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THE DEVELOPMENT OF INDOLEAMINE DERIVATIVES SELECTIVE FOR SUBTYPES OF SEROTONIN RECEPTORS

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THE DEVELOPMENT OF INDOLEAMINE DERIVATIVES
SELECTIVE FOR SUBTYPES OF SEROTONIN RECEPTORS

by

Ethan Will Taylor

A Dissertation Submitted to the Faculty of the
PROGRAM IN PHARMACOLOGY AND TOXICOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1 9 8 5
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Ethan Will Taylor entitled "THE DEVELOPMENT OF INDOLEAMINE DERIVATIVES SELECTIVE FOR SUBTYPES OF SEROTONIN RECEPTORS" and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Dissertation Director

11/25/85

Date
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DEDICATION

To my parents, in appreciation for their significant role in encouraging my interest and education in science.
ACKNOWLEDGEMENTS

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ABSTRACT

Central serotonin (5-hydroxytryptamine, 5-HT) receptors are classified into 5-HT$_1$ (defined by [³H]5-HT binding) and 5-HT$_2$ (defined by [³H]ketanserin binding). Antagonists selective for 5-HT$_1$ receptors or 5-HT$_{1A}$ and 5-HT$_{1B}$ subtypes are currently unavailable. To develop such antagonists, a study of derivatives of tryptamine (TRYF) (which are generally selective for 5-HT$_1$ sites) was undertaken. For amino-N-substituted TRYFs at 5-HT$_1$ sites, although overall potency decreases with increased N-alkyl substituent size (up to N,N-di-iPr), discrimination between subtypes of 5-HT$_1$ sites increases. Compounds such as N,N-di-iPr-TRYF (DIPT) and 3-(2-morpholinoethyl)indole (MEI) recognize 30% of [³H]5-HT binding sites with high affinity ($K_i$<50 nM), the rest with low affinity ($K_i$>4000 nM). For both the DIPT (agonist) and MEI (antagonist) series, incorporating 5-oxy substituents resulted in rank order of overall 5-HT$_1$ potency of 5-OH>MeO=5H>BzO; however, the 5-oxy compounds lost the high-affinity recognition component shown by DIPT and MEI. Incorporation of an additional hetero-aromatic moiety gave amino-N-aryl substituted TRYFs (prototype AHR 1709). These were 1) highly selective for 5-HT$_{1A}$ sites ($K_i$=10-200 nM) over 5-HT$_{1B}$ sites ($K_i$>3000 nM), 2) potent at the 5-HT$_2$ site & 3) vascular antagonists of 5-HT. Pharmacophoric differences between 5-HT$_1$, 5-HT$_{1A}$ and 5-HT$_2$ sites were studied with rigid analogs. Racemic partial ergolines (PEs) RU 27849 and RU 28306 showed diminished potency compared to TRYFs at 5-HT$_1$ sites, but
were equipotent to homologous TRYPs at the 5-HT$_2$ site. At all three sites, 3-(tetrahydropyridyl)indoles (THPIs) were the most potent rigid analogs. A non-ergoline-like constrained analog of TRYP was synthesized and was even less potent than RU 27849 at 5-HT$_1$ sites, but was 4-5 times as potent as TRYP & RU 27849 at the 5-HT$_2$ site. While enhancing affinity for 5-HT$_1$ sites, the 5-MeO group can give reduced affinity for 5-HT$_2$ sites, thus enhancing 5-HT$_1$ selectivity. 5-Unsubstituted compounds may be best for 5-HT$_2$ selectivity. A study of 5-HT agonists in the canine basilar artery (CBA) suggests the contraction in vitro is mediated by a receptor similar to the 5-HT$_{1A}$ binding site; 5-HT$_2$ receptors may also be present. Theoretical models for the production of apparent noncompetitive antagonism to 5-HT in the CBA are also examined. Syntheses of tryptamine derivatives and a conformationally constrained analog of TRYP are described.
CHAPTER 1

INTRODUCTION

Background of 5-HT Receptor Research

Serotonin (5-hydroxytryptamine, 5-HT; Fig. 1) is a neurotransmitter which has been of great significance in the development of neuropsychopharmacology as a scientific discipline. For a compound with such a long history, it is surprising how much still remains to be discovered about its multifaceted role in both the central nervous system (CNS) and the periphery.

Although its structure was undetermined, the existence of 5-HT was known to physiologists (as early as the nineteenth century) as a vasoconstrictor substance present in clotted blood which also possessed the ability to cause powerful contractions of smooth muscle in various organs. The identification of the chemical nature of the compound was achieved independently by two groups in the late 1940's.

The serum vasoconstrictor was to some extent initially viewed primarily as a nuisance compound both in perfusion experiments and, later, in attempts to identify other hormonal pressor agents. This led to the isolation of the compound (which had formerly been known by various names such as vasotonin) as a crystalline complex by researchers at the Cleveland Clinic, who proposed the name serotonin (Rapport et al., 1948) and subsequently deduced that 5-hydroxytryptamine was the structure of the active component (Rapport, 1949).
Meanwhile, and totally independently, extended studies in Italy by Erspamer and his colleagues had led to the isolation of a gut stimulating factor from the intestinal mucosa, which they named enteramine; the compound was suggested to be an indolealkylamine (Erspamer, 1946).

With the synthesis of 5-HT by several labs in 1951, the realization of the identity of serotonin and enteramine was bound to follow (Erspamer and Asero, 1952). The identification of 5-HT in the CNS (e.g. Amin, Crawford and Gaddum, 1954) and the realization of the structural similarity between 5-HT and hallucinogens such as d-lysergic acid diethylamide (d-LSD; Fig.1) set the stage for the first experiments demonstrating the antagonism (by d-LSD) of 5-HT-induced smooth muscle contractions (Gaddum, 1953) and the suggestion that a similar action in the brain might account for the psychotomimetic effects of d-LSD.

The availability of antagonists such as d-LSD and related ergot alkaloid derivatives led to the first recognition of the existence of multiple types of 5-HT receptors (Gaddum and Hameed, 1954) and, naturally, to the first attempt at 5-HT receptor classification, in a guinea pig isolated ileum preparation (Gaddum and Picarelli, 1957). The designation of two types of peripheral receptors as "M" and "D" referred to the preferential blockade by dibenzyline (phenoxybenzamine) and morphine respectively. The D receptor was located on smooth muscle whereas the M receptor was a neuronal receptor. Although this classification has been criticized because of the general lack of specificity of phenoxybenzamine as well as the indirect mechanism of M receptor blockade by morphine (which works by blocking acetylcholine release rather than direct 5-HT receptor antagonism), it is still a basis for
Figure 1. Structures of 5-hydroxytryptamine (5-HT) and d-lysergic acid diethylamide (d-LSD).
comparison of subsequent classification systems, particularly where peripheral receptors are concerned. The recent development of a new class of peripheral neuronal M receptor antagonists (Richardson et al., 1985) exemplifies the renewed interest in this receptor type.

Although almost 30 years have passed since this first demonstration of 5-HT receptor heterogeneity, research in this field is only now reaching a stage comparable to that in the area of adrenergic receptors some 20 years ago, when the development of selective pharmacologic agents made possible the identification of subtypes of alpha and beta receptors. The therapeutic and research benefits achieved by the subsequent development of highly selective adrenergic agents have been incalculable. It is reasonable to expect that, with the development of highly selective serotonergic agents, similar results can be achieved in some of the many areas where 5-HT is known to play a significant physiologic role.

Testimony to our profound understanding of the biosynthesis and metabolism of 5-HT as well as the anatomical localization of the serotonergic neuronal system can be found in any current textbook of biochemistry or neuroanatomy; however, it is the very diffuseness of this neuronal system, and the plethora of functions it modulates, that make its pharmacological analysis so difficult, particularly where selective antagonists are unavailable.

Some of these diverse functions attributed to 5-HT include involvement in fundamental behaviors such as sleep, appetite, pain perception and sexual response; the control of physiologic parameters such as body temperature and blood pressure, and a role in disease
states such as migraine, depression, anxiety and schizophrenia (See
reviews edited by Barchas and Usdin, 1973; Essman, 1978; Bollin, 1978;
Osborne, 1982). It is also widely accepted that the serotonergic system
is of considerable importance in the mechanism of action of many
hallucinogens (Glennon and Rosecrans, 1981, 1982; Jacobs, 1982).

Since many of these areas in which new selective serotonergic
agents might have potential research or therapeutic uses involve actions
of 5-HT within the CNS, it seems that a reasonable approach to the
development of such agents would be to concentrate on the study of
central 5-HT receptors. These have been most effectively characterized
by the radioligand binding technique (which is explained in a subsequent
section of this chapter). However, the study of peripheral 5-HT
receptors, as well as being of intrinsic interest, is also relevant to
any study of central 5-HT receptors. As discussed below, there is a
growing body of evidence that suggests there are considerable similar­
ities between certain central 5-HT binding sites and at least some
peripheral 5-HT receptors. Since binding studies alone cannot reveal
whether a new drug is an agonist or antagonist, peripheral receptor
preparations (or electrophysiological and biochemical studies of central
neurons) are important for obtaining additional pharmacological data on
such compounds. Furthermore, if a correlation can be drawn between the
pharmacology of a central binding site and a functional receptor
preparation, the case for the central site as an actual receptor is
supported.
Pharmacological and Electrophysiological Evidence for Heterogeneity of 5-HT Receptors

In addition to the early studies of Gaddum and others discussed above, some striking evidence for the existence of multiple 5-HT receptor types has been obtained from invertebrate preparations. In electrophysiological studies with Aplysia ganglia, Gerschenfeld and Paupardin-Tritsch (1974) observed six different types of ionic responses (both excitatory and inhibitory) to the intophoretic application of 5-HT. This led them to postulate the existence of multiple types of 5-HT receptors, based on the effects of various antagonists for antagonizing the observed membrane conductance changes.

In the mammalian CNS, electrophysiologic studies have also suggested the existence of both excitatory and inhibitory responses to 5-HT mediated by pharmacologically distinct postsynaptic receptors (Roberts and Straughan, 1967; Boakes et al., 1970; Haigler and Aghajanian, 1974). In these studies, it was found that "classical" 5-HT antagonists such as d-LSD and methysergide could block the excitatory responses to 5-HT, but failed to antagonize (or in some cases potentiated) the inhibitory effects of 5-HT. A presynaptic receptor has also been described (Aghajanian and Haigler, 1975) and pharmacologically differentiated from the postsynaptic receptors, since d-LSD and related tryptamine hallucinogens were more potent in inhibiting the firing of 5-HT neurons of the raphe nucleus than that of post-synaptic cells.

The vasculature of various species has also been a source of pharmacological evidence for the existence of multiple types of 5-HT receptors. Differences have been found between various canine arteries
in their sensitivity to different 5-HT antagonists. For example, differences in the potencies of classical 5-HT antagonists such as methysergide and cyproheptadine have been observed between the canine femoral and saphenous veins (Apperly et al., 1980). Differences in vascular 5-HT receptor types between species have also been reported, such as those observed between rat and canine vasculature (Cohen et al., 1984). Pharmacological differences have been observed between 5-HT receptors in the cerebral and the peripheral vasculature by various investigators (see review by Peroutka, 1984b) but this question remains controversial and will be discussed further later, as the nature of the controversy can only be appreciated in the context of attempts to correlate functional 5-HT receptors with central 5-HT bindings sites.

**Heterogeneity of Central 5-HT Binding Sites**

The technique of receptor binding assays using radiolabeled compounds, in essence, allows the convenient quantification of the amount of receptor-bound ligand. This is accomplished by the rapid separation (e.g., by filtration) of an incubated mixture of labeled ligand, inhibiting drug and membrane suspension in a buffer solution at equilibrium. This makes possible the kinetic analysis of drug-receptor interactions and the determination of dissociation constants and receptor density, allowing the pharmacological characterization of neurotransmitter receptor binding sites. (For a complete exposition of the techniques and uses of neurotransmitter receptor binding assays, see Yamamura, Enna and Kuhar, 1985).
Almost since it was first found in the CNS, 5-HT has been suspected of being a neurotransmitter, a speculation that was fueled by 1) the structural relationship that was perceived between 5-HT and indole hallucinogens such as d-LSD, 2) the observed antagonism of 5-HT at peripheral 5-HT receptors, and 3) the observation that the tranquilizer reserpine was a potent depletor of brain 5-HT. These observations led naturally to the development of hypotheses regarding a possible role for disturbances in 5-HT synthesis, metabolism or function in the etiology of various mental illnesses (Woolley, 1962).

Thus, with the development of the ligand-binding technique, it is not surprising that both d-[3H]LSD and [3H]5-HT were chosen to probe brain 5-HT receptors.

Initial studies with these two ligands suggested that their binding sites were very similar, possibly being different affinity states of the same receptor (Bennet and Snyder, 1976). Subsequently, studies with the neuroleptic spiperone suggested that this compound interacts with 5-HT receptors in the rat frontal cortex (Leysen et al., 1978). Using spiperone as a third ligand for 5-HT binding sites, Peroutka and Snyder (1979) suggested that [3H]spiperone labeled sites in the frontal cortex that were different from those labeled by [3H]5-HT, but that both of these sites were labeled by d-[3H]LSD.

These observations were the basis for the generally accepted classification by Peroutka and Snyder (1979) of central 5-HT receptors into two major types: 5-HT1 receptors, defined by the high affinity binding of [3H]5-HT, and 5-HT2 receptors, defined by the high affinity
binding of \[ ^3\text{H} \]spiperone or later by \[ ^3\text{H} \]ketanserin (Leysen et al., 1982). There has been, however, some resistance to the acceptance of the 5-HT\(_1\) binding site as an actual 5-HT receptor (Leysen, 1983). Evidence for correlations for both sites with functional 5-HT receptors will be discussed in the next section.

The 5-HT\(_2\) site is generally characterized by a high affinity for "classical" 5-HT antagonists and a rather low affinity for 5-HT and its agonists, whereas the 5-HT\(_1\) site generally has a higher affinity for 5-HT agonists (tryptamines) and a lower affinity for antagonists (Peroutka and Snyder, 1979), with the exception of a few ergoline derivatives such as d-LSD (which may be an agonist at some 5-HT\(_1\) sites) and metergoline.

Several lines of evidence have suggested that the 5-HT\(_1\) sites are themselves heterogeneous. These include regional differences in the inhibition of \[ ^3\text{H} \]5-HT binding by various drugs as well as shallow inhibition curves for the inhibition of \[ ^3\text{H} \]5-HT binding (Pedigo, Yamamura and Nelson, 1981; Nelson et al., 1981; Nelson et al., 1983). The observation of regional differences in the potency of spiperone to inhibit \[ ^3\text{H} \]5-HT binding, as well as its ability to produce biphasic inhibition curves for \[ ^3\text{H} \]5-HT binding, led to the classification of \[ ^3\text{H} \]5-HT binding sites into 5-HT\(_{1A}\) and 5-HT\(_{1B}\) types, based on the greater potency of spiperone at the former site (Pedigo et al., 1981; Nelson et al., 1981). This classification has also gained considerable acceptance, particularly since the demonstration of the 5-HT\(_{1A}\) selectivity of the 5-HT agonist 8-hydroxy-2-(d1-n-propylamino)tetralin (8-OH-DPAT) by Middlemiss and Fozard (1983). This compound has recently
come into use as a tritiated agonist ligand for 5-HT$_{1A}$ sites. There is now evidence that the 5-HT$_{1A}$ sites may also be heterogeneous, based on regional differences in the potencies of various drugs for the inhibition of $[^3\text{H}]8$-OH-DPAT binding (Gozlan et al., 1983; also see the Discussion of Ch.8).

In saturation studies of the inhibition of $[^3\text{H}]5$-HT binding by cold 5-HT, Sills et al. (1984a) observed curvilinear Scatchard plots, which would normally suggest that a heterogeneous population of sites was involved. However, in the presence of guanosine-5'-triphosphate (GTP), linear Scatchard plots were observed. This suggested that some of the observed heterogeneity of $[^3\text{H}]5$-HT binding sites could be due to the existence of multiple affinity states of the receptor, since GTP eliminates the high affinity state. However, the additional observation (Sills et al., 1984b) that a number of compounds have shallow $[^3\text{H}]5$-HT binding inhibition curves even in the presence GTP suggests that there are at least two distinct subtypes of 5-HT$_1$ receptors, rather than there being only one receptor type existing in different affinity states.

The subject of indoleamine binding sites has been made even more confusing by the discovery of a high affinity binding site for $[^3\text{H}]$tryptamine (Kellar and Cascio, 1982). However, this site appears to be quite distinct from the 5-HT binding sites, having a low affinity for tryptamines that are 5-oxy substituted or with bulky substituents on the amino nitrogen. It shows greatest affinity for tetrahydro-beta-carbolines (Casio and Kellar, 1982). Nonetheless, if and when specific tryptamine derivatives are developed as potential selective 5-HT$_1$ ligands, it is important that they be also checked for activity at this
site, since this would interfere with their usefulness as serotonergic ligands.

