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SUBTYPES OF RECEPTORS FOR SEROTONIN

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INTRODUCTION

Interest in the physiological functions of 5-hydroxytryptamine (5-HT; *serotonin*) has increased steadily since its discovery in the intestine (1) and in serum (2). Its identification in brain in the early 1950s (3), followed a decade later by the initial studies of its distribution in brain by histofluorescence (4), stimulated investigations of the possible behavioral functions of this indolealkylamine. In addition to studies of its function in brain, a steady stream of investigations have described its possible functions in the periphery, particularly in the gastrointestinal tract (see 5) and, more recently, in the cardiovascular system (see 6). Indeed, the initial suggestion that there might be more than one type of receptor for 5-HT came from experiments on the isolated guinea pig ileum. Gaddum & Picarelli (7) demonstrated that only a portion of its contractile response to 5-HT could be blocked by high concentrations of morphine but that the remainder of the response could be

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antagonized by low concentrations of dibenzyline (phenoxybenzamine). Similarly, when maximally effective concentrations of dibenzyline were present, the remaining contractile response elicited by 5-HT was blocked by low concentrations of morphine. They speculated that this result occurred because there were two different receptors for 5-HT in the ileum, termed D (blocked by dibenzyline) and M (blocked by morphine) receptors. The D receptor was thought to be on the smooth muscle of the ileum whereas the M receptors were considered to be on nerve ganglia or nerves within the muscle.

In the 1970s, the development of radioligand binding methods furthered our understanding of subtypes of receptors for 5-HT. More recently, the application of the tools of molecular biology to the study of serotonin receptors has given even greater impetus to our understanding of the subtypes of receptors for this indolealkylamine. Not surprisingly, therefore, an explosive growth of research reports has occurred on 5-HT in general and its receptors in particular (see 8). Consequently, this chapter is not intended to be encyclopedic, but rather focuses on selected issues that we think will promote our understanding of the receptors for 5-HT and their links to signal transduction processes and second messengers. In particular, we review current classification schemes for subtypes of receptors for 5-HT, and we discuss possible ways that results from studies of signal transducing systems and cloning of receptors might be incorporated into classification schemes. Also, we detail the approaches used to clone several subtypes of 5-HT receptors. Linkages of receptor subtypes with second-messenger systems are reviewed, as well as certain functional responses mediated by the subtypes of receptors for 5-HT. More comprehensive reviews of many aspects of 5-HT receptor function may be found in recent reviews or books on this subject (9–12). The molecular structures and chemical names of many of the drugs mentioned throughout this review can be found in the Appendix of *The Serotonin Receptors* (10).

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SUBTYPES OF RECEPTORS FOR 5-HT

Ligand Binding Experiments

As we are reviewing a type of receptor, it makes sense to start by defining the term "receptor." Erlich first used the term to describe a group of atoms (haptophore) in a cell that combines or unites with a similar group of atoms in toxins or other substances. For neurotransmitter receptors, functions they subserve have not only been emphasized in their definition but also have been used historically to classify them. We agree with Leff & Martin (13) that Stephenson's definition for neurotransmitter receptors (14) is a good one: "that small spatial arrangement of atoms to which a substance endogenous to the organism attaches itself as an essential step in modifying cellular function." This definition appropriately emphasizes endogenous compounds and

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their physiological function. Stephenson (14) argued that the receptor is only the binding site for an endogenous substance. More recent results from molecular biological and other approaches have isolated the proteins that comprise particular subtypes of receptors (see below). Although "binding sites" for endogenous agonists have tentatively been identified on these proteins (see 15), different parts of the receptor clearly take part in the spatial arrangement of the binding or recognition site. We, therefore, would modify the Stephenson (14) definition by interpreting "small" to mean atoms of the entire protein that constitutes the receptor and not just that portion of the protein to which the endogenous ligand binds. Binding sites (or "acceptor" or "recognition" sites) for 5-HT have been identified using radioligand binding methods. These binding sites may have been termed "receptors" for 5-HT; indeed, many have subsequently been shown to mediate functional responses elicited by the indolealkylamine. In this review,[1] though, we refer to such binding sites as "sites related to 5-HT receptors" to distinguish them from membrane proteins that modify cellular function when activated by 5-HT (i.e. 5-HT receptors).

Early work used radioligand binding methodology to study sites related to 5-HT receptors. The first use of such methodology to provide reasonable evidence for the labeling of the site related to a receptor for 5-HT was that of Farrow & Van Vunakis (17, 18) who used ^{3}H-LSD and an equilibrium dialysis technique to detect specific binding. They found high-affinity, stereospecific binding of this radioligand in rat cortex that was more potently inhibited by 5-HT than by other known neurotransmitters. The development of ^{3}H-5-HT as a radioligand with relatively high specific activity led to its use in further studies of sites related to serotonin receptors. Bennett & Snyder (19) were the first to demonstrate high-affinity (K_D = 7nM), saturable binding of ^{3}H-5-HT to membranes prepared from rat brain. Nelson and his colleagues (20) later demonstrated that preincubation of the tissue at 37°C for 10 minutes removes endogenous serotonin, with a resultant lowering of the K_D to 2nM without affecting the Bmax.

Several years later, another radioligand was found to label sites related to serotonin receptors; Leysen and her associates (21) observed that ^{3}H-spiroperidol, but not ^{3}H-haloperidol, labeled sites related to serotonin receptors in the frontal cortex of the rat. In a key experiment reported in 1979, Peroutka & Snyder (22) examined the binding of three radioligands, ^{3}H-5-

[1]In this review, the following terms and definitions relating to potencies of drugs in either binding or functional assays are used: (*a*) K_D, the apparent dissociation constant obtained for a radioligand in a binding assay; (*b*) K_i, the apparent dissociation constant obtained by inhibiting competitively the binding of a radioligand after correction for the concentration of the radioligand (16); (*c*) EC_{50}, the concentration of an agonist that causes 50% of the maximal response elicited by the agonist in a functional assay; (*d*) K_b, the dissociation constant calculated for a competitive antagonist in a functional assay.

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HT, ^{3}H-spiroperidol, and ^{3}H-LSD, to membranes of the frontal cortex of the rat. The assumptions underlying their experiment were that if all three radioligands bind to the same population of receptors, then (*a*) the maximum density of binding sites (Bmax) for all three radioligands should be the same; (*b*) 5-HT, spiroperidol and LSD should exhibit monophasic displacement curves in competing for all three radioligands; and (*c*) the affinity of, for example, ^{3}H-5HT for the binding site, as measured by its K_D, should be equivalent to its affinity for the binding site as determined by its K_i for the inhibition of either ^{3}H-spiroperidol or ^{3}H-LSD binding. This was not found. Furthermore, competition experiments showed that tryptamine agonists competed for the binding of ^{3}H-5-HT with high affinity whereas 5-HT antagonists and neuroleptics exhibited low affinity. By contrast, when the binding of ^{3}H-spiroperidol was studied, antagonists and neuroleptics demonstrated high affinity whereas tryptamine agonists exhibited low affinity. All compounds showed an intermediate affinity for the binding site labeled with ^{3}H-LSD. The maximum density of binding sites labeled with ^{3}H-LSD was equivalent to the sum of the Bmax values with ^{3}H-5-HT and ^{3}H-spiroperidol. Based on these and other findings, Peroutka & Snyder (22) suggested that ^{3}H-5-HT and ^{3}H-spiroperidol label two distinct, noninterconvertible classes of receptor sites, designated 5-HT_1 and 5-HT_2 receptors, respectively. They suggested that ^{3}H-LSD labels both classes of receptor with similar affinities. The designation of the ^{3}H-spiroperidol binding site as a 5-HT receptor was based primarily on the affinity of 5-HT for this site in the frontal cortex, which was about 30 times greater than that of DA, the other neurotransmitter known to have high affinity for ^{3}H-spiperidol binding sites. As is discussed below, evidence has been provided that the D receptor described by Gaddum & Picarelli (7) and the 5-HT_2 receptor are indistinguishable pharmacologically.

