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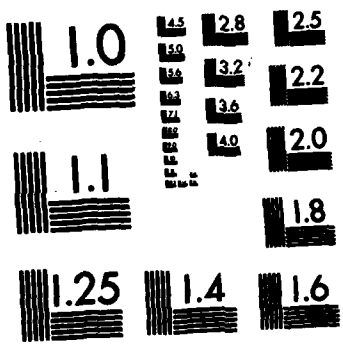

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INTERACTIONS BETWEEN PSYCHOTROPIC DRUGS AND MEMBRANES

FINAL COMPREHENSIVE REPORT

S. T. CHRISTIAN, PH.D.

February 10, 1979

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University of Alabama in Birmingham
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The objectives of this investigation were directed toward understanding both the biophysical and biochemical events which may occur at the neuronal level when selected drugs of abuse interact with this tissue. From the beginning of this study in 1973 several interesting and important observations with respect to various properties of the neuron have been forthcoming. For example, it has been shown that serotonin (5-HT), 1) has multiple binding sites on the synap-

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tosomal membrane and 2) that 5-HT binding to mature rat brain preprations stimulates cyclic nucleotide production. The binding of serotonin to its high affinity synaptosomal binding sites could not be displaced by physiological concentrations of LSD. However, the binding of LSD to this tissue could be blocked both in vivo and in vitro by non-psychoactive structural analogues of this hallucinogen. New analytical techniques developed in this laboratory have allowed the detection of an endogenous hallucinogen, N,N-dimethyltryptamine, and its metabolites in mammalian brain. The finding of this compound has important implications in at least three areas of neurochemistry. For example, 1) it appears to exhibit many of the properties of a neurotransmitter and, 2) its presence in the CNS together with its metabolic disposition may have a direct relationship to certain emotional disorders. Recent studies have demonstrated a specific DMT binding site in the CNS. These facts suggest that hallucinogenic drugs (of the indole class)such as LSD may act by binding to the endogenous DMT binding site and/or interfering with its "normal" metabolism.

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Abstract

The objectives of this investigation were directed toward understanding both the biophysical and biochemical events which may occur at the neuronal level when selected drugs of abuse interact with this tissue. From the beginning of this study in 1973 several interesting and important observations with respect to various properties of the neuron have been forthcoming. For example, it has been shown that serotonin (5-HT), 1) has multiple binding sites on the synaptosomal membrane and 2) that 5-HT binding to mature rat brain preparations stimulates cyclic nucleotide production. The binding of serotonin to its high affinity synaptosomal binding sites could not be displaced by physiological concentrations of LSD. However, the binding of LSD to this tissue could be blocked both in vivo and in vitro by non-psychoactive structural analogues of this hallucinogen. New analytical techniques developed in this laboratory have allowed the detection of an endogenous hallucinogen, N,N-dimethyltryptamine, and its metabolites in mammalian brain. The finding of this compound has important implications in at least three areas of neurochemistry. For example, 1) it appears to exhibit many of the properties of a neurotransmitter and, 2) its presence in the CNS together with its metabolic disposition may have a direct relationship to certain emotional disorders. Recent studies have demonstrated a specific DMT binding site in the CNS. These facts suggest that hallucinogenic drugs (of the indole class) such as LSD may act by binding to the endogenous DMT binding site and/or interfering with its "normal" metabolism.

Introduction

The original objective of this investigation was to determine the mode of action of various drugs of abuse at the neuronal membrane level. As the project evolved special emphasis has been placed on the interaction of hallucinogenic agents with synaptosomal membranes. The basic research plan originally proposed has been closely followed and considerably expanded. This report is being written after the contract has been terminated. However, since it is most difficult to pinpoint, on a chronological basis, exactly when an idea was conceived we will take the liberty in this report of summarizing our research findings to date, which are directly related to experimentation initiated during the contract period.