In addition, two other pharmacologically distinct central 5-HT binding sites have recently been observed: a proposed 5-HT$_{1C}$ site (Pazos et al., 1984) and another site distinct from 5-HT$_1$ and 5-HT$_2$ (Robaut et al., 1985). The significance of these sites in relation to the other 5-HT binding sites has yet to be determined. Clearly, the possibility of cross-labeling exists and will have to be taken into consideration in the design and interpretation of binding experiments.

**Correlations Between 5-HT Binding Sites and Functional Receptors**

The demonstration of functional correlates for binding sites on membrane preparations is an essential step in any attempt to define those sites as true receptors.

A number of such correlations have been observed for both 5-HT$_1$ and 5-HT$_2$ sites, but those for the 5-HT$_2$ sites have gained wider acceptance. Even before they were named 5-HT$_2$ sites, a correlation was observed between affinities of drugs for the [$^3$H]spiperone binding site in frontal cortex and their potencies for the inhibition of tryptamine induced clonic seizures in rats (Leysen et al., 1978). A correlation between drug potencies at the 5-HT$_2$ site and the "head shake" component of the 5-HT behavioral syndrome was demonstrated by Peroutka et al. (1981). The role of 5-HT$_2$ receptors in the mediation of peripheral 5-HT induced vasoconstriction has also been convincingly demonstrated (Van Neuten et al., 1981), and a 5-HT$_2$ receptor site has also been found on
cat blood platelets (Leysen et al., 1983). Functional correlates for 5-HT₂ sites have recently been reviewed by Leysen et al. (1984).

For 5-HT₁ sites, evidence for correlations to functional preparations has been slower to appear. This is at least partly due to the difficulty of demonstrating correlations to a binding site which is not homogeneous. Recent advances in the development of selective compounds for at least the 5-HT₁A subtype are helping to resolve this problem.

One function that was originally suggested for 5-HT₁ sites (Peroutka and Snyder, 1979) was the modulation of a 5-HT sensitive adenylate cyclase. There appear to be several such cyclase systems, at least one of which is not related to the 5-HT₁ receptor (Nelson et al., 1980). However, Fillion et al. (1979) demonstrated a high-affinity 5-HT cyclase system which, based on the results of lesion studies, appeared to be associated with 5-HT₁ sites. More recently, a 5-HT sensitive cyclase has been described in the guinea pig hippocampus which has 5-HT₁ characteristics (Shenker et al., 1983; 1985) and used as a 5-HT₁A preparation (Yocca and Maayani, 1985).

A number of labs have demonstrated correlations between 5-HT autoreceptor preparations (modulation of 5-HT release) and 5-HT₁ type pharmacology (Gothert, 1980; Martin and Sanders-Bush, 1982; Engel et al., 1983). More recently, it has been suggested that the autoreceptor controlling the release of 5-HT may be of the 5-HT₁B subtype (Middlemiss, 1984; Monroe and Smith, 1985). However, those on serotonergic cells and/or dendrites (but not nerve terminals) may be of the 5-HT₁A subtype (Verge et al., 1985).
The 5-HT receptor in the canine basilar artery (CBA) has been claimed to correlate both with 5-HT$_1$ (Peroutka et al., 1983) and with 5-HT$_2$ binding sites (Muller-Schweinitzer and Engel, 1983). An attempt has been made to resolve this question (Taylor, Duckles and Nelson, 1985); this study suggests that the 5-HT induced contraction of the CBA is mediated primarily by a receptor similar to a 5-HT$_{1A}$ type, but that 5-HT$_2$ receptors may also be present. These findings form the subject matter of the last chapter of this Dissertation.

Recent studies suggest that, as opposed to the "head shake" response noted above, the rest of the 5-HT behavioral syndrome appears to be mediated by 5-HT$_1$ receptors (Lucki et al., 1984). The observation that the syndrome is produced by 8-OH-DPAT (Tricklebank, 1984) suggests mediation by 5-HT$_{1A}$ receptors.

These and other possible functional correlates for 5-HT$_1$ sites have recently been reviewed by Peroutka (1984a).

**The Lack of Selective 5-HT$_1$ Antagonists; Goals of this Research**

A limiting factor for progress in the field of 5-HT$_1$ receptors and their associated functions is the lack of selective 5-HT$_1$ antagonists. The availability of such compounds would not only make the definitive identification of 5-HT$_1$ receptors and their subtypes easier: in addition, the availability of antagonist ligands for the binding sites would be theoretically and practically advantageous, making binding data less variable and correlations more significant. There are also other unresolved problems associated with the use of [³H]5-HT as a ligand, related to its tendency to oxidize (Andresen et al., 1985) and
problems with the use of ascorbic acid as an antioxidant (Nelson, 1981). Towards the ultimate end of developing such antagonists, the goals of this research are to obtain information about the structure-activity relationships (SAR) for serotonin analogs at (primarily) 5-HT\textsubscript{1} sites, although as the study progresses, interactions at 5-HT\textsubscript{2} sites will also be examined; also, the availability of the 5-HT\textsubscript{1A} agonist ligand \(^{3}\text{H}\)8-OH-DPAT during the last year of the project has allowed a more precise analysis of the selectivity of the 5-HT analogs for 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B} and 5-HT\textsubscript{2} sites.

Vascular models were chosen as the most convenient for the examination of certain analogs in functional receptor systems; this led to the necessity of analysing the pharmacology of the controversial CBA 5-HT receptor preparation, an objective which has proven both challenging and rewarding, although perhaps at the expense of more progress in the area of drug development.

The approach has been for the most part a simplistic one, that is, analogs have been chosen on the basis of a high degree of structural homology to 5-HT, with deliberate avoidance of (perhaps more active) compounds whose structural dissimilarity to 5-HT might make the integration of SAR information more difficult. This approach has the added advantage that most of the compounds synthesized were therefore tryptamines, which as a class are generally somewhat selective for 5-HT\textsubscript{1} sites.
CHAPTER 2

GENERAL EXPERIMENTAL METHODS

Chemicals and Drugs

8-hydroxy-2-(di-n-propylamino)tetratin (8-OH-DPAT) was obtained from Research Biochemicals Incorporated, Wayland, MA. The compounds RU 24969 (5-methoxy-3(1,2,5,6-tetrahydro-4-pyridyl)1H indole and RU 28253, its 3-pyridyl isomer, as well as RU 27849 (4-amino-1,3,4,5-tetrahydro-benz[c,d]indole) and RU 28306, its N,N-dimethyl analog, were generously supplied by Roussel-Uclaf, Romainville, France. N'-methyl-3(1,2,5,6-tetrahydro-4-pyridyl)1H indole (THPI) and its 5-methoxy analog (5-MeO-THPI) were synthesized by Dr. Sham Nikam. A sample of 5-methoxy-diisopropyltryptamine (5-MeO-DIPT) was kindly provided by Dr. Richard Glennon of Virginia Commonwealth University. Metergoline was donated by Farmitalia Carlo Erba; methysergide by Sandoz; ketanserin by Janssen Pharmaceutica; phenoxybenzamine by Smith, Kline and French, and AHR 1709 by A.H. Robbins. \(^{3}H\)8-OH-DPAT was obtained from Research Products International, Mount Prospect, Illinois. \(^{3}H\)5-HT and \(^{3}H\)ketanserin were obtained from New England Nuclear Corporation. All other chemicals used were from standard commercial sources.

5-HT\(_{1}\)-Binding Assay

This was performed essentially as described by Nelson et al. (1983). Male Sprague-Dawley rats were killed by decapitation; the
brains were then rapidly removed and dissected over ice. The cortex dorsal to the rhinal sulcus was homogenized in 40 volumes of Tris-HCl (50mM, pH 7.4) using a Brinkmann Polytron (setting 5 for 15 sec). The homogenates were centrifuged at 48,000g for 10 min, the pellet was resuspended in buffer and this process repeated 3 more times. Between the second and third washes, the resuspended homogenates were incubated for 10 min at 37°C to facilitate the removal of endogenous 5-HT (Nelson et al., 1978). The final pellet was resuspended in Tris buffer for use in the binding assay to a final concentration of 10 mg (original wet weight) of tissue per ml. To each assay tube was added the following in sequence: 0.1ml of inhibitor dilution, 0.9ml of binding buffer (containing 50mM Tris and CaCl₂, paraglyline and [³H]5-HT to achieve final concentrations of 3mM, 100μM and 2nM respectively), and finally 1ml of tissue suspension. The tubes were then incubated for 10 min at 37°C, placed in ice water for 10 min, and then filtered through Whatman GF/B filters using a Brandel cell harvester. The filters were first cooled by a pre-wash of cold buffer, and each filtration was followed by 2 rinses with 4ml of cold buffer. After drying overnight, the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Specific [³H]5-HT binding was defined as the difference between binding in the absence and in the presence of 10μM cold 5-HT.

**5-HT₁A-Binding Assay**

This was essentially identical to the [³H]5-HT binding assay except that [³H]8-OH-DPAT to a final concentration of 1.0nM was used as ligand (the commercial product was diluted with cold 8-OH-DPAT to a
specific activity of 30-40 Ci/mmmole). In addition, the GF/B filters were pre-treated with polyethyleneimine as described below for the 5-HT\textsubscript{2} assay. As in the 5-HT\textsubscript{1} assay, non-specific binding was defined using 10\mu M 5-HT.

\textbf{5-HT\textsubscript{2} Binding Assay}

This was modified from the assay described by Leysen et al. (1982). Tissue was obtained as for the 5-HT\textsubscript{1} assay except that only frontal cortex was used. The tissue was homogenized in 10 volumes of ice cold 0.25M sucrose, using a teflon-glass homogenizer. After centrifugation for 10 min at 1,100g the pellet was rehomogenized in 5 volumes of 0.25M sucrose as above and again centrifuged at 1,100g for 10 min. The supernatant was diluted in Tris buffer (50mM, pH 7.6) to 1:80, w/v (based on the original wet weight of tissue), and the suspension centrifuged for 10 min at 48,000g. The pellet was resuspended in the same volume of Tris buffer and centrifuged again under the same conditions. The final pellet was resuspended in Tris buffer for use in the binding assay, to a final concentration of 5 mg (original wet weight) of tissue per ml. Since ketanserin was found to bind significantly to glass and other materials, the assays were conducted in disposable polypropylene tubes, and the GF/B filters were pre-treated with polyethyleneimine (soaked in a 0.1% v/v solution for 2 hr, then dried) to reduce nonspecific binding to the filters. To each assay tube was added 0.1ml inhibitor dilution, 0.9ml binding buffer (50mM Tris containing [\textsuperscript{3}H]ketanserin to a final concentration of about 0.3nM), and 1ml of tissue suspension. The assay tubes were incubated at 37°C for 15
min and then filtered immediately as described above. Non-specific binding was defined using 1 µM methysergide.

**Determination of Free Ligand Concentration**

For the 5-HT2 binding assay, 1ml of the tissue suspension was placed in a polypropylene tube and diluted with 0.1ml of water and 0.9ml of binding buffer containing the tritiated ligand and then incubated for 15 min at 37°C (all this is identical to an assay for total binding). This sample was then centrifuged at 2,000g for 10 min and a small aliquot of the supernatant dried in a scintillation vial. The actual free ligand concentration in the 5-HT2 assay could then be calculated from the amount of radioactivity in the aliquot of supernatant. (This procedure was carried out to take into account the ligand lost by binding to the tubes and the membranes).

For the 5-HT1 and 5-HT1A assays, the free ligand concentration as measured by a similar procedure was found to be essentially identical to that estimated by a direct count of the [3H]ligand added, so the latter method was routinely used.

**Analysis of Binding Data**

For the 5-HT1A ([3H]8-OH-DPAT) and 5-HT2 ([3H]ketanserin) binding assays, potencies of inhibiting drugs are reported as $K_i$ values, calculated from inhibitor $IC_{50}$ values using the equation

$$K_i = \frac{IC_{50}}{1 + (L/K_d)}$$

where $L$ is the radioligand concentration and $K_d$ is the dissociation
constant of the ligand-receptor complex (determined by the inhibition of the cold ligand for its own binding; for the 5-HT\textsubscript{1A} assay, the \(K_d\) for \(^{3}\text{H}\)8-OH-DPAT = 5.5\text{nM}; for the 5-HT\textsubscript{2} assay, the \(K_d\) for \(^{3}\text{H}\)ketanserin = 0.4\text{nM}).

Since \(^{3}\text{H}\)5-HT labels both 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} (and possibly other) sites, it is not always very meaningful to attempt to report inhibitor \(K_i\) values, since different inhibitors may have \(^{3}\text{H}\)5-HT binding inhibition curves with varying degrees of shallowness. Therefore, unless such curves are subjected to 2 site analysis (see below), only "overall" \(IC_{50}\) values will be reported for inhibition of \(^{3}\text{H}\)5-HT binding. If it is necessary to attempt to compare these values to \(K_i\) values from the 5-HT\textsubscript{2} or 5-HT\textsubscript{1A} assays, division of the 5-HT\textsubscript{1} \(IC_{50}\) by 2 gives the "apparent" \(K_i\) for the 5-HT\textsubscript{1} site (since the \(K_d\) of 5-HT for inhibition of its own binding is about 2\text{nM}).

The \(IC_{50}\) values for all three assays are determined from logit-log ("Hill") plots, directly from the curves themselves, or by use of nonlinear regression analysis (see below). All three methods give approximately the same values.

For multisite nonlinear regression analysis of \(^{3}\text{H}\)5-HT binding data, the computer program NONLIN (or the IBM PC version PC NONLIN) was used, based on the following models:

One site: \(b=B_H/(1+x/K'_{H})\)

Two site: \(b=B_H/(1+x/K'_{H}) + B_L/(1+x/K'_{L})\)

where \(b\) is the proportion of radioligand bound, \(B_H\) and \(B_L\) are the proportion of high and low affinity sites, respectively (expressed as
percentages in Table 3) and x is the concentration of the unlabelled inhibiting drug. $K'_H$ and $K'_L$ are the apparent dissociation constants (equivalent to IC$_{50}$ values) for the high- and low-affinity sites, respectively. The actual dissociation constant ($K_1$) is then calculated from the radioligand concentration using the equation given previously.

The statistical method of DeLean et al. (1982) using a partial F-test was used to determine if the data fit a two site model significantly better than a one site model.

Unless otherwise indicated, all tabular and other data represent the mean ± standard error of the mean (SEM) for 3 or more separate experiments. However, in some cases where literature values are used, standard errors are unavailable.

In Vitro Vascular Smooth Muscle Preparations

Both canine basilar artery (CBA) and rabbit femoral artery (RFA) preparations were used. Basilar arteries were dissected from mongrel dogs of either sex which had been killed by barbiturate overdose; femoral arteries were dissected from rabbits immediately after decapitation. The arteries were then immediately placed in Kreb's solution (at room temperature) which was composed as follows (mM):

- $Na^+$, 147.6; $K^+$, 6.4; $Ca^{++}$, 1.6; $Mg^{++}$, 1.2; $Cl^-$, 130; $HCO_3^-$, 26; $SO_4^{2-}$, 1.2; $H_2PO_4^-$, 1.2; glucose, 11; $Na_2EDTA$, 0.027. Circular artery segments with a length of 4mm (canine basilars) or 2mm (rabbit femorals) were then placed on parallel platinum wire stirrups and mounted in 50ml Krebs-Henseleit baths containing the Krebs buffer gassed with a mixture of 95%$O_2$-5%$CO_2$. After mounting, the temperature of the baths was raised to
37°C. The segments were connected to a force transducer and chart recorder and stretched to 1 g resting tension after equilibrating in the bath for 1 hr, at which time the bath solution was replaced. The optimal resting tension had been determined as described previously (Duckles, 1979). Cumulative concentration-response curves to agonists were obtained by the addition of 50 μl aliquots of drug dilutions, successive additions being made only after a stable or maximal response to the previous concentration had been attained. For each preparation, 1 or 2 control curves for 5-HT were first obtained to establish a stable response. Washout was always for a period of at least 15 min with at least 4 changes of buffer during this time.

Phenoxybenzamine (PBZ) was used as the irreversible antagonist. Although use of this compound is usually associated with adrenergic receptors, it has also been shown to irreversibly block vascular 5-HT receptors (Furchgott, 1972). PBZ has apparent Kᵢ values of 1400nM for 5-HT₁ binding and 110nM for 5-HT₂ binding (Leysen et al., 1981). The exposure to PBZ which was used (in the CBA, up to 1000nM for 15-20 min) produces a marked diminution of response which persists even after repeated washout. In the RFA, doses of 100-300nM were found to be sufficient.