Interestingly, in their report, Peroutka & Snyder (22) provided the initial evidence that what they termed the 5-HT_1 receptor might be heterogeneous. They showed that the inhibition of ^{3}H-5-HT binding caused by spiroperidol (hereafter termed spiperone—SPIP) was complex and not monophasic in nature. Although they mentioned that the Hill coefficient of the spiperone competition curve was less than unity (0.64), not until two years later did Pedigo et al (23) repeat this experiment using the frontal cortex and corpus striatum of the rat; they interpreted their similar results as favoring the existence of two populations of binding sites for ^{3}H-5-HT. That component of binding for which spiperone showed high affinity (about 10 nM) was termed the 5-HT_{1A} subtype, whereas the component for which spiperone displayed low affinity (about 4μM) was called the 5-HT_{1B} subtype. Even though shallow displacement curves can be due to factors other than multiple types of receptors (see 24), many other types of experiments (see below) apparently have validated the interpretation of Pedigo et al (23).

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Additional evidence indicates more than two binding sites related to 5-HT receptors in the 5-HT_1 subclass. In autoradiographic studies with ^{3}H-5-HT, Pazos et al (25) found a high density of binding sites in the choroid plexus that did not show the pharmacological characteristics expected of the 5-HT_{1A} or 5-HT_{1B} binding site, or the 5-HT_2 binding site for that matter. They termed this binding site the 5-HT_{1C} subtype. This result has been confirmed by others (26).

A fourth binding site for ^{3}H-5-HT that may be linked to 5-HT receptors has been identified recently. In bovine brain, which does not contain the 5-HT_{1B} subtype (27), "specific" binding of ^{3}H-5-HT remained in the presence of concentrations of drugs that should inhibit over 90% of the binding of the radioligand to binding sites related to the 5-HT_{1A} and 5-HT_{1C} subtypes (28). The pharmacological characteristics of this "left-over" binding did not correspond to that of the known subtypes of 5-HT receptors. This recognition site for ^{3}H-5-HT was called the 5-HT_{1D} binding site. Similar results were obtained by others using brain membrane preparations from other species, including humans (29, 30). This site shows high affinity for tryptamine analogs such as 5-HT, 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeOT); ergoline derivatives such as metergoline, dihydroergotamine, and methysergide; as well as α_2 adrenoceptor antagonists such as yohimbine and its isomer rauwolscine. Selective 5-HT_{1A} agonists such as DPAT, buspirone, and ipsapirone have low affinity for this binding site as does the 5-HT_2 antagonist ketanserine and drugs with high affinity for 5-HT_{1B} sites [(−)propranolol and (−) pindolol]. In addition, drugs such as mianserin and mesulergine, which have high affinity for sites linked to the 5-HT_{1C} receptor subtype, exhibit low affinity for this binding site, as does MDL 72222, a selective antagonist of 5-HT_3 receptors (previously M receptors; see below). In some areas of brain such as the caudate nucleus and substantia nigra, the 5-HT_{1D} subtype appears to be the predominant 5-HT_1 receptor (30). The 5-HT_{1B} subtype is either not present or is barely detectable in the central nervous system (CNS) of those species in which the 5-HT_{1D} subtype occurs (28–30). It has been hypothesized that in such species (e.g. calf, guinea pig, pig, human) the 5-HT_{1D} subtype subserves functions mediated by the 5-HT_{1B} subtype in other species, such as being the serotonergic terminal field autoreceptor (31). Recently, the 5-HT_{1D} subtype has been shown to be negatively coupled to adenylyl cyclase (32), providing further evidence that this binding site may function as a receptor in that its activation triggers a second-messenger response.

The M receptor, originally described in nerves of guinea pig ileum, is pharmacologically distinct from all the binding sites associated with 5-HT receptors just described, and has been renamed the 5-HT_3 receptor (33). Radioligands used to study all these binding sites as well as others associated with 5-HT receptors are presented in Table 1.

Table 1 Ligands for subtypes of 5-HT receptors

Receptor	Ligand	Comments	References
5-HT_1	^{3}H-LSD	Partial agonist; also labels the 5-HT_2 receptor	17, 18, 22
	^{3}H-5-HT	Agonist; labels all 5-HT_1 subtypes (by definition)	19
5-HT_{1A}	^{3}H-5-HT	Agonist; in presence of drugs to prevent its binding to other subtypes of the 5-HT_1 receptor	23
	^{3}H-8-OH-DPAT	Agonist	34
	^{3}H-ipsapirone	Agonist	35
	^{3}H-WB 4101	Partial agonist; in presence of prazosin to block its binding α_1 adrenoceptors	36
	^{3}H-PAPP	Agonist	37
	^{3}H-spiroxatrine	Partial agonist	38
5-HT_{1B}	^{3}H-5-HT	Agonist; in presence of drugs to block its binding to other subtypes of the 5-HT_1 receptor	23
	^{125}I-ICYP	Antagonist; in presence of isoproterenol to block its binding to β-adrenoceptors	39, 40
	^{3}H-dihydroergotamine	Partial agonist (?); in presence of phentolamine to block its binding to α adrenoceptors and DPAT to block its binding to the 5-HT_{1A} subtype	41
5-HT_{1C}	^{3}H-5-HT	Agonist; in presence of drugs to block its binding to other subtypes of the 5-HT_1 receptor	42
	^{3}H-mesulergine	Antagonist; also labels the 5-HT_2 receptor	25
	^{125}I-LSD	Partial agonist; also labels the 5-HT_2 receptor	26
5-HT_{1D}	^{3}H-5-HT	Agonist; in presence of drugs to block its binding to other subtypes of receptors for 5-HT	28
5-HT_{1P}	^{3}H-5-OHIP	Agonist	43
5-HT_2	^{3}H-LSD	Partial agonist; also labels the 5-HT_1 receptor	19, 22
	^{3}H-spiperone	Antagonist; also labels D2 dopamine receptors and α_1 adrenoceptors	21
	^{3}H-ketanserin	Antagonist;	44
	^{3}H-mesulergine	Antagonist; also labels the 5-HT_{1C} receptor	45

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Table 1 (*Continued*)

Receptor	Ligand	Comments	References
	^{125}I-LSD	Partial agonist; selectivity for the 5-HT_2 over the 5-HT_1 receptor	46, 47
	^{125}I-MIL	Antagonist (?)	48
	^{3}H-DOB	Partial agonist;	49
	^{3}H-DOI	Agonist; 50	
5-HT_3	^{3}H-GR65630	Antagonist	51
	^{3}H-ICS 205-930	Antagonist	52
	^{3}H-quaternised ICS 205-930	Antagonist	53
	^{3}H-zacopride	Antagonist	54
	^{3}H-quipazine	Antagonist	55

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For the sake of completeness, two additional recently described binding sites for ^{3}H-5-HT should be mentioned. Leonhardt et al (56) reported the presence of a binding site for ^{3}H-5-HT in human cortical tissue that persisted in the presence of pindolol and mesulergine (which should block the binding of the radioligand to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-T_{1C} and 5-HT_2 receptors). The remaining binding could be divided into two components, one with the pharmacological characteristics of the 5-HT_{1D} subtype, the other, by contrast, with low affinity for 5-CT, but high affinity for ^{3}H-5-HT (K_D, about 5nM) and inhibited by guanyl nucleotides. This binding site has been termed the 5-HT_{1E} subtype. Similarly, Xiong & Nelson (57) recently found a binding site for ^{3}H-5-HT in homogenates of rabbit caudate nucleus that had a pharmacological profile different from the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_2 subtypes. Although this binding site exhibited some similarities to the 5-HT_{1D} subtype, it differed significantly from this subtype in its quantitative affinity for certain serotonergic antagonists and its modulation by guanyl nucleotides and calcium ion. This binding site has been termed the 5-HT_{1R} site.

