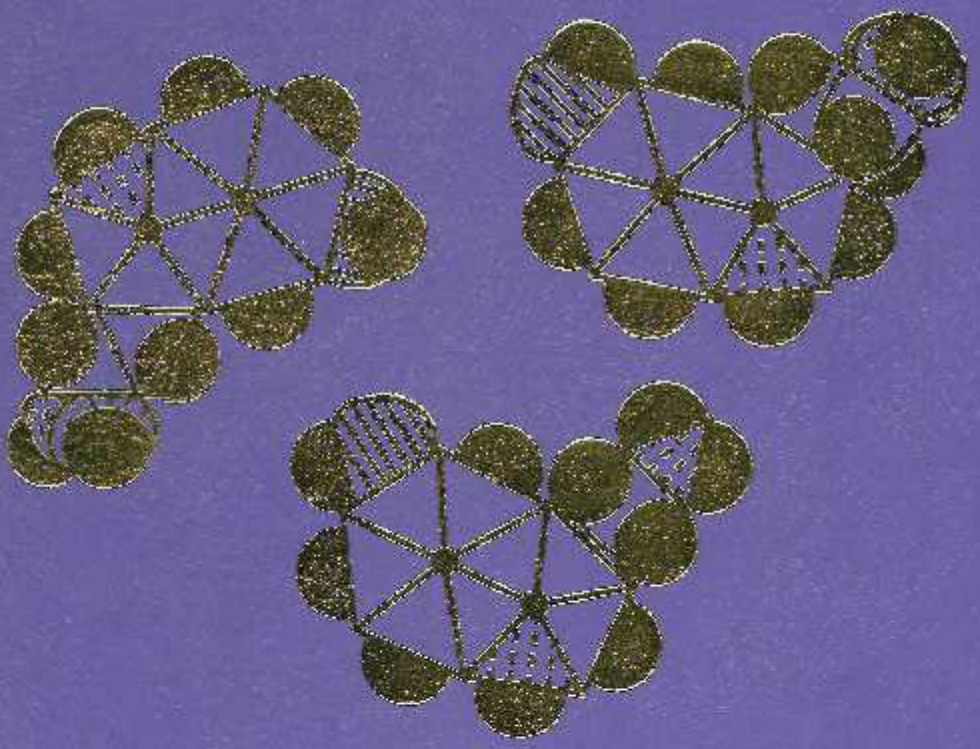
5-HT AGONISTS AS PSYCHOACTIVE DRUGS



RICHARD H. REICH
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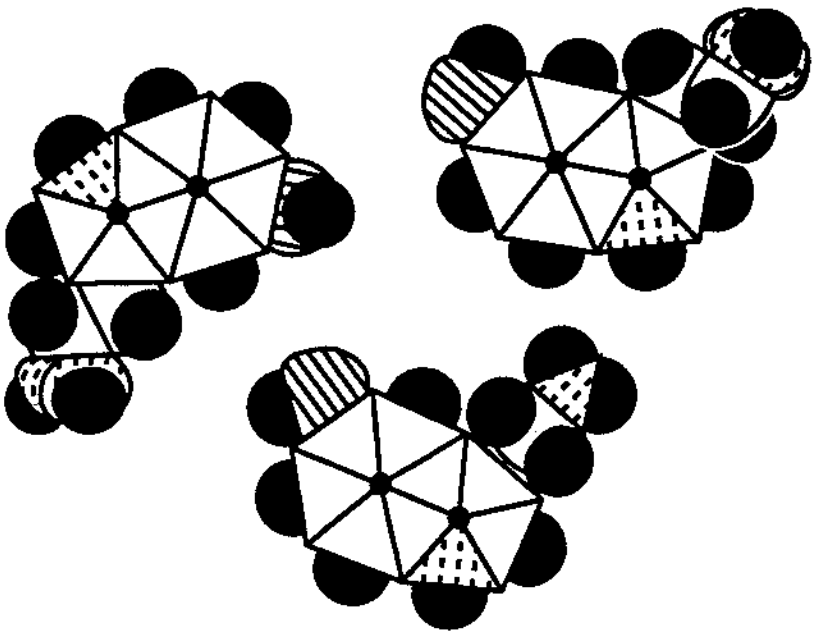

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DEDICATION

It seems only proper to dedicate this book to the early pioneers of research into serotonin: L.H. Page, V. Erspamer, J.H. Gaddum, D.W. Woolley and E. Shaw, and B.B. Brodie come easily to mind. These investigators dared to combine their analytical skills and intuition to draft hypotheses about the essence and workings of this neurotransmitter even before the chemical nature of the substance was clearly understood. But they also had the courage to test their hypotheses, risking impasses, failures and skepticism from colleagues in pursuit of a vision for which the path was uncertain and poorly defined. This ability to rise above the pedestrian efforts and pragmatic designs of fellow researchers distinguishes these men as trailblazers for the scientists of succeeding generations. In this volume, we reaffirm their heroic efforts and trust that our endeavors revere in some small measure the gifts that they bestowed.

PREFACE

Many of the papers in this book were based upon presentations at the 24th Annual Meeting of the American College of Neuropsychopharmacology (ACNP), held in Maui, Hawaii, in December, 1985. They were contained in two symposia: (1) Mechanisms of Indole and Phenalkylamine Hallucinogens, chaired by R.H. Rech; (2) Serotonin Receptor Subtypes: Biochemical, Physiological, Behavioral and Clinical Implications, co-chaired by G.A. Gudelsky. The results presented were based largely on the differential effects of various 5-HT agonists, many of which have been developed only recently. Further development and utility of 5-HT antagonists and methodology to characterize 5-HT mechanisms also contributed to the presentations at the ACNP. The success of these Symposia and the obvious interrelationships of the studies reviewed prompted the publication of this volume. However, a number of other investigators have contributed to this book in areas not represented at the Maui Symposia, and the chapters derived from the ACNP presentations contain much additional data, some developed very recently.

