goni and Mechoulam (1964) identified delta-9-THC as the major active component of cannabis. Following their research, several other active ingredients were identified. Later, a number of analogs of delta-9-THC, cannabidiol and several closely related compounds, were synthesized and evaluated for therapy. Some examples of these synthetic compounds are nabilone, nabitan, levonantradol, and BRL 4664. Nabilone is currently available in Canada and Switzerland for the treatment of emetic effects of cisplatin in cancer chemotherapy. In the United States, the only two newly approved drugs to date for treatment of drug-induced emesis are delta-9-THC and nabilone.

Although pharmacological evaluations of several hundred analogs for various therapeutic purposes are not highly encouraging, considerable research is being carried out. Some of the therapeutic aspects that are considered for cannabinoids are:

- Antiemetic effects in cancer chemotherapy
- Reduction of intraocular pressure in glaucoma
- Anticonvulsant effects; and
- Miscellaneous minor uses--as an antiasthmatic, analgetic, and appetite stimulant, and for the treatment of insommia, hypertension, and anxiety.

It is expected that in the near future at least some synthetic analogs that are devoid of "cannabimimetic" (psychotropic) effects may be approved in the United States for therapeutic purposes. Hence, this is an opportune time to present the regulatory aspects of drug approval by the Food and Drug Administration.

REGULATORY ASPECTS OF DRUG APPROVAL

In order to understand the extensive procedures involved for FDA approval, a brief account of the FDA regulatory aspects will be helpful. Federal regulatory legislation started with the passage of the 1848 Import Drug Act. This was a result of finding that the quinine used by American troops in Mexico was adulterated. In 1906, the original Food and Drug Act was passed, which specified standards for purity, strength, and quality. In 1938, the Federal Food, Drug and Cosmetic Act was enacted. This legislation required clearance for safety of new drugs prior to distribution. Later, following the thalidomide tragedy in Europe in 1962, the Kefauver-Harris amendments were passed. These amendments require that, in addition to the safety of the drugs, their effectiveness must be demonstrated. For final approval of a drug by FDA for commercial distribution, the manufacturer must go through the IND and NDA stages.

INVESTIGATIONAL NEW DRUG (IND)

In order for a researcher or a commercial firm to establish the safety and efficacy of a compound, a "Notice of Claimed Investigational Exemption for a New Drug" (an IND) must be filed. The IND contains information on (1) all the components; (2) quantitative composition; (3) methods of preparation; (4) analytical methodology to assure identity, strength, and quality; (5) results of preclinical investigations; (6) a detailed clinical protocol of phase I, II, and III studies; and (7) other related information. After the sponsor or the applicant is confident from the animal and human trials that the product is safe and effective, an NDA may be filed.

NEW DRUG APPLICATIONS (NDA)

In 1985 the Food and Drug Administration published a comprehensive revision of the Code of Federal Regulations relating to the procedure for NDA submission and approval (Federal Register, Vol. 50, No. 36, p. 7542). The NDA contains information on each of the following disciplines: chemistry, clinical medicine, pharmacology, statistics, biopharmaceutics, and microbiology. The chemistry section should include:

1. Drug Substance--Names, formulas, physical stability, manufacturers, methods of manufacture, process controls, specifications, and analytical methods.

2. Drug Product--components, composition, specifications and analytical methods for components, manufacturers, methods of manufacture, packaging procedures, and in-process controls, specifications and analytical methods for drug product container--closure system, and stability.

All the material supplied is reviewed by FDA, and only after approval may the drug be sold in interstate commerce. For additional information, see Hoiberg and Rapaka (1986).

CONCLUSION

At present, research on the medicinal chemistry of cannabinoids is being extensively carried out. It is hoped that, in the not too distant future, this research will lead to the introduction of useful new therapeutic drugs to alleviate human suffering.

REFERENCES

Abrams, R.M.; Cook, C.E.; Davis, K.H.; Niedureither, K.; Jaeger, M.J.; and Szeto, H.H. Plasma-delta-9-tetrahydrocannabinol in pregnant sheep and fetus after inhalation of smoke from a marijuana cigarette. Alcohol Drug Res 6:361-369, 1985.

Agurell, S.; Carlsson, J.E.; Lindgren, J.-E.; Ohlsson, A.; Gillespie, H.; and Hollister, L. Interactions of delta-1-tetrahydrocannabinol with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography. *Experientia* 37:1090-1091, 1981.

Agurell. S.; Halldin, M.; Lindgren, J.-E.; Ohlsson, A.; Widman, M.; Gillespie, H.; and Hollister, L. Pharmacokinetics and metabolism of delta-1-tetrahydrocannabinoids with emphasis on man. *Pharmacol Rev* 38:21-43, 1986.

Barnett, G.; Chiang, C.N.; Perez-Reyes, M.; and Owens, S.M. Kinetic study of smoking marijuana. *J Pharmacokinet Biopharm* 10:495-506, 1982.

Barnett, G.; Licko, V.; and Thompson, T. Behavioral pharmacokinetics of marijuana. *Psychopharmacology* 85:51-56, 1985.

Blackard, C., and Tennes. K. Human placenta transfer of cannabinoids. *N Engl J Med* 311:797, 1984.

Burstein, B. Biotransformation of the cannabinoids. Barnett, G., and Chiang, C.N., eds. *Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs.* Foster City, CA: Biomedical Publications, 1985. pp. 396-414.

Camp, W.H. The antiquity of hemp as an economic plant. *J NY Bot Gard* 37:110-114, 1936.

Chiang, C.N., and Barnett, G. Marijuana effect and delta-9-tetrahydrocannabinol plasma level. *Clin Pharmacol Ther* 36:234-238. 1984.

Chiang, C.N.; Barnett, G.; and Brine, D. Systemic absorption of delta-9-tetrahydrocannabinol after ophthalmic administration to the rabbit. *J Pharm Sci* 72:136-138, 1983.

Davis, K.H., Jr.; McDaniel, I.A., Jr.; Cadwell, L.W.; and Moody, P.L. Some smoking characteristics of marijuana cigarettes. In: Agurell, S.; Dewey, W.L.; and Willette, R.E., eds. *Cannabinoids: Chemical, Pharmacologic and Therapeutic Aspects*. New York: Academic Press, 1984. pp. 97-109.

Dewey, W. Cannabinoid pharmacology. *Pharmacol Rev* 38:151-178, 1986.

Fehr, K.O., and Kalant, H. Fate of ^{14}C-delta-1-THC in rat plasma after intravenous injections and smoking. *Eur J Pharmacol* 25:1-8, 1974.

Garrett, E.R., and Hunt. C.A. Physicochemical properties, solubility, and protein binding of delta-9-tetrahydrocannabinol. *J Pharm Sci,* 63:1056-1064, 1974.

Garrett, E.R.. and Hunt, C.A. Pharmacokinetics of delta-9-tetrahydrocannabinol in dogs. *J Pharm Sci* 66:395-407, 1977.

Goni, Y., and Mechoulam, R. *J Am Chem Soc* 86:1646, 1964.

Halldin, M.M.; Andersson, L.K.R.; Widman, M.; and Hollister, L.E. Further urinary metabolites of delta-1-tetrahydrocannabinol in man. *Arzeimittelforsch* 32:1135-1138, 1982a.

Halldin, M.M.; Carlsson, S.; Kanter, S.L.; Widman, M.; and Agurell, S. Urinary metabolites of delta-1-tetrahydrocannabinol in man. *Arzeimittelforsch* 32:764-768, 1982b.

Halldin, M.M.; Widman, M.; Bahr, C.V.; Lindgren, J.E.; and Martin, B.R. Identification of in-vitro metabolites of delta-1-tetrahydrocannabinol formed by human liver. *Drug Metab and Dispos* 10:297-301, 1982c

Harvey, D.J. Chemistry, metabolism and pharmacokinetics of cannabinoids. In Nahas, G.,ed. *Marijuana in Science and Medicine*. New York: Raven Press, 1984. pp. 38-108.

Harvey, D.J .; Leuschner, J.T.A .; and Paton, W.D.M. Pharmacokinetics of delta-1-tetrahydrocannabinol in the mouse In: Harvey, D.J., ed. *Marijuana '84.* Oxford: IRL Press, 1984. pp. 77-84.

Herman, T.S.; Einhorn, L.H.; Jones, S.E.; Nagy, C.; Chester, A.B.; Dean, J.C.; Furnas, B.; Williams, S.D.; Leigh, S.A.; Dorr, R.T .; and Moon, T.E. Superiority of nabilone over prochlorperazine as an antiemetic in patients receiving cancer chemotherapy. *N Engl J Med* 300:1295-1297, 1979.

Hoiberg, C.P., and Rapaka, R.S., *Opioid peptides as drug products: FDA regulatory requirements*. In: *"Opioid Peptides: Molecular Pharmacology, Biosynthesis, and Analysis*". NIDA Research Monograph 70. DHHS Pub. No. (ADM)86-1455. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1986, pp. 385-404.