As noted, it is premature to characterize as a receptor a binding site defined in this manner. Nevertheless, essentially all the original speculations about serotonergic receptor subtypes generated from radioligand-binding experiments appear to have been substantiated by many other types of experiments, including the cloning of several subtypes.

Confounding Variables in the Assessment of Ligand Binding Data

Prior to the advent of ligand-binding methodology, receptors were classified in large measure by the use of drugs and by functional responses. This is important since there clearly can be problems in attaching too much im-

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portance to *drug specificity of binding sites* as a major criterion for receptor classification. This is especially true as agonists have been used to label the 5-HT_1 receptor, whereas antagonists historically were used for the 5-HT_2 receptor. As both receptor types have been linked to second-messenger systems associated with guanine nucleotide-binding proteins (G proteins; see below), most likely multiple-affinity states of each receptor exist (see 58) and labeled agonists and labeled antagonists bind to different species of the same receptor. Consistent with this view are the observations that guanyl nucleotides decrease the binding of ^{3}H-5-HT to the 5-HT_1 receptor (59, 60) as well as that of agonist radioligands to the 5-HT_{1A} subtype (61–63), the 5-HT_{1B} and 5-HT_{1C} subtypes (64), and the 5-HT_{1D} subtype (28, 65), but not that of a selective antagonist radioligand for the 5-HT_{1B} subtype (40). Also, guanyl nucleotides decrease the apparent affinity of agonists for the 5-HT_1 receptor (60) or the 5-HT_{1B} subtype (41). Furthermore, when the agonist ^{3}H-DPAT was used to label the 5-HT_{1A} subtype, K_i values obtained for agonists in the absence of GTP were 10–100 times lower than the EC_{50} values of the same agonists in eliciting a 5-HT_{1A}-mediated response linked to a G protein, namely inhibition of adenylyl cyclase (66, 67). A similar result has been reported recently for the 5-HT_{1C} and 5-HT_{1D} subtypes as well (29, 68). It appears that multiple affinity states that probably involve formation of ternary complex are observed when competition experiments with agonists are carried out on the 5-HT_1 receptor.

The 5-HT_2 receptor may have multiple affinity states also. When antagonists such as ^{3}H-spiperone or ^{3}H-ketanserin have been used to label this receptor, GTP has little effect on their binding (59, 69). Whereas antagonists compete with ^{3}H-ketanserine in a monophasic manner, agonist competition curves are complex and cannot be described by a one-site model. Also, the presence of GTP affects agonist but not antagonist, competition curves (69). An important new tool for the study of 5-HT_2 receptors has been the development of agonist radioligands, specifically ^{3}H-DOB (49) and ^{125}I-DOI (50). When ^{3}H-DOB was used to label 5-HT_2 receptors in the rat cortex (49), several interesting observations were made: (*a*) although fewer binding sites exist for ^{3}H-DOB than for ^{3}H-ketanserin, there was an excellent correlation between the regional distribution of each in rat brain; (*b*) GTP potently inhibited the binding of ^{3}H-DOB in contrast to its lack of effect on the binding of ^{3}H-spiperone or ^{3}H-ketanserin; (*c*) despite high correlations between the potencies of either agonists or antagonists for inhibiting the binding of ^{3}H-DOB and ^{3}H-ketanserin, agonists were 10–400-fold more potent when the 5-HT_2 receptor was labeled with ^{3}H-DOB than with ^{3}H-ketanserin, but antagonists showed similar affinities (49, 70).

Note that this latter observation is similar to that made originally by Peroutka & Snyder (22) in which they postulated (correctly) the existence of

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subtypes of receptors for 5-HT. Thus, the observations of Lyon et al (49) may indicate either that subtypes of 5-HT_2 receptors exist or, just as likely, that ^{3}H-agonist binding to 5-HT_2 receptors reflects formation of ternary complex because it can be inhibited by guanyl nucleotides. This is currently a matter of controversy (71, 72). Thus, agonist inhibition of ^{3}H-agonist binding reflects primarily the ability of an agonist to promote formation of ternary complex. By contrast, agonist inhibition of ^{3}H-antagonist binding reflects not only the potency of the agonist to interact directly with the receptor alone but also its ability to form ternary complex. It follows then that agonist inhibition of ^{3}H-antagonist binding will give lower apparent potency values than those measured using ^{3}H-agonist (see 73). Thus, either the presence of subtypes of a receptor or multiple affinity states of that receptor can produce the same experimental result; consequently, the interpretation of such data obtained with agonists is problematic.

Despite alternative explanations for the effects of guanyl nucleotides on agonist potencies for ^{3}H-5-HT binding (74, 75), we think that the wealth of data linking subtypes of receptors for 5-HT with G proteins favors the view that the effects of GTP on agonist binding most likely is a reflection that these receptors can exist in multiple-affinity states. If so, this emphasizes the importance of the characteristics of the radioligand (i.e. agonist or antagonist) in the quantitative values obtained for agonist potencies and illustrates why the quantatitive use of such potencies may be problematic when classifying receptors, at least those linked to G proteins. If quantitative relationships are desired, drug affinities as measured by ligand binding should be compared with a functional measure of receptor activation. In 1979, if Peroutka & Snyder (22) had ^{3}H-DOB rather than ^{3}H-spiperone available to label the 5-HT_2 receptor, then they would have shown that 5-HT itself has essentially the same affinity for the 5-HT_1 and 5-HT_2 receptor, rather than a very low affinity for the 5-HT_2 subtype. Had this occurred, a decade of claims could have been avoided over whether a receptor belonged in the 5-HT_1 class primarily because it showed high affinity for 5-HT and other tryptamine agonists. Also, the claim would probably not have been made that the 5-HT_1 receptor was not a "physiological" receptor since, because of its apparent high affinity for 5-HT, it alone would be saturated with 5-HT at "physiological" concentrations of the neurotransmitter, whereas the 5-HT_2 receptor would not be (76).

MOLECULAR BIOLOGICAL STUDIES OF SEROTONIN RECEPTOR SUBTYPES

Information obtained from molecular biological studies of subtypes of 5-HT receptors may be incorporated into their classification. To understand the

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rationale behind these newer schemes of classification, this section reviews in some detail current information on the approaches used to clone and the structure of several of the subtypes of receptors for 5-HT.