Several important findings have been published as a direct result of the techniques developed and/or mastered during the course of the contract period. Each of these studies will be briefly reviewed with representative references given. It should be pointed out that even though most of these studies were published after termination of the contract, much of the work was nevertheless initiated during the period in which the contract was active. That is, many of our studies (even those we are presently engaged in) can be traced to the initial contractual investment. A brief summary of each area of investigation is as follows:

Interaction of Psychoactive Drugs with Synaptosomal Membranes - A study was designed to determine the effects of various psychoactive drugs on the Ca++, Mg++ ATPase found in synaptic vesicles isolated from rat brain synaptosomes. The results of this study showed that certain antipsychotic drugs (e.g. dibenzoxapine) were non-competitive inhibitors of this enzyme with K_i's in the range of 10^{-5} M. However, cocaine was also an inhibitor of this enzyme system with a similar K_i. No effect was observed with the opiates, barbiturates, stimulants or hallucinogens at concentration ranges up to 5×10^{-4} M. One possible interpretation of these data is that at least selected antipsychotic agents may act in part at the vesicular level (1).

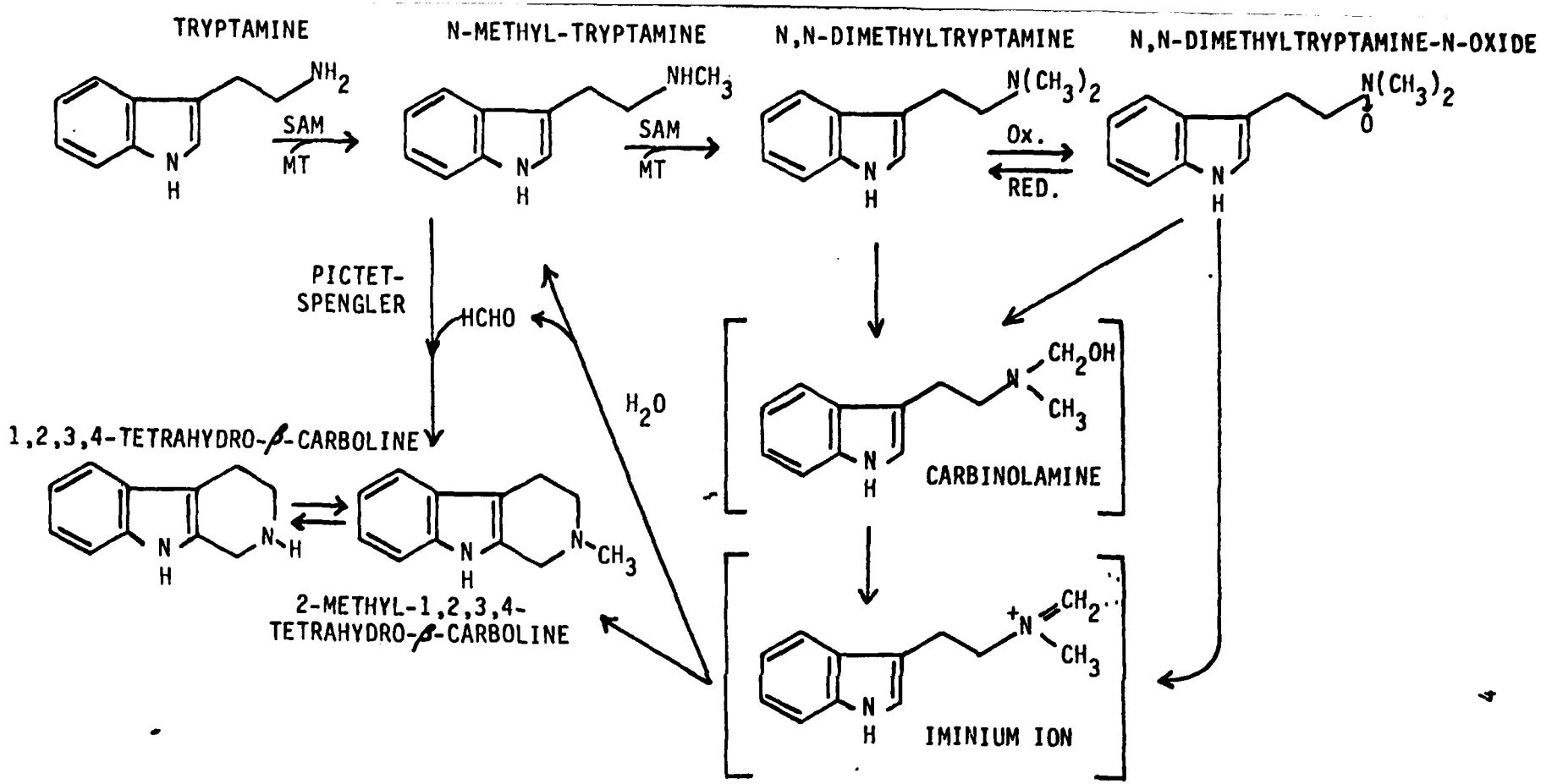
Blockage of LSD Binding at its High Affinity Site on Synaptosomal Membranes - Early in our studies of LSD binding to synaptosomal membranes a specific high affinity site was identified with a K_i of 2.9×10^{-9} M. The hallucinogen could not be displaced from this site by 10^{-5} M serotonin, several phenothiazines or tryptamine. Displacement could be affected, however, with other hallucinogenic agents (e.g. N,N-dimethyltryptamine). A compound, 1-methyl-1,2,5,6-tetrahydropyridine-N,N-diethylcarboxamide (THPC) had previously been reported to be a "blocking" agent with respect to the behavioral disruptive effects of LSD. We evaluated this compound and found that at a concentration of 1×10^{-5} M it completely blocked the binding of d-LSD to its high affinity binding site. Consequently, the data suggest that compounds like THPC may find use in the therapeutic ablation of LSD induced hallucinations in humans (2).