The book is divided into four sections on the basis of the major thrust of the various chapters: Biochemical Approaches, Neurophysiological Approaches, Behavioral Approaches, and Neuroendocrine Aspects. It will become obvious to the reader that these divisions are rather arbitrary, and that many of the tools utilized, results obtained, and general interpretations advanced, have commonalities across chapters in different sections as well as within sections. Despite the current explosive progress in this field identifying yet more subtypes of 5-HT receptors and uncovering greater complexities in functional aspects of 5-HT mechanisms, we anticipate that the contributions contained in this volume will remain a valuable resource for serotonin research for some years to come.

June 15, 1988

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INTRODUCTION

5-Hydroxytryptamine (5HT) bas been known to occur in various plant and animal species for over 3 decades, including its presence in the mammalian nervous system (Twarog and Page, 1953; Amin et al., 1954).

The proposed functional significance of 5HT has been related to skin pigmentation in frogs (Davey, 1959), ciliary motion in mussels (Gosselin, 1966), blood platelet functions (Paasonen and Pletscher, 1959), ACTH release from the pituitary (Saffran and Vogt, 1960), edema formation after cutaneous tissue damage (Dorofeev and Polushkin, 1967), cardiac (Erspamer and Ghiretti, 1951; Trendelenburg, 1960) and cardiovascular stimulant properties (Haddy et al., 1959; Walsh, 1967), and migraine (Freedman, 1961), to mention a few of the earlier proposals for roles of this biological substance.

The term "serotonin" was coined by Page's group as they searched for humoral pressor substances (Rapport et al., 1948). They also synthesized 5-hydroxytryptamine a few years later to demonstrate its identity to serotonin. Erspamer and coworkers had first described a substance in the enterochromaffin cells of the gastrointestinal mucosa which induced smooth muscle stimulation and was called "enteramine" (Erspamer, 1954). They identified enteramine as 5-hydroxytryptamine in 1952. The effects of 5HT on alimentary smooth muscle are very complex, involving actions on intramural nerve components and various muscle components for a mixture of stimulatory and inhibitory responses. Increased 5HT levels can induce gastrointestinal ulceration that is prevented by atropinization, vagotomy or LSD (Blackman et al., 1959). McCall and Harris review more recent studies on the influences of central 5HT pathways on vasomotor control mechanisms in this volume, showing both excitatory and inhibitory effects on blood pressure controls. The activation of $5HT_{1A}$ receptors appeared to reduce central sympathetic nerve discharge whereas $5HT_{1B}$ excitation and $5HT_2$-selective agonists caused some increase.

Tryptaminergic receptors in smooth muscle were identified early by Gaddum (1958). "M" receptor activity was found to be antagonized by morphine (also atropine and cocaine), while "D" receptors were blocked by Dibenzyline® (phenoxybenzamine, as well as by other α-adrenergic blockers). It appeared that M receptor activity was mediated via nerve components, while D receptors involved direct myogenic influences of 5HT (Kosterlitz and Robinson, 1958; Day and Vane, 1963).

The highest concentration of 5HT in mammalian tissue is found in the pineal gland, and its derivative melatonin is synthesized only in this structure (Axelrod et al., 1961). The synthesis of melatonin was found to be entrained diurnally by the amount of light exposure of the retina, the pathways involved coursing through the brain, spinal cord and superior cervical ganglion and culminating in sympathetic fibers to the pineal gland (Axelrod et al., 1966). Further elaborations of this system have led to theories of at least partial control of diurnal patterns of behavior through changes in melatonin levels elaborated by the gland. The role of brain 5HT in sleep-wake patterns (see Koella, 1983) is

obviously broader than this, since the activity of serotonergic raphe neurons are clearly implicated in the slow-wave sleep mechanisms and their cycling with REM-sleep stages. The study of effects on basic sleep mechanisms of recently developed drugs acting on serotonergic systems appears to have been neglected to some extent. Gudelsky et al. have reviewed in their chapter 5HT modulation of brain thermoregulatory controls. Controversies in the earlier literature on this topic have been resolved to a considerable extent by these investigators, $5HT_2$ receptor agonists causing hyperthermia while $5HT_{1A}$ agonists were hypothermic. Second-generation anxiolytics (buspirone, etc.) exerted hypothermic responses that were blocked by pretreating with spiperone or (-)-pindolol, as predicted by their presumed $5HT_{1A}$ activity.

The anatomical distribution of 5HT-containing neurons was early documented by Hillarp and Falck (Falck et al., 1962), though considerable refinements were added later (see Azmitia, 1987; Ho et al., 1982; Parent et al., 1981). The finding by Aghajanian (see Ho et al., 1982) that 5HT cell bodies were inhibited by the action of LSD as well as 5HT reflected back on the earlier speculations of Gaddum (1953) and Woolley and Shaw (1954) that LSD and other hallucinogens may exert their effects by interfering with tonic brain 5HT modulations. The hypothesis by Brodie and Shore (1957) that release of brain 5HT by reserpine contributed to the depressant effects seen, as well as observations such as that of Koe and Weissman (1968), that reducing brain 5HT levels by the synthesis inhibitor PCPA resulted in hyperreactivity, were also effective in orienting the scientific community to potential brain neurotransmitter roles of 5HT in mental processes. While 5HTP in smaller doses caused sedation and EEG synchronization (Monnier and Tissot, 1958), much larger doses or combination with an MAO inhibitor caused excitation, tremors and convulsions (Tedeschi et al., 1960). Thus, there appeared to be a biphasic effect of 5HT on brain activity, depending upon concentration.