The first report of the cloning of a cDNA encoding a serotonin receptor came from Lubbert et al (77). In this interesting study, mRNA isolated from choroid plexus tumor cells was injected into frog oocytes and produced a serotonin-stimulated chloride ion current. This electrophysiological assay was used as a screening tool, initially to identify specific sizes of mRNA responsible for the serotonin stimulation and later to screen the resultant clones of a cDNA library constructed from the mRNA. Choroid plexus tumor cells were chosen because of their high (6.6 pmol/mg) density of 5-HT_{1C} binding sites. A hybrid depletion technique was used to identify candidate clones. Thus, single-stranded antisense DNA (i.e. DNA complementary to the strand coding for protein) from pools of 20 cDNA clones isolated from the size-selected cDNA library was hybridized with RNA isolated from the choroid plexus tumor. The RNA was separated from the DNA and the RNA–DNA hybrids by density gradient centrifugation and was subsequently injected into oocytes to determine if the RNA encoding the serotonin receptor was depleted. One clone has been identified by this method and it apparently encodes at least part of the 5-HT_{1C} receptor. Since the entire sequence was not present in this clone, no sequence data were presented (77).

Julius et al (78) presented this information in a later manuscript in which they described a slightly different strategy for cloning the 5-HT_{1C} receptor. These investigators, starting with mRNA from choroid plexus, constructed a size-fractionated cDNA library using the serotonin-stimulated Cl^--current in frog oocytes described above to determine which fractions contained the mRNA encoding a serotonin receptor. The library was constructed in λ_{zap}, a vector that allows easy production of RNA transcripts. It was then divided into five parts and RNA transcripts prepared from each were injected into oocytes, which were tested for their sensitivity to serotonin. Positive fractions were further subdivided and retested. The process was repeated until a single clone was obtained which, when transcribed and injected into oocytes, conferred serotonin-stimulated Cl^--channel gating properties. The pharmacological profile of this receptor was consistent with its being a 5-HT_{1C} receptor in that 1 μM mianserin blocked the effects of 10 nM serotonin, whereas 1 μM spiperone was nearly ineffective. The nucleotide sequence and the deduced protein sequence indicate that this protein is, as expected, a member of the G-protein-coupled receptor family and is a single polypeptide of 460 amino acids with 7 hydrophobic, presumably membrane-spanning, regions. Using ^{35}S-labeled antisense RNA from this clone as a probe, in situ hybridization studies have revealed intense labeling of epithelial cells in rat choroid plexus as well as neuronal cell bodies in the lateral habenula. Northern blot analyses have indicated that 5-HT_{1C} or 5-HT_{1C}-like mRNA is produced in the

hypothalamus, hippocampus, pons-medulla, basal ganglia, spinal cord, and choroid plexus of the rat.

The cloning of the gene encoding the 5-HT_{1C} receptor subtype has enabled possible new functions of this receptor to be studied. Introducing this gene into fibroblasts (NIH3T3) and continuous activation of the expressed receptor by 5-HT resulted in foci formation (79); furthermore, injection of cells from transformed foci into mice resulted in tumor generation. Julius et al (79) proposed that the cascade of events elicited by activation of the 5-HT_{1C} subtype is consistent with the "multistage" hypothesis of carcinogenesis. This approach illustrates the power of the types of novel studies possible once genes for receptors have been cloned.

Using a completely different strategy, Kobilka et al (80) have isolated a cDNA clone that was shown later (81) to code for the 5-HT_{1A} receptor. They (80) noted that high-stringency (i.e. washing of labeled blots at high temperature and low salt concentration to minimize retention of the probe where a significant number of base mismatches exist) Southern blot analysis of human genomic DNA that had been cut with the endonuclease EcoR1, using a full-length β_2-adrenergic receptor cDNA probe, revealed only a single species of DNA that corresponded to the gene encoding the β_2-adrenergic receptor. On the other hand, when using lower stringency conditions that allowed the probe to remain bound to mRNA species that had a significant number of base-pair mismatches, another approximately 3.8kb band was found that hybridized with the probe. Thus, a size-selected (3–4.5 kb) genomic library was constructed and screened at low stringency with the β_2-adrenergic receptor cDNA probe. The isolated and sequenced clone has high homology to the β_2-adrenergic receptor cDNA and encodes a single polypeptide of 421 amino acids. Hydropathicity analysis (i.e. estimates of whether a series of amino acids are hydrophobic or hydrophilic) revealed seven hydrophobic regions postulated to be membrane-spanning regions. Thus, this clone, named G-21, has been suggested to code for a member of the G-protein-coupled receptor family. Initial attempts to determine which receptor this cDNA encodes were unsuccessful because no specific binding could be detected when the protein was expressed in Xenopus laevis oocytes, using ligands for β_1- or β_2-adrenergic receptors, as well as D_1- or D_2-dopamine receptors. Fargin et al (81) subsequently demonstrated that the G-21 clone does code for the 5-HT_{1A} receptor. These workers demonstrated that when the mRNA derived from G-21 was expressed in oocytes, high-affinity binding of ^{3}H-DPAT was present; furthermore, this binding was inhibited by appropriate concentrations of drugs that interact with 5-HT_{1A} receptors. The binding of ^{3}H-DPAT was sensitive to guanine nucleotides, and solubilized ^{3}H-DPAT binding sites were immunoprecipitated by an antiserum directed against peptide predicted to be present in the receptor.

Pritchett et al (82) have used a strategy somewhat similar to that of Kobilka

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et al (80) in isolating a clone encoding the 5-HT_2 receptor. Two oligonucleotides directed against the sequences of membrane spanning regions 2 and 3 of the 5-HT_{1C} receptor, regions expected to be highly conserved between receptor subtypes, were labeled and used to probe a cDNA library from rat forebrain, an area especially rich in 5-HT_2 receptors (see 83). Several clones have been isolated and sequenced. One such clone encodes a 449 amino acid polypeptide resembling, in hydropathicity profile and sequence homology, many of the G-protein-coupled receptors. In particular, this clone demonstrates high (51%) sequence identity to the 5-HT_{1C} receptor. When expressed in a mammalian cell line, these receptors display a pharmacological profile consistent with the 5-HT_2 assignment. Thus, ^{3}H-spiperone binds with high affinity (0.5nM) and this binding is potently inhibited by ketanserin and mianserin, but not by haloperidol, DPAT, or the 5-HT_3 receptor antagonist, MDL-72222. The expressed clone also has been shown to mediate serotonin stimulation of intracellular calcium levels as well as phosphoinositide hydrolysis, consistent with published data indicating that 5-HT_2 receptors mediate these responses (see below).

Clearly, recent progress in our knowledge of the primary structure of at least some subtypes of serotonin receptors has been considerable. This rate of data acquisition will likely continue and before long we may know the sequences of each subtype. This knowledge will solidify, for each subtype that is cloned, the concept that these molecules are indeed distinct proteins mediating specific second-messenger functions and having discrete pharmacological profiles.

SECOND-MESSENGER LINKAGES TO SUBTYPES OF 5-HT RECEPTORS

5-HT Receptors Linked to Adenylyl Cyclase Activity

Recently, several reviews on this topic have been published (84, 85) and a summary is presented here of these and more recent studies reported through June, 1989. This review is restricted to studies on mammals.

Three distinct 5-HT receptors linked to adenylyl cyclase activity in vertebrate brain have been positively classified to date by pharmacological methods: the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptor subtypes. By positive classification we mean that drugs selective for a particular subtype are available that have allowed the 5-HT-mediated response to be assigned conclusively to the 5-HT receptor subtype in question. In addition to these three receptor subtypes, other subtypes of receptors for 5-HT linked to adenylyl cyclase (e.g. 88) exist but cannot be classified positively with the use of our current drugs.