For comparison of responses before and after treatment with PBZ, after the first cumulative concentration-response curve, PBZ was added to the bath for 15-20 min, followed by a 15 min washout. For experiments with other antagonists, the second agonist concentration-response curve was obtained in the presence of the antagonist after a 20
min preincubation. Data were analysed according to methods described in the following section.

**Analysis of Vascular Data**

Antagonist data were analysed by methods described by Van Rossum (1963) and specific protocols described in Ch.8. For partial agonists such as l-(m-trifluoromethylphenyl) piperazine (TFMPP), the $K_F$ was determined by the method of Waud (1969) as modified by Gero and Tallarida (1977).

Determination of $K_A$ Using an Irreversible Antagonist

The method of "partial irreversible blockade" (Furchgott and Bursztyn, 1967) was used to determine agonist dissociation constants ($K_A$ values). In this method, no unwarranted assumptions are made about 1) the function relating receptor occupancy to effect or 2) the amount of "spare receptors" present.

Concentration-effect curves were obtained before and after treatment with an irreversible antagonist. Concentrations of the agonist (A) producing equal responses before ([A]) and after ([A]') treatment were determined. It can be shown that a plot of $1/[A]$ (y axis) vs. $1/[A]'$ (x axis) gives:

$$\text{slope} = \frac{1}{q}$$

$$\text{y intercept} = \frac{(1-q)}{qK_A}$$

where $q$ is the fraction of receptors remaining functional after treatment. Thus

$$K_A = \frac{\text{slope} - 1}{\text{intercept}}.$$
General Procedure for the Syntheses of Tryptamine Derivatives

All the tryptamine derivatives were synthesized by the LiAlH₄ reduction of the indole glyoxyloyl amides, which were synthesized by the oxalyl chloride method (Speeter and Anthony, 1954). The synthetic scheme is shown as Fig. 2.

Either indole, 5-methoxy (5-MeO) indole or 5-benzyloxy (5-BzO) indole were used as starting materials; the 5-BzO tryptamine products were subsequently cleaved to form the 5-OH tryptamines by catalytic hydrogenation in ethanol with 10% palladium on carbon as catalyst. Cleavage of the benzyl ethers was confirmed by comparison of the proton NMR spectra of the 5-BzO and 5-OH tryptamines, as well as by elemental analysis of the products.

The experimental and analytical data on the synthesized compounds will be reported in the appropriate chapters, except for the synthesis of the constrained tryptamine analog 3-aminomethyl-1,3,4,5-tetrahydrobenz[cd]indole, which will be reported separately in Appendix A. The general synthetic procedure follows. Details of instrumentation, etc. are given in the experimental section of Appendix A.

Synthesis of Indole Glyoxyloyl Amides

Ten mmol of substituted indole is dissolved in about 20 ml of anhydrous diethyl ether and the flask is cooled on ice. Oxalyl chloride (15 mmol) in 15 ml ether is added dropwise with stirring, which is continued for 20 min after the addition is complete. The precipitated yellow-orange indole glyoxyloyl chloride is filtered on a fritted glass
funnel and washed with 20 ml of cold ether, then returned to the rinsed flask and resuspended in 50 ml dry THF. The amine component (25-35 mmol), dissolved in 25 ml THF, is added slowly at room temperature and stirred for 30 min, then refluxed for an additional 30 min. After cooling, the solvent is removed under vacuum and the residue is partitioned between 60 ml ethyl acetate and 60 ml water containing 2 ml of conc. HCl. The organic layer is washed with water and then saturated NaCl, then dried over Na₂SO₄. After removal of the solvent under vacuum, the product can be crystallized from MeOH/hexane or THF/ether.

Reduction of Indole Glyoxyloyl Amides

To 1 gm (26 mmol) of LiAlH₄ suspended in 40 ml dry THF, 5 mmol of the amide in 30 ml THF is added dropwise at room temperature. The mixture is refluxed for 90 min, then cooled and neutralized (with vigorous stirring) by 4 ml of MeOH (dropwise) followed by 6 ml of saturated aqueous Na₂SO₄. Stirring is continued for 15 min, followed by filtration and wash with additional THF. The filtrate is evaporated under vacuum and the product is taken up in ether or ethyl acetate, washed with water, saturated NaCl and then dried. The product can then be purified by sublimation, chromatography or formation of an amine salt (see below) and subsequent recrystallization. Usually the latter method is preferred.

Catalytic Hydrogenation of 5-BzO Tryptamines

The pure 5-BzO-tryptamine free base (100 mg) is dissolved in 50 ml of absolute ethanol. Fifty mg of 10% palladium on carbon is added
and the mixture shaken in a Parr hydrogenation apparatus, using a hydrogen pressure of 50-60 lb/sq.in. After 6 hr, the mixture is filtered through a compressed Celite pad in a fritted glass funnel. The ethanol is removed from the filtrate under vacuum and the residue is taken up in dry ethyl ether and again filtered. This is used directly for amine salt formation as described below.

Formation of Amine HCl or Hydrogen Oxalate Salts

To a solution of the amine in ethyl ether (or ether containing the minimum of THF if solubility is a problem) either anhydrous HCl in MeOH or oxalic acid in ether is added until a basic reaction is undetectable with pH paper. The salt usually precipitates immediately, but stirring is maintained for 5-30 min before filtration. The product is well rinsed with ether and placed immediately in a dessicator, taking care not to let condensation form in the funnel by the rapid evaporation of ether. If necessary, the product can be recrystallized from alcohol-ether mixtures.
Figure 2. Synthetic scheme for substituted tryptamine derivatives.
CHAPTER 3

EFFECTS ON [3H]5-HT BINDING OF INCREASING SUBSTITUENT SIZE IN A SERIES OF N-SUBSTITUTED TRYPTAMINES

At the outset of this research project it was apparent that, although a number of tryptamine (TRYP) derivatives were commercially available and had been examined in previous studies, the lack of complete homologous series of compounds made it difficult to draw general conclusions on the SAR for 5-HT binding sites, since published studies often included somewhat randomly substituted collections of analogs.

Thus, as a first approach to the problem, the study of a series of tryptamines substituted at the amino nitrogen was chosen, since this part of the molecule is easily amenable to synthetic alteration. The effects of increasing alkyl substituent size could then be studied independently of other molecular alterations (such as at the important indole 5 position).

Methods

A series of N-substituted tryptamines (Fig. 3) with increasing complexity of amino function was obtained from commercial sources (TRYP, DMT, DET) or synthesized by the standard method (DIPT, PEI, MEI). The compounds were assayed using the 5-HT₁ assay procedure. The IC₅₀ and slopes of logit-log plots were determined. For compounds which appeared
Figure 3. Structures of N-substituted tryptamines named in Table 2.
to discriminate between subtypes of 5-HT\textsubscript{1} binding sites, 2-site analysis was performed.

**Results**

Synthesized Compounds

The compounds are listed in Table 1 along with experimental data. Since these three compounds have been synthesized previously and studied by various authors (Barlow and Kahn, 1958; Bradley and Johnston, 1970; Shulgin, 1980), analytical data for the intermediate amides will not be reported. The tryptamine free bases were purified by repeated vacuum sublimation; C,H,N analyses were within <0.4% of theoretical values. Melting points of the free bases have been included, since these have not been previously reported. Melting point ranges of the hydrochlorides were essentially identical to literature values.

\[^3H\]5-HT Binding Assays

Table 2 lists the chemical names of the compounds along with the overall IC\textsubscript{50}s for the inhibition of \[^3H\]5-HT binding. Although these values suggest a general decrease in potency as the bulk of the amino substituent is increased (with the exception that differences in IC\textsubscript{50} values for tryptamine, DMT and DET are not statistically significant), examination of the binding inhibition curves (Fig. 4A-4C) reveals that the IC\textsubscript{50} values alone do not give a complete picture of the interaction of these compounds with 5-HT\textsubscript{1} sites. Several of the compounds had distinctly biphasic binding inhibition curves, compared to the monophasic curve shown for the potent 5-HT antagonist metergoline (MET).
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>YIELD FROM LAH REDUCTION</th>
<th>SALT</th>
<th>RECRYSTALLIZED FROM</th>
<th>MELTING POINT °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diisopropyl Tryptamine (DIPT)</td>
<td>73%</td>
<td>Free base</td>
<td>Sublimed</td>
<td>73-74</td>
</tr>
<tr>
<td>3-(2-morpholinoethyl) indole (MEI)</td>
<td>64%</td>
<td>HCl</td>
<td>EtOH/Et₂O</td>
<td>194-196</td>
</tr>
<tr>
<td>3-(2-piperidinoethyl) indole (PEI)</td>
<td>69%</td>
<td>HCl</td>
<td>EtOH/Et₂O</td>
<td>215-216</td>
</tr>
<tr>
<td>COMPOUND</td>
<td>[3H]5-HT BINDING, IC$_{50}$, nM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>---------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptamine (TRYP)</td>
<td>176 ± 27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N,N-Dimethyl Tryptamine (DMT)</td>
<td>137 ± 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N,N-Diethyl Tryptamine (DET)</td>
<td>176 ± 42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N,N-Diisopropyl Tryptamine (DIPT)</td>
<td>961 ± 272</td>
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</tr>
<tr>
<td>3-(2-Piperidinoethyl) indole (PEI)</td>
<td>1000 ± 78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-(2-Morpholinoethyl) indole (MEI)</td>
<td>1421 ± 262</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Data from Nelson, Weck and Taylor, 1983)
<table>
<thead>
<tr>
<th>DRUG</th>
<th>$K_H$ (nM)</th>
<th>$K_L$ (nM)</th>
<th>$B_H$ (%)</th>
<th>$B_L$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRYP</td>
<td>33.4</td>
<td>&gt;10,000</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>DMT</td>
<td>84.5</td>
<td>-</td>
<td>93</td>
<td>-</td>
</tr>
<tr>
<td>DET</td>
<td>2.6</td>
<td>268</td>
<td>28</td>
<td>71</td>
</tr>
<tr>
<td>DIPT</td>
<td>31.3</td>
<td>4,356</td>
<td>37</td>
<td>61</td>
</tr>
<tr>
<td>PEI</td>
<td>89.2</td>
<td>7,321</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>MEI</td>
<td>48.9</td>
<td>4,606</td>
<td>28</td>
<td>57</td>
</tr>
</tbody>
</table>

$^a$ See Ch.2, 'Analysis of Binding Data', for definitions of $K_H$, $K_L$, $B_H$ and $B_L$.

(Data from Nelson, Weck and Taylor, 1983)
Figure 4. 5-HT binding inhibition curves for N-substituted tryptamines and metergoline (MET).
The slopes of logit-log plots for all the tryptamines were significantly less than unity (except for TRYP itself, for which the logit-log plot was nonlinear), which is also consistent with the possibility that these compounds discriminate between subtypes of \[^{3}H\]5-HT binding sites.

The parameter estimates from nonlinear regression analysis of the \[^{3}H\]5-HT binding inhibition curves produced by TRYP and its analogs are shown in Table 3. All of the curves except that for DMT fit a 2-site model significantly better than a 1-site model (p<0.01).

**Discussion**

It is apparent that the 5-hydroxyl group of 5-HT is of considerable importance for its potency at 5-HT\(_1\) sites, since TRYP has an IC\(_{50}\) value approximately 35-60 times greater than cold 5-HT. In addition, even at very high concentrations, TRYP fails to inhibit about 25% of specific \[^{3}H\]5-HT binding. The results of 2-site analysis suggest that, at the sites it does recognise, TRYP may actually be slightly more potent than DMT, which has the most monophasic curve of all the tryptamines tested.

There is little difference between DMT and DET (Fig. 4B), but increasing the substituent bulk to form DIPT or the heterocyclic amines PEI or MEI results in an almost tenfold increase in overall IC\(_{50}\), while at the same time enhancing the discrimination between a low affinity site and a high affinity site comprising about 30-40% of the sites. Thus, despite the overall lower potency (as defined by IC\(_{50}\) values) observed for compounds such as DIPT and MEI, these results suggest that
these compounds do recognise a subset of $[^3\text{H}]5$-HT binding sites with comparatively high affinity ($K_i < 50 \text{ nM}$).

These findings are also consistent with previous studies which have demonstrated that $[^3\text{H}]5$-HT binds to a heterogeneous population of sites (Nelson et al., 1981; Pedigo et al., 1981). Although the curves do not contain enough points for a first-rate 3-site analysis, it should be noted that for both DIPT and PEI it was found that the data fit a 3-site model significantly better than a 2-site model. This is at best only suggestive of the possibility that $[^3\text{H}]5$-HT labels more than two subtypes of binding sites, but nonetheless is in agreement with a previous study comparing the effects of various drugs in different species (Nelson et al., 1982), as well as the more recent introduction of the $5$-HT$_{1C}$ receptor subtype into the literature (Pazos et al., 1984)
Analogs of MEI and DIPT were chosen for further study because out of the six simple tryptamine derivatives studied in the previous chapter, these two compounds showed the greatest discrimination between subsets of the 5-HT₁ binding site. This was also a fortuitous choice in that, as described below, the DIPT series proved to be vascular 5-HT agonists, whereas several of the analogs of MEI proved to be vascular 5-HT antagonists in the CBA preparation. This is consistent with the observations of previous investigators regarding DIPT and MEI. Barlow and Kahn (1958) found that DIPT was very active in causing contractions of the rat fundic strip preparation, whereas MEI was essentially inactive as an agonist. Using a behavioral disruption test (an animal model of hallucinogenic action) Bradley and Johnston (1979) reported that, in a similar series of compounds, DIPT was the most active; MEI was found to be inactive.

The importance of the substituent at the indole 5 position for potency at 5-HT₁ sites was pointed out in Ch.3. Thus, comparisons of the potency of TRYP, 5-HT and 5-methoxytryptamine(5-MeO-TRYP) suggest that the 5-OH group is most favorable, closely followed by 5-MeO, with the 5-unsubstituted compound being least potent. This suggested the approach of synthesizing some 5-oxy analogs of DIPT and MEI, in the hope
that some analogs might retain the discriminative ability of the parent compounds but that greater potency (conferred by a 5-MeO or 5-OH group) might be obtained.

**Methods**

A series of tryptamine derivatives substituted at the indole 5 position were synthesized by the standard method. The compounds were 5-OH and 5-BzO derivatives of DIPT and MEI, and the 5-MeO derivative of MEI (5-MeO-DIPT was provided by Dr. Richard Clennon). The derivatives were assayed using the 5-HT₁ assay procedure and the IC₅₀ values determined. About half of the compounds were tested for activity in the CBA and RFA vascular preparations using procedures described in Ch.2.

**Results**

**Synthesized Compounds**

The compounds are listed in Table 4 (the intermediate indole glyoxyloyl amides) and Table 5 (the tryptamine salts), along with the experimental data. The compounds were purified by recrystallization from the solvent pairs indicated. Melting points and C,H,N analyses are reported for all the compounds.