A number of studies in the past have supported a role of 5HT in anxiety neurosis (Stein et al., 1975) and the speculation that the anxiolytic activity of benzodiazepines relates to the capacity of these agents to reduce brain 5HT turnover. The conflict model of anxiety (Geller and Seifter, 1960) has been used to demonstrate a purported anti-anxiety activity of agents with 5HT antagonistic or attenuating properties (Stein et al., 1973; Graeff, 1974; Cook and Sepinwell, 1975; Geller et al., 1974; Schoenfeld, 1976). It has also been demonstrated in conflict models that depletion of brain 5HT by pretreating with p-chlorophenylalanine results in a release from punishment suppression (Robichaud and Sledge, 1969; Geller and Blum, 1970; Stein et al., 1973), although others failed to confirm this (Blakely and Parker, 1973; Cook and Sepinwall, 1975). Pretreatment with 5,6- or 5,7-dihydroxytryptamine also appeared to exert anticonflict activity (Stein et al., 1975; Lippa et al., 1977; Tye et al., 1977). However, these tests generally have used food as reinforcement, and 5HT has been implicated in the motivational aspects of feeding behavior (Samanin et al., 1977).

Kilts et al. (1982), Commissaris et al. (1981), and Commissaris and Rech (1982) developed evidence that diazepam, pentobarbital and methaqualone did not release punishment suppression by a primary action on 5HT mechanisms, although agents affecting brain 5HT did have some influence on conflict behavior. These findings are discussed in more detail in the chapter by Rech et al. Use of the acoustic startle reflex in the analysis of centrally-active 5HT drugs has been explored by Michael Davis and colleagues (see their chapter in this book). Modulatory roles of 5HT systems on the nerve pathways involved in the startle reaction were identified, though differing in direction of change as a function of forebrain vs. spinal cord 5HT innervations. Moreover, the subtype of 5HT receptor involved was different for each category of influence on the startle reflex. It was also discovered that second-generation anxiolytics with serotonergic activity would attenuate fear-potentiated startle, although the association with 5HT mediation is problematical.

A more extensive examination of second-generation anxiolytics (SGAs) and 5HT activity is presented in the chapter by Young and Glennon. As emphasized by Cunningham and Appel, Young and Glennon also believe that these agents act mainly at $5HT_{1A}$ sites. Buspirone and close relatives differ from benzodiazepines not only in 5HT receptor binding characteristics, but also in their lack of prominent sedation or significant anticonvulsant properties. Earlier studies on the effects of serotonergic drugs on types of conflict behavior are reviewed to support a role of brain 5HT in the mediation of punishment-suppressed behavior. The effect of SGAs on conflict behavior is very variable and appears to relate to subtle aspects of the particular conflict procedure and dose parameters (see also McCloskey et al., 1987). The effects of SGAs in certain other behaviors (light-dark exploration, social interaction) also support anxiolytic activity. Drug discrimination studies, however, demonstrate that SGAs differ in their mechanism from benzodiazepines (see Cunningham and Appel, this volume).

LSD has been found to label both $5HT_1$ and $5HT_2$ receptors in the brain (Peroutka and Snyder, 1982), and $5HT_1$ activity has been equated with inhibitory effects while excitatory influences have been associated with $5HT_2$ receptors (Jacobs, 1984). As indicated earlier, LSD and other hallucinogens may act directly or indirectly on brain 5HT mechanisms to bring about their effects (see Cunningham and Appel, and Rech et al., this volume), and these effects appear to relate to an increased activity at certain brain 5HT receptors. Indeed, Cunningham and Appel, Rech et al., and Glennon et al. (1984) have all implicated $5HT_2$ receptors in actions of hallucinogens, and this theme is reiterated in the chapter by Heym and Jacobs. An alteration in the balance of activities at various 5HT receptor subsystems may also be involved. 5HT precursors, as well as LSD, DMT, mescaline, and related hallucinogens, produce the "serotonin syndrome": tremor, rigidity, forepaw treading, hindlimb abduction (Lucki and Frazer, 1982). This behavior appears to relate to $5HT_1$ receptors, since non-specific antagonists block it while $5HT_2$ antagonists do not (Lucki et al., 1984; Frazer et al.,

this volume). Contrariwise, head shaking induced by 5HTP or quipazine was antagonized by the $5HT_2$ antagonist ketanserin. In their chapter in this volume, Frazer et al. concentrate on the definition of agonists and antagonists affecting $5HT_1$ receptor subtypes. In this analysis, they have used a clever mixture of biochemical, pbarmacological and behavioral approaches to distinguish activities associated with $5HT_{1A}$ or $5HT_{1B}$ receptors. Pierce and Peroutka have also emphasized the study of $5HT_1$ receptors in their review of ligand binding characteristics, although they have previously presented evidence that there are at least 5 different classes of 5HT hinding sites in mammalian brain.

Sanders-Bush and Conn use a biochemical approach in their chapter to discuss the transmembrane coupling of the 5HT receptor systems. As also indicated by Frazer et al. and Pierce and Peroutka, adenylate cyclase has been identified as the second-messenger coupling mechanisms for some inhibitory and stimulatory effects. Sanders-Bush and Conn are concerned with piperazine derivatives having agonistic activity at various types of $5HT_1$ and $5HT_2$ receptors, these being linked to systems for phosphoinositide hydrolysis. The chapter by Fuller also deals with piperazine derivatives with 5HT agonistic activities. Included in this analysis are buspirone and related derivatives and structure-activity relationships selecting for one or another subclass of 5HT receptor. The complications introduced by the production of active metabolites, as well as indirect mechanisms, with some drugs in this class are discussed. Aspects of endocrinological, appetitive and behavioral effects of these piperazines are concisely reviewed, including the proposition that $5HT_{1A}$ agonists may have anxiolytic properties.