Three lines of evidence point to the crucial role played by G proteins in

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coupling 5-HT receptors to adenylyl cyclase activity: (*a*) Both stimulation and inhibition of adenylyl cyclase are dependent on the presence of GTP (see below); (*b*) GTP and its nonhydrolyzable analogs affect the binding of serotonergic agonists to these receptor recognition sites (see 89) and (*c*) pertussis toxin (PT)-sensitive G proteins participate in adenylyl cyclase responses mediated by activation of the 5-HT_{1A} and 5-HT_{1B} receptor subtypes (90–96). Because ADP-ribosylation by pertussis toxin involves different and distinct G proteins (Gi; 97) (Go; 98) and other protein(s) as well (99, 100), the type of G protcin which couples each of these 5-HT_1 receptor subtypes to adenylyl cyclase activity is not yet known.

5-HT Receptors Linked to Stimulation of Adenosine 3', 5'-Monophosphate (cyclic AMP) Formation

The reports by Kakiuchi & Rall (101, 102) demonstrating that serotonin can increase cyclic AMP levels in slices of rabbit cerebellum were the first indication that mammalian brain had a 5-HT receptor positively linked to adenylyl cyclase activity. Several years later, Von Hungen et al (103) described a 5-HT receptor in colliculi from newborn rats that was subsequently studied thoroughly by Enjalbert et al (104, 105) and Nelson et al (106, 107). However, because receptor classification has been dependent upon the development of selective drugs, the 5-HT receptors mediating these responses could not be classified positively. For positive classification, the response must also be sufficiently robust to allow detailed and accurate pharmacological studies. Starting in the early 1980s, Shenker et al (108–110) attempted to characterize the 5-HT receptors that stimulate adenylyl cyclase activity in membrane preparations from hippocampus of adult guinea pig. In this preparation, 5-HT stimulated adenylyl cyclase activity about twofold over basal values. One receptor was found with high affinity (R_H) for 5-HT and could be classified positively as the 5-HT_{1A} subtype; the other receptor had low affinity (R_L) for 5-HT (EC_{50} = 400 nM) and has not been classified positively. Classification of these receptors was aided by the newly available drug 5-carboxamidotryptamine (5-CT) as well as by spiperone. The high-affinity receptor (R_H) could be activated by 5-CT (EC_{50} = 4 nM) and such activation could be blocked in a competitive manner by spiperone (K_b = 25 nM), whereas the low-affinity receptor (R_L) was insensitive to spiperone and 5-CT exhibited much lower potency (EC_{50} = 2 μM) for it. Also, selective 5-HT_{1A} drugs such as DPAT inhibited cyclic AMP formation through the R_H receptor, whereas drugs selective for other 5-HT receptors (e.g. ketanserin, MDL 72222) were not active even at concentrations as high as 1 μM on the response mediated by the high-affinity receptor. In addition to this response in the guinea pig hippocampus, stimulation of cAMP formation in membrane preparations from adult rat hippocampus has been described (111) and posi-

tively classified as the 5-HT_{1A} subtype (112). However, the percent stimulation of basal adenylyl cyclase activity by serotonergic agonists (20–30%) renders the rat preparation difficult for pharmacological studies.

The reports of Shenker et al (108–110) on two 5-HT receptor subtypes positively linked to adenylyl cyclase activity in adult guinea pig hippocampus were confimred recently by Dumuis et al (113, 114); in addition, these investigators found and studied the R_L receptor subtype in primary culture cells from embryonic mouse colliculi. In this preparation, some serotonergic agonists can increase the conversion of ^{3}H-ATP to ^{3}H-cAMP from a basal conversion rate of 1% to above 3%. 5-MeOT and 5-HT had EC_{50} values of about 100 nM, whereas 5-CT (EC_{50} = 3 μM) and the N,N dimethyl analog of 5-MeOT (EC_{50} = 18 μM) were much less potent. A series of potent (though not necessarily selective) serotonergic agonists, such as DPAT, buspirone, ipsapirone, and RU 24969, as well as numerous serotonergic antagonists—such as spiperone, methiothepin, pindolol, mesulergine, ketanserin, MDL 72222, and cocaine—were inactive at inhibiting this response at concentrations as high as 1 μM. Only the 5-HT_3 antagonist, ICS 205–930, exhibited competitive antagonism, but it had a relatively low affinity (K_b = 1 μM), which exludes its classification as the 5-HT_3 receptor subtype (see 115). Certain benzamide derivatives acted as either full or partial agonists in this preparation and the potent effect of the full agonist BRL 24924 could be blocked by high concentrations of ICS 205-930 (116). On the basis of these data, Dumuis et al (116) considered the 5-HT receptor mediating this response to be a novel subtype and proposed to classify it as the 5-HT_4 subtype. Unfortunately, the lack of additional selective drugs does not really allow a positive classification of this receptor, but rather allows only a description of what it is not.

5-HT Receptors Linked to Inhibition of Cyclic AMP Formation

Initial reports on 5-HT-induced inhibition of basal levels of cyclic AMP in blood vessels (see 84) were followed by studies in which forskolin was used to stimulate adenylyl cyclase activity. In membrane preparations from brain regions (including the hippocampus) from guinea pigs and rats, forskolin-induced stimulation of adenylyl cyclase could be inhibited by low concentrations of 5-HT and certain serotonergic agonists, notably DPAT and 5-CT (86, 117). Spiperone (K_b = 24 nM) and methiothepin (K_b = 13nM) were potent, competitive antagonists of the inhibitory effect of 5-HT, which was classified as being mediated by the 5-HT_{1A} subtype on the basis of the action of these antagonists, as well as the high correlation of agonists for ^{3}H-DPAT binding (52, 61) with their EC_{50} values for inhibition of forskolin-stimulated adenylyl cyclase activity.

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Thus, the 5-HT_{1A} receptor subtype has been classified positively as being linked both to stimulation and to inhibition of adenylyl cyclase. This can occur even in the same tissue, such as rat hippocampus. It remains to be determined in what type of hippocampal cells these two opposite effects of 5-HT occur. Furthermore, Zgombick et al (95) recently provided evidence that the 5-HT_{1A} and the adenosine A_1 receptors in rat hippocampus share some elements of the same signal transduction system. These investigators found that treatment of rats in vivo with pertussis toxin caused a similar decrease in (*a*) the amount of outward current evoked by 5-CT or by adenosine under voltage clamp conditions in the same hippocampal pyramidal cells; and (*b*) the Emax values for the inhibition of forskolin-stimulated adenylate cyclase activity caused by either 5-CT or adenosine. It will be important to determine whether this phenomenon occurs in a homogeneous population of cells.

Dumuis et al (92) studied 5-HT receptor(s) negatively linked to adenylyl cyclase activity in hippocampal and cortical neurons in primary culture cells from mouse embryos. Quantitative differences in the affinities of serotonergic drugs were observed in the two types of neuronal cells. For example, DPAT acted as a low-potency partial agonist (EC_{50} = 277 nM) in the cortical cells but was a highly potent full agonist in the hippocampal cells (EC_{50} = 7 nM). WB 4101 and cyanopindolol were highly potent at the hippocampal receptor and were 30 times less potent on the cortical receptor; by contrast, propranolol was ten times more potent in cortical neurons than in hippocampal cells. Because of this Dumius et al (92) proposed that the 5-HT receptor that inhibits cyclic AMP production in hippocampal neurons is the typical 5-HT_{1A} subtype, whereas that found in embryonic cortical neurons exhibits a pharmacology similar to, but not identical with, that of the 5-HT_{1A} subtype. However, similar variations of drug affinity and efficacy across tissues have been reported in other systems, such as α-adrenoceptors in mammalian blood vessels (see 119), and should not necessarily be the sole reason for invoking additional subtypes of receptors.