Hollister, L.E. Health aspects of cannabis. *Pharmacological Reviews* 38:1-20, 1986.

Hollister, L.E., and Gillespie, H. Interactions in man of delta-9-tetrahydrocannabinol. I. cannabinol and cannabidiol. *Clin Pharmacol-Ther* 18: 80-93, 1975.

Hollister, L.E.; Gillespie, H.K.; Ohlsson, A.; Lindgren, J-E.; Wahlen, A; and Agurell, S. Do plasma concentrations of delta-9-THC reflect the degree of intoxication? *J Clin Pharmacol* 21:171s-177s, 1981.

Hunt. C.A., and Jones. R.T. Tolerance and disposition of tetrahydrocannabinol in man. *J Pharmacol Exp Ther* 215:35-44, 1980.

Hunt, C.A .; Reese, R.T.; Herning, R.I.; and Bachman, J. Evidence that cannabidiol does not significantly alter the pharmacokinetics of tetrahydrocannabinol in man: *J Pharmacokin Biopharm* 9:245-260, 1981.

Karniol, I.G .; Shiralawa, I.; Kasinski, N.; Pfeferman, A.; and Carlini, I.G. Cannabidiol interferes with the effects of delta-9-tetrahydrocannabinol in man. *Eur J Pharmacol* 28:172-177. 1974.

Kreuz, D.S., and Axelrod, J. Delta-9-tetrahydrocannabinol: Localization in body fat. *Science* 179:391-393, 1973.

Lee, C.C., and Chiang. C.N. Maternal-fetal transfer of abused substances: Pharmacokinetic and pharmacodynamic data. In: Chiang, C.N. and Lee, C.C., eds. *Prenatal Drug Exposure: Kinetics and Dynamics.* NIDA Research Monograph 60. DHHS Pub. No. (ADM)85-1413. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1985. pp. 110-147.

Leighty, E.G. Metabolism and distribution of cannabinoids in rats after different methods of administration. *Biochem Pharmac* 22:1613-1621, 1973.

Lemberger, L.; Silberstein, S.D.; Axelrod, J.; and Kopin. I.J. Marijuana: Studies on the disposition and metabolism of delta-9-tetrahydrocannabinol in man. *Science* 170:1320-1322, 1970.

Lemberger, L .; Axelrod, J.; and Kopin, I.J.,Metabolism and disposition of tetrahydrocannabinol in naive subjects and chronic marijuana users. Annals New York Academy of Science 191:p.152-154, 1971a.

Lemberger, L.; Tomarkin, N.R.; Axelrod, J.; and Kopin, I.J. Delta-9-tetrahydrocannabinol: Metabolism and disposition in long-term marijuana smokers. Science 173;72-73, 1971b.

Lemberger, L .; Crabtree, R.E .; and Rowe, H.M. 11-hydroxy-delta-9-tetrahydrocannabinol: Pharmacology, disposition, and metabolism of a major metabolite of marijuana in man. Science 177:62-64, 1972.

Lemberger, L.; Martz, R.; Rodda, B.; Forney, R.; and Rowe, H. Comparative pharmacology of delta-9-tetrahydrocannabinol and its metabolite, 11-OH-delta-9-tetrahydrocannabinol. Journal of Clinical Investigation 52:2411-2417, 1973.

Leuschner, J.T.A.; Harvey, D.J.; Bullingham, R.E.S.; and Paton, W.D.M. Pharmacokinetics of delta-9-tetrahydrocannabinol in rabbits following single or multiple intravenous doses. Drug Met Disposition 14:230-238, 1986.

Lindgren, J-E.; Ohlsson, A.; Agurell, S.; Hollister, L.E.; and Gillespie, H. Clinical effects and plasma levels of delta-9-tetrahydrocannabinol in heavy and light users of cannabis. Psychopharmacology 74:208-212, 1981.

Martin, B.R. Cellular effects of cannabinoids. Pharmacological Reviews 38:45-74, 1986.

Martin, B.R.; Dewey, W.L.; Harris, L.S.; and Beckner, J.S. ^{3}H-delta-9-tetrahydrocannabinol tissue and subcellular distribution in the central nervous system and tissue distribution in peripheral organs of tolerant and nontolerant dogs. J Pharmacol Exp Thr 196:128-144, 1976.

Martin, B.R.; Dewey, W.L.; Harris, L.S.; and Beckner, J.S. ^{3}H-delta-9-tetrahydrocannabinol distribution in pregnant dogs and their fetuses. Res Commun Chem Pharmacol 17:457-470, 1977

Martin, B.R .; Harris, L.S.; and Dewey, W.L. Pharmacological activity of delta-9-THC metabolites and analogs of CBD, delta-8-THC, and delta-9-THC. In: Agurell, S.; Dewey, W.L.; and Willette, R.E., eds. Cannabinoids: Chemical, Pharmacologic and Therapeutic Aspects. New York: Academic Press, 1984. pp. 97-109.

Mason, A.P., and McBay, A.J. Cannabis: Pharmacology and interpretation of effects. J Forensic Sci 30:615-631, 1985.

Ohlsson, A.; Agurell, S.; Leander, K.; Dahmen, J.; Edery, H.; Porath, G.; Levy, S.; and Mechoulam, R. Synthesis and psychotropic activity of side chain hydroxylated delta-6-tetrahydrocannabinol metabolites. Acta Pharm Suec 16:21-33, 1979.

Ohlsson, A.; Agurell, S.; Lindgren, J-E.; Gillespie, H.K.; and Hollister, L.E. Pharmacokinetic studies of delta-1-tetrahydrocannabinol in man. In: Barnett, G., and Chlang, C.N., eds. Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs. Foster City, CA: Biomedical Publications, 1985. pp. 75-92.

Ohlsson, A.; Johansson, E.; Lindgren. J.E.; Agurell, S.; Gillespie, H.; and Hollister, L.E., Kinetics of cannabinol in man. In: Harvey, D.J., ed. Marijuana '84, Oxford: IRL Press Limited, 1984. p.63-68.

Ohlsson, A.; Lindgren, J-E.; Wahlen, A.; Agurell, S.; Hollister, L.E.; and Gillespie, H.K. Plasma delta-9-tetrahydrocannabinol concentrations and clinical effects after oral and intravenous administration and smoking. Clin Pharmacol Ther 28:409-416. 1980.

Ohlsson, A.; Lindgren, J.-E.; Andersson. S.; Agurell, S.; Gillespie, H.K.; and Hollister, L.E. Single dose kinetics of deuterium labeled cannabidiol in man after smoking and intravenous administration. Biomed Environ Mass Spectrom 13:77-83, 1986.

Ohlsson. A.; Lindgren, J.-E.; Wahlen, A.; Agurell, S.; Hollister, L.E.; and Gillespie, H.K. Single-dose kinetics of deuterium-labelled delta-1-tetrahydrocannabinol in heavy and light users. Biomedical Mass Spectrometry 9:6-10, 1982.

Perez-Reyes, M. Pharmacodynamics of certain drugs of abuse. In: Barnett, G., and Chiang, C.N., eds. Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs. Foster City, CA: Biomedical Publications, 1985. pp. 287-310.

Perez-Reyes. M. Presence of delta-9-tetrahydrocannabinol in human milk. N Engl J Med 307:819-820, 1982.

Perez-Reyes, M.; DiGuiseppi, S.; Davis, K.H.; Schindler, V.H.; and Cook, C.E. Comparison of effects of marijuana cigarettes of three different potencies. Clinical Pharmacology and Therapetics 31(5): 617-624, 1982.

Perez-Reyes, M.; Timmons, M.C.; Lipton, M.A.; Davis, K.H.; and Wall, M.E. Intravenous injection in man of delta-9-tetrahydrocannabinol and 11-OH-delta-9-tetrahydrocannabinol. Science 177:633-635, 1972.

Perez-Reyes, M.; Timmons, M.S.; Lipton. M.A.; Christensen, H.D.; Davis. K.H.; and Wall, M.E. A comparison of the pharmacological activity of delta-9-tetrahydrocannabinol and its monohydroxylated metabolites in man. Experientia 29:1009-1010, 1973.

Perlin, E.; Smith, C.G.; Nichols A.I.; Almirez, R.; Flora, K.P.; Cradock, J.C.; and Peck, C.C. Disposition and bioavailability of various formulations of tetrahydrocannabinol in rhesus monkey. J Pharm Sci 74:171-174, 1985.

Razdan, R.K. Structure-activity relationships in cannabinoids. Pharmacological Reviews 38:75-149, 1986.

Rubin, A.; Lemberger. L.; Warrick, P.; Crabtree, R.E.; Sullivan, H.; Rowe, H.; and Obermeyer, B.D. Physiologic disposition of nabilone, a cannabinol derivative in man. Clin Pharmacol Therap 22:85-91, 1977.