The capacity of [^{3}H]PAPP and its azido derivative to act as selective ligands for $5HT_{1A}$ receptors, as well as influencing coupled adenylate cyclase in Aplysia neurons has been examined in this volume by Shih et al.. Several other chapters deal with clinical aspects of 5HT receptor activation. Evidence is accumulating to support a role of serotonin in hypothalamic-pituitary controls of hormonal secretion (see the chapter by Mellow et al.). A comparison of neuroendocrine effects of 5HT agonists in the rodent has been presented in the last chapter of the hook by Koenig et al.

A number of important areas of serotonin research have been barely mentioned in this book. For example, certain 5HT agonists have prominent anorectic activity, presumably by influencing 5HT mechanisms operating at hypothalamic feeding centers that normally relate to satiating mechanisms (Blundell, 1977; Garattini et al., 1978; Samanin and Garattini, 1982; Thurlby et al., 1983; Rech et al., 1984; Henck et al., 1985). These studies and much other recent literature have exhaustively reviewed this appetite-suppression concept of central 5HT activity. Another area relates to 5HT mechanisms that modulate pain sensory processes involving opioid receptors in brain and spinal cord (Harvey and Yunger, 1973; Samanin and Valzelli, 1971; Tilson and Rech, 1974). More recent work with kappa opioid analgesics indicate a major role of brain serotonergic systems in their pharmacological effects (Von Voigtlander et al., 1984; Henck et al., 1983; Rech et al., 1984). However, the relationship of brain or spinal cord 5HT mechanisms to

kappa opioid effects is still poorly understood and will require considerable research in various aspects before a clear understanding is achieved.

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5-HT Agonists as Psychoactive Drugs
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ANALYSIS OF 5-HT$_1$ BINDING SITE SUBTYPES AND POTENTIAL FUNCTIONAL CORRELATES

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INTRODUCTION

In the forty years since the discovery of the neuroactive compound serotonin (5-hydroxytryptamine; 5-HT), biochemical, neurophysiological and behavioral data have suggested that multiple 5-HT receptors exist in the central nervous system. In the past decade, radioligand binding studies have been instrumental in characterizing multiple subtypes of putative 5-HT receptors in brain membranes. The identification and characterization of 5-HT receptor subtypes may be useful in the clinical analysis of certain disease states. For example, neuropsychiatric disorders such as anxiety, depression and hallucinosis have been specifically linked to specific 5-HT receptor subtypes in the central nervous system. Knowledge of drug interactions with specific receptor subtypes may allow for the prediction of drug effects in certain diseases and may elucidate the pathophysiology of central nervous system disorders.

However, altered 5-HT neurotransmission has never been directly linked to a specific human disease. To a large extent, this failure relates to the multiplicity of 5-HT actions in the central nervous system. The first investigators to suggest that multiple 5-HT receptors exist were Gaddum and Picarelli (1957) when they identified M and D receptor subtypes in gut muscle. The application of radioligand binding studies in the 1970's led to the characterization of at least 5 distinct subtypes of central 5-HT binding sites (Peroutka, 1987). Moreover, other subtypes of 5-HT receptors appear to exist in the periphery (Bradley et al., 1986).

Radioligand studies focus on the analysis of "binding sites" for drugs as opposed to "receptors". In order for a 5-HT binding site to be classified as a 5-HT receptor, a specific physiological,

biochemical or behavioral correlate must be identified. In the present report, an attempt will be made to correlate the known pharmacological characteristics of central 5-HT binding sites (as defined in radioligand binding studies) with a variety of physiological, biochemical and behavioral correlates.

Differentiation of 5-HT Receptor Subtypes

In 1974 Bennett and Aghajanian reported the first successful radioligand analysis of 5-HT receptors by using ^{3}H-d-lysergic acid diethylamide (d-LSD). These results were confirmed by other laboratories (Bennett and Snyder, 1975; Lovell and Freedman, 1976). The next radioligand used for 5-HT receptor analysis was ^{3}H-5-HT (Bennett and Snyder, 1976). However, radioligand binding analysis revealed discrepancies between the data derived using these two ligands. Shortly thereafter ^{3}H-spiperone was found to label 5-HT sites in addition to its known binding to dopamine D_2 receptors (Leysen et al., 1978). Furthermore, 5-HT was found to displace ^{3}H-5-HT at a concentration (3.8 nM) which was three orders of magnitude lower than was needed to displace ^{3}H-spiperone (2700 nM). Spiperone displaced ^{3}H-spiperone at a concentration (0.51 nM) which was fourteenhundred fold lower than was needed to displace ^{3}H-5-HT (730 nM). When 5-HT sites were labeled with ^{3}H-LSD, 5-HT had an apparent K_i of 110 nM while the K_i for spiperone was 18 nM. From the above results, Peroutka and Snyder (1979) suggested the existence of two distinct 5-HT receptor sites in the central nervous system. Those labeled by ^{3}H-5-HT were referred to as 5-HT_1 sites and those labeled by ^{3}H-spiperone were designated 5-HT_2 sites. ^{3}H-LSD labeled both sites since its affinity was similar for each site. With the radioligands used to date, it appears that a generalized pattern exists in which 5-HT_1 sites have a high affinity for 5-HT and tryptamine derivatives while 5-HT_2 sites display high affinity for 5-HT antagonists such as cyproheptadine, ketanserin and mianserin.