Two reports on the positive classification of the 5-HT_{1B} receptor subtype linked to inhibition of adenylyl cyclase activity have been performed in cell culture; (*a*) in fibroblasts from chinese hamster lung (93); and (*b*) in oppossum kidney (OK) cells (96, 120). The first system has been reviewed (85) and the second system is discussed here. Murphy & Bylund (120) in their initial report concluded that a 5-HT_1-like receptor mediates the inhibition of parathyroid hormone-stimulated adenylyl cyclase activity in OK cells by oxymetazoline. In their second report, 5-HT and certain other serotonergic agonists were found to inhibit parathyroid hormone-stimulated adenylyl cyclase activity (96). Selective 5-HT_{1A} agonists (DPAT, buspirone and ipsapirone), 5-HT_2 drugs (ketanserin, spiperone, DOI), 5-HT_3 antagonists (ICS 205-930, MDL

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72222) and nonselective serotonergic drugs (DMT, cyproheptadine) were not active in this preparation, even at concentrations as high as 100 μM. The pharmacology of the receptor mediating the inhibitory response in the OK cells was determined by comparing the EC_{50} values of the drugs in the adenylyl cyclase assay with their K_i values in a radioligand-binding assay for the 5-HT_{1B} subtype. In the binding assay, ^{125}I-iodocyanopindolol (^{125}I-ICYP) was used as the radioligand (39, 40) and GTP was present. Its high specific activity enables an extensive characterization to be made of the sites and compared to the results of functional studies. These investigators found (*a*) most of the tested drugs were either full or partial serotonergic agonists, including the β-adrenergic antagonists propranolol and cyanopindolol, piperazine analogs (quipazine, TFMPP and mCPP), as well as methysergide and oxymetazoline; (*b*) a high correlation between the affinities of 12 of the 16 drugs in the binding (occupancy) and adenylyl cyclase (response) assays (r = 0. 88).

Comparative studies of drug affinity for a receptor subtype assessed by both functional (adenylyl cyclase) and occupancy (binding) assays, coupled with the effect of modulators (e.g. GTP), should provide valuable information on the initial steps of signal transduction. For example, most of the 19 drugs studied by Murphy & Bylund (96) in both the adenylyl cyclase and the binding assays exhibited EC_{50}/K_i ratios close to unity (K_i values were calculated by assuming a simple competitive behavior for an homogeneous population of sites). It seems likely that by using ^{125}I-ICYP, which retains high affinity in the presence of GTP (40), Murphy & Bylund (96) studied a state of the 5-HT_{1B} receptor that is the same state studied in the adenylyl cyclase assay. By contrast, comparison of the K_i values for drugs obtained in binding assays for the 5-HT_{1A} or 5-HT_{1D} subtypes (28, 61) with the EC_{50} values of the same drugs in the adenylyl cyclase assay (29, 86) reveal EC_{50}/K_i ratios of around 10 or more. However, the occupancy of the 5-HT_{1A} and the 5-HT_{1D} sites was studied with agonist radioligands (^{3}H-DPAT and ^{3}H-5-HT, respectively) in the absence of GTP analogs, which favors formation of a state of the receptor different from that present in the adenylyl cyclase assay where GTP is an absolute requirement. The possibility of multiple states of the 5-HT_{1A} and the 5-HT_{1B} subtypes was thoroughly tested by Sills et al (60) by studying the high-affinity binding of ^{3}H-5-HT. However, the lack at that time of high-affinity selective drugs and of functional assays for the 5-HT_{1A} and the 5-HT_{1B} receptors did not allow them to probe directly their multistate proposal. Nevertheless, subsequent data on the EC_{50} values of serotonergic agonists for inhibiting adenylyl cyclase (66, 86) reveal that such values are in much better quantitative agreement with the K_i values for these agonists for the 5-HT_{1A} receptor when measured in the presence of GTP (60) than with values found with GTP was not present (122).

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Because rat substantia nigra has been reported to contain spiperone-insensitive ^{3}H-5-HT binding sites, best described as the 5-HT_{1B} subtype (see 83), Bouhelal et al (87) studied the 5-HT receptor that mediates inhibition of forskolin-stimulated adenylyl cyclase activity in membrane preparations from this tissue to determine whether the 5-HT-mediated inhibition was linked to the 5-HT_{1B} subtype. This response was inhibited by serotonergic agonists with the following rank order of potency: RU 24969 > 5-HT > 5-CT > CGS 120 66B = TFMPP > tryptamine > DPAT >> ipsapirone; also, spiperone was unable to antagonize the inhibitory effect of the serotonergic agonists. Such data support the notion that this response is not mediated by the 5-HT_{1A} or 5-HT_{1D} subtypes. However, unlike the 5-HT_{1A} receptor, where very selective agonists and some potent antagonists are available for positive classification, the classification of the 5-HT receptor subtype linked to inhibition of adenylyl cyclase activity in this tissue cannot be assigned positively until more selective drugs for the 5-HT_{1B} subtype become available.

After initial reports by Peroutka (123) and Heuring & Peroutka (28) on binding sites associated with the 5-HT_{1D} receptor subtype, Waeber et al (124) characterized these sites in membrane preparations from humans, cows, and pig. The anatomical distribution of these sites and their pharmacological profile was similar in these species. The best source for this receptor is calf substantia nigra, where an extensive characterization of the 5-HT receptor subtype linked negatively to adenylyl cyclase has recently been reported (32). The rank order of EC_{50} values for a series of serotonergic agonists was: 5-CT > 5-HT > 5-MeOT > RU 24969 > N,N, dipropyl 5-CT > DPAT > buspirone > ipsapirone. Some other drugs, such as metergoline, methysergide, rauwolscine and cyanopindolol, known to act on 5-HT receptors (often as antagonists) were agonists on this response. Methiothepin, mianserin, and spiperone behaved as antagonists. This pharmacological profile is best described as the 5-HT_{1D} receptor subtype because the correlation coefficient between K_i values (occupancy) and EC_{50} or K_b values (functional studies) was 0.94. Still, the lack of selective antagonists for all three receptor subtypes that are negatively linked to adenylyl cyclase activity forces the classification to rest mostly on serotonergic agonists.

Physiological Role for 5-HT Receptors Linked to Adenylyl Cyclase Activity

Possible physiological roles of 5-HT–stimulated cyclic AMP formation in neurons are the phosphorylation of synapsin (protein I) in brain slices containing the facial motor nucleus (125) and the gil withdrawal reflex in aplysia (see 126; see also 84). Another example of a putative role for a 5-HT receptor subtype that mediates increases in cyclic AMP level has been described (88, 127, 128). A relaxation response of porcine vena cava to 5-HT and its analogs

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was compared to the ability of these drugs to increase cyclic AMP levels in this tissue. Rings of isolated blood vessels were precontracted with either PGF2α or with αmethyl-5-HT and the relaxation to cumulative concentrations of 5-HT was assessed. The relaxation response was very rapid as were the kinetics of cyclic AMP accumulation (127). Methysergide, methiothepin, and spiperone were potent, competitive antagonists of both effects; 5-CT was a more potent agonist than 5-HT on both responses (88, 128). Selective 5-HT_{1A} agonists were inactive at concentrations up to 1 μM as were drugs with high affinity for 5-HT_1 (RU 24969, cyanopindolol), 5-HT_2 (ketanserin), or 5-HT_3 (GR 38032 binding sites. It appears that this 5-HT_1-like receptor is unlike any of the 5-HT_1 receptor subtypes mentioned above. The relationship between relaxation of vascular smooth muscle and cyclic AMP formation may also apply to other smooth muscle, such as that in cat saphenous vein and guinea pig ileum (129, 130).