**[^H]5-HT Binding Assays**

Table 6 lists the two parent compounds and their 5-substituted analogs, along with their overall IC₅₀ for the inhibition of[^H]5-HT binding. In both cases, the addition of the 5-BzO group leads to a significant loss in potency. The two 5-OH analogs, as expected, are
# TABLE 4

**INDOLE GYLOXYLOYL AMIDES FOR 5-OXY ANALOGS OF DIPT AND MEI**

**STRUCTURE:**

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>YIELD</th>
<th>MELTING POINT, °C</th>
<th>(\text{C,H,N} ) (THEORY)</th>
<th>(\text{C,H,N} ) (FOUND)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_1 = \text{MeO-} ), ( R_2 = \text{O} )</td>
<td>71%</td>
<td>191-193</td>
<td>C=62.49, H=5.59, N=9.72</td>
<td>C=62.65, H=5.50, N=9.50</td>
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<tr>
<td>( R_1 = \text{BzO-} ), ( R_2 = \text{O} )</td>
<td>76%</td>
<td>172</td>
<td>C=69.22, H=5.53, N=7.69</td>
<td>C=68.85, H=5.48, N=7.59</td>
</tr>
<tr>
<td>( R_1 = \text{BzO-} ), ( R_2 = \text{iPr}_2\text{N}^- )</td>
<td>64%</td>
<td>213-214</td>
<td>C=72.99, H=6.92, N=7.40</td>
<td>C=72.76, H=7.16, N=7.34</td>
</tr>
</tbody>
</table>

(The amides were recrystallized from MeOH/hexane)
TABLE 5
5-OXY ANALOGS OF DIPT AND MEI

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>YIELD FROM LAH REDUCTION OR CATALYTIC HYDROGENATION</th>
<th>AMINE SALT</th>
<th>MELTING POINT, °C</th>
<th>C,H,N (THEORY)</th>
<th>C,H,N (FOUND)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MeO-MEI</td>
<td>75%</td>
<td>HYDROGEN OXALATE</td>
<td>196-197</td>
<td>C=58.28</td>
<td>C=58.45</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H= 6.33</td>
<td>H= 6.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N= 8.00</td>
<td>N= 7.86</td>
</tr>
<tr>
<td>5-BzO-MEI</td>
<td>77%</td>
<td>HCl</td>
<td>232</td>
<td>C=67.64</td>
<td>C=67.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H= 6.76</td>
<td>H= 6.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N= 7.51</td>
<td>N= 7.33</td>
</tr>
<tr>
<td>5-OH-MEI</td>
<td>91%</td>
<td>HYDROGEN OXALATE</td>
<td>226-228</td>
<td>C=57.14</td>
<td>C=57.87</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>H= 5.99</td>
<td>H= 6.26</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>N= 8.33</td>
<td>N= 8.47</td>
</tr>
<tr>
<td>5-BzO-DIPT</td>
<td>93%</td>
<td>HCl</td>
<td>221-222</td>
<td>C=71.39</td>
<td>C=71.69</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>H= 8.07</td>
<td>H= 8.24</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>N= 7.24</td>
<td>N= 7.16</td>
</tr>
<tr>
<td>5-OH-DIPT</td>
<td>84%</td>
<td>HYDROGEN OXALATE</td>
<td>233-235</td>
<td>C=61.70</td>
<td>C=61.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H= 7.48</td>
<td>H= 7.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N= 7.99</td>
<td>N= 7.82</td>
</tr>
</tbody>
</table>

(The compounds were recrystallized from MeOH/ether)
more potent than the parent compounds, but, by this overall measure of potency, the 5-MeO group only confers a slight advantage in the MEI series, and actually appears to have led to a slight loss in potency in the DIPT series.

The actual binding inhibition curves are exemplified for the MEI series in Fig. 5 (results for the DIPT series are essentially similar). A closer examination of the results by inspection of the shapes of these curves reveals that the addition of the 5-MeO group to MEI actually does confer a significant potency increase relative to the low affinity portion of the MEI binding inhibition curve, but that the simultaneous loss of the high affinity component results in an approximate net equality of IC$_{50}$ values for the two compounds. Although 5-OH-MEI is overall the most potent of the series, it too has lost the high affinity binding component.

Underlying these results is the fundamental observation that for both these series of compounds, the 5-OH and 5-MeO analogs have much more monophasic [$^3$H]5-HT binding inhibition curves than the parent compounds, and thus the most interesting attribute of the parent compounds appears to have been lost by these substitutions.

Vascular Studies

The most significant result of this study was probably the finding that the 5-MeO and 5-OH analogs of MEI are both vascular 5-HT antagonists in the CBA preparation, with approximate $K_B$ values of 900 ± 100 nM and 860 nM respectively. (The latter value was based on only one determination because at this time the 5-OH-MEI, which is not very
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>[(^{3}\text{H})]5-HT BINDING, IC(_{50}), nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIPT</td>
<td>960 ± 270</td>
</tr>
<tr>
<td>5-MeO-DIPT</td>
<td>2000 ± 600</td>
</tr>
<tr>
<td>5-BzO-DIPT</td>
<td>7200 ± 800</td>
</tr>
<tr>
<td>5-OH-DIPT</td>
<td>740 ± 260</td>
</tr>
<tr>
<td>MEI</td>
<td>1420 ± 260</td>
</tr>
<tr>
<td>5-MeO-MEI</td>
<td>1100 ± 400</td>
</tr>
<tr>
<td>5-BzO-MEI</td>
<td>9400 ± 2000</td>
</tr>
<tr>
<td>5-OH-MEI</td>
<td>400 ± 70</td>
</tr>
</tbody>
</table>
Figure 5. 5-HT₁ binding inhibition curves for MEI and its 5-oxy analogs.
stable, already appeared to have undergone some decomposition, so the experiment was not repeated). It was also possible to demonstrate 5-HT antagonism in the CBA using MEI itself, but only at very high concentrations, suggesting a $K_B$ of about 10,000 nM.

Both DIPT and 5-MeO-DIPT were found to be agonists in the CBA, with $K_A$ values of $5,300 \pm 700$ nM and $1,010 \pm 410$ nM respectively. DIPT was also an agonist in the RFA (5-HT$_2$) preparation, with an apparent $K_A$ of about $3,300$ nM (N=2). The 5-OH and 5-MeO analogs of DIPT were not tested in the RFA.

**Discussion**

In terms of the original objectives, the results of this study can only be termed disappointing, but nonetheless significant in that the possibility of developing 5-HT antagonists from simple tryptamine derivatives was demonstrated. This ultimately led to the development of the aryl substituted tryptamine 5-HT antagonists described in Ch.6.

The potencies of the compounds tested in the CBA preparation were all quite close to their potencies at the 5-HT$_1$ binding site, or the low affinity 5-HT$_1$ sites for MEI and DIPT. These data were incorporated in a more detailed study of the pharmacological profile of the CBA 5-HT receptor (Taylor et al., 1985: see Ch.8).

The failure to increase the potency of MEI and DIPT without a concomitant loss of selectivity was also a stimulus to the development of an alternate approach to the design of selective and potent 5-HT analogs, based on the consideration of conformationally constrained analogs of 5-HT.
CHAPTER 5

RELATIVE SELECTIVITY OF SOME CONFORMATIONALLY CONSTRAINED 5-HT ANALOGS AT 5-HT\textsubscript{1}, 5-HT\textsubscript{1A}, AND 5-HT\textsubscript{2} BINDING SITES

The results of the study described in the previous chapter seemed to offer little hope for the possibility of developing potent as well as selective tryptamine derivatives. It seemed that, although some simple tryptamine derivatives were capable of discriminating between different types and subtypes of 5-HT binding sites, most such compounds were not potent enough to be useful as selective ligands or drugs. The problem is that the few simple tryptamines which do show very high affinity for 5-HT receptors (e.g., 5-methoxydimethyltryptamine) are generally so structurally homologous to 5-HT that their activity and binding profiles are also very similar: thus their selectivity is unlikely to be much better than that of 5-HT itself. (Fortunately, exceptions to this generalization were discovered in the class of compounds discussed in Ch.6).

One of the properties of tryptamines is that they can assume different conformations, by rotations around the bonds of the ethylamino side chain. If it is assumed that perhaps 5-HT is recognised in different conformations by different receptor types, then clearly the problem with trying to use other tryptamines as selective ligands for 5-HT receptors is that they can assume all the same conformations as 5-HT: thus, unless considerably altered, they are unlikely to be much more selective than 5-HT itself. Therefore, the development of
compounds which are potent and selective may be contingent upon the
determination of the precise side chain conformation of 5-HT recognised
at each receptor site (i.e. the pharmacophore for that receptor). In
theory, an analog incorporating such a conformation into its structure,
by eliminating the time spent in non-optimal conformations, would show
enhanced affinity for that particular receptor type.

The comparative study of various conformationally constrained or
rigid analogs is one method which can be used to attempt to determine
the pharmacophore for a particular receptor. It must be kept in mind,
however, that the interpretation of the results of such studies can
involve the consideration of more than just conformational factors
alone, since other structural features are inevitably introduced into
the molecule in the process of restricting the conformation. These
changes can alter the electronic and other physical properties of the
molecule, obscuring the effect of the conformational change. Despite
this caveat, the study of rigid analogs is probably the most effective
way to map the pharmacophore of a receptor, short of x-ray studies of
the ligand-receptor complex.

The choice of some prototype rigid analogs of 5-HT is not
difficult. Compared to most other indole derivatives, the high potency
shown by ergolines such as d-LSD, metergoline and methysergide at all
the 5-HT binding sites would suggest that an ergoline-like conformation
of the amino nitrogen might be optimal for recognition at 5-HT receptor
sites, and thus correspond to the pharmacophore for those receptors.
This view has been either tacitly or explicitly advocated by many,
particularly in the hallucinogen field (eg. Kang and Green, 1970;
Nichols et al., 1978). It has also been used to explain the activity of at least one useful selective serotonergic agent: the 5-HT$_{1A}$ agonist 8-OH-DPAT (Arvidsson et al., 1984), which is a partial ergoline without the pyrrole ring, with a hydroxy group in a position corresponding to the indole 5 position.

Just as the ergolines are a promising class of compounds for this purpose, other classes of compounds can be ruled out on the basis of previous studies. In particular, derivatives of tetrahydro-beta-carboline (THBC) have been found to have very low affinities for both 5-HT$_1$ and 5-HT$_2$ binding sites (Casio and Kellar, 1982). At both 5-HT binding sites, THBC is roughly an order of magnitude less potent than tryptamine, its corresponding non-rigid analog. Hydroxy and methoxy THBC analogs are even less potent. In addition, indole alkaloids of the yohimbine type are conformationally similar to THBC; yohimbine also has rather low affinity ($K_i$ > 600 nM) for both 5-HT$_{1A}$ and 5-HT$_2$ sites (Gozlan et al., 1983; Leysen et al., 1981). Thus, these types of rigid analogs have not been included in this study.

Fig. 6 shows the structures of some of the analogs included in the present study, along with d-LSD for purposes of comparison. 2 partial ergolines were examined: RU 27849 (A, R=H) which is a rigid analog of tryptamine, and RU 28306 (A, R=Me), a rigid analog of DMT.

The partially constrained tetrahydropyridylindole analog RU 28253 (B) is very potent at both 5HT sites: it can assume an ergoline-like conformation (inset), or, by rotation, one such as that shown as B. Thus, its active conformation could vary for different 5-HT receptor types. For the purposes of comparison, RU 28253 can be
Figure 6. Some conformationally constrained analogs with structural homology to tryptamines.
considered a constrained analog of 5-MeO-tryptamine. A related compound was also tested: N'-methyl-3(1,2,5,6-tetrahydro-4-pyridyl)1H indole (THPI; structure not shown). This compound is an N'-methyl, des-methoxy, 4-pyridyl analog of RU 28253. For the sake of comparison THPI can be regarded as a constrained analog of DMT; however, since in the 4-pyridyl indoles there are 3 carbons between the indole and the amino nitrogen, the analogy to the tryptamine structure is not precise.

An analog such as C, a 3-aminomethyl-1,3,4,5-tetrahydrobenz-[cd]indole (3-aminomethyl-THBI), which can approximate only the non-ergoline conformations of RU 28253, would be useful as an analog for conformations intermediate between the ergoline and beta-carboline types. One analog of this type was synthesized and tested (Fig. 6C, R=H); like the partial ergoline RU 27849, this compound can be regarded as a rigid analog of tryptamine.

**Methods**

A series of constrained analogs of tryptamine, DMT and 5-MeO-tryptamine was obtained from various sources (noted in Ch.2); 3-aminomethyl-THBI was synthesized as described in Appendix A. These compounds and the corresponding free chain tryptamines were assayed using the 5-HT<sub>1</sub>, 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> assay procedures. Since none of the compounds appeared to discriminate very significantly between subtypes of [³H]5-HT binding sites (as discussed below), 2 site analysis of the [³H]5-HT binding data was not performed.
Results

For all of the compounds, IC$_{50}$ or $K_i$ values against all 3 tritiated ligands are given in Table 7. None of the compounds show a high degree of selectivity for 5-HT$_{1A}$ over 5-HT$_{1B}$ binding, since for all compounds the $K_i$ values vs. the 5-HT$_{1A}$ ligand $[^3H]8$-OH-DPAT are close to the "apparent" $K_i$ values for 5-HT$_1$ sites obtained by dividing by 2 the listed IC$_{50}$s for inhibition of $[^3H]5$-HT binding.

Since the object of this study was to find rigid or semi-rigid analogs which showed enhanced potency relative to the corresponding free chain compounds, the compounds grouped within each section of the table are to be compared because of their structural homology (as discussed previously). Thus, comparison of the partial ergolines RU 27849 and RU 28306 to tryptamine and DMT respectively shows that at both 5-HT$_1$ and 5-HT$_{1A}$ sites, the partial ergoline analogs are generally several times less potent than the corresponding tryptamines; at 5-HT$_2$ sites, however, the partial ergolines are approximately equipotent to their corresponding tryptamine analogs. Surprisingly, the partial ergolines do not show significantly enhanced potency at any of the 5-HT binding sites.

In contrast, the 3-aminomethyl-THBI analog of tryptamine (Fig. 6C, R=H), although even less favorable than the partial ergolines at the 5-HT$_1$ sites, shows significantly enhanced potency (4-5 fold) at the 5-HT$_2$ site, relative to both tryptamine and RU 27849.

The two tetrahydropyridyl indole analogs THPI and RU 28253 also showed enhanced potency at the 5-HT$_2$ site relative to their non-rigid
TABLE 7

5-HT₁, 5-HT₁A AND 5-HT₂ BINDING PARAMETERS FOR CONFORMATIONALLY
CONSTRAINED ANALOGS COMPARED TO THE HOMOLOGOUS TRYPTAMINES

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>5-HT₁:</th>
<th>5-HT₁A</th>
<th>5-HT₂:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OVERALL IC₅₀</td>
<td>[³H]8-OH-DPAT</td>
<td>[³H]KETANSERIN</td>
</tr>
<tr>
<td></td>
<td>FOR [³H]5-HT</td>
<td>Kᵢ, nM</td>
<td>Kᵢ, nM</td>
</tr>
<tr>
<td>RU 27849</td>
<td>520 ± 130</td>
<td>325 ± 57</td>
<td>2900 ± 1200</td>
</tr>
<tr>
<td>TRYPTAMINE</td>
<td>176 ± 27</td>
<td>125 ± 29</td>
<td>2200 ± 140</td>
</tr>
<tr>
<td>3-AMINOMETHYL-THBI</td>
<td>800 ± 150</td>
<td>369 ± 42</td>
<td>465 ± 58</td>
</tr>
<tr>
<td>RU 28306</td>
<td>390 ± 50</td>
<td>338 ± 51</td>
<td>309 ± 60</td>
</tr>
<tr>
<td>DMT</td>
<td>137 ± 30</td>
<td>232 ± 77</td>
<td>225 ± 90</td>
</tr>
<tr>
<td>THPI</td>
<td>490 ± 90</td>
<td>154 ± 29</td>
<td>73 ± 10</td>
</tr>
<tr>
<td>RU 28253</td>
<td>20 ± 8</td>
<td>5.7 ± 2.2</td>
<td>84 ± 9</td>
</tr>
<tr>
<td>5-MeO-TRYPTAMINE</td>
<td>13 ± 10</td>
<td>6.1 ± 2.4</td>
<td>1695⁺</td>
</tr>
</tbody>
</table>

⁺ Data from Martin and Sanders-Bush (1982), based on the
inhibition of [³H]spiperone binding.
analogs. At this site, THPI is approximately 3 times as potent as the (somewhat) analogous compound DMT, and RU 28253 is many times more potent than its homolog 5-MeO-tryptamine. In addition, at the 5-HT₁A site, THPI and RU 28253 are at least equipotent to DMT and 5-MeO-tryptamine, respectively. Interpretation of the [³H]5-HT binding data for this set of compounds is complicated by the fact that, of all the compounds in this table, the two tetrahydropyridylindoles (particularly THPI) have the most shallow inhibition curves for inhibition of [³H]5-HT binding. However, it is apparent that the 3-pyridyl analog RU 28253 is a potent inhibitor of the binding of all three ligands.

Discussion

The results of this study can be briefly summarized as follows:

1) At the 5-HT₁ sites, none of the rigid analogs show significantly enhanced potency relative to the homologous free chain compounds; however, the tetrahydropyridylindoles are equipotent to the related tryptamines, at least at the 5-HT₁A site.

2) Although the partial ergolines do not appear to have an optimal conformation for 5-HT₁ binding sites, since they are generally several times less potent than TRYP and DMT, the non-ergoline conformations attainable by 3-aminomethyl-THBI appear to be even less favorable, since it is even less potent than RU 27849. This is true for the [³H]5-HT binding site, and, to a lesser extent, for the [³H]8-OH-DPAT binding site.
3) At the 5-HT₂ site, the partial ergolines RU 27849 and RU 28306, although not showing enhanced potency relative to their free chain homologs (TRYP and DMT, respectively), are nonetheless approximately equipotent to them.

4) At the 5-HT₂ site, the non-ergoline-like analog 3-aminomethyl-THBI is significantly more potent (4-5 times) than the two homologous compounds, tryptamine and the partial ergoline RU 27849.

5) The tetrahydropyridylindoles THPI and RU 28253 both show considerably enhanced potency at 5-HT₂ sites relative to the homologous tryptamines DMT and 5-MeO-tryptamine.