5-HT_1 Receptor Subtypes

The non-sigmoidal displacement of ^{3}H-5-HT by spiperone suggested that subtypes of 5-HT_1 binding sites exist. 5-HT_1 sites which displayed high affinity (K_i = 2 - 13 nM) for spiperone were designated 5-HT_{1A} sites while those which displayed lower affinity for spiperone (K_i = 35 uM) were

designated 5-HT_{1B} sites (Pedigo et al., 1981). Lending support to this subclassification was the difference in regional localization between these two subtypes, a distinction which has been identified in many species (Schnellmann, 1984). Identification of a third 5-HT_1 site highly localized in the choroid plexus led to the characterization of the 5-HT_{1C} site (Pazos et al., 1984). The fourth 5-HT_1 subtype to be classified is the recently identified 5-HT_{1D} site in bovine brain (Heuring and Peroutka, 1987). A comparative summary of all 5-HT_1 binding site subtypes is provided in Table 1.

5-HT_{1A} RECEPTORS

The 5-HT_{1A} receptor subtype was first identified by Nelson and colleagues (Pedigo et al., 1981; Schnellmann et al., 1984). However, it was the introduction of ^{3}H-8-OH-DPAT by Gozlan et al. (1983) which enabled an extensive characterization of this site. 8-OH-DPAT is approximately 10,000 times more potent at the 5-HT_{1A} receptor subtype than at any other known 5-HT receptor subtype. Importantly, ^{3}H-8-OH-DPAT also labels glass fiber filter paper under certain assay conditions in the absence of the anti-oxidant, ascorbate (Peroutka and Demopulos, 1986). In addition, ^{3}H-8-OH-DPAT has been reported to label a 5-HT "autoreceptor" (Gozlan et al., 1983; Hall et al., 1985; 1986) and the "5-HT transporter" (Schoemaker and Langer, 1986). However, in the presence of ascorbate, the 5-HT_{1A} binding site is the only site that has been labeled by ^{3}H-8-OH-DPAT (Peroutka, 1985; 1986; Hoyer et al., 1986b). Subsequently, the site has been selectively labeled by a variety of other radioligands including ^{3}H-5-HT, ^{3}H-ipsapirone, ^{3}H-buspirone, ^{3}H-WB 4101, ^{3}H-PAPP and ^{3}H-spiroxatrine (Peroutka, 1987).

Due to the variety of agents which show potent and selective actions at this site, the 5-HT_{1A} site has been the most extensively analyzed 5-HT_1 binding site subtype. Anatomically, the 5-HT_{1A} receptor is found in extremely high density in the raphe nuclei and in the dentate gyrus and CA1 region of the hippocampus (Deshmukh, 1983; Marcinkiewicz, 1984; Pazos and Palacios, 1985; Glaser et al., 1985; Hoyer et al., 1986a).

Adenylate Cyclase

Serotonergic modulation of adenylate cyclase

TABLE 1

CHARACTERISTICS OF 5-HT_1 BINDING SITE SUBTYPES

Data given are derived from Peroutka and Snyder (1979), Peroutka (1986), Hoyer et al. (1985b), Heuring and Peroutka (1987) and unpublished observations.

	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_{1D}
RADIOLABELED BY	^{3}H-5-HT	^{3}H-5-HT	^{3}H-5-HT	^{3}H-5-HT
	^{3}H-8-OH-DPAT	^{125}I-CYP	^{3}H-Mesulergine	
	^{3}H-Ipsapirone	(Rat and	^{125}I-LSD	
	^{3}H-WB 4101	Mouse only)		
	^{3}H-Buspirone			
	^{3}H-PAPP			
	^{3}H-Spiroxatrine			
HIGH DENSITY REGIONS	Raphe nuclei	Substantia Nigra	Choroid Plexus	Basal Ganglia
	Hippocampus	Globus Pallidus		

TABLE 1

DRUG POTENCIES (K_i, nM)				
< 10 nM	5-CT	RU 24969	Mesulergine	5-CT
	8-OH-DPAT	5-CT	Metergoline	5-HT
	5-HT	5-HT	Methysergide	Metergoline
	RU 24969			
	d-LSD			
10-1000 nM	Metergoline	Metergoline	Mianserin	Methysergide
	Methysergide	Methysergide	5-HT	Mianserin
	Spiperone	d-LSD	RU 24969	8-OH-DPAT
	Mesulergine		5-CT	d-LSD
			d-LSD	RU 24969
> 1000 nM	Mianserin	Mianserin	Spiperone	Mesulergine
		Spiperone	8-OH-DPAT	Spiperone
		Mesulergine		
		8-OH-DPAT		

has been linked to the 5-HT_{1A} site. The first 5-HT-sensitive adenylate cyclase detected in mammalian brain was in newborn rats (Von Hungen et al., 1974; Von Hungen et al., 1975). More recently, nanomolar concentrations of the 5-HT_{1A} selective drugs 5-carboxyamidotryptamine (5-CT) and 8-OH-DPAT stimulated a 5-HT-sensitive adenylate cyclase in guinea pig hippocampal membranes (Shenker et al., 1985). Additional evidence linking the 5-HT_{1A} binding site to the adenylate cyclase second-messenger system derives from studies determining the effect of guanine nucleotides on the binding of ^{3}H-8-OH-DPAT. The effect of guanine nucleotides on agonist binding of a receptor is often an indication that an adenylate cyclase system is linked to the receptor (Maguire et al., 1977; Rodbell, 1980; Limbird, 1981). It was found that GTP and GDP, but not GMP, inhibit the binding of ^{3}H-8-OH-DPAT to brain homogenates (Hall et al., 1985; Schlegel and Peroutka, 1986).