Some examples for tentative physiological roles for 5-HT receptors negatively linked to adenylyl cyclase activity have been discussed previously (see 84, 85). Another example is the autoreceptor for 5-HT in serotonergic terminal fields in mammalian brain. These receptors can be assayed by preloading ^{3}H-5-HT into brain slices and by measuring the inhibition produced by the test drug of either potassium ion- or electrical-evoked release of the labeled 5-HT. A good correlation was reported between the inhibition of forskolin-stimulated adenylyl cyclase activity in membranes from calf substantia nigra by serotonergic drugs (mediated by the 5-HT_{1D} subtype) and the potency of the same drugs to inhibit evoked ^{3}H-5-HT release in guinea pig ($r = 0.70$) and pig cortical slices ($r = 0.82$) (see 31). Because the 5-HT receptor mediating these responses in these species is best described as the 5-HT_{1D} subtype, and since a similar good correlation was reported between 5-HT_{1B}-binding sites and the serotonergic terminal field autoreceptor in rat brain (131), Hoyer & Middlemiss (31) proposed that the 5-HT_{1B} and the 5-HT_{1D} receptor subtypes that are negatively linked to adenylyl cyclase activity share similar functions in different species.

5-HT Receptors Linked to Phosphoinositide (PI) Hydrolysis

Two distinct 5-HT receptors linked to PI hydrolysis have been classified positively—the 5-HT_{1C} and the 5-HT_2 subtypes. This area has been reviewed recently (132) as has the general subject of receptors linked to PI hydrolysis through phospholipase C activity (133). This part of the review summarizes some selected studies reported through June, 1989.

The initial reports on methysergide-sensitive 5-HT receptor(s) linked to PI hydrolysis were done with strips of guinea pig ileum (134) and in blowfly salivary gland (135). Several years later, the availability of selective drugs enabled a positive identification of the 5-HT–mediated stimulation of phos-

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phoinositide hydrolysis to 5-HT_2 receptors in muscle pieces (136), smooth muscle cells (137, 138), human and rabbit platelets (139, 140), and in astrocytes from different regions of rat brain (141). The first report on positive classification of *neuronal* 5-HT_2 receptors linked to stimulation of PI hydrolysis (142) was followed by several studies from different groups on the second-messenger linkage of this receptor subtype (see 132). A substantial body of information regarding PI hydrolysis linked to the 5-HT_2 receptor has been gathered using cultured cerebellar granule cells from the rat (143), astrocytes (141), C_6 glioma cells (144), a rat mammary tumor cell line (145), platelets from several species (139, 146) and various smooth muscle cells (137, 138).

Cory et al, (145) who studied this response in a rat mammary tumor cell line (WRK1), produced some of the clearest evidence linking the 5-HT_2 receptor to stimulation of PI hydrolysis. In these intact cells, the kinetics of 5-HT–stimulated inositol phosphates was linear with time up to 20 minutes (in the presence of 20 mM LiCl) and the increase in PI hydrolysis by a maximal concentration of 5-HT was more than 400% higher than basal activity. Consequently, concentration-response curves to eight serotonergic agonists, in the absence and presence of antagonists, could be assayed accurately. Two tryptamine analogs, 5-HT and 5-MeOT, were full agonists, whereas the other tested drugs were partial agonists, all with a slope index of the concentration response curves greater than unity. For example, quipazine (EC_{50} = 1.23 μM; intrinsic activity = 0.44; slope index = 1.62) not only acted as a partial agonist but also as a competitive antagonist of the response to 5-HT. Ketanserin, spiperone, and methiothepin were all potent antagonists of the response elicited by 5-HT. However, the inhibition caused by ketanserin could not be fully surmounted by increasing concentrations of 5-HT, and only a long wash of the cells recovered the authentic E_{max} value to 5-HT. This phenomenon is likely a consequence of the slow dissociation rate of ketanserin, which is a reflection of its high affinity. In addition to working with the intact WKR1 cells, Cory et al (145) were the first to demonstrate that 5-HT_2 receptors are linked to PI hydrolysis in a cell-free system. In membrane preparations from these cells, 5-HT stimulated a GTP-dependent, ketanserin-sensitive hydrolysis of PI. In contrast to the robust effect of 5-HT in the intact cells, 5-HT-stimulated PI hydrolysis in the membrane preparations was meager (about 50%) and its concentration response curve was very shallow. If other membrane receptors exist on these cultured cells, which is quite likely, then these cells may provide an excellent model for studying the interactions between receptor systems.

The initial report linking the 5-HT_{1C} receptor subtype to stimulation of PI hydrolysis in rat choroid plexus was that of Conn & Sanders-Bush (147). Two reports linking PI hydrolysis to the 5-HT_{1C} receptor in freshly dissociated

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cells from pig choroid plexus have been published recently (68, 148). In the study of Hoyer et al (68), a pharmacological characterization of the 5-HT_{1C} receptor linked to PI hydrolysis in freshly dissociated cells from pig choroid plexus was done along with occupancy (binding) studies in membrane preparations from the same tissue using ^{3}H-mesulergine. In this study, several intriguing characteristics of the 5-HT_{1C} subtype were reported: (*a*) although it was a full agonist, 5-HT was not the most potent drug; two other full agonists, α-methyl-5-HT and 1-methyl-5-HT, along with a partial agonist, DOI, were more potent in eliciting the PI response than was 5-HT; (*b*) three drugs that exhibited partial agonist activity on this receptor—DOI, bufotenine, and 5-MeOT—have been reported to act as hallucinogenic drugs in humans (see 149). This finding confirms a report by Sanders-Bush et al (150) that other hallucinogenic drugs, such as DOM and d-LSD, are partial 5-HT_{1C} agonists; (*c*) three ergoline derivatives, metergoline, mesulergine, and LY 53857, as well as ritanserin, methiothepin, and mianserin were potent, competitive antagonists of the PI response.

Obviously, selective 5-HT_{1C} drugs remain to be developed; the close similarity between the pharmacological profie of the 5-HT_2 and the 5-HT_{1C} receptor subtypes that mediate PI hydrolysis can be accounted for by the reported homology of the cloned 5-HT_2 (82) and 5-HT_{1C} (78) receptors.

Similar to the combined occupancy and efficacy study of the 5-HT_{1B} receptor in OK cells (96), Hoyer et al (68) compared data collected in functional studies of the 5-HT_{1C} receptor in intact cells to the observations made from using radioligand binding to membrane preparations from the same cells. Nine of the tested drugs acted as 5-HT_{1C} antagonists, with similar K_i values in the functional and the occupancy studies. However, the average EC_{50}/K_i ratio was about 10 for the tested agonists. This ratio between affinity determined by occupancy and EC_{50} values assessed by functional assay resembles the data reported on the 5-HT_{1A} and the 5-HT_{1D} receptor subtypes (see above) and may indicate that multiple affinity states of the 5-HT_{1C} receptor exist also.