Ryrfeldt, A.; Ramsay, C.H.; Nilsson, I.M.; Widman, M.; and Agurell, S. Whole-body autoradiography of delta-1-tetrahydrocannabinol and delta-8-tetrahydrocannabinol in mouse. Acta Pharm Suec 10:13-28, 1973.

Siemens Doyle, O.L.; and Pryor, G.T. Determination of the disposition of ^{14}C-delta-9-tetrahydrocannabinol and ^{3}H-delta-9-tetrahydrocannabinol. Life Sciences 24:1261-1274, 1979.

Turner, C.E.; Elsohly, M.A.; and Boeren, E.G. Constituents of cannabis sativa L., XVII: A review of the natural constituents. Journal of Natural Products 43: 169-234, 1980.

Wall, M.E.; Brine, D.R.; and Perez-Reyes, M. Metabolism of cannabinoids in man. In: Braude, M.C., and Szara, S. eds. Pharmacology of Marijuana. New York: Raven Press, 1976. pp 93-113.

Wall, M.E., and Perez-Reyes, M. The metabolism of delta-g-tetrahydrocannabinol and related cannabinoids in man. J Clin Pharmacol 21:178s-189s, 1981.

Wall, M.E.; Sadler, B.M.; Brine, D.; Harold, T.; and Perez-Reyes, M. Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. Clin Pharmacol Ther 34:352-363, 1983.

Widman, M.; Agurell, S.; Ehrnebo, M.; and Jones, G. Binding of (+)-and (-)-delta-1-tetrahydrocannabinols and (-)-7-hydroxy-delta-1- tetrahydrocannabinol to blood cells and plasma proteins in man, Journal of Pharmacy and Pharmacology 26(11):914-916, 1974.

Widman, M.; Halldin, M.M.; and Agurell, S. Metabolism of delta-1-tetrahydrocannabinol in man. In: Barnett, G., and Chiang. C.N, eds. Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs. Foster City, CA:Biomedical Publications, 1985. pp. 415-426.

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Drug Abuse in India: Progress in Research

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INTRODUCTION

Intensive studies on drug abuse in India have been undertaken during the last two decades, but surveys on the use of cannabis and opium have been undertaken for about 100 years. One of the earliest and most exhaustive studies on drug dependence was conducted on the abuse of cannabis by the Indian Hemp Commission (published in 1894). Chopra and Chopra (1935) undertook a detailed survey of the opium habit in the country. In recent years, there have been changing trends in usage of drugs, and age-old culturally circumscribed barriers are being thrown aside.

It is proposed to include work done after 1970 only in the present review due to constraints of space, etc. These studies can be broadly classified in three major categories: 1) Epidemiological surveys; 2) Adverse effects of drug abuse; and 3) Experimental studies to investigate mechanisms involved in drug abuse, particularly in the case of cannabis and methaqualone. Some other miscellaneous problems have also been investigated.

EPIDEMIOLOGICAL STUDIES

The epidemiological surveys undertaken during this period differ widely in their sample size, parts of the country covered, type of population, etc. The studies can be classified in three major groups, depending upon the type of population surveyed: 1) Rural population; 2) Urban population; and 3) Select categories, like students, psychiatric patients, etc.

Chopra (1971) has analysed the factors motivating persons to drug abuse and has identified certain "beneficial" factors which promote drug abuse. The abuse in most cases starts with such objectives as facilitation of social interaction, enhancement of sensual pleasures, mystical or religious experience, and quasi-medical use (cannabis leaves are used to sharpen appetite, to relieve muscle and joint pains, etc.). Modern adolescents often use cannabis as part of a purely exploratory activity without any religious overtones.

Rural Population

These surveys have collected data on the variety of drugs being abused as well as the type of community structures, etc. According to an ICMR report (Drug

Addiction - An Overview) published in 1977, 40% of the rural population consumed tobacco. The major agents of abuse were alcohol (25.6%). opium (18.9%), barbiturates (6.2%). and cannabis (2.2%). The rather excessive use of barbiturates was surprising. The females had a much lower incidence than the males with all the agents. Several reports of various sample sizes have been published during the last few years. Their results have been summarised in table 1. In all of these surveys, the incidence has been much lower in the females. In an earlier survey of rural adolescents of Punjab, Mohan et al. (1978a) had interviewed 281 male subjects. The incidence of alcohol usage was 32.9% and another 0.8% were using opium, cannabis, or other agents. The abuse incidence was higher in illiterate youth. The overall percentage of abusers varied between 15% and 40%. Nandi et al. (1982) have brought out the difference in incidence between restrictive (where society has a strong disapproval) and permissive communities. Further, in the restrictive communities, the abuse was almost totally confined to the psychopaths (antisocial behaviour, from police records) as opposed to a more uniform distribution in the permissive societies. Mohan et al. (1980) point out that more than 50% of alcohol abusers start drinking before the age of 25 years.

TABLE 1

Incidence of Drug Abuse in Rural Communities of India

Investigator	Year	Sample size	Incidence of abuse	
			No.	%
Lal and Singh	1978	443	124	29.3
Sethi and Trivedi	1979	2,010	427	21.2
Mohan et al.	1980	3,600	1,309	36.4
Varma et al.	1980	1,031	409	39.7
Nandi et al.	1982	5,423	834	15.4
a. Restrictive community		2,804	45	1.6
b. Nonrestrictive community		2,619	789	30.1

Urban Population

Less data are available about the incidence of drug abuse in the general urban population, though specific subgroups, like students, have been more extensively studied. Thacore et al. (1971) investigated the incidence of drug abuse in 2,696 members of 497 families in Lucknow. The survey revealed an incidence of 18.55 per 1,000 population, most of which were cases of alcohol abuse. A significantly higher incidence was observed in the poor population, in unitary or small families, and in males; the majority of abusers fell in the age group of 26 to 45 years. Similar findings from other investigators have been reviewed by Sethi et al. (1984). Among sociodemographic correlates, the incidence is highest in semi-rural areas, followed by rural and urban areas in decreasing order. It is also common in industrial areas. A study team of the Planning Commission made a nationwide survey of the alcohol consumption habits and the results have been summarised in the ICMR report of 1977. It analysed the budgets of 21,197 working class families of 50 different centres. Between 10% and 24% of all working class families were given to drinking. The incidence was lowest among factory workers (9.6%) and highest among mine workers (23%). The incidence was higher in the northwestern part of the country and in those urban centres where there was a greater trend toward "pseudo-westernisation."

Drug Abuse in Students

A comprehensive survey of incidence of drug abuse among students in various parts of the country was undertaken by the Department of Social Welfare, Government of India, in 1976. Their results indicate that pain killers, tobacco, and alcohol had the highest incidence, followed by cannabis. The relative incidence as well as the total incidence varied and was higher in bigger cities like Bombay, Delhi, and Madras. Cannabis had the highest proportion of users at Varanasi, partly reflecting its widespread use and social acceptance in that region of the country. Mohan and his colleagues have undertaken a systematic study of drug abuse in school and college students. Their results, published in a series of papers, have been summarised in table 2. The data on tobacco have not been included in this and subsequent tables. The table also includes data on pre-university students from Chandigarh, where the incidence is much higher. A similar study of school children from other parts of the country will be useful. A comparison of findings in students of different age groups indicates only

TABLE 2

Incidence of Drug Abuse in University and College Students at Delhi

Agents	% Users			
	School	Pre-University*	University Colleges	University
Alcohol	27.9	51.2	24.1	23.5
Amphetamines	4.9	19.1	2.0	3.6
Cannabis	9.8	18.1	4.4	8.4
Barbiturates	3.1	21.7	0.6	3.4
Opium	1.3	11.8	2.0	2.7
Tranquillisers	6.7	17.4	3.2	7.8
LSD	-	12.6	-	2.6
Others	-	15.3	3.5	12.1
Sample Size	225	117	882	502
Authors	Mohan et al. 1978	Varma and Dang 1978	Mohan and Arora 1976	Mohan et al. 1977

*Data from Chandigarh (ages 13-20 yr)

marginal changes after the habit has been acquired at a young age. The increased use of tranquillisers and other agents as well as exposure to LSD may be related to the residential character of the university wherein a large number of students stay in the university hostels. Mohan et al. (1978) have found a significantly higher incidence of drug use among students residing in a hostel. A major change appears to be a much higher percentage of students becoming regular users as they move from the school to the university (table 3), as well as introduction to harder stuff like barbiturates, tranquillisers, etc. There is also a marked increase in regular use of alcohol among college students. It is possible that many of these students come from families where other family members also use alcohol and, as they grow up, it becomes permissible for them also to drink. Such data, however, are not available. Mohan et al. (1977) have provided comparative data on the two sexes. A summary of their results in table 4 clearly indicates that the incidence of both occasional and regular use is much higher in the male students. As a matter of fact, there was hardly any regular use of drugs in the female students.