The observation that the partial ergolines do not show significantly enhanced potency at any of the 5-HT binding sites is somewhat unexpected, considering the high affinity shown by ergoline derivatives such as d-LSD and metergoline for those sites. This observation is particularly difficult to explain for the 5-HT₁A site, since the compound now used to label those sites, 8-OH-DPAT, is a partial ergoline without the pyrrole ring. One factor that must be considered in interpreting the binding data for the partial ergolines (and also for 3-aminomethyl-THBI) is that they are racemates. Thus, if one enantiomer is almost inactive, the potency of the other enantiomer could be as much as twice as that estimated for the mixture. Also, it may be that the 5-HT₁ sites are exquisitely sensitive as far as the exact conformation required for the amino nitrogen, and that these partial ergoline derivatives are sufficiently different from that optimal conformation to explain the observed lack of potency. In this
case, the very lack of rigidity posessed by the free chain compounds may confer an advantage because, although they can assume many unfavorable conformations, the exact optimal conformation can be induced during their interaction with the receptor.

The tetrahydropyridylindoles (particularly the 3-pyridyl series) may be useful prototypes for the design of other rigid analogs for 5-HT$_1$ receptors, since they are the most promising of all the analogs examined in terms of showing high potency at 5-HT$_1$ sites. As discussed previously, the compounds in the 3-pyridyl indole series are of considerable interest because of their ability to assume both ergoline-like and non-ergoline-like conformations. It is possible that RU 28253 may bind optimally to 5-HT$_1$ sites in some conformation in which the two rings are roughly perpendicular (intermediate between the two rotations shown in Fig. 6). However, it is also possible that the potency of these compounds originates at least in part from electronic effects inherent in structural features such as the conjugated double bond, and that conformational parameters are of secondary importance. In this regard, it is interesting that in the case of d-LSD, the conjugated double bond of the so-called D ring is known to be extremely important, at least as far as hallucinogenic potency is concerned (Kang and Green, 1970; Fanchamps, 1978). It is also known that the stereochemistry and alkyl substituent size on the amide of d-LSD is critically important for psychoactivity and 5-HT antagonism (Fanchamps, 1978). Thus, it is possible that factors such as these, rather than the position of the ergoline nitrogen, are most responsible for the high affinity of d-LSD
and its analogs for 5-HT binding sites. This question must remain open until more rigid analogs of 5-HT become available for study.

The finding that both RU 28253 and 3-aminomethyl-THBI show enhanced potency at the 5-HT\textsubscript{2} site is exciting, for the following reason. If the indole rings of these two structures are superimposed, there is only one possible conformation for each enantiomer of 3-aminomethyl-THBI where the amino nitrogens of the two compounds can be made to superimpose. Since this conformation represents only one of many possible conformations for both RU 28253 and 3-aminomethyl-THBI (neither of which is completely rigid), it would be interesting to synthesize a completely rigid analog with this conformation. The synthesis of such an analog has recently been proposed, but has not yet been executed (Martin, 1985). These observations also provide a strong incentive for the resolution of 3-aminomethyl-THBI, because if its enhanced potency at 5-HT\textsubscript{2} sites is genuinely related to conformational factors, one would expect to see a distinct preference for one of the enantiomers. This would give insight into the stereochemistry of the 5-HT\textsubscript{2} receptor site.
DISCRIMINATION BETWEEN 5-HT BINDING SITE SUBTYPES SHOWN BY ARYL SUBSTITUTED TRYPTAMINES: VASCULAR ANTAGONISTS OF 5-HT

The primary incentive for this research has been the fact that although there are a number of antagonists available that are selective for 5-HT receptors of the 5-HT\textsubscript{2} type, no antagonists selective for 5-HT\textsubscript{1} receptors or the 1A and 1B subtypes have been developed. Although some compounds have been demonstrated to have 1A or 1B selectivity, they have either not been demonstrated to be antagonists, or else they also have high potency at 5-HT\textsubscript{2} or other monoamine receptors, and thus are not true selective antagonists. For example, spiroxatrine, a compound related to spiperone, is selective for 5-HT\textsubscript{1A} sites (Nelson and Taylor, 1985) but also has high affinity for D-2 receptors (Dan Burns, personal communication). In this chapter, a series of compounds that show a high degree of selectivity between 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} sites, and are also vascular antagonists of 5-HT, is described.

Preliminary binding studies with the aryl substituted tryptamine AHR 1709 (Fig. 7) indicated that this compound was capable of discriminating between 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} binding sites (Nelson et al., 1981). Since \(^3\text{H}\)5-HT labels both 1A and 1B sites, the \(^3\text{H}\)5-HT binding inhibition curve for such a compound is extremely shallow or biphasic. Subsequent experiments have confirmed that AHR 1709 has high affinity for both 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptors, but very low affinity for 5-HT\textsubscript{1B} receptors (Fig. 8A). Preliminary results with a rabbit femoral artery
preparation suggested that this compound was a vascular 5-HT antagonist.

The structure of this compound is interesting because it has certain features in common with DIPT and MEI, two tryptamine derivatives which had also been observed to have very shallow \([^3H]5-HT\) binding inhibition curves (Ch.3; Nelson et al., 1983). Specifically, all three of these compounds are tryptamine derivatives with bulky or heterocyclic amino substituents; AHR 1709 has the added feature of an attached aromatic ring. A characteristic not shared by DIPT and MEI is the high affinity shown by AHR 1709 for 5-HT\(_2\) receptors \((K_A = 4.8\text{nM})\); to our knowledge it is by far the most potent tryptamine analog at the 5-HT\(_2\) site, being about 60 times as potent as 5-HT. This unfortunately is a drawback as far as its potential as a selective 5-HT\(_{1A}\) antagonist is concerned; however, because of the generally low affinities shown by tryptamines for the 5-HT\(_2\) site, there are reasonable prospects for the possibility of altering the pharmacological profile of AHR 1709 in the desired direction.

Using AHR 1709 as a lead compound, several other aryl substituted tryptamines were synthesized (Fig. 7) to see if they also showed 1A-1B selectivity, and to see if it was possible to reduce the affinity at 5-HT\(_2\) receptors so that a selective 5-HT\(_{1A}\) antagonist might be obtained. The design of the last analog, compound 4, was chosen because the benzodioxane group occurs in two other compounds known to have very high affinity for 5-HT\(_{1A}\) receptors: the alpha-1 antagonist WB4101 (Norman et al., 1985) and spiroxatrine. All the synthesized derivatives contain the 5-methoxy group because we have found that in most cases the 5-methoxylation of tryptamines or related compounds
actually decreases affinity for 5-HT$_2$ receptors while increasing affinity for 5-HT$_1$ receptors. (This observation will be examined in more detail in Ch. 7).

**Methods**

A series of aryl substituted 5-methoxytryptamines (Fig. 7, compounds 2–4) was synthesized by the standard method. In the case of compound 4, reduction of the secondary indole glyoxyloyl amide with LiAlH$_4$ gave the desired product in extremely poor yield (<10%); the yield indicated in Table 9 was obtained by using a variant of the reduction procedure. This consisted of adding 0.5 equivalents of 100% H$_2$SO$_4$ to the LiAlH$_4$ in ether to generate AlH$_3$; the rest of the procedure was essentially as described for the LiAlH$_4$ reduction.

The synthesized compounds and AHR 1709 were assayed using the 5-HT$_1$, 5-HT$_{1A}$ and 5-HT$_2$ assay procedures. Since all the compounds appeared to discriminate between subtypes of $[^3H]$5-HT binding sites, 2 site analysis was performed on the $[^3H]$5-HT binding data in order to obtain estimates for the $K_i$ values at the 5-HT$_{1B}$ site, since there is no ligand available for labeling this site alone. In all cases, the $K_i$ for the high affinity site obtained by 2 site analysis of $[^3H]$5-HT binding was within one standard deviation of the $K_i$ for the 5-HT$_{1A}$ site as measured by $[^3H]8$-OH-DPAT binding. Therefore the $K_i$ for the low affinity site obtained from 2 site analysis of $[^3H]$5-HT binding was taken as the $K_i$ for the 5-HT$_{1B}$ site.

The compounds were tested for their ability to antagonize the effects of 5-HT in the vascular preparations as described previously.
In the CBA preparation, a protocol was used whereby all responses (control and otherwise) were measured during exposure to 300nM ketanserin, to eliminate the possibility that 5-HT$_2$ receptors were contributing to the response. The rationale for this approach is discussed at length in Ch.8.

Results

Synthesized Compounds

The compounds are listed in Table 8 (the intermediate indole glyoxyloyl amides) and Table 9 (the tryptamine salts and free bases) along with experimental data. The compounds were purified by recrystallization from the solvent pairs indicated. C,H,N analyses were all within <0.4% of the theoretical values.

Binding Data

For each of the 4 compounds, summary curves for the receptor binding profiles against all 3 ligands are shown as Fig. 8A-8D. All show a high degree of selectivity for 5-HT$_{1A}$ over 5-HT$_{1B}$ binding, since for all compounds the binding inhibition curves for the 5-HT$_{1A}$ ligand [³H]8-OH-DPAT (triangles) are significantly to the left of the [³H]5-HT binding inhibition curves (squares), which are also less steep in all cases.

It is apparent that relatively minor structural changes are capable of altering the relative potency at 5-HT$_{1A}$ vs. 5-HT$_2$ receptors. Thus, compounds 1 and 3 are most potent at 5-HT$_2$ binding (crosses) whereas compounds 2 and 4 are most potent at 5-HT$_{1A}$ binding (triangles).
Figure 7. Structures of aryl substituted tryptamines.
# TABLE 8

**INDOLE CYLINDLOYL AMIDES FOR ARYL SUBSTITUTED TRYPAMINES**

![Structure Diagram]

<table>
<thead>
<tr>
<th>COMPOUND &amp; NUMBER</th>
<th>YIELD %</th>
<th>MELTING POINT °C</th>
<th>C, H, N (THEORY)</th>
<th>C, H, N (FOUND)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#2; R= -NH-</td>
<td>78%</td>
<td>199-200</td>
<td>C=73.32</td>
<td>C=73.53</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>H= 5.59</td>
<td>H= 5.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N= 7.77</td>
<td>N= 7.53</td>
</tr>
<tr>
<td>#3; R= -N-</td>
<td>89%</td>
<td>221-222</td>
<td>C=66.13</td>
<td>C=66.23</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>H= 5.29</td>
<td>H= 5.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N= 11.02</td>
<td>N= 10.71</td>
</tr>
<tr>
<td>#4; R= -N-</td>
<td>69%</td>
<td>191-193</td>
<td>C=65.57</td>
<td>C=65.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H= 4.95</td>
<td>H= 4.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N= 7.65</td>
<td>N= 7.43</td>
</tr>
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</table>
### TABLE 9

EXPERIMENTAL & ANALYTICAL DATA FOR ARYL SUBSTITUTED TRYP TAMINES

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>YIELD FROM LAH REDUCTION</th>
<th>AMINE SALT</th>
<th>MELTING POINT, °C</th>
<th>C&lt;sub&gt;H&lt;/sub&gt;N&lt;sub&gt;N&lt;/sub&gt; (THEORY)</th>
<th>C&lt;sub&gt;H&lt;/sub&gt;N&lt;sub&gt;N&lt;/sub&gt; (FOUND)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMPOUND 2</td>
<td>87% FREE BASE</td>
<td>196-197</td>
<td>C=79.48, H=7.28, N=8.43</td>
<td>C=79.75, H=7.36, N=8.33</td>
<td></td>
</tr>
<tr>
<td>COMPOUND 3</td>
<td>87% FREE BASE</td>
<td>232</td>
<td>C=71.36, H=6.84, N=11.89</td>
<td>C=71.70, H=7.01, N=11.78</td>
<td></td>
</tr>
<tr>
<td>COMPOUND 4</td>
<td>73% HYDROGEN OXALATE</td>
<td>226-228</td>
<td>C=61.68, H=5.65, N=6.54</td>
<td>C=61.54, H=5.47, N=6.39</td>
<td></td>
</tr>
</tbody>
</table>

(Compounds 2 & 3 were recrystallized from EtOH; #4 from EtOH/ether)
TABLE 10

DISSOCIATION CONSTANTS FOR ARYL SUBSTITUTED TRYPHTAMINES
AT 5-HT$_{1A}$, 5-HT$_{1B}$ AND 5-HT$_{2}$ BINDING SITES

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>5-HT$_{2}$</th>
<th>5-HT$_{1A}$</th>
<th>5-HT$_{1B}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_{i}$, nM</td>
<td>$K_{i}$, nM</td>
<td>$K_{i}$, nM</td>
</tr>
<tr>
<td>COMPOUND 1</td>
<td>4.8 ± 1.6</td>
<td>27 ± 2</td>
<td>8300 ± 2900</td>
</tr>
<tr>
<td>COMPOUND 2</td>
<td>21 ± 5</td>
<td>9.2 ± 0.7</td>
<td>3900 ± 600</td>
</tr>
<tr>
<td>COMPOUND 3</td>
<td>10.4 ± 1.3</td>
<td>188 ± 12</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>COMPOUND 4</td>
<td>135 ± 10</td>
<td>28 ± 4</td>
<td>3000 ± 1300</td>
</tr>
</tbody>
</table>

(Calculated from binding data in Fig. 8)
Figure 8, A&B. Summary binding inhibition curves for aryl substituted tryptamines, compounds 1 and 2.
Figure 8, C&D. Summary binding inhibition curves for aryl substituted tryptamines, compounds 3 and 4.
A simple 5-methoxylolation of compound 1 to form compound 2 reverses the rank order of potency at these 2 binding sites.

The calculated dissociation constants for each compound at all 3 binding sites are given in Table 10. These results suggest that in general compounds of this type have very low affinity for the 5-HT\textsubscript{1B} site, and that appropriate choices for the substituent groups can yield compounds that range from an almost 20 fold selectivity for 5-HT\textsubscript{2} over 5-HT\textsubscript{1A} (compound 3) to a 5 fold selectivity for 5-HT\textsubscript{1A} over 5-HT\textsubscript{2} (compound 4).

Vascular Antagonism

All the compounds failed to produce any agonist type response in the \textit{in vitro} vascular preparations at any concentrations tested (up to 1000 nM). Compounds 2 and 4, the most 5-HT\textsubscript{1A} selective, were tested in the canine basilar artery, a proposed model for functional 5-HT\textsubscript{1} receptors. When all responses were measured after exposure to 300 nM ketanserin, to eliminate the possibility that 5-HT\textsubscript{2} receptors were contributing to the response, the additional presence of the tryptamine antagonists produced a shift to the right of the concentration-effect curve (plus some diminution in maximal effect). Based on measurements of dose ratios, the following estimated dissociation constants were obtained:

\begin{align*}
\text{COMPOUND 2:} & \quad 70 \pm 29 \text{ nM} \\
\text{COMPOUND 4:} & \quad 235 \pm 59 \text{ nM}
\end{align*}

Because of the theoretical problems associated with the measurement of antagonist potencies in this preparation (see Ch.8),
these values are probably only of qualitative significance, since this method effectively ignores the noncompetitive element of the antagonism and may thus be underestimating the antagonist potency.

The finding that these compounds are 5-HT antagonists (at least in vasculature) suggests that they may be useful prototypes for the future development of selective 5-HT$_{1A}$ antagonists.

**Discussion**

The results of this study suggest the following generalizations (admittedly based on a small number of compounds): if the tryptamine amino group is incorporated into a heterocyclic ring to which an aromatic moiety is attached, the compounds obtained are capable of discriminating between 5-HT$_{1A}$ and 5-HT$_{1B}$ binding sites. They have extremely shallow inhibition curves for [$^3$H]-5-HT binding (which labels 1A and 1B sites), but show high affinity monophasic inhibition of [$^3$H]-8-OH-DPAT binding, which labels only 5-HT$_{1A}$ sites. These compounds also show high affinity for 5-HT$_2$ sites, but it is apparent that the selectivity for either 5-HT$_{1A}$ or 5-HT$_2$ sites can be enhanced by alterations in the substituent groups.

The related secondary amine analog (compound 4) containing the benzodioxane group (which occurs in other compounds with high affinity for 5-HT$_{1A}$ sites) had a binding profile similar to the tertiary amines and showed the greatest selectivity for 5-HT$_{1A}$ sites (5 fold over 5-HT$_2$). The opposite selectivity is exemplified by compound 3, which has an almost 20 fold selectivity for 5-HT$_2$ over 5-HT$_{1A}$.
It is probable that the observed antagonist activity of these compounds is related to increased affinity produced by the binding of the additional hetero-aromatic group to an accessory site on the receptor. It is also interesting that they are structurally related to other 5-HT antagonists such as spiperone and ketanserin in the general sense that all of these compounds consist of two hydrophobic hetero-aromatic moieties connected by an alkyl chain.