Serotonin Sensitive Adenylate and Guanylate Cyclase Activity Associated with Isolated Synaptic Membranes - This laboratory was the first group to clearly demonstrate the occurrence of a serotonin activated adenylate cyclase located on mammalian synaptosomal membranes (3). In addition, further studies indicated that multiple (four) binding sites for serotonin (5-HT) with K_d's of 5×10^{-10} M, 5×10^{-9} M, 1×10^{-8} M, and 5×10^{-8} M respectively, could be demonstrated. Each of these binding sites appears to have a specific adenylate cyclase associated with the binding sites. It was found that fluphenazine, a very potent antipsychotic drug which has been reported to block dopamine sensitive adenylate cyclase activity, was found to have virtually no effect on 5-HT associated cAMP production in this system.

Further, research in this area has resulted in the identification of what appears to be a serotonin sensitive guanylate cyclase likewise associated with synaptosomal membranes (4). The enzyme appears to have multiple activation sites for 5-HT with specific activity maxima at 5-HT concentrations of 5×10^{-10} M and 7×10^{-8} M, respectively. We have not as yet been able to explain the occurrence of concentration specific activation sites. Work in this area is still in progress.

Identification and Separation of Endogenous Hallucinogens in Mammalian Brain and Human CSF - Work over the past several years which was first initiated during the period of DADA 17-73-C-3088 was designed to answer the question as to whether or not endogenous hallucinogens of the indole class existed in the mammalian central nervous system. Highly specific and sensitive analytical techniques were developed to separate and identify such compounds (5,6). Analysis of various tissues using high resolution GLC with Ni^{63} electron capture detection revealed the presence of the following compounds: N,N-dimethyltryptamine, tryptamine, N-methyltryptamine, 5-methoxy-N,N-dimethyltryptamine and 5-methoxytryptamine. These compounds were found both in rat brain (7) and in human CSF (8).

Further development of our methodology using GC/MS not only confirmed our original findings but also allowed the detection of 2-methyltetrahydro-β-carboline, tetrahydro-β-carboline and dimethyltryptamine-N-oxide. Quantitation of these compounds was achieved by the addition of tetradeutero standards before tissue extraction. The presence of these compounds in brain led to the metabolic pathway shown in Figure 1. Experimentation designed to confirm these findings has now been carried out using both C^{14}-DMT and C^{14}-DMT-NO (9). The occurrence of a potent hallucinogenic agent such as DMT in normal brain suggests that it may have a role in normal brain function. Studies have been designed and carried out to validate this point (7,10). The results of these studies suggest that DMT is, in fact, a neuroregulatory agent (7). We have now been able to further demonstrate what appears to be a specific high affinity binding site for DMT on synaptosomal membranes (11). DMT bound to this site can be displaced by LSD but not by 5-HT or tryptamine. Work in this area is continuing as funds permit. Regional brain concentrations are being determined and peripheral organ presence and concentrations are likewise under investigation.