TABLE 3

Frequency of Abuse in Students

Agents	% Frequency of Abuse							
	School		Pre-University*		University Colleges		University	
	O	R	O	R	O	R	O	R
Alcohol	24	3.5	37.2	14.0	3.6	20.5	20.1	3.4
Amphetamines	4.9	-	17.3	1.9	1.8	2.0	2.8	0.8
Cannabis	8	1.8	16.3	1.8	3.8	0.6	6.4	2.0
Barbiturates	3.1	-	17.4	4.3	0.6	-	3.0	0.4
Opium	1.3	-	8.1	3.7	1.4	0.7	2.7	-
Tranquillisers	6.7	-	13.1	4.3	2.6	0.6	7.0	0.8
Others	-	-	23.8	5.0	3.5	-	13.1	2.4

O = Occasional, R = Regular.
*Data from Chandigarh (ages 13-20 yr)

Varma et al. (1977) investigated the incidence of abuse of five specific drugs in 408 college students in Chandigarh. Their results have been summarised in table 5 and the incidence (18.87%) is higher than the rates obtained by Mohan and his colleagues at Delhi or by Thacore et al. (1971) at Lucknow. One of the reasons for this could be their differing definition of drug abuse. Varma et al. (1977) and (1980) have defined it as even occasional intake without medical prescription. The percentage of regular users is low even in this study. In this study also, the percentage in female students was much lower.

TABLE 4

Sex-wise Distribution of Drug Usage in University Students*

Agents	Male students %		Female students %	
	Occasional	Regular	Occasional	Regular
Alcohol	28.9	6.6	10.6	
Amphetamines	4.7	1.6	0.8	
Cannabis	11.3	3.9	1.2	
Barbiturates	5.1	0.8	0.8	
Opium	4.7		0.8	
LSD	4.3	0.4	0.4	
Tranquillisers	9.8	1.6	4.1	
Others	13.3	2.3	7.3	0.4

*Sample size was 256 male and 246 female students.

TABLE 5

Incidence of Abuse to Specific Agents in Students in Chandigarh

Agents	% Students' use		
	Never	Occasional	Regular
Amphetamines	91.9	5.4	2.7
Barbiturates	96.3	3.7	0
Cannabis	95.1	4.4	0.5
LSD	99.3	0.7	0
Mandrax*	90.9	8.1	1.0

*Mandrax is a combination of methaqualone and diphenhydramine; its production has now been banned in India.

Agarwal et al. (1980) have analysed the interrelationship between drug abuse, anomie, alienation, and authoritarianism in 197 postgraduate students at Chandigarh. Anomie can be defined as deregulation or normlessness; alienation indicates distance between mind and reality leading to loss of identity and depersonalization; while authoritarianism represents antidemocratic and fascist social attitudes. Their results indicate a negative correlation between anomie and authoritarianism and drug use, particularly in the younger age group, in those with an urban background and high parental education, and in female students. A positive correlation under similar conditions was observed between alienation and drug use. The psychosocial correlates in adolescents have also been investigated by Mohan and Thomas (1977). Mohan et al. (1978a) have found a highly significant correlation between the father's income, education, and place of residence as well as the adolescent's course of study and the drug abuse in both sexes.

Medical Students and Interns

At least three detailed studies on prevalence and pattern of drug abuse in medical students have been published in recent years following the survey by Agarwal et al. in 1975. Singh (1979) has investigated 672 undergraduate students in Punjab, and 576 students have been studied by Sethi and Manchanda (1977) at Lucknow. In both studies, the ratio of male to female students involved in drug abuse was 6:1, conforming to the pattern obtained in other studies. Agarwal and Pande have recently (1982) undertaken a similar study in 270 medical students at Gorakhpur. The ratio of prevalence in males and females was 3:1. The relative abuse of different agents in the three studies has been summarised in table 6.

TABLE 6

Incidence of Drug Abuse in Medical Students

Agents	% Students' abusing		
	Sethi and Manchanda (1977)	Singh (1979)	Agarwal and Pande (1982)
Alcohol	14.2	12.2	11.85
Amphetamines	7.6	4.8	–
Barbiturates	0.7	1.5	–
Cannabis	5.6	4.0	14.45
Nonbarbiturate sedatives	2.8	3.9	9.25
Tranquillisers	17.7	12.0	14.81

A rather surprising finding is the high incidence of use of tranquillisers, which may in part be related to their availability in the hospital. In the sample of Agarwal and Pande, the greater use of cannabis is related to its widespread use in that part of the country. The relative frequency of use of various agents has not been described in Agarwal and Pande, but the data from the other centres appear to be comparable (table 7).

There have been two recent surveys concerning drug abuse in interns and resident doctors in medical college. The survey at Jammu (Choudhary et al. 1980) covered 105 doctors, while that at Madras (Ponnudurai et al. 1984) covered 116 interns. In both surveys, the incidence was about sixfold lower in females. The data in table 8 indicate the marked increase in drug abuse by interns in comparison with the figures reported for medical students (table 6), particularly in the case of alcohol and tranquillisers. Choudhary et al. (1980) have reported that similar results were obtained by Joshi and Bharadwaj in a survey in

TABLE 7

Frequency of Drug Abuse in Medical Students

Agents	% Frequency of use							
	Never		Occasional		Frequent		Regular	
	A	B	A	B	A	B	A	B
Alcohol	87.8	85.8	2.4	23.5	0.6	0.7	3.1	-
Amphetamines	95.2	92.4	2.5	1.6	2.2	3.3	-	2.7
Barbiturates	98.5	99.3	1.0	-	0.5	-	-	0.7
Cannabis	96.0	94.4	3.4	5.0	0.6	0.6		
Nonbarbiturate sedatives	96. 1	97.2	3.3	2.8	0.4	-	0.2	-
Tranquillisers	90.0	82.3	5.4	15.8	4.3	1.0	0.3	0.7

A: Data of Singh (1979), 672 students
B: Data of Sethi and Manchanda (1977), 576 students

Rajasthan in 1978. The increase is largely among those taking it occasionally and is much less marked in regular users (see tables 9 and 7). The possible reasons appear to be availability of tranquillisers in the hospital and financial independence from their parents due to their stipends. To this also must be added the long hours of work and stress and the urge to get temporary respite from all this.

TABLE 8

Incidence of Drug Abuse among Interns and House Officers

Agents	% Ever used	
	Choudhary et al. 1980	Ponnudurai et al. 1984
Alcohol	55.2	53.5
Amphetamines	12.6	7.8
Cannabis	16.7	18.1
Hallucinogens	-	7.8
Pain killers	12.0	-
Tranquillisers	36.5	14.7
Others	17.2	-

TABLE 9

Frequency of Drug Abuse in Interns and House Officers (Choudhary et al. 1980)

Agents	% Frequency of use			
	Never	Occasional	Frequent	Regular
Alcohol	44.8	44.8	4.2	6.3
Amphetamines	87.6	10.5	1.0	1.0
Analgesics	87.5	5.2	2.1	5.2
Cannabis	83.4	15.6	-	1.0
Tranquillisers	63.5	34.4	-	2.1

Psychiatric Patients

Even though there is no uniform pre-addictive personality, individuals with personality problems are more likely to look for relief with drugs; therefore, chronic users may have a higher proportion of insecure personality. There have been global efforts to establish any possible relationship between drug abuse and psychiatric disorders. One approach has been to find out the prevalence of drug abuse in patients coming to psychiatric hospitals. Some interesting observations have been made in the country during the last few years. Trivedi and Sethi (1978) investigated 1,000 consecutive newly registered male psychiatric outpatient cases for drug abuse to find any possible correlation with psychiatric illness. The pattern of drug abuse in their sample has been summarised in table 10. They reported an overall incidence of 16.4%. There was a slightly higher incidence (22.8%) of drug abuse in patients suffering from affective disorders in comparison to those suffering from schizophrenia (16.5%) or neurosis (15.4%). The incidence in other diseases was much lower (12.4%). There appeared to be a possible correlation between abuse of alcohol and cannabis with affective disorders and neurosis, but further studies are necessary to establish a definitive relationship and to offer an explanation. Venkoba Rao et al. (1978) have analysed cases of drug addiction seen in a psychiatric hospital over a 5-year period. They obtained a sample of 178 cases from 10,023 patients (1.8%) attending the outpatient clinic and only three of these were female. The relative distribution in various drug categories has been given in table 10. A majority of these patients (53.4%) came for the treatment of drug dependence, 39% were suffering from schizophrenia, and the remaining few had associated affective disorders or neuroses. More than a third of these cases had a family history of psychiatric morbidity. Ray and Chandrashekhar (1982) have examined 1,984 consecutive admissions to male psychiatric wards at the National Institute of Mental Health and Neuro Science, Bangalore, for evidence of alcoholism by administering to them the Michigan Alcoholism Screening Test (MAST). During the period, 6.1% of all admissions to that hospital were due to alcohol-related problems. The results indicated that 19% of the patients were alcoholic and

TABLE 10

Incidence of Drug Abuse in Psychiatric Patients

Agents	% Incidence	
	Sethi and Trivedi 1979	Venkoba Rao et al. 1978
Alcohol	9.3	24.2
Amphetamines	1.1	-
Barbiturates	0.6	2.2
Cannabis ± alcohol	-	29.2
Opium	0.3	3.4
Tranquillisers	2.1	-
Multiple	-	11.1
Patient population	Outpatient cases	Addicted cases
Sample size	1,000	178

another 22% were social drinkers. The remaining patients had not been exposed to alcohol. The alcoholics had more first-degree relatives afflicted with alcoholism, sociopathy, and depression.