The fact that the secondary amine compound 4 has a similar pharmacological profile to the other compounds and yet is the most 1A selective opens the door to the design of other secondary amine analogs in which the length of the chain between the amino nitrogen and the heteroaromatic moiety might be extended. If this chain were extended by one methylene group and the benzodioxane replaced by a 3-indolyl group, a type of tryptamine dimer would be obtained. This type of analog might be advantageous because either end of the molecule could be recognized by the indole recognition site of the receptor, thus conferring an increased ability for binding. One such tryptamine dimer has been tested in preliminary experiments which suggest that its pharmacologic profile is very similar to the other aryl substituted tryptamines.

The finding that all of these compounds are vascular antagonists of serotonin is encouraging and suggests that more analogs of this type should be developed. With some perseverance it should be possible to further increase the selectivity and potency for 5-HT$_{1A}$ type receptors.
The 5-Methoxy Group as a Means of Enhancing Selectivity For 5-HT\textsubscript{1} Over 5-HT\textsubscript{2} Receptors

As stated previously in Ch. 4, at 5-HT\textsubscript{1} sites, the rank order of potency for 5-substituted tryptamines is 5-OH > 5-MeO > 5-H. Since, based on inhibition of [\textsuperscript{3}H]ketanserin binding, 5-HT is about 5 times as potent as tryptamine, one might assume that the same rank order of potency also pertains to 5-HT\textsubscript{2} receptors. However, some published data for the potency of 5-MeO-tryptamine at the 5-HT\textsubscript{2} site (see Table 11) suggested that it did not show very greatly enhanced potency relative to tryptamine. However, the data of Peroutka et al. (1983), based on the inhibition of [\textsuperscript{3}H]spiperone binding, actually suggests that tryptamine is almost twice as potent as 5-MeO-tryptamine at the 5-HT\textsubscript{2} site. This suggested the possible usefulness of the 5-MeO substituent on indole as a means of enhancing selectivity for 5-HT\textsubscript{1} receptors, which is illustrated for 4 pairs of compounds in Table 11. The increased 5-HT\textsubscript{1} selectivity produced by 5-methoxylation is clearly a two-fold effect. For all the compounds, 5-methoxylation significantly enhances the affinity of a compound for 5-HT\textsubscript{1} (and particularly 5-HT\textsubscript{1A}) receptors. At the 5-HT\textsubscript{2} site, however, 5-methoxylation appears to produce either:
**TABLE 11**

5-HT₁, 5-HT₁A AND 5-HT₂ BINDING PARAMETERS FOR 5-MeO SUBSTITUTED ANALOGS COMPARED TO THE 5-UNSUBSTITUTED COMPONDS

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TRYPTAMINE</td>
<td>176 ± 27</td>
<td>125 ± 29</td>
<td>1482ᵃ</td>
</tr>
<tr>
<td>5-MeO-TRYPTAMINE</td>
<td>13 ± 10</td>
<td>6.1 ± 2.4</td>
<td>742ᵃ</td>
</tr>
<tr>
<td>DMT</td>
<td>137 ± 30</td>
<td>232 ± 77</td>
<td>225 ± 90</td>
</tr>
<tr>
<td>5-MeO-DMT</td>
<td>98 ± 26</td>
<td>31.8 ± 3.9ᵇ</td>
<td>290ᶜ</td>
</tr>
<tr>
<td>THPI</td>
<td>490 ± 90</td>
<td>154 ± 29</td>
<td>73 ± 10</td>
</tr>
<tr>
<td>5-MeO-THPI</td>
<td>92 ± 20</td>
<td>25 ± 7</td>
<td>910 ± 220</td>
</tr>
<tr>
<td>AHR 1709</td>
<td>650 ± 126</td>
<td>27 ± 2</td>
<td>4.8 ± 1.6</td>
</tr>
<tr>
<td>5-MeO ANALOG</td>
<td>346 ± 63</td>
<td>9.2 ± 0.7</td>
<td>21 ± 2</td>
</tr>
</tbody>
</table>

ᵃ Data from Leysen et al., 1981.
ᵇ Calculated from the pIC₅₀ value of Gozlan et al., 1983.
ᶜ N=1; however, based on the inhibition of [³H]spiperone binding, Martin and Sanders-Bush (1982) suggest a value of 1145nM.
1) a comparatively small enhancement of affinity (eg. for tryptamine, based on the [H]$^3$ ketanserin binding data of Leysen et al., 1981), or

2) an essentially neutral effect (eg. for DMT), or

3) a distinct decrease in affinity, opposite to the effect seen at 5-HT$_1$ sites (eg. for THPI and AHR 1709).

Since in all 4 cases the 5-methoxylation enhances the affinity for 5-HT$_1$ sites, the net effect is either an enhancement of 5-HT$_1$ (and 5-HT$_1^A$) selectivity, or, in the case of THPI and AHR 1709, an actual reversal of the selectivity of the parent compounds, since THPI and AHR 1709 are most potent at 5-HT$_2$ sites, whereas the 5-MeO analogs are most potent at 5-HT$_1^A$ sites.

This effect appears to be unique to the methoxy group, at least in a study of 5-substituted analogs of THPI (Nelson and Nikam, unpublished data). Its potential usefulness in the design of 5-HT$_1^A$ selective indoleamines was illustrated in Ch.6.

Conclusions on the SAR of Indoleamine Analogs for 5-HT$_1$ Binding Sites

1) In 5-unsubstituted tryptamines, increase in non-aromatic substituent bulk on the amino nitrogen is accompanied by a general loss of overall potency for 5-HT$_1$ binding, but is accompanied by an increased discrimination between subsets of the 5-HT$_1$ binding site. However, unlike the aryl substituted tryptamines, the high affinity site discriminated by compounds such as DIPT and MEI does not appear to correspond to the 5-HT$_1^A$ site, based on the micromolar affinity of DIPT
for inhibition of $[^3\text{H}]8$-OH-DPAT binding and the low potency of DIPT and MEI in the CBA preparation (see Ch.8).

2) If the bulk of the amino substituent is increased further by the addition of an aromatic group, either attached to a heterocyclic ring incorporating the amino nitrogen, or to an alkyl chain attached to the tryptamine amino group (forming a secondary amine), the compounds obtained are highly selective for the $5$-$\text{HT}_{1A}$ as compared to the $5$-$\text{HT}_{1B}$ binding site. This also confers the property of antagonism, probably associated with the enhancement of binding produced by the binding of the aromatic moiety to an accessory site on the receptor.

3) An additional difference between the $N$-alkyl substituted tryptamines such as DIPT and the aryl substituted tryptamines is that in the former, the 5-oxy analogs lost the discrimination for subtypes of $5$-$\text{HT}_1$ sites shown by the 5-H analogs, whereas all the 5-methoxylated aryl substituted tryptamines were highly selective for $5$-$\text{HT}_{1A}$ as compared to $5$-$\text{HT}_{1B}$ sites. This is further evidence that the nature of the sites discriminated by the two types of compounds are different.

4) At $5$-$\text{HT}_1$ sites, the rank order of potency for $5$-substituted tryptamines (and probably also for related compounds such as tetrahydropyridylindoles) is 5-OH > 5-MeO > 5-H. However, for the design of compounds selective for $5$-$\text{HT}_1$ sites, the 5-MeO group may be optimal if it is desired to minimize the affinity for $5$-$\text{HT}_2$ receptors.

5) Of all the rigid analogs so far studied, only tetrahydropyridylindoles such as RU 28253 show promise in terms of slightly enhanced potency at $5$-$\text{HT}_1$ sites, but their potential as prototypes for
5-HT\textsubscript{1} selective compounds is seriously compromised by their high affinity for 5-HT\textsubscript{2} receptors.

6) Although the partial ergolines RU 27849 and RU 28306 do not appear to have an optimal conformation for 5-HT\textsubscript{1} (and 1A) binding sites, being generally several times less potent than TRYP and DMT, the non-ergoline-like conformations attainable by 3-aminomethyl-THBI appear to be even less favorable, since it is even less potent than RU 27849. Given the high potency of the partial ergoline 8-OH-DPAT at the 5-HT\textsubscript{1A} site, it is difficult to draw any conclusions regarding the 5-HT\textsubscript{1A} pharmacophore from this data. 5-MeO analogs of RU 27849 and RU 28306 have recently been synthesized (Kruse and Meyer, 1984); because of their greater homology to 5-HT, these compounds would be very useful in resolving the question of a possible non-ergoline-like pharmacophore for 5-HT\textsubscript{1} sites. Other rigid 5-HT analogs that currently do not exist may be necessary to definitively demonstrate that an alternate amino conformation is preferred.

Conclusions on the SAR of Indoleamine Analogs for 5-HT\textsubscript{2} Receptors

The development of 5-HT\textsubscript{2} selective compounds, except possibly as agonists, was not a primary goal of this research. Thus, the pharmacologic profile of this site was not studied as intensely as that of the 5-HT\textsubscript{1} sites. However, in the course of this research we have made some interesting observations regarding the SAR of some indoleamine derivatives and related rigid analogs at the 5-HT\textsubscript{2} site. Some of these can be summarized as follows:
1) Despite the low potency of tryptamines and 5-HT agonists generally at the 5-HT₂ site, the high affinity of aryl substituted tryptamines such as AHR 1709 ($K_i = 4.8\text{nM}$) demonstrates that some tryptamines can be highly potent at this site. However, this class of compounds does not offer much promise for the development of selective 5-HT₂ agonists, as all the aryl substituted tryptamines studied so far have been antagonists. A number of selective 5-HT₂ antagonists are of course already available, so there seems little point in trying to develop a new class of such compounds.

2) As discussed above, the 5-MeO group is unfavorable for high potency at the 5-HT₂ site; unsubstituted compounds may be best for 5-HT₂ selectivity, because of the associated lower potency at 5-HT₁ sites.

3) The tetrahydropyridylindoles as a class are the most potent rigid analogs tested at the 5-HT₂ site; this includes both 3-pyridyl analogs such as RU 28253 and 4-pyridyl analogs such as THPI. Both here and at the 5-HT₁ site, the observed high affinity of these compounds may be related to conformational factors and/or electronic characteristic conferred on the molecule by the conjugated double bond. Like RU 28253 and RU 24969, THPI is probably an agonist. To our knowledge, there are no known 5-HT agonists that have a higher affinity for 5-HT₂ receptors than RU 28253 ($K_i = 84\text{nM}$) or THPI ($K_i = 73\text{nM}$). Examination of the 5-HT receptor binding parameters for THPI given in Table 11 reveals that compounds of this type may have potential as prototypes for the development of selective 5-HT₂ agonists.
4) The partial ergolines RU 27849 and RU 28306 (Fig. 6A), although not showing enhanced potency relative to their free chain homologs (TRYF and DMT, respectively), are nonetheless approximately equipotent to them at the 5-HT$_2$ site. Assuming that one enantiomer is significantly less active, this might suggest that the other enantiomer has a somewhat enhanced affinity relative to the free chain homologs. This is at least consistent with the possibility of an ergoline-like pharmacophore for the 5-HT$_2$ site.

5) However, the partially constrained non-ergoline-like analog 3-aminomethyl-THBI (Fig. 6C) is significantly more potent (4-5 times) than the two homologous compounds (tryptamine and the partial ergoline RU 27849). This suggests that it may be worthwhile to examine totally rigid analogs of the non-ergoline-like conformations attainable by 3-aminomethyl-THBI, particularly those that overlap with possible conformations of the tetrahydropyridylindole RU 28253.

Relevance to Future Drug Development

As stated at the outset, this study has concentrated on compounds that are highly homologous to 5-HT, primarily because of the difficulty in drawing comparisons between the potencies of structurally dissimilar compounds. However, it is quite possible that, as in the case of the 5-HT$_2$ receptor, the most useful selective ligands and antagonists that may be developed for 5-HT$_1$ receptor subtypes may not be indole derivatives.

For example, the 1-phenylpiperazines are an important class of compounds that show a varied spectrum of 5-HT receptor selectivity,
including activity at 5-HT\textsubscript{1} sites (Fuller et al., 1980), analogs showing selectivity for 5-HT\textsubscript{1B} sites (Sills et al., 1984b) and analogs showing selectivity for 5-HT\textsubscript{1A} sites (Ransom et al., 1985). Analogues such as spiroxatrine, containing the 1-phenyl-1,3,8-triazaspiro(4.5)decan-4-one (PTSD) nucleus of spiperone may also have considerable potential as selective 5-HT\textsubscript{1A} antagonists (Nelson and Taylor, 1985). Analogues of the novel anxiolytic buspirone also appear to be quite selective for the 5-HT\textsubscript{1A} site (Dompert et al., 1985; Yocca and Maayani, 1985).

A fruitful area for future research will be the integration of this information, perhaps by the use of computer programs capable of determining the common structural features in these different types of compounds. The successful design of one of the aryl substituted tryptamines (Ch.6, compound 4) as a 5-HT\textsubscript{1A} selective hybrid of 5-MeO-tryptamine and the PTSD derivative spiroxatrine demonstrates that common elements can be found in the mode of action of these different classes of compounds.

It is hoped that the results of this research will be of use in the future design of useful selective compounds for 5-HT receptor subtypes. Although no highly potent and completely selective compounds were produced, several of the antagonists described in Ch.6 may have some use as research tools because of their ability to effectively block both 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptors at concentrations having negligible effects on 5-HT\textsubscript{1B} receptors. This could be useful for probing the physiologic roles of both 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptor subtypes.
CHAPTER 8

CORRELATIONS BETWEEN CENTRAL 5-HT BINDING SITES AND
5-HT-INDUCED CONTRACTIONS OF THE CANINE BASILAR ARTERY

The canine basilar artery (CBA) has recently been the focus of controversy regarding the characterization of the receptor mediating its contraction in response to 5-HT. Attempts to correlate the pharmacological characteristics of this receptor with those of central 5-HT binding sites have led to discrepant claims. Peroutka et al. (1982) initially reported mediation by 5-HT\(_1\) receptors; this was countered by the report of Muller-Schweinitzer and Engel (1983), who offered evidence for mediation by 5-HT\(_2\) receptors. Data for the potencies of certain antagonists comprise the most striking discrepancy between the two papers: Peroutka et al. report potencies for ketanserin and spiperone more than a thousand fold lower than those reported by Muller-Schweinitzer and Engel.

Both groups have reported that 5-HT antagonism in the CBA is almost universally noncompetitive and accordingly measure the potency of antagonists in terms of the concentration producing a 50% reduction of the maximal contraction. This corresponds to the \(pA_h\) of Arunlakshana and Schild (1959) or the \(pD_2\)' of Van Rossum (1963) which is based on the theoretical model of Ariens et al (1956; 1964).

However, neither group addressed the theoretical problem associated with this apparent noncompetitive antagonism. In the Ariens
model, the noncompetitive antagonist does not interact at the agonist binding site, but instead acts only at a second site, having no effect on the binding of the agonist. Since these antagonists are known to inhibit agonist binding at 5-HT binding sites (and in fact were selected for study because of this property), and have also been observed to act competitively at other smooth muscle 5-HT receptors (e.g. some peripheral vascular 5-HT₂ receptors), clearly this Ariens model is inapplicable if the CBA receptor is identical to either the 5-HT₁ or 5-HT₂ binding site. If it is similar to one of the binding sites, the $p_{A_h}$ as an empirical measure of vascular antagonist potency cannot necessarily be expected to correspond to the antagonist $K_i$ for the inhibition of agonist binding as measured in a ligand binding assay.

These problems regarding the measurement and interpretation of antagonist data in the CBA have led us to concentrate on the measurement of agonist dissociation constants ($K_A$ values) using the method of 'partial irreversible blockade' (Furchgott and Bursztyn, 1967). The values obtained give a significant correlation only with the 5-HT₁ₐ binding site subtype (Pedigo et al., 1981; Nelson et al., 1981; Sills et al., 1984b).

It will also be shown that a true noncompetitive antagonist (i.e. conforming to the Ariens model) can be used in the same way as an irreversible antagonist to measure an agonist $K_A$. Values obtained by both methods will be compared (using several different apparent non-competitive antagonists). However, since (for reasons discussed above) the Ariens model cannot apply if the receptor is a 5-HT₁ₐ type,
alternate hypotheses for the production of apparent noncompetitive antagonism will be considered.

Methods

A series of seven 5-HT agonists (Table 12) was studied in the 5-HT$_1$, 5-HT$_{1A}$ and 5-HT$_2$ binding assays using the procedures described in Ch.2. For the same series of compounds, $\text{ED}_{50}$ and $K_A$ values in the CBA were measured, also as described in Ch.2. In order to assess various theoretical models for the production of apparent noncompetitive antagonism, $K_A$ values for 5-HT and DIPT were also measured using several different apparent noncompetitive antagonists (metergoline, ketanserin, and 5-MeO-MEI) using the methods described below.