TRYPTAMINE
N-METHYL-TRYPTAMINE
N,N-DIMETHYLTRYPTAMINE
N,N-DIMETHYLTRYPTAMINE-N-OXIDE
SAM
MT
SAM
MT
Ox.
RED.
PICTET-
SPENGLER
HCHO
H2O
1,2,3,4-TETRAHYDRO-β-CARBOLINE
2-METHYL-1,2,3,4-
TETRAHYDRO-β-CARBOLINE
CARBINOLAMINE
IMINIUM ION

At this point in our research we would propose that the site of action of hallucinogens (e.g. LSD) may be at the endogenous DMT neuronal binding site. From a teleological point of view this hypothesis appears to be most attractive. In addition, our data strongly suggest that DMT and/or its active metabolites are, in fact, normal neuroregulatory agents. Additional work in this area is, of course, needed. The data already points to the identification of a new neuroregulatory agent about which little is known with respect to its proposed role in such a capacity. These data also open up new questions that need answers with respect to the hypothesis of endogenous hallucinogens as the cause of schizophrenia. Further, LSD-DMT binding site interactions appear to bring us one step closer to the mechanism of action of hallucinogens.

Unfortunately, current funding for research in this area is either scarce or non-existant. We do, however, hope that this situation will change in the future. The possible benefit to the public is impressive. The awarding of an Army contract to me, as a young investigator, some 6-7 years ago is, I believe, an excellent example of how a small amount of money can, in science, lead to both the establishment of a laboratory and to the generation of important scientific data. Although still working in the drug abuse field, this laboratory has expanded its projects to include the study of the properties of cell membranes. From such studies data has been generated which, we believe, will lead to the in utero diagnosis of cystic fibrosis (12). In addition, preliminary data suggest that we may be able to develop tests for carriers of this disease. Further, recent studies on malignant cell membranes show promise in helping to understand some of the molecular changes which take place in the malignant cell membrane (13). This in turn may lead to new treatment methods.

Although only two abstracts were published during the contract period, per se, some 30+ publications have since been accepted and published. Consequently, we are most grateful to the staff of Walter Reed and to the U.S. Army Research and Development Command for having the wisdom to understand and support our original proposal.

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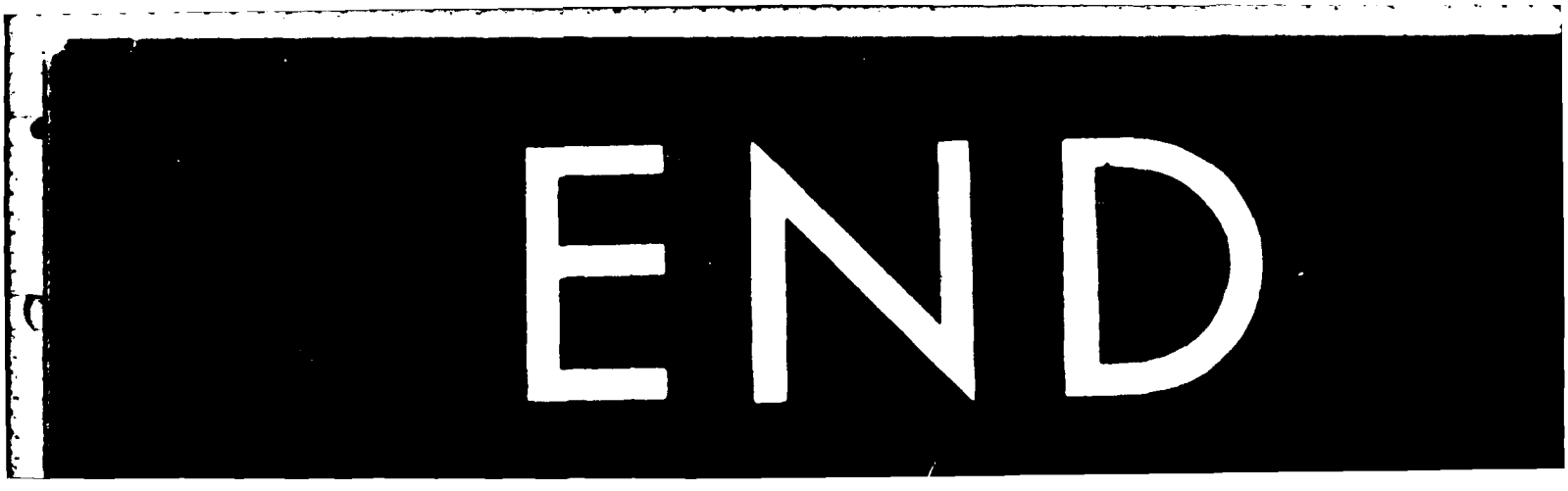
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