Markstein et al. (1986) have shown that 5-HT-stimulated cyclase activity in rat hippocampus is mediated by the 5-HT_{1A} receptor. By contrast, DeVivo and Maayani (1986) have shown that 5-HT inhibits forskolin-stimulated adenylate cyclase in both guinea pig and rat hippocampal membranes. Although their studies show that 5-HT inhibits rather than stimulates cyclase activity under this condition, the results of DeVivo and Maayani (1986) are consistent with the inhibition being mediated by a single, homogeneous population of 5-HT_{1A} receptors. Selective 5-HT_{1A} agonists inhibited cyclase activity and spiperone competitively blocked this inhibition. Ketanserin had no effect on cyclase activity, neither directly nor by modulating 5-HT-induced inhibition of the cyclase activity.

Inhibition of Raphe Nuclei

The inhibition of dorsal raphe cell firing by 5-HT, d-LSD and related tryptamines has been extensively studied for almost two decades (Rogawski and Aghajanian, 1981). More recently, VanderMaelen and Wilderman (1984) suggested that 5-HT_{1A} receptors inhibit raphe firing since buspirone, a selective 5-HT_{1A} agent, completely inhibited firing of the dorsal raphe in the rat. This same effect of buspirone has also been seen in the mouse (Trulson and Arasteh, 1986). Evidence supporting a role for 5-HT_{1A} receptors in the inhibition of raphe neuronal

firing has also been reported by other laboratories. For example, ipsapirone, 8-OH-DPAT and 5-CT were also found to mimic the effect of 5-HT on raphe cell firing (Sprouse and Aghajanian, 1985; Sinton and Fallon, 1986; VanderMaelen et al., 1986). Further evidence supporting a role of 5-HT_{1A} receptors in raphe inhibition is the ability of (-)propranolol to reversibly block the inhibitory effects of ipsapirone and 8-OH-DPAT on raphe cell firing (Sprouse and Aghajanian, 1987). The antagonist, (-)propranolol, is a beta-adrenergic agent which also displays a high affinity for the 5-HT_{1A} receptor (Hiner et al., 1986).

Inhibition of raphe neuronal firing has recently been implicated as a "final common pathway" for the anxiolytic actions of benzodiazepines and the two novel anxiolytics, buspirone and ipsapirone (Peroutka, 1985). Benzodiazepines are presumed to assert their effects via the benzodiazepine/GABA receptor complex where they potentiate the effect of the inhibitory neurotransmitter, GABA. In addition to their anxiolytic properties, benzodiazepines also produce many undesirable side effects such as sedation, incoordination, and addictive potential (Rickels, 1983). Clinically, buspirone is equipotent to diazepam in relieving anxiety but has only slight effects on levels of alertness, coordination or memory (Goldberg and Finnerty 1979; Moskowitz and Smiley 1982; Schuckit 1984). The benzodiazepine/GABA receptor complex is present not only on the dorsal raphe nuclei (Gallager and Aghajanian, 1976), but is ubiquitous in the central nervous system. By contrast, 5-HT_{1A} receptors are concentrated on the raphe nuclei and hippocampus. Importantly, benzodiazepine effects on dorsal raphe neurons mimic the action of buspirone. Therefore, the specific anxiolytic actions of these drugs could be mediated by their effects on raphe cell firing, while the side effects of benzodiazepines may be due to their widespread actions in other parts of the brain.

Effects on Hippocampal Neurons

The effects of 5-HT have also been studied at the cellular level in hippocampus. The high density of 5-HT_{1A} receptors in the dentate and CA1 region and the paucity of 5-HT_{1B} and 5-HT_2 sites (Pazos and Palacios, 1985) makes the hippocampus an excellent region in which to study the neurophysiological effects of selective 5-HT_{1A}

agents. After stimulation of Schaffer collaterals in the stratum radiatum, field potentials can be recorded in the pyramidal cell layer of CA1, The effects of 5-HT and related agents on the population spike can then be determined. Bath application of the selective 5-HT_{1A} putative agonists 8-OH-DPAT, buspirone or ipsapirone decreases the elicited field potential after 5 minutes. However, 5-HT causes an increase in the amplitude of the population spike within the first 5 minutes after bath application (Peroutka et al., 1987). The effect of 5-HT on CA1 field potentials correlates with its hyperpolarizing effect (15 - 20 mV) on CA1 pyramidal cell membranes (Segal, 1980; Andrade and Nicoll, 1985). In contrast, buspirone causes only a 1 - 2 mV hyperpolarization of CA1 pyramidal neurons (Andrade and Nicoll, 1985). The above results suggest that the effects of 5-HT and 5-HT_{1A} selective agonists on the CA1 population spike are not mediated by the same receptor population. Studies are in progress to more clearly determine the receptors(s) which mediate the neurophysiological effects of 5-HT_{1A} drugs in the hippocampus (Mauk et al., 1987; Hiner et al., unpublished observations).

Vascular Effects of 5-HT

The 5-HT_{1A} receptor, along with the 5-HT_2 receptor, has also been implicated in vascular smooth muscle contractions. Although the effect of 5-HT on smooth muscle varies greatly depending on the vasculature being studied, the effects observed consistently fall into two categories. Blood vessels which display a K_{ed50} greater than 100 nM for 5-HT are competitively inhibited by the classical antagonists. This response to 5-HT appears to be mediated by the 5-HT_2 receptor (Peroutka, 1984).