OTHER CLINICAL STUDIES

There has been considerable interest in abuse of cannabis and methaqualone. Chopra et al. (1942) were the earliest investigators to draw attention to a possible relationship between cannabis use, mental diseases, and crime. The problem of cannabis abuse has been reviewed recently by Sethi et al. (1984). They have also evaluated 50 pure chronic cannabis users for their physical, mental, cognitive, and socioeconomic function and compared the results with a matched control group. On clinical evaluation, most (64%) of the subjects had no psychiatric problem; indeed, they had significantly low anxiety scores in comparison to controls on Taylor's Manifest Anxiety Scale. The other tests also failed to reveal any impairment. Their earlier study (Sethi et al. 1981) also had failed to detect any particular identifiable personality trait in chronic cannabis users. Clinical study of volunteers on the effect of chronic cannabis use did not reveal any serious harmful effects even though some subjects reported withdrawal effects (Singh et al. 1981a). Similarly, no differences were observed in cognitive functions between chronic users of cannabis and nonusers (Ray et al. 1979). The results indicate that cannabis does not appear to be as injurious in the Indian setting as has been suggested by reports from Western countries.

There are, however, a few reports which indicate that chronic use of cannabis may not be as harmless as made out by studies on the cognitive functions. There have been reports of psychoses in chronic cannabis users. In a well-controlled study, Thacore and Shukla (1974) compared cases of cannabis psychoses with those of paranoid schizophrenia. Cannabis psychoses were characterised by more bizarre behaviour, more violence and panic, but an absence of schizophrenic thought disorders. These cases cleared more rapidly with hospitalisation and relapsed when drug was resumed. Chopra and Smith (1974) reported that cases of cannabis psychoses were predominantly instances of toxic delirium, but those who had schizoid features became overtly schizophrenic during the period of intoxication. Uppal et al. (1981) have observed a significant decrease in antipyrine elimination half-life compared to control subjects. It was more marked than observed even in cigarette smokers (Uppal et al. 1980). A similar decrease in theophylline clearance has been reported from Western countries (Jusko et al. 1978).

Thacore et al. (1971) undertook a detailed study of addiction to methaqualone. The clinical tolerance developed in 4 to 6 months and the withdrawal symptoms included depression, sleep disturbances, anxiety, and restlessness in addition to an intense craving for the drug. In most cases, they were first prescribed the drug by a physician for some ailment. Mahal and Nayar (1978) reported 15 cases of petrol dependence from Bangalore. All of these were young boys in the second decade of life, most of them from unsettled families. They suffered from perceptual distortion and hallucination, in some cases of a wish-fulfilling quality and having some resemblance with LSD-psychosis. There was no evidence of tolerance or report of withdrawal symptoms, but there was intense psychological craving following deprivation.

There seems to have been little effort at innovative treatment of drug addiction even though institutional care is available at many places. Earlier work in this area has been reviewed by Wig and Varma (1977). Gangadharan et al. (1982) investigated the use of clonidine in management of cases of opiate dependence (morphine or pethidine). In their studies, clonidine controlled the nonpurposive withdrawal symptoms effectively within 2 to 3 days following abrupt opiate withdrawal, but the purposive symptoms took 8 to 10 days to be adequately controlled. The patients could be discharged earlier from the hospital. The drug merits further studies. Bagadia et al. (1982) have evaluated the usefulness of disulfiram in management of chronic alcoholics in the Indian settings. They have concluded that older patients and those brought by relatives or friends did better and, except for psychotic episodes in 3 out of 75 patients, other side effects were of mild nature.

Shukla (1980) has investigated the sociocultural aspects of abuse of drugs in male adults in a rural community, including the use of alcoholic drinks and drugs as a sociocultural phenomenon. Only 27% of the respondents in the village surveyed by him were nonusers and about 32% were habitual users of alcohol or cannabis; the use of opium was rare. This had gone on for generations without posing any sociocultural crisis or any increase in number of addicts in the village. The use was more frequent in unskilled labourers, and regular use was more common in nonfamilial households or in children whose fathers used drugs. Hasan (1975) investigated social aspects of use of cannabis and concluded that there was no evidence of increase in dosage or of physical dependence. In most cases, persons in that rural community used it on occasions of festivity and ceremonial functions.

ANIMAL STUDIES

The major interest in recent years has centered around methaqualone, which was discovered in India (Gujral et al. 1955, 1956), and cannabis, which has a widespread use.

Methaqualone

Khanna et al. (1971) have investigated development of tolerance to methaqualone in rats following once daily oral administration. Maximum reduction of hypnotic response was obtained in 6 days. There was a cross-tolerance between phenobarbitone and methaqualone. It appears that induction of microsomal enzymes in the liver play a role in development of tolerance, since normal hypnotic response has been restored by SKF 525-A, an inhibitor of liver microsomal enzyme system. These studies have been subsequently extended by Kohli et al, (1974). Tolerance was discernible during the first week to hypothermia and reduction in spontaneous motor activity. Withdrawal resulted in increased susceptibility to audiogenic seizures, hyperactivity, and precipitious decrease in body weight. Nath et al. (1980) compared the dependence liability of methaqualone alone and when used in combination with diphenhydramine (a combination which is widely abused). Their results indicate a more severe degree of physical dependence to the combination than to methaqualone alone.

Cannabis

Singh and Das (1977) undertook a detailed study on tolerance to the resin extracted from Cannabis indica in rats. The resin contained 17% delta-9-tetrahydrocannabinol (THC) and was administered in a dose of 50 to 200 mg/kg. The animals developed tolerance to hypothermic, analgesic, and anticonvulsant activities very rapidly. The potentiating effect on barbiturate hypnosis was also concomitantly reduced. Tolerance did not develop to either increased or decreased locomotor activity or to hyperthermia in tolerant rats.

The disappearance of tolerance on stoppage of cannabis was very slow. Tolerance to hypothermic activity was the first to disappear and that to analgesic activity was the last. Daily administration of cannabis did not affect rate of learning or rate of extinction of learned behaviour. This is in agreement with the clinical results reviewed earlier. Singh et al. (1981) have reported similar tolerance to petroleum ether extract of flowering tops of Cannabis *indica.* On withdrawal, treated animals showed increased sensitivity to audiogenic seizures. Dixit et al. (1974) found a reduction in the size of testis and a regression of Leydig cells following chronic administration of cannabis extract in mice.

Investigations have also been directed toward biochemical changes following chronic administration of cannabis and its constituents. Singh et al. (1980) allowed rats to inhale marijuana fumes daily for a period of up to 90 days. Exposure for up to 15 days decreased brain acetylcholine levels but increased the catecholamine and serotonin levels. After a prolonged administration, the effects were found to be reversed and associated with a decrease in body weight due to a decrease in food intake. Ghosh and his colleagues have studied the effects of acute and chronic administration of cannabis and THC on subcellular components. Chronic treatment with cannabis or THC was devoid of effect on dopamine and norepinephrine turnover (Poddar and Ghosh 1976b) and on the metabolism of other biogenic amines (Poddar et al. 1975). Acute treatment resulted in either increased mitochondrial (Na^+K^+)- and Mg^{++} ATPase or a decrease in Na^+K^+ ATPase activity in synaptosomal preparation. The effect disappeared following chronic treatment (Poddar and Ghosh 1976a). Similarly, acute treatment led to a marked increase in sialoglyco-proteins and ganglioside content in both microsomal and synaptosomal fractions, but the effects were not observed after chronic treatment (Sarkar and Ghosh 1976). Sarkar and Ghosh (1975) also investigated the effect of THC on lipid constituents of subcellular fractions in rat brain. Their results have been summarised in table 11. In contrast to other biochemical parameters, the effect on several lipid components remained unaltered following chronic administration. It has been suggested that chronic administration of THC stimulates its own metabolism, leading to its conversion to hydroxylated polar metabolites which may not interact strongly with membrane components. This can explain some but not all of the differences between the effects of acute and chronic administration.