Determination of $K_A$ Using an Ariens Type Noncompetitive Antagonist

It will now be demonstrated that a noncompetitive antagonist conforming to the Ariens model can be used in a manner essentially identical to that described previously (Ch.2) for an irreversible antagonist to determine an agonist $K_A$. (The only experimental difference is that the second agonist concentration-effect curve is obtained in the presence of the antagonist, rather than after washout).

The theoretical model of Ariens, Van Rossum and Simmonis (1956) for noncompetitive antagonism is based on a linked dual receptor system in which agonist A acts at receptor R and a noncompetitive antagonist B acts at receptor R', producing an antagonistic effect proportional to the occupation of R':
(B interacts competitively with R': it is noncompetitive with respect to the production of E by the action of A at R).

In this model, the agonist $K_A$ corresponds to the dissociation constant for AR and the antagonist $K_B'$ corresponds to the dissociation constant for BR'. The $pD_2'$ of Van Rossum is the negative logarithm of this antagonist dissociation constant and is thus unrelated to any dissociation constants that may be measured competitively at the agonist receptor site.

Ariens et al. demonstrate that, if $E_m$ is the maximal effect, $E_a$ the effect produced by A, and $E_{ab}$ the effect produced by A in the presence of B (a fully efficacious noncompetitive antagonist with an intrinsic activity of -1), then

$$\frac{E_{ab}}{E_m} = \frac{E_a}{E_m} \left( \frac{K_B'}{[B]+K_B'} \right)$$

Thus if $[A]'$ is required to produce the same effect in the presence of $[B]$ as the effect $E_a$ produced in the presence of $[A]$ alone, then, since the stimulus is the same in each case, by analogy to the approach of Furchgott and Burstyn,

$$\frac{[A]}{[A]+K_A} = \frac{[A]'}{[A]'+K_a} \left( \frac{K_B'}{[B]+K_B'} \right)$$
This can be rearranged to

\[
\frac{1}{[A]} = \frac{1}{[A]'} \left( 1 + \frac{[B]}{K_B'} \right) + \frac{[B]}{K_A K_B'}
\]

Therefore, as with the irreversible antagonist method, for a plot of \(1/[A]\) vs. \(1/[A]'\),

\[K_A = \frac{\text{slope}-1}{\text{intercept}}\]

Thus, if the mechanism of noncompetitive antagonism observed in the CBA for 5-HT antagonists corresponds to Ariens' theoretical model, any data set used to obtain an antagonist pD₅₀' can also be used to calculate the \(K_A\) of the agonist used.

However, it must be emphasized that this Ariens model precludes the possibility of any inhibition of agonist binding by the antagonist. Thus, any attempt to claim homology between the CBA receptor and a 5-HT binding site where these same antagonists do inhibit 5-HT binding necessitates the rejection of this model and the examination of alternate hypotheses for the production of this effect.

Mixed Competitive and Noncompetitive Antagonism

A closely related model is that in which the antagonism is mixed in a literal sense, with both a competitive action at the agonist receptor site and a simultaneous noncompetitive action according to the Ariens model discussed above. In this situation, with the addition of increasing concentrations of antagonist, one would observe a shift to the right of the log concentration-response curve in addition to the decrease in maximal response observed for a pure noncompetitive antagonist.
This model has also been analysed by Ariens et al. (1964, p.310 and equation on p.297). We will now show a simple way in which the applicability of this model to the experimental data can be tested.

With such a mixed antagonist, using an experimental situation identical to that described above for a pure noncompetitive antagonist, if doses of agonist producing identical responses in the absence ([A]) and presence ([A]') of a mixed antagonist B are obtained, then

\[
\frac{[A]}{[A]+K_A} = \frac{[A]'}{[A]'+(1+[B]/K_B)K_AK_B'+[B]}
\]

where \(K_B\) is the competitive dissociation constant of the antagonist at the agonist receptor site and \(K_B'\) is the 'noncompetitive' dissociation constant at the accessory receptor site, as described in the model given above for pure noncompetitive antagonism.

It can be shown that, in this situation, for a plot of \(1/[A]\) (y axis) vs. \(1/[A]'\) (x axis), the value of

\[
\frac{(\text{slope}-1)}{\text{y intercept}} = K_A \left(1 + \frac{[B]+K_B'}{K_B} \right)
\]

Thus, although this calculation would give the agonist \(K_A\) if a pure noncompetitive antagonist were used (independent of antagonist concentration), for a true mixed antagonist one would observe an increase in the value of \((\text{slope}-1)/\text{intercept}\) as the concentration of antagonist was increased, and this value would always be greater than the actual agonist \(K_A\).
Selectivity of Compounds Between 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} sites

5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} sites were originally defined by differences in the potency of spiperone (Pedigo et al., 1981; Nelson et al., 1981). This selectivity makes it possible to estimate the 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} potencies of compounds by incorporating cold spiperone in the binding assays; this method has been used to demonstrate the 5-HT\textsubscript{1A} selectivity of 8-OH-DPAT (Middlemiss and Fozard, 1983). Similarly, Sills et al. (1984b) have combined the use of such methods with 2 site analysis of \(^3\text{H}\)5-HT binding inhibition curves measured in the presence of guanosine 5'-triphosphate (GTP) (which is used to eliminate the high affinity state of the receptor) to obtain estimates of \(K_i\) values at 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} binding sites. Since the functional significance of high and low affinity states of \(^3\text{H}\)5-HT binding sites is unknown, in this study the inhibition of \(^3\text{H}\)8-OH-DPAT binding was used to obtain \(K_i\) values for the 5-HT\textsubscript{1A} site. All of the seven compounds showed monophasic inhibition of \(^3\text{H}\)8-OH-DPAT binding.

5-HT and 5-MeO-DIPT were found to be nonselective between 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} sites, based on their monophasic inhibition of \(^3\text{H}\)5-HT binding. Similarly, although RU 24969 and RU 28253 have somewhat shallow \(^3\text{H}\)5-HT binding inhibition curves, there is no significant difference in their \(IC_{50}\) values at \(^3\text{H}\)5-HT and \(^3\text{H}\)8-OH-DPAT binding sites. Thus, we have no evidence that either of these compounds has significant selectivity between 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} sites. Although Sills et al. (1984b) have found that, in the presence of GTP, RU 24969 appears
to be selective for 5-HT$_{1B}$ sites, the low $K_i$ (11.3nM) observed vs. 
$[^3H]8$-OH-DPAT binding suggests that RU 24969 cannot practically be used
as a selective 5-HT$_{1B}$ agonist. Nonetheless, it is interesting that
Sills' estimate of the $K_i$ of RU 24969 at the 5-HT$_{1A}$ site (200nM),
compared to its $K_i$ vs. $[^3H]8$-OH-DPAT binding (11.3nM), is much closer to
the $K_A$ value measured in the CBA (144nM).

The remaining 3 compounds (8-OH-DPAT, DIPT and TFMPP) have
shallow or biphasic $[^3H]5$-HT binding inhibition curves and also have
significantly different IC$_{50}$ values at $[^3H]5$-HT and $[^3H]8$-OH-DPAT
binding sites, suggesting selectivity between 5-HT$_{1A}$ and 5-HT$_{1B}$ sites.
The $K_i$ for DIPT vs. $[^3H]8$-OH-DPAT binding (1960nM) approximately
corresponds to the value previously reported for the low affinity site
(4610nM) obtained by 2 site analysis of $[^3H]5$-HT binding data (Nelson et
al., 1983).

### Correlations Between Cerebral Vascular Data and 5-HT Binding Sites

Table 12 shows vascular and binding data for the series of 5-HT
agonists. In all cases, the negative logs of the dissociation constants
or IC$_{50}$ values were used in the linear regression analyses. The first
column lists the apparent dissociation constants measured in the CBA.
The second column contains the overall IC$_{50}$s for $[^3H]5$-HT binding, and
illustrates the lack of correlation ($r=0.5732$, $p>0.05$) between binding
data obtained using this ligand and the CBA data: this is not
surprising, since, as discussed above, about half of these compounds
have the ability to discriminate between subtypes of $[^3H]5$-HT binding
sites.
The third column of Table 12 gives $K_i$ values for the 5-HT$_{1A}$ receptor subtype, based on [$^3$H]8-OH-DPAT binding (except for the nonselective compound 5-MeO-DIPT, for which a value based on [$^3$H]5-HT binding is used, since our sample of this compound was exhausted before this assay could be performed). The correlation between the vascular log $K_A$ values and the log $K_i$ values for the 5-HT$_{1A}$ site is excellent ($r=0.9456$, $p<0.01$; Fig. 9) and, except for RU 24969, the $K_A$ and $K_i$ values for each compound are within less than an order of magnitude of each other.

The last column of Table 12 gives apparent $K_i$ values for the 5-HT$_2$ binding site, based on inhibition of [$^3$H]ketanserin binding. No correlation was found between these values and the vascular data ($r=0.2253$, $p>0.05$).

Comparison of Vascular $K_A$ and ED$_{50}$ Values

Table 13 again shows the agonist $K_A$ values measured in the CBA by the irreversible antagonist method (except for TFMPP, which was measured as a partial agonist) and gives a comparison to their ED$_{50}$ values observed in the same tissue. These two values are in many cases essentially identical, and in the most extreme case (5-MeO-DIPT) only differ by a factor of 3.

The Use of Apparent Noncompetitive Antagonists to Estimate $K_A$ values

Like the previous investigators, we have observed that there is a decrease in the maximal response after preincubations with antagonists (Fig. 10). If the mechanism for the production of this noncompetitive
TABLE 12
Correlations between the CBA receptor and 5-HT binding sites

<table>
<thead>
<tr>
<th>AGONIST</th>
<th>DISSOCIATION CONSTANT IN CBA, nM&lt;sup&gt;b&lt;/sup&gt;</th>
<th>5-HT&lt;sub&gt;1&lt;/sub&gt;: OVERALL IC&lt;sub&gt;50&lt;/sub&gt; FOR [&lt;sup&gt;3&lt;/sup&gt;H]5-HT BINDING, nM</th>
<th>5-HT&lt;sub&gt;1A&lt;/sub&gt;: APPARENT K&lt;sub&gt;i&lt;/sub&gt;, nM&lt;sup&gt;c&lt;/sup&gt;</th>
<th>5-HT&lt;sub&gt;2&lt;/sub&gt;: APPARENT K&lt;sub&gt;i&lt;/sub&gt;, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>46 ± 7</td>
<td>4 ± 0.7</td>
<td>7.4 ± 1.6</td>
<td>300 ± 85</td>
</tr>
<tr>
<td>RU 28253</td>
<td>11 ± 4</td>
<td>20 ± 8</td>
<td>5.7 ± 2.2</td>
<td>84 ± 9</td>
</tr>
<tr>
<td>RU 24969</td>
<td>144 ± 15</td>
<td>22 ± 4</td>
<td>11.3 ± 2.3</td>
<td>299 ± 55</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>31 ± 13</td>
<td>1,180 ± 180</td>
<td>5.5 ± 0.1</td>
<td>5,000 ± 400</td>
</tr>
<tr>
<td>5-MeO-DIPT</td>
<td>1,010 ± 410</td>
<td>2,000 ± 600</td>
<td>1,000 ± 300</td>
<td>-d</td>
</tr>
<tr>
<td>DIPT</td>
<td>5,300 ± 700</td>
<td>960 ± 270</td>
<td>1,960 ± 260</td>
<td>1,000 ± 320</td>
</tr>
<tr>
<td>TFMPP</td>
<td>520 ± 210</td>
<td>146 ± 47</td>
<td>404 ± 16</td>
<td>310 ± 90</td>
</tr>
<tr>
<td></td>
<td>CORRELATION TO CBA VALUES&lt;sup&gt;e&lt;/sup&gt;:</td>
<td></td>
<td>r=0.5732</td>
<td>r=0.9456 r=0.2253</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&gt;0.05</td>
<td>p&lt;0.01 p&gt;0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent the mean ± S.E. for 3 or more separate experiments.

<sup>b</sup> Determined by the method of irreversible blockade, except for the partial agonist TFMPP, for which the K<sub>p</sub> was determined by the method of Gero and Tallarida (1977).

<sup>c</sup> Based on inhibition of [<sup>3</sup>H]8-OH-DPAT binding, except for the non-selective (1A-1B) compound 5-MeO-DIPT, for which the value is based on inhibition of [<sup>3</sup>H]5-HT binding (see note d).

<sup>d</sup> Our sample of 5-MeO-DIPT was exhausted after the vascular studies and the [<sup>3</sup>H]5-HT binding assay were performed.

<sup>e</sup> The negative logs of the dissociation constants or IC<sub>50</sub> values were used in the linear regression analyses.
TABLE 13

Comparison of dissociation constants and ED$_{50}$ values

<table>
<thead>
<tr>
<th>AGONIST</th>
<th>DISSOCIATION CONSTANT IN CBA, nM</th>
<th>ED$_{50}$ IN CBA, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>46 ± 7</td>
<td>42 ± 8.3</td>
</tr>
<tr>
<td>RU 28253</td>
<td>11 ± 4</td>
<td>12 ± 1.2</td>
</tr>
<tr>
<td>RU 24969</td>
<td>144 ± 15</td>
<td>150 ± 13</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>31 ± 13</td>
<td>78 ± 11</td>
</tr>
<tr>
<td>5-MeO-DIPT</td>
<td>1,010 ± 410</td>
<td>3,000 ± 700</td>
</tr>
<tr>
<td>DIPT</td>
<td>5,300 ± 700</td>
<td>3,200 ± 500</td>
</tr>
<tr>
<td>TFMPP</td>
<td>520 ± 210</td>
<td>650 ± 170</td>
</tr>
</tbody>
</table>

*a All values are means ± S.E. for three or more separate experiments.

*b See Table 12, note b.
<table>
<thead>
<tr>
<th>AGONIST</th>
<th>METERGOLINE (nM)</th>
<th>KETANSERIN (μM)</th>
<th>5-MeO-MEI (μM)</th>
<th>AVERAGE OF ALL DETERMINATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>48 ± 5</td>
<td>39 ± 4</td>
<td>30 ± 13</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>DIPT</td>
<td>5,800 ± 1,000</td>
<td>6,400 ± 400</td>
<td>-</td>
<td>6,000 ± 700</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E. for 3 or more separate experiments.
Figure 9. Correlation between the negative logs of agonist dissociation constants measured in the CBA and Ki values for the 5-HT$_{1A}$ binding site.

(The data are from Table 12. The correlation coefficient obtained from linear regression analysis was 0.9456 (p<0.01), with a slope of 1.15 and y intercept of -0.53).
Figure 10. An example of a single experiment showing the decrease in the maximal response to 5-HT that can be observed with 5-HT antagonists in the CBA.

(In this case, the antagonist was 5-MeO-MEI after a 20 min preincubation).
effect conforms to the Ariens model (see methods), such antagonists can be used to determine an agonist $K_A$.

To assess the applicability of this or other models for the production of noncompetitive antagonism, apparent $K_A$ values for 5-HT and DIPT were determined using several apparent noncompetitive antagonists; these values were then compared to those obtained by the irreversible antagonist method using PBZ. The method of data analysis was identical for both methods. Three apparent noncompetitive antagonists were used: metergoline over a range of concentrations from 10-1000 nM, ketanserin at 1-10 µM, and 5-MeO-MEI at 1-10 µM. Table 14 gives the apparent $K_A$ values obtained for the 2 agonists using the different antagonists and, in the last column, the average of all the determinations. For both agonists, the $K_A$ determined by this method is not significantly different from that determined by the irreversible antagonist method. Also, the $K_A$ values obtained for a particular agonist are essentially identical regardless of which apparent noncompetitive antagonist was used. The $K_A$ values obtained were independent of the concentration of antagonist used, at least over the concentration ranges indicated (this is reflected in the small standard errors for the values in Table 14).

Discussion

The observation that, at least for the 2 agonists studied, the different apparent noncompetitive antagonists give estimates of agonist $K_A$ values identical to those obtained by the irreversible antagonist method suggests at least two possible conclusions:
1) The antagonism is genuinely noncompetitive according to the Ariens model, in which case the antagonists must have no effect on the binding of agonists, and therefore the CBA receptor cannot correspond to any known 5-HT binding site. (The possibility of an Ariens type non-competitive action in combination with a competitive effect, i.e. true mixed antagonism, is mitigated against by the observation that $K_A$ estimates did not increase as higher concentrations of apparent non-competitive antagonists were used. Such an increase is predicted by the theoretical analysis of mixed antagonism given in the methods section).

2) The apparent noncompetitive antagonism is produced by some other mechanism.