By contrast, nanomolar concentrations of 5-HT contract vessels such as the human basilar artery, human pial vessels, canine basilar artery and saphenous vein (Peroutka et al., 1983; Peroutka, 1984). Methysergide appears to act as a partial agonist in these vessels (Curro et al., 1978; Apperley et al., 1980; Muller-Schweinitzer, 1980; Forster and Whalley, 1982). Moreover, classical 5-HT antagonists and neuroleptics non-competitively inhibit contractions at micromolar concentrations. Taken together, these data indicate that certain vascular effects of 5-HT are mediated by the 5-HT_1 class of receptors. Furthermore, autoradiographic studies indicate that 5-HT_1, and not 5-HT_2, binding

sites are located on the tunica media of canine and human basilar arteries (Peroutka and Kuhar, 1984). Recent studies correlating drug affinities for 5-HT_{1A} binding sites with force rankings of agonist-induced contractions strongly suggest that the 5-HT_{1A} receptor mediates 5-HT-induced contractions of the canine basilar artery (Peroutka et al., 1986; Taylor et al., 1986).

The vasoactive effects of 5-HT have implicated this neurotransmitter in the pathogenesis of headache. Furthermore, 5-HT may alter pain sensation during a migraine attack, since 5-HT has been shown to lower pain thresholds when injected into inflammed tissue (Moskowitz, 1984). Methysergide, cyproheptadine, pizotifen and (-)propranolol are antimigraine drugs which inhibit radioligand binding to 5-HT_{1A} receptors (Hiner et al., 1986). The antimigraine effects of these drugs have been attributed to their ability to modulate 5-HT actions (Lance, 1981; Raskin, 1981; Fozard, 1982; Hiner et al., 1986).

Behavioral Studies

Central 5-HT stimulation with drugs such as 5-hydroxytryptophan (5-HTP) results in a complex behavioral response (Jacobs, 1976; Green, 1984). The syndrome includes resting tremor, forepaw treading, flattened body posture, head weaving, hindlimb abduction, Straub tail and head twitching. Studies have attempted to relate each of these components to the 5-HT_1 or 5-HT_2 binding sites. Head twitching appears to be mediated by the 5-HT_2 receptor since antagonism of this component of the syndrome correlates with blockade of the 5-HT_2 receptors (Peroutka et al., 1981; Leysen et al., 1982; Yap and Taylor, 1983; Colpaert and Janssen, 1983). Many components of the 5-HT behavioral syndrome may be related to 5-HT_1 binding sites since only the non-selective 5-HT antagonists, metergoline and methysergide, block the entire set of behavioral responses. Higher concentrations of 5-HT_2 antagonists are needed to block the forepaw treading, head-weaving, tremor, hindlimb abduction, flattened posture and Straub tail components of the 5-HT behavioral response (Lucki et al., 1984).

Recent correlations between specific components of the 5-HT behavioral syndrome and the 5-HT_{1A} receptor have been made possible by development of selective 5-HT_{1A} agents such as 8-OH-DPAT and 5-methoxydimethyltryptamine (5-MeODMT). Behavioral

studies with these agents indicate that forepaw treading and flattened body posture are correlates of the 5-HT$_{1A}$ receptor (Tricklebank et al., 1984, 1985; Tricklebank, 1985; Middlemiss et al., 1985). Additional studies (Smith and Peroutka, 1986) indicate that 8-OH-DPAT and MeODMT are "full agonists" with respect to six of the seven components of the response. In contrast, buspirone and ipsapirone act as antagonists in relation to forepaw treading, head-weaving and tremor.

Other Systems

The 5-HT$_{1A}$ receptor subtype has also been implicated in the mediation of many other physiological and behavioral effects. Studies in male rats suggest that 5-HT$_{1A}$ selective agonists facilitate seminal emissions and/or ejaculations (Kwong et al., 1986). In addition, the 5-HT$_{1A}$ site appears to mediate certain hypotensive effects of tryptamine derivatives (Kalkman et al., 1983; Doods et al., 1985). The 5-HT$_{1A}$ receptor has also been correlated with the thermoregulatory effects of 8-OH-DPAT and RU 24969 (Middlemiss et al., 1985; Tricklebank et al., 1986; Gudelsky et al., 1986).

5-HT$_{1B}$ RECEPTORS

Interestingly, the 5-HT$_{1B}$ site, as defined by radioligand binding studies, has only been identified in rat and mouse brain (Heuring et al., 1986; Hoyer et al., 1986a). Sills et al. (1984) defined 5-HT$_{1B}$ binding as specific ^{3}H-5-HT binding in rat brain observed in the presence of 1 mM GTP and 2000 nM spiperone. They concluded that RU 24969 and TFMPP were selective 5-HT$_{1B}$ agents. The 5-HT$_{1B}$ site has been more directly labeled in rat brain with ^{125}I-cyanopindolol (Pazos et al., 1985; Hoyer et al., 1985a; 1985b). The ^{125}I-cyanopindolol binding site has high affinity for 5-HT and RU 24969 and relatively low affinity for d-LSD and 8-OH-DPAT. The highest densities of 5-HT$_{1B}$ sites in rat brain are found in the globus pallidus, dorsal subiculum and substantia nigra (Pazos and Palacios, 1985). Recent data has demonstrated that this site can also be labeled with ^{3}H-5-HT in rat frontal cortex (Peroutka, 1986; Blurton and Wood, 1986). The 5-HT$_{1B}$ site has been observed in rat and mouse brain but not in guinea pig, cow, chicken, turtle, frog or human brain membranes (Heuring et al., 1986; Hoyer et al., 1986a).

Autoreceptors

To date, functional correlates of the 5-HT$_{1B}$ site have been limited to studies of the serotonin "autoreceptor". Briefly, 5-HT autoreceptors are studied in synaptosomal or slice preparations in which depolarization-evoked release of stored ^{3}H-5-HT is measured by superperfusion techniques. The release of ^{3}H-5-HT can be inhibited by 5-HT and related agonists, presumably through a presynaptic "autoreceptor". Engel et al. (1986) have convincingly demonstrated that in rat brain synaptosomes, the effects of 5-HT and other agents are mediated by the 5-HT$_{1B}$ receptor. No significant correlation was observed between drug potencies at the 5-HT "autoreceptor" and drug affinities for 5-HT$_{1C}$ or 5-HT$_2$ binding sites. However, a significant correlation was obtained between drug affinities for 5-HT$_{1B}$ sites and the rat 5-HT "autoreceptor". As a result, the 5-HT$_{1B}$ binding site appears to be the receptor which mediates release of 5-HT from nerve terminals in rat brain.