TABLE 11

Effect of Delta-9-Tetrahydrocannabinol on Lipid Constituents of Rat Brain Subcellular Fractions

Lipid component	Subcellular fraction							
	Mitochondria		Microsome		Synaptosomes		Myelin	
	A	B	A	B	A	B	A	B
1. Total lipids	-	-	-	0	-	-	-	-
2. Total cerebroside	-	-	-	-	-	-	-	-
3. Total cholesterol	-	-	+	0	-	-	-	-
4. Total phospholipids	-	-	+	0	-	-	-	-
i Phosphatidylcholine	-	-	+	0	-	-	-	0
ii Phosphatidylethanol	-	0	+	0	-	0	-	0
iii Phosphatidylserine	-	-	+	0	-	-	-	-
iv Sphingomyelin	0	0	0	0	0	0	0	0

A = Acute treatment, B = Chronic treatment, - = Decrease, + = Increase,
O = No change.

CONCLUDING REMARKS

The problem of drug abuse in India has taken a different shape and presented different dimensions than in most of its neighbouring countries. Opium abuse has not become a major problem due to a well-defined government policy which has controlled cultivation of opium since 1895. Also, there has been no ban or control on cannabis. The use of alcohol has undergone a period of rigid controls, but the laws are more relaxed now. Due to closed borders with Pakistan, the heroin traffic from Afghanistan has not posed a major problem until very recently. This situation has also determined the research activity on drug abuse in the country.

Recent studies suggest a change in the traditional pattern of drug usage in two main directions (Mohan et al. 1978). One of these is a movement away from nonusage of drugs as a cultural norm, even in restrictive societies, beginning at an earlier age, particularly with respect to alcohol and tobacco. Second, and a somewhat redeeming feature, is that the social strata involved in such deviation is the small affluent upper socioeconomic group and the pattern has not undergone a major change in the rural areas. Such a change had been predicted (Nayar 1972) since the concerned group of urban population tends to confuse modernisation with westernisation and tends to adopt symbols rather than substances of Western societies. Fortunately, this does not appear to have assumed subculture or counterculture overtones or a symbolic protest value against accepted social norms as has happened in many Western countries.

REFERENCES

Agarwal, A.K.; Singh, N.; and Kohli, R.P. A survey of drug dependence in medical students. Indian Pract 28:505-510, 1975.

Agarwal, J.L., and Pande, C.D. Deviant drug use. Indian J Med Sci 36:151-154, 1982.

Agarwal, R.K.; Varma, V.K.; and Dang, R. Interrelationship between drug use, anomie, alienation and authoritarianism amongst university students. Indian J Psychiat 22:103-107, 1980.

Bagadia, V.N.; Dhawale, K.M.; Shah, L.P.; and Pradhan, P.V. Evaluation of disulfiram in the treatment of alcoholism. Indian J Psychiat 24:242-247, 1982.

Chopra, G.S., and Smith, J.W. Psychotic reactions following cannabis use in East Indians. Arch Gen Psychiatry 30:24-27, 1974.

Chopra, I.C. Drug addiction. Indian J Pharmacol 3:43-50, 1971.

Chopra, R.N., and Chopra, G.S. Opium habit in India. Indian J Med Res 23:359-389, 1935.

Chopra, R.N.; Chopra, G.S.; and Chopra, I.C. Cannabis sativa in relation to mental diseases and crime in India. Indian J Med Res 30:155-171, 1942.

Choudhary, R.K.; Singh, R.; Avasthi, A.; and Gupta, R. Non-medical drug use among interns and house officers. Indian J Psychiat 22:301-303, 1980.

Dixit, V.P.; Sharma, V.N.; and Lohiya, N.K. The effects of chronically administered cannabis extract on the testicular function in mice. Eur J Pharmacol 26:111-114, 1974.

Drug addiction - An overview. ICMR Bulletin 7(2):1-6, 1977.

Gangadharan, B.N.; Subrahmanya, B.; Venkatesh, H.S.; and Channahasavanna, S.M. Clonidine in opiate detoxification. Indian J Psychiat 24:387-388, 1982.

Gujral, M.L.; Kohli, R.P.; and Saxena, P.N. A preliminary report on some new synthetic narcotics. J Assoc Physicians India 2:29-35, 1955.

Gujral, M.L.; Kohli, R.P.; and Saxena, P.N. Experimental study of hypnotic potency, toxicity and safety margin of 2,3-disubstituted quinazolones. Indian J Med Sci 10:871-876, 1956.

Hasan, K.A. Social aspects of the use of cannabis in India. In: Rubin, V., ed. Cannabis and Culture. Hague, Paris: Mouton Publishers, 1975. pp. 235-246.

Jusko, W.J.; Schentag, J.J.; Clark, J.H.; Gardener, M.; and Yuchak, A.M. Enhanced biotransformation of theophylline in marihuana and tobacco smokers. Clin Pharmacol Ther 24:406-410, 1978.

Khanna, V.; Kulshreshtha, V.K.; and Bhargava, K.P. Experimental studies on methaqualone dependence. Indian J Pharmacol 3:66-70, 1971.

Kohli, R.P.; Singh, N.; and Kulshreshtha, V.K. An experimental investigation of dependence liability of methaqualone in rats. Psychopharmacology 35:327-334, 1974.

Lal, B., and Singh, G. Alcohol consumption in Punjab. Indian J Psychiat 20:212-216, 1978.

Mahal, A.S., and Nair, M.C. Dependence on petrol - A clinical study. Indian J Psychiat 20:15-19, 1978.

Mohan, D., and Arora, A. Prevalence and pattern of drug abuse among Delhi University college students. J Indian Med Assoc 66:28-33, 1976.

Mohan, D., and Thomas, M.G. Adolescent drug abuse: Some psychosocial correlates. In: Ghai, O.P., ed. New Development in Paediatric Research. New Delhi: Interprint Publishers, 1977. pp. 1007-1011.

Mohan, D.; Prabhakar, A.K.; and Sharma, P.N. Prevalence and pattern of drug abuse among Delhi University students. Indian J Med Res 66:627-634, 1977.

Mohan, D.; Prabhakar, A.K.; Mohun, M.; and Chitkara, B. Factors associated with the prevalence of drug abuse among Delhi University students. Indian J Psychiat 20:332-338, 1978a.

Mohan, D.; Sharma, H.K.; Darshan, S.; Sundaram, K.; and Neki, J.S. Prevalence of drug abuse in young rural males in Punjab. Indian J Med Res 68:689-694, 1978b.

Mohan, D.; Thomas, M.G.; and Prabhu, G.G. Prevalence of drug abuse in high school population. Indian J Psychiat 20:20-24, 1978c.

Mohan, D.; Sharma, H.K.; Sundaram, K.R.; and Neki, J.S. Pattern of alcohol consumption of rural Punjab males. Indian J Med Res 72:702-711, 1980.

Nandi, D.N.; Banerjee, G.; Chowdhury, A.; Boral, G.C.; Ghosh, A.; Datta, P.; and Bera, S. Certain aspects of drug abuse - An epidemiological study. Indian J Psvchiat 24:227-229, 1982.

Nayar, B.R. Modernisation Imperative and Indian Planning. New Delhi: Vikas Publishers, 1972. 250 pp.

Poddar, M.K., and Ghosh, J.J. Effect of cannabis extract and delta-9-tetrahydrocannabinol on brain adenosine triphosphate activity. Indian J Biochem Biophys 13:267-272, 1976a.

Poddar, M.K., and Ghosh. J.J. Effect of cannabis extract and delta-9-tetrahydrocannabinol on rat brain catecholamines. Indian J Biochem Biophys 13:273-277, 1976b.

Poddar, M.K.; ‘Biswas, B.; and Ghosh, J.J. Delta-9-tetrahydrocannabinol and brain biogenic amines. In: Bradley, P.B., and Dhawan, B.N., eds. Drugs and Synaptic Transmission. London: Macmillan Press, 1975. pp. 192- 199.

Ponnudurai, R.; Somasundaram, O.; Indira, T.P.; and Gunasekar, P. Alcohol and drug abuse among internees. Indian J Psychiat 26:128-112, 1984.

Ray, R., and Chandrashekhar, K. Detection of alcoholism among psychiatric inpatients. Indian J Psychiat 24:389-393, 1982.

Ray, R.; Prabhu, G.G.; Mohan, D.; Nath, L.M.; and Neki, J.S. Chronic cannabis use and cognitive functions. Indian J Med Res 69:996-1000, 1979.

Report of the Indian Hemp Commission (1893-1894), Vol. 7. Simla: Government Printing House, 1894.

Sarkar, C., and Ghosh, J.J. Effect of delta-9-tetrahydrocannabinol administration on the lipid constituents of rat brain subcellular fractions. J Neurochem 24:381-385, 1975.