The first possibility seems unlikely, since a significant correlation is observed between the CBA receptor and the 5-HT$_{1A}$ binding site. However, any alternate hypothesis should be able to explain why the $K_A$ values estimated using the 2 types of antagonist are identical.

The approximate equality observed between $K_A$ and $ED_{50}$ values for 5-HT agonists in the CBA (Table 13) suggests that in this tissue there is essentially no receptor reserve, since virtually all the receptors must be occupied to produce a maximal response. This suggests an alternate hypothesis for the production of apparent noncompetitive antagonism.

There is a well documented phenomenon in classical pharmacology which has been variously called 'anomalous' or 'mixed' antagonism, of which the so-called 'atropine anomaly' is perhaps the best known example. In essence, a tissue shows very slow recovery to an apparently competitive antagonist following repeated washout, and/or, at very high
antagonist concentrations, the antagonism becomes noncompetitive. This has been explained in terms of the kinetics of antagonist- (slow) vs agonist- (fast) receptor association and dissociation, combined with the presence of a receptor reserve which becomes exhausted at high antagonist concentrations.

This phenomenon has been described as far back as Clark (1937) for atropine and acetylcholine and Gaddum (1937) for ergotoxine and adrenaline. The explanation in terms of slow antagonist-receptor dissociation and the role of receptor reserve (eg. Arunlakshana and Schild, 1959) has become a standard argument in favor of the existence of spare receptors.

In the case of 5-HT antagonism in the CBA, it is possible that the antagonists, although essentially competitive, are acting in a pseudo-irreversible manner, due to just such a slow dissociation of their complex with the receptor (which accumulates during the relatively long pre-incubation). In the absence of a receptor reserve, this would result in an apparent noncompetitive effect. By this hypothesis, the similarity of the agonist $K_A$ estimates to those obtained by the irreversible antagonist method is to be expected.

Paton and others have stated the theoretical basis for the production of apparent noncompetitive antagonism by competitive antagonists, under similar kinetic conditions, which they call a "hemi-equilibrium" state (Paton and Rang, 1966; Paton and Waud, 1967). The nature of this effect has been succinctly described in a recent review as follows: "This is most commonly encountered with persistent
(low rate of off-set) antagonists and low efficacy agonists... the equilibrium of the antagonist is not changed by the presence of the agonist and the agonist equilibrates with only a portion of the total receptor population. Under these conditions, the antagonist behaves as an essentially irreversible blocker and produces insurmountable antagonism." (Kenakin, 1984). Note that low efficacy, by definition, implies the absence of spare receptors.

A possible factor in the production of this effect is related to the established sensitivity of some 5-HT$_1$ receptors to GTP. Using a computer simulation of the ternary complex model, involving agonist and antagonist high and low affinity states and the presence of a regulatory component, Wreggett and DeLean (1984) have shown that apparent non-competitive antagonism can be produced under certain conditions in which kinetics favor the accumulation of inactive high-affinity antagonist-receptor complex. This effect would definitely be more observable in the absence of a receptor reserve.

In any case, by this interpretation the depression in maximal response produced by antagonists in this preparation, although definitely related to antagonist potency, is probably very dependent upon kinetic factors (e.g. time of pre-incubation), and there seems to be little theoretical basis for relating this depression of maximal response to antagonist dissociation constants measured at the agonist binding site.

At fairly low antagonist concentrations (up to 10 times the $K_1$) with shorter incubation times (10-20 min) the depression in the maximal
effect is small enough that a distinct parallel shift in the log concentration-response (LCR) curve can be observed and thus dose ratios can be at least roughly estimated at the midpoint of the shifted LCR curve. This method was used in a preliminary examination of several antagonists in the CBA (Taylor et al., 1983). Nevertheless, the approach taken in this study reflects the belief that in this preparation, the measurement of agonist potencies has a firmer experimental and theoretical basis, at least where accurate quantitative data are required (as for correlations to 5-HT binding sites).

The finding of primary importance is that the pharmacologic profile observed for agonists in the CBA fails to correlate with the 5-HT$_2$ binding site, but shows a highly significant correlation to the 5-HT$_{1A}$ binding site. Thus, the present data contribute to the growing list of studies suggesting that the 5-HT$_1$ binding sites actually correspond to functional receptors. In addition, these findings agree with the suggestion, based on binding studies, that the 5-HT$_1$ sites represent a heterogeneous group of sites that can be further subdivided (Pedigo et al., 1981; Schnellmann et al., 1984; Sills et al., 1984b). However, as discussed below, there are significant tissue differences in the pharmacology of [³H]8-OH-DPAT binding sites. Thus 5-HT$_{1A}$ receptors are somewhat ill-defined, and at most one can say that the CBA receptor shows pharmacology of the 5-HT$_{1A}$ type. Since the CBA provides a means of measuring the actions of functional 5-HT$_{1A}$ type receptors in vitro, it should prove to be a valuable system for the characterization
of the pharmacological and biochemical properties of this group of receptors.

The present results are consistent with the previous findings of Peroutka et al. (1982) and the more recent demonstration, by autoradiographic techniques, of the presence of 5-HT\textsubscript{1} but not 5-HT\textsubscript{2} receptors in the CBA vascular wall (Peroutka and Kuhar, 1984). One discrepancy in the present data is that 5-HT, 8-OH-DPAT and RU 24969 were found to be respectively 6.2, 5.6 and 12.7 times less potent in the CBA than at 5-HT\textsubscript{1A} sites defined by [\textsuperscript{3}H]8-OH-DPAT binding using whole rat cortex. However, as noted above, there is already evidence for heterogeneity of 5-HT\textsubscript{1A} sites defined by the high affinity binding of [\textsuperscript{3}H]8-OH-DPAT, as significant differences have been observed between the hippocampal and striatal binding sites for this ligand (Gozlan et al., 1983). At the striatal site, potencies for 5-HT (47nM), 8-OH-DPAT (20nM) and RU 24969 (910nM) are less than those observed at the hippocampal site and are also much closer to those observed in the CBA. Whether this is merely coincidental has yet to be determined. It should also be noted that in this study neither a monoamine oxidase inhibitor or a 5-HT uptake blocker were used, which may have caused an underestimation of the potency of 5-HT. Aside from these possibilities, the \( K_A \) values measured for these 3 compounds are admittedly somewhat high for a 5-HT\textsubscript{1} type receptor; however, they are so far below the 5-HT\textsubscript{2} \( K_A \) values that it is evident that the response cannot be explained by the latter receptor alone.
It has not been definitively demonstrated, however, that there are not also 5-HT$_2$ receptors in the CBA. In the autoradiographic study that demonstrated the presence of 5-HT$_1$ receptors in the CBA (Peroutka and Kuhar, 1984), the conditions used to label 5-HT$_2$ receptors ([$^3$H]LSD plus 300nM 5-HT) may not have been optimal, since current estimates of the $K_d$ of 5-HT at the 5-HT$_2$ site, based on [$^3$H]ketanserin binding, are about 300nM (Leysen et al., 1982). Thus, under the conditions used, as many as half of any available 5-HT$_2$ receptors would be occupied by cold 5-HT and thus be unavailable for labeling. Therefore, the possibility of 5-HT$_2$ receptors also being present should not be totally excluded. Actually, this possibility has recently been acknowledged by Peroutka (1984a), based upon the observation that 5-HT$_2$ antagonists do have small but significant effects in the CBA at low concentrations. Furthermore, since agonists are generally much more potent at 5-HT$_1$ receptors, in this situation (both receptors being present) one would still expect the overall agonist profile to be of the 5-HT$_1A$ type. One might also expect experimental $K_A$ values for some compounds to lie between the 5-HT$_1A$ and 5-HT$_2$ $K_i$ values. Thus, the present results are not inconsistent with this possibility.

Although we have generally observed ketanserin to have only slight effects at concentrations below 1µM, and another selective 5-HT$_2$ antagonist has recently been reported to be essentially inactive in the CBA (Cohen et al., 1984), it would be erroneous to assume that this is sufficient proof of the absence of 5-HT$_2$ receptors. If both receptor types are present in the CBA, and the activation of either is
independently capable of causing a maximal (or near maximal) contraction, one would still expect to observe that 5-HT$_2$ antagonists would be virtually inactive against 5-HT, as a maximal response could still be produced by activation of 5-HT$_1$ receptors. The converse would apply for a selective 5-HT$_1$ antagonist: its effect would be largely masked by the activation of 5-HT$_2$ receptors at higher 5-HT concentrations (>100nM).

The possibility that some 5-HT$_2$ receptors may be present does not seriously interfere with the usefulness of the CBA as a 5-HT$_1$ receptor preparation, as the former can be selectively occluded with any of several highly potent 5-HT$_2$ antagonists. Thus, the protocol described previously (Ch.6, Methods) has been used for the investigation of potential 5-HT$_{1A}$ antagonists: all responses are obtained in the presence of 300nM ketanserin, to eliminate the possibility that 5-HT$_2$ receptors are contributing to the response.

If, as suggested by Peroutka (1984b), 5-HT$_1$ type receptors predominate in intracranial vessels, whereas 5-HT$_2$ receptors predominate in peripheral vessels, it is possible that in the basilar artery both receptors are present because it is near the interface of these regions. Alternatively, both receptors may be present throughout the intracranial vessels. This might not involve redundancy of function if one of them is involved in neurogenic control of the cerebral circulation and one of them is a luminal receptor responsive to circulating 5-HT.

In summary, the 5-HT agonist profile of the CBA was found to correlate very well with the 5-HT$_{1A}$ receptor subtype, which is
consistent with the original finding of Peroutka et al. (1982) that the contraction to 5-HT is mediated by a 5-HT\textsubscript{1} type receptor. However, preliminary experiments suggest that some 5-HT\textsubscript{2} receptors may also be present. Assuming that the simultaneous activation of both receptors sometimes produces a larger maximal response than that produced by either receptor alone, this may partially explain why Muller-Schweinitzer and Engel were able to reach the opposite conclusion. Also, if the hypothesis about the mechanism of noncompetitive antagonism is correct, this tissue may be useful as a model for studying the kinetics of drug-receptor interactions in the absence of a receptor reserve. It also suggests that there may sometimes be problems associated with the assumption that equilibria are actually attained in tissue bath preparations when preincubations with antagonists are used.
APPENDIX A

THE SYNTHESIS OF 3-AMINOMETHYL-1,3,4,5-TETRAHYDROBENZ[c,d]INDOLE

The conformationally constrained tryptamine analog 3-aminomethyl-THBI (IV, R=H) was synthesized from the commercially available indole-3-aldehyde in 8 steps, according to the scheme shown as Fig. 11. The desired starting material, keto acid I, had been previously synthesized by Szmuszkovicz (1964) from 3-indolesuccinic acid, which was synthesized by the method of Perron and Minor (1959). Acid II was also synthesized by Szmuszkovicz, by the Wolff-Kishner procedure, in 7.6% yield. This was considered an unacceptable loss of starting material, so several alternate methods of reducing the benzylic ketone of I were investigated. Both diborane and mixtures of LiAlH₄ and AlCl₃ gave unsatisfactory results. Reduction of the tosylhydrazone of I by NaCNBH₃ in acidic medium, followed by alkaline hydrolysis, was satisfactory, but still involved overall losses close to 50%, as well as being somewhat inconvenient. Excellent results (83%) were obtained by the use of triethylsilane in trifluoroacetic acid (West et al., 1973), followed by hydrazinolysis of the 1-acetyl group without purification of the intermediate. Amide III was synthesized using carbonyl diimidazole, and the amide reduced with diborane in THF according to the procedure of Brown and Heim (1972).
Figure 11. Scheme for the synthesis of 3-aminomethyl-THBI from the commercially available indole-3-carboxaldehyde (R=H).
Experimental

Infrared spectra were recorded on a Beckman IR-33 spectrophotometer. Proton NMR spectra were recorded on a Varian Model EM360 or a Jeol FX90Q spectrophotometer using tetramethysilane as an internal standard (chemical shifts are reported as delta values). Melting points were determined on an electrothermal apparatus and are uncorrected. High resolution mass spectra were recorded on a Varian MAT 311 A double focusing mass spectrometer at 70eV. Elemental analyses were performed by the University of Arizona Analytical Centre or by MicAnal, Inc. (Tucson, AZ).

1,3,4,5-Tetrahydrobenz[cd]indole-3-COOH (II):

In a 25 ml roundbottom flask 1 gm of 1-acetyl-5-oxo-1,3,4,5-tetrahydrobenz[cd]indole (I) was dissolved in 10 ml of trifluoroacetic acid and stirred mechanically. Triethylsilane (2.5 gm) was then added dropwise and the mixture was stirred at room temperature for 2 hours. The mixture was then evaporated under vacuum at 35°; the residue was partitioned between water and ethyl acetate. The organic layer was washed once with water and dried over Na$_2$SO$_4$, then the solvent was again removed under vacuum and replaced with ethanol containing about 5% hydrazine. This was refluxed for 1 hr, allowed to cool and evaporated under vacuum. The residue was taken up in ethyl acetate and washed first with dilute HCl, then saturated NaCl, and dried over Na$_2$SO$_4$. The crude product was purified by flash chromatography on silica gel (9:10:1 ethyl acetate:hexane:acetic acid). The product was recrystallized from
THF-toluene to give 650 mg (83%), m.p. 202-203° (lit. 203°). An analytical sample was recrystallized out of chloroform. NMR (deuterioacetone): 10 (b, 1H, indole N-1), 6.7-7.2 (m, 4H, aryl), 3.9 (t, 1H, methine), 2.9 (dt, CH₂), 2.2 (m, 2H, CH₂). (Found: C, 71.42; H, 5.49; N, 6.92. C₁₂H₁₁N₂O₂ requires C, 71.63; H, 5.51; N, 6.96%).

1,3,4,5-Tetrahydrobenz[cd]indole-3-carboxamide (III, R=H):

Acid II (500 mg) and 403 mg of carbonyl diimidazole were dissolved in 100 ml dry THF under argon and warmed at 60° for 1 hr. After cooling for 15 min, excess anhydrous ammonia in THF was added and stirred for 45 min. The THF was then evaporated under vacuum and the residue was taken up in 150 ml ethyl acetate and washed with about 100 ml of water, then about 50 ml each of 1) dil. HCl 2) 5% NaHCO₃ 3) saturated NaCl. After drying over Na₂SO₄, the ethyl acetate was removed under vacuum and the residue was crystallized from ethanol to give 310 mg of product, m.p. 199-200°; on recrystallization from ethanol, m.p. 201°. An additional 46 mg was obtained as a second crop; total yield, 356 mg (72%). IR (KBr): 3420, 3185 (NH stretch), 1665 (C=O), 1640 (NH bend, amide II). NMR (CDCl₃): 8.0 (s, 1H, indole N-1), 6.8-7.3 (m, 4H, aryl), 5.3-5.7 (b, 2H, amide NH₂), 3.8 (t, 1H, methine), 2.9 (dt, 2H, CH₂), 2.45 (m, 2H, CH₂). (Found: C, 71.16; H, 6.07; N, 13.88. C₁₂H₁₂N₂O requires C, 71.98; H, 6.04; N, 13.99%).

3-Aminomethyl-1,3,4,5-tetrahydrobenz[cd]indole (IV, R=H):

Four ml of 1.0 molar borane-THF was added by syringe to 100 mg of amide III in 30 ml THF. The solution was stirred under argon and
refluxed for 3 hours. After cooling to room temperature, 12 ml of 0.5N HCl was added dropwise, then the THF was distilled off at atmospheric pressure. The flask was then placed on ice and the solution slowly saturated with NaOH pellets, followed by extraction with 3 x 40 ml of ethyl acetate. After drying and evaporation, the product was taken up in a warm mixture of THF and ether, filtered, and used immediately to make the oxalate salt (91 mg, 66%), m.p. 203-205° (dec.) (The procedure for salt formation was described in Ch. 2). As the oxalate proved to have very poor solubility, the hydrochloride was made for use in bioassays and for analysis. NMR (CD$_3$OD) for HCl salt: 6.7-7.2 (m, 4H, aryl), 3.5 (q, 2H, alpha CH$_2$ coupled to NH$_3^+$, J=7 Hz), 3.2 (partially obscured by methanol, methine?), 3.0 (t, 2H, benzylic CH$_2$), 2.0 (m, 2H, ring CH$_2$), 1.2 (t, 3H, NH$_3^+$). Anal. molecular weight calcd. C$_{12}$H$_{14}N$: 186.1157. Found (high resolution mass spectrum): 186.1147. (Found: C, 62.59; H, 6.80; N, 11.70. (C$_{12}$H$_{15}$N$_2$Cl)$_2$.CH$_3$OH requires C, 62.89; H, 7.17; N, 11.70%).
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