A similar conclusion was reached from an analysis of 5-HT and related drug effects on the release of ^{3}H-5-HT induced by depolarization of rat cerebellum synaptosomes (Raiteri et al., 1986). By contrast, the 5-HT heteroreceptor mediating release of endogenous glutamate induced by depolarization of rat cerebellum synaptosomes did not conform to the previously described pharmacological characteristics of 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$ and 5-HT$_2$ binding sites.

5-HT$_{1C}$ RECEPTORS

The 5-HT$_{1C}$ site was first discovered in autoradiographic analysis of ^{3}H-5-HT binding. Pazos et al. (1984) noted that ^{3}H-5-HT densely labeled choroid plexus membranes. Simultaneously, Yagaloff and Hartig (1985) analyzed ^{125}I-LSD binding and also noted an extremely dense labeling of the choroid plexus. Pharmacologically, both radioligands labeled a similar site which has been designated the 5-HT$_{1C}$ receptor. This site has high affinity for certain 5-HT$_2$ antagonists such as mesulergine and mianserin and a lower affinity for spiperone.

Phosphatidylinositol Hydrolysis

Recent work of Sanders-Bush and colleagues

(Conn et al., 1986) has convincingly demonstrated that the 5-HT_{1C} receptor is linked to the phosphatidylinositol second messenger system. Phosphatidylinositol is a membrane phospholipid that is cleaved when receptors which are linked to this system are activated. The cleavage products (inositol with a varying number of phosphates and diacylglycerol) then activate other biochemical pathways in the cell. For example, inositol phosphates are believed to trigger the release of calcium from the endoplasmic reticulum while diacylglycerol activates protein kinase C, a molecule which mediates numerous cellular functions. A third messenger, arachidonic acid, is released as a product of diacylglycerol, leading to an increased production of prostaglandins, thromboxanes and leukotrienes.

5-HT has been shown to cause a 6-fold increase in phosphatidylinositol hydrolysis in rat choroid plexus (Conn et al., 1986). The rank order potencies of antagonists are consistent with antagonist affinities for the 5-HT_{1C} receptor. For example, mianserin and ketanserin were more potent antagonists of phosphatidylinositol hydrolysis than spiperone. By contrast, PI studies in rat cerebral cortex are most consistent with 5-HT effects being mediated by 5-HT_2 receptors. Ketanserin and spiperone are equipotent antagonists of 5-HT-mediated cortical PI hydrolysis (Kendall and Nahorski, 1985). Binding affinities of these antagonists at the 5-HT_2 receptor correlate with these results. These data suggest that both the 5-HT_{1C} and 5-HT_2 receptor are linked to the phosphatidylinositol second messenger system.

5-HT_{1D} RECEPTORS

Most recently, a fourth subtype of the 5-HT_1 class of 5-HT receptors has been identified. Using bovine brain (which lacks 5-HT_{1B} sites), Heuring and Peroutka (1987) analyzed ^{3}H-5-HT binding in the presence of 100 nM 8-OH-DPAT and 100 nM mesulergine to block the binding of ^{3}H-5-HT to 5-HT_{1A} and 5-HT_{1C} sites. Under this condition, a large amount of residual ^{3}H-5-HT binding could be identified which had a unique pharmacological profile. Scatchard analysis revealed that the B_{max} of ^{3}H-5-HT binding was reduced by 10 - 15% without affecting the K_D value (1.8 $\pm$ 0.3 nM).

5-HT_{1D} sites are extremely dense in the basal ganglia but are present in all brain regions including the hippocampus and choroid plexus.

5-HT and 5-CT are the most potent inhibitors of ^{3}H-5-HT binding to bovine 5-HT_{1D} sites. All other 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 agents are essentially inactive at this radioligand binding site. The only pharmacologic agent which is disproportionately potent at this site is yohimbine (K_i = 59 nM).

There have been no direct correlations between this binding site and physiological effects of 5-HT. However, it has been suggested that the effects of 5-HT on the isolated perfused rat kidney preparation (Charlton et al., 1986) and the rat fundus strip (Leysen and Tollenaere, 1982; Clineschmidt et al., 1985; Cohen and Wittenauer, 1986) may share pharmacological similarities with this radioligand binding site (Heuring and Peroutka, 1987).

FUTURE DIRECTIONS

The identification and characterization of multiple 5-HT receptors has largely been based on radioligand binding data. However, these studies are limited in many ways. For example, radioligand studies cannot differentiate agonist versus antagonist effects of drugs. Secondly, the functional relevance of radioligand sites can also be difficult to determine especially if the radioligand labels a heterogeneous population of sites.

Therefore, future progress in the analysis of 5-HT receptor subtypes will depend upon the identification of homogeneous subtypes of 5-HT receptors. Alternatively, radioligands which label multiple binding site subtypes could be useful if regions of the brain are selected which contain only a single population of subtypes. Once homogeneous binding site subtypes are characterized, attempts must be made to correlate the data with second messenger systems such as adenylate cyclase or phosphatidylinositol hydrolysis. Such correlations would allow for the determination of agonist versus antagonist properties of drugs at the receptor. More detailed characterization of all 5-HT receptor subtypes should elucidate the role of 5-HT in neuropsychiatric disorders such as depression and anxiety.

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SUBJECT INDEX