Sarkar, C., and Ghosh, J.J. Effect of delta-9-tetrahydrocannabinol on gangliosides and sialoglycoproteins in subcellular fractions of rat brain. J Neurochem 26:721-723, 1976.

Sethi, B.B., and Manchanda, R. Drug abuse among medical students. Indian J Psychiat 19(4):31-35, 1977.

Sethi, B.B., and Trivedi, J.K. Drug abuse in rural population. Indian J Psychiat 21:211-216, 1979.

Sethi, B.B.; Trivedi, J.K.; and Singh, H. Long term effect of cannabis. Indian J Psychiat 23:224-229, 1981.

Sethi, B.B.; Tiwari, S.C.; Kumar, P.; and Trivedi, J.K. Drug abuse in India: An overview with special reference to cannabis. Indian J Psychiat 26:55-66, 1984.

Shukla, B.R.K. Drinks and Drugs in a North Indian Village - An Anthropological Study. Lucknow: Ethnographic and Folk Culture Society, 1980. 198 pp.

Singh, G. Drug use among medical students I. Prevalence and pattern of use. Indian J Psychiat 21:332-338, 1979.

Singh, L.B.; Mazumdar, S.; and Prasad, G.C. Neurohumoral responses to marijuana fume inhalation. Indian J Exp Biol 18:513-515, 1980.

Singh, N.; Nath, R.; Kulshreshtha, V.K.; and Kohli, R.P. An experimental evaluation of dependence liability of methaqualone diphenhydramine (combination) and methaqualone in rats. Psychopharmacology 67:203-207, 1980.

Singh, N.; Agarwal, A.K.; Shankar, K.; and Bhargava, K.P. Evidence of abstinence syndrome in cannabis-treated albino rats. Indian J Crude Drug Res 19:65-74, 1981a.

Singh, N.; Vrat, S.; Ali, B.; and Bhargava, K.P. An assessment of biological effects of chronic use of cannabis (marihuana) in human subjects. O J Crude Drug Res 19:81-91, 1981b.

Singh, P.P., and Das, P.K. Tolerance to cannabis in albino rats. Indian J Exp Biol 15:280-284, 1977.

Thacore, V.R., and Shukla, S.R.P. Cannabis psychosis and paranoid schizophrenia. Arch Gen Psychiatry 33:382-386, 1974.

Thacore, V.R.; Saxena, R.C.; and Kumar, R. Epidemiology of drug abuse in Lucknow with special reference to methaqualone. Indian J Pharmacol 3:58-65, 1971.

Trivedi, J.K., and Sethi, B.B. Drug abuse in psychiatric patients. Indian J Psychia 20:345-348, 1978.

Uppal, R.; Garg, S.K.; Sharma, P.R.; Nair, C.R.; and Chaudhary, R.R. Enhanced antipyrine metabolism in cigarette smokers in Indian population. Int J Clin Pharmacol Ther Toxicol 18:269-271, 1980.

Uppal, R.; Garg, S.K.; Sharma, P.R.; Varma, V.K.; and Chaudhary, R.R. Antipyrine kinetics in cannabis smokers. Br J Clin Pharmacol 11:520-523, 1981.

Varma, V.K., and Dang, R. A study of attitudes, perception and exposure to drug use and its relation to socio-demographic variables. Indian J Psychiat 20:318-323, 1978.

Varma, V.K.; Ghosh, A.; and Wig, N.N. Drug abuse among college students. Indian J Psychiat 19(1):1-10, 1977.

Varma, V.K.; Singh, A.; Singh, S.; and Mehrotra. A. Extent and pattern of alcohol use and alcohol related problems in north India. Indian J Psychiat 22:331-337, 1980.

Venkoba Rao, A.; Sukumr, A.; and Neelambaradharan, C. Drug addiction - A report from Madurai, India. Indian J Psychiat 20:310-317, 1978.

Wig, N.N., and Varma, V.K. Present state of drug dependence treatment in India. In: International survey of drug abuse and its treatment in 25 nations. Addict Dis 3:79-85, 1977.

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The Medicinal Chemistry of Cannabinoids: An Overview

Alexandros Makriyannis, Ph.D., and Rao S. Rapaka, Ph.D.

Since the early 1940s, when Roger Adams synthesized the first cannabinoid analogs and subjected them to pharmacological testing, cannabinoid research has maintained a sustained progress involving the synthesis of a large number of analogs with a varied degree of resemblance to the natural analogs. At the same time, the pharmacological properties of the principal cannabis constituents were studied in detail, while less extensive studies were reported on their synthetic analogs. A good effort was also made to study biochemical effects of cannabinoids on a variety of biochemical preparations and the use or potential use of those compounds as therapeutic agents.

Over four decades later and after the successful synthesis and testing of several hundred analogs a number of important questions related to the function of cannabinoids still remain unanswered. Nevertheless, we have made progress in defining correlations between structure and function while recognizing that such correlations should be made in the context of the biological function being measured. It has also become clear that differences between the species tested are an important consideration. More recently testing of the cannabinoids on discrete biochemical systems has allowed us to better dissect the molecular events involved in the "cannabinoid effect." Parallel to such tests, we should note that increased sophistication in behavioral testing has allowed for more accurate structure-function correlations.

Still, the specific details involved in the cannabinoid molecular mechanism of action are far from clear. The "receptor" versus "membrane" site of action arguments have not been resolved, and there are indications that each of the two hypotheses can provide partial answers, while no unifying theory has yet been articulated. The recent informative review by Martin (1986) does a very good job of outlining these different points of view. The present overview is not intended to be all inclusive, nor does it seek to define all the unanswered questions related to cannabinoid research. Instead, it seeks to highlight some of the important developments in this interesting multidisciplinary field of research, drawing to a large extent from the presentations made during the recent NIDA technical review on structure-activity relationships of cannabinoids.

STRUCTURE-FUNCTION CORRELATIONS

Structure-function correlations in cannabinoids have recently been reviewed in great detail by Razdan (Razdan 1986, Razdan, this volume; Mechoulam et al., this volume). From the emerging picture, we can identify the key structural features required for activity. These should also provide us with insights about the molecular mechanism of cannabinoid activity.

Phenolic Hydroxyl

First, the presence of a free phenolic group is an absolute requirement for cannabinoid activity. Phenolic esters of delta-9-tetrahydrocannabinol (THC) are active *in vivo*, presumably after hydrolysis, and are inactive in *in vitro* preparations. However, the phenolic ethers are totally inactive. The role of the phenolic group during its interaction with the active site could be either as hydrogen bonding donor or a hydrogen bond acceptor through its lone pair of electrons. The latter hypothesis has been elaborated by Reggio (this volume) who points out that the orientation of the phenolic lone pair is the key molecular determinant and that only the free phenolic group has the opportunity to align in the proper "active orientation" while substituted phenols are unable to do so because of steric considerations. The ability of the phenolic OH to act as a hydrogen donor with one or more of the polar membrane sites (either at the phospholipid or the protein component) has been investigated by Makriyannis and colleagues and is discussed later in this review.

The role of hydroxyl substitution in cannabinoid molecules becomes more intriguing in the case of analogs having an additional one or two hydroxyl groups in the C ring. Several of these analogs have been synthesized, and their structure-functions correlations can be clearly visualized. Certainly, the OH orientations must have an important molecular role during the cannabinoid interaction with the site(s) of action. Because of the large differences in activity between individual analogs, the di- and trihydroxy cannabinoids may prove to be very good tools for probing the molecular requirements of the drug-active site interactions.

Ring C Geometry

An important molecular determinant is the geometry of the C ring. It is now being recognized that compounds where the C ring is totally coplanar with the aromatic ring, as in the case of delta 9- and 11-THC and cannabinol, result in inactive compounds. A certain deviation from this coplanarity between rings A and C appears to be one off the crucial molecular requirements (Razdan, this volume; Mechoulam et al., this volume; Makriyannis et al., this volume). This may be related to specific geometric requirements for interaction with a specific "receptor protein." On the other hand, this "out-of-plane" conformation may be required for the molecule to produce perturbations at the level of the phospholipid bilayer (Makriyannis et al., this volume).

Side Chain in Ring A

The role of this molecular fracture is a crucial one, pointing to some requirement for hydrophobic interactions with the membrane components. However, these hydrophobic interactions still allow for the presence of some polar groups such as that of a phenolic ether oxygen or a hydroxy substituent in certain positions of the chain. The length of the chain is critical, with the n-heptyl group being optimal. Also, methyl substituents (e.g., 1,1- or 1.2-dimethyl) enhance activity not only by interfering with the drug metabolism but apparently by a direct effect at the site(s) of action as well.

Absolute Configuration

Although it was early recognized that tetrahydrocannabinol enantiomers differ in potency, accurate values for enantiomeric potency ratios were not available. This, to a large extent, was due to the fact that the enantiomeric starting materials were not of high enough purity. This situation has been remedied through careful purification of these compounds. More recently, starting materials possessing high optical purity have become available commercially.

Recent work by Mechoulam et al. (this volume) involving the 11-hydroxy-delta-8-THC enantiomers showed a potency difference of two to three orders of magnitude between the enantiomers. This difference is much higher than anything reported earlier and does not appear to be solely due to metabolic factors. In all likelihood, it is to a large extent due to the abilities of individual cannabinoids for productive interaction with their site(s) of action.

Nonclassical Cannabinoids

The term "nonclassical cannabinoids" includes a group of compounds that were developed by Melvin and Johnson (this volume). These resemble the tetrahydrocannabinols but do not incorporate the pyran ring. These analogs, developed as potential analgesics, possess pharmacolgical profiles resembling those of the classical cannabinoids. First among these analogs possessing pharmacological activity is a hydroxyphenyl cyclohexanol with a 1.1-dimethylheptyl side chain. It represents the simplest of the cannabinoid structures and incorporates the required phenolic group and an appropriate side chain. The presence of a secondary hydroxy group on the cyclohexane ring enhances activity; as with the "classical cannabinoids," its orientation is crucial in determining activity. This compound also shows a modest enantiomeric potency ratio. The nonclassical bicyclic structure introduces a hydroxypropyl group on the cyclohexanol ring that results in dramatic increases both in potency and in enantiomeric potency ratio. The highest enantiomeric potency ratio is obtained when the hydroxypropyl group is incorporated into a six-membered ring to yield the corresponding hydroxymethyl decalin tricyclic analog.

Melvin and Johnson (this volume) synthesized a relatively large number of such analogs and examined the structural and stereochemical correlates with activity in detail. Based on their studies, they formulated a structure for the hypothetical receptor site. The correlations made here do not necessarily advance our knowledge about the nature of the cannabinoid site of action. However, they do provide very interesting molecular templates, beyond the classical cannabinoid structure, which may serve as important tools in the search for the cannabinoid active sites.

MOLECULAR MECHANISMS OF CANNABINOID ACTIVITY

The idea of a specific receptor through which cannabinoids produce some of their effects has traditionally received favorable attention (Mechoulam et al., this volume; Mechoulam et al 1985; Martin 1986; Martin et al., this volume). Recently, evidence for a cannabinoid receptor has been advanced from two laboratories. In one case, the water soluble nonhydrolyzable cannabinoid trimethylammonium delta-8-tetrahydrocannabinol (TMA) was used by Snyder and his coworkers as a radiolabeled ligand to test for the presence of "cannabinoid receptors" (Martin et al., this volume; Nye and Snyder, this volume). Indeed, the compound showed some specific binding to rat brain membranes. Furthermore, delta-9-THC and other cannabinoids competed successfully with TMA for its binding site. Snyder and coworkers also demonstrated some enantiomeric selectivity among the THC antipodes and lack of binding for other non-cannabinoid structures. There was no good correlation between cannabinoid "affinity" for the TMA binding receptor and for the abilities of the cannabinoiids to displace TMA from its binding site.

One shortcoming of the above approach rests with TMA's most attractive feature, namely its water solubility. Although, this molecule maintains the key cannabinoid structural features, it introduces a permanently charged bulky group in the molecule. This alters one of the key characteristics of the cannabinoid structure, i.e., its overall hydrophobic properties, especially at the level of the side chain. The other data favoring a specific protein site for cannabinoid activity by Howlett (this volume) comes from experiments with murine neuroblastoma cells showing that cannabinoids decrease cyclic AMP production. This response can be attributed to the inhibition of adenylate cyclase activity. Since adenylate cyclase is regulated by a number of receptors through the intermediacy of the ubiquitous G protein, the data suggest that cannabinoids may act either at the receptor or the G-protein level. After having shown that cannabinoids do not act at the receptor protein level, Howlett suggests that the G protein is, indeed, their site of action. The results from this biochemical system are encouraging because there are good correlations between adenylate cyclase inhibition and cannabinoid potency. This hypothesis for the site of action of cannabinoids is yet to be proved through direct binding experiments.

A different type of evidence pointing to a role played by membrane lipids in cannabinoid activity comes from the laboratories of Makriyannis and coworkers (this volume). Using model membranes, and with the help of solid state nuclear magnetic resonance and other biophysical techniques, they showed differences in the manner in which cannabinoid enantiomers and cannabinoid isomers having different potencies interact with model membranes. Such data refute the general notion that cannabinoid phospholipid interactions are "nonspecific" and totally dependent on the drug's oil/water partitioning properties. Makriyannis also showed that cannabinoid analogs orient in the membrane in such a manner that the phenolic hydroxy group serves as the anchor at the membrane bilayer polar/nonpolar interphase and has an important role in determining the geometry of the cannabinoid-membrane amphipathic interaction. The above studies are also congruent with a hypothesis involving more specific cannabinoid-lipid interactions which would, in turn, affect the function(s) of the neighboring proteins. Although one may expect some common general features in the interaction of cannabinoids with membranes, one also expects differences in the molecular details of these interactions depending on the nature and composition of the membrane. Such differences may explain the observed variations in cannabinoid selectivity with different membrane preparations.

Additional evidence for cannabinoid action at the membrane level comes from the laboratories of Burstein (this volume). Using a cell culture model to test cannabinoid activity, Burstein showed that cannabinoids have marked stimulatory action on eicosanoid synthesis and that the cannabinoid interaction does not occur at the level of the protein catalytic site. Also, the model system shows a fair degree of correlation between structure and activity relationships, tolerance, and stereospecificity for the cannabinoids tested. These effects of cannabinoids on the eicosanoid biochemical systems may be related to their CNS activity. Indeed, delta-9-THC when administered orally was found to increase PGE_2 content in the mouse brain and also increased phospholipase activity in brain synaptosomes. A more recent interesting finding from the same laboratories is that the 9-carboxylic acid metabolite of delta-9-THC inhibits delta-9-THC effects on eicosanoid synthesis, apparently through its action on prostaglandin synthetase. This metabolite may thus prove useful as a nonsteroidal antiinflammatory agent.

CANNABINOID PHARMACOLOGY, TOXICOLOGY, AND PHYSIOLOGY

Based on extensive pharmacological evidence, Martin et al. (this volume) pointed out that the cannabinoid behavioral syndrome represents a composite rather than a single event. To dissect these different effects, which he classifies in two general categories as membrane- and receptor-related effects, he used a variety of behavioral test systems. These include spontaneous activity, rectal temperature and analgesia in the mouse, static ataxia in the dog, and drug discrimination and conditioned behavior in monkeys. This approach is very useful and should contribute towards putting the correlations between cannabinoid structure and function on more solid ground. In the same vein, detailed studies by Karler and Turkanis (this volume) have served to point out the dramatic differences in cannabinoid activity among different species. These studies are important because they help to show the possible discrepancies when correlations are made between cannabinoid structure and function using pharmacological data from difference species. Also, from the laboratories of Karler and Turkanis (this volume), toxicological and electrophysiological data demonstrate the wide diversity of action between different cannabinoids. Again, correlations between cannabinoid structure and function should be made judiciously, taking into account the details of pharmacodynamics, toxicology, and electrophysiological testing.

Last but not least, the use of cannabinoids as anticonvulsants is an important therapeutic application. In this regard, Consroe and Mechoulam (this volume) have made good contributions towards elucidating the correlations between cannabinoid structure and anticonvulsant properties. The testing is done with epilepsy-prone rats, and the compounds are tested for their abilities to prevent audiogenic seizures (AGS) and to cause rotorod neurotoxicity (ROT). From these studies, it is clear that the anticonvulsant SAR for cannabinoids does not overlap with the corresponding correlations for psychotropic or analgesic activity. This observation holds a good deal of promise for the use of cannabinoid analogs as anticonvulsants.

Excellent research is being carried out all over the world on the SAR of cannabinoids. This research has resulted in the development of several compounds of therapeutic use and, more importantly, provided tools for further research on cannabinoid biochemistry and pharmacology. It is not unrealistic to hope that in the near future some additional compounds of potential therapeutic value will be developed.

REFERENCES

Martin, B.R. Cellular effects of cannabinoids. Pharmacological Reviews 38:45-74, 1986.

Mechoulam. R.; Srebnik. M.; and Burstein. S. Cannabinoid chemistry; biochemistry and therapeutic applications: An overview. In: Harvey, D.J., ed. Marihuana '84. Proceedings of the Oxford Symposium on Cannabis. Oxford: IRL Press, 1985. pp. 1-12.

Razdan, R.K. Chemistry and structure-activity relationships of cannabinoids: An overview. In: Agurell, S.; Dewey, W.L.: and Willete, R.E.. eds. The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects. Orlando: Academic Press, 1985. pp. 63-78.

Razdan, R.K. Structure-activity relationships in cannabinoids. Pharmacological Reviews. 38:75-149, 1986.

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