Several studies point to unsolved problems in this field. For example, it might be expected that pharmacological evidence for the presence of activated 5-HT_2 receptors should be accompanied by evidence of 5-HT_2 receptor-linked PI hydrolysis. However, Cohen & Wittenauer (151) tested the PI response to serotonin on five smooth muscle preparations—two blood vessels, uterus, rat stomach fundus, and guinea pig trachea—all containing 5-HT receptors that mediate contraction. A robust PI response to serotonin was found in the jugular vein and uterus and a moderate response in the rat aorta, but no response was measured in the rat stomach fundus or guinea pig trachea. Although the 5-HT receptor that mediates contraction in the stomach is not the 5-HT_2 subtype, the negative results with the guinea pig trachea are puzzling

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because in smooth muscle cells from dog trachea a good PI response was observed (152). Also, the contractile response to 5-HT in guinea pig trachea preparations is ketanserin-sensitive (153). Thus, the guinea pig trachea contains 5-HT_2 receptors that mediate contraction even though no PI response could be detected. A plausible explanation for this apparent discrepency may be the observed rapid desensitization of the 5-HT_2 receptor in the guinea pig trachea (t1/2 = 3 min; 154). The protocol used by Cohen & Wittenauer (151) for measuring the PI response calls for 90 minutes of incubation with the tested compound. Consequently, this long time of incubation may not reflect the actual initial receptor activation by 5-HT.

Another unresolved question is apparent from the work of Conn & Sanders-Bush (155); they assayed 5-HT receptor(s) that mediate PI hydrolysis in several regions of rat brain and compared the E_{max} values of this response to the density of 5-HT_2 receptors, measured using ^{3}H-ketanserin. In the cortex, the 5-HT receptor mediating PI hydrolysis could be classified as the 5-HT_2 subtype based, for example, on the rank order of antagonists in the functional and the occupancy assays. By contrast, in other brain areas, notably the hippocampus and the hypothalmus, a low density of ^{3}H-ketanserin binding sites was accompanied by a cortex-like PI response. Also, in these brain areas, the PI response to serotonin was only poorly antagonized by ketanserin. One possibility is that 5-HT receptors other than the 5-HT_2 subtype are linked to the PI hydrolysis response in these subcortical areas (156), but the available pharmacological data do not permit a positive receptor classification.

In summary, similar to what has been found with subtypes of receptors for other biogenic amines (e.g. cholinergic, dopaminergic, adrenergic), subtypes of receptors for 5-HT are linked to a variety of second-messenger systems. Whether one receptor subtype is linked exclusively to one second-messenger system or is able to link to multiple second-messenger systems is an important question for future research.

CLASSIFICATION SCHEMES AND FUNCTIONS OF SEROTONERGIC RECEPTOR SUBTYPES

Pharmacological Classification

Bardley et al (33) proposed three major types of receptors for serotonin: "5-HT_1-like," 5-HT_2, and 5-HT_3. The term "5-HT_1-like" was proposed for that heterogeneous group of receptors whose activation by 5-HT elicits functional responses that are: (*a*) potently antagonized by methiothepin and/or methysergide (two drugs that are not selective for the 5-HT_1 binding site but show high affinity for it); (*b*) not antagonized by compounds with high affinity and selectivity for other 5-HT receptor sites (such as ketanserin, a selective 5-HT_2 antagonist; or MDL 72222, ICS 205-930 and (−)cocaine-

selective antagonists of the 5-HT_3 receptor); (*c*) mimicked by 5-CT, a selective 5-HT_1 agonist, at concentrations equal to or less than that of serotonin.

As has been indicated, results from radioligand binding studies indicate at least four subtypes of 5-HT_1-like receptors (1A, 1B, 1C, and 1D). In general, activation of the 5-HT_1-like class of receptors produces the presynaptic inhibitory effects of 5-HT on neurons (131) and relaxation of smooth muscle (129), although it can produce contraction of smooth muscle too (157). More specifically, the somatodendritic autoreceptor for 5-HT in the cells of the dorsal raphe nucleus may be the 5-HT_{1A} subtype as these cells are inhibited potently and rather selectively by 5-HT_{1A} selective agonists (158–160). It is possible, though, that other types of serotonergic receptors function as the somatodendritic autoreceptor in addition to the 5-HT_{1A} subtype. Sprouse & Aghajanian (160) found that (−)propranolol was very effective in inhibiting the effects of DPAT or ipsapirone on the firing rate of raphe cells, but not that of 5-HT itself. Certain electrophysiological effects produced by 5-HT in serotonergic terminal field areas, particularly the CA_1 region of the hippocampus, may be mediated through activation of the 5-HT_{1A} subtype (161–165). In particular, both in the hippocampus and in the raphe nucleus, 5-HT can activate a K^+ current so as to cause neuronal hyperpolarization (166) and this effect appears to be mediated by activation of the 5-HT_{1A} subtype (163–165). Furthermore, this electrophysiological consequence of 5-HT_{1A} receptor activation may involve a G protein as it can be blocked by the administration of pertussis toxin (which is presumably inactivating Gi by ADP-ribosylation) (167, 168).

Serotonin has been implicated as a transmitter that is involved in temperature regulation. It is interesting, therefore, that DPAT causes hypothermia (169, 170) that may be linked to its activating 5-HT1A receptors (171). Another unconditioned behavior that appears to be linked to activation of central 5-HT1A receptors is a complex behavioral syndrome, commonly termed the serotonin syndrome, that can be produced by administration of either indirectly-—or directly—acting serotonergic agonists. The syndrome consists of hindlimb abduction, forepaw treading, lateral headweaving, resting tremor, hindlimb rigidity, and Straub tail (172, 173). Evidence has been presented that this syndrome, or at least some components of it, are caused by activation of central 5-HT1A receptors (174–176).

In addition to these central effects of 5-HT_{1A} receptor activation, some peripheral effects of 5-HT seem to be mediated by this subtype of receptor. Evidence has been presented, for example, that the 5-HT-induced contraction of the canine basilar artery is due to 5-HT_{1A} receptor activation (177, 178). However, 5-HT_2 receptors also appear to be involved in the contractile effects of 5-HT_2 receptors also appear to be involved in the contractile effects of 5-HT on this tissue (179). Thus, even though definitive assignments of all of

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these responses to the 5-HT_{1A} subtype must await the development of a potent and selective antagonist of this receptor, a considerable body of data has accumulated from which it may be inferred that 5-HT_{1A} receptors do mediate certain functional responses to 5-HT.

Presently, the best-established function mediated by activation of the 5-HT_{1B} subtype is the inhibition of the neuronal release of 5-HT in serotonergic terminal fields, i.e. it functions as an "autoreceptor" in such areas. As with other neurotransmitters, there is evidence that presynaptic receptors on serotonergic terminals modulate the release of 5-HT (see 180, 181). Such autoreceptors may function physiologically to decrease the release of 5-HT, especially at high frequencies of serotonergic nerve-stimulation (182). Both in the rat brain and the canine saphenous vein, these terminal field autoreceptors are of the 5-HT_1-like class (183, 184). More recently, it was shown that methiothepin and (−)-propranolol, but not ketanserin, methysergide, mianserin or spiperone, blocked the inhibitory effect of 5-HT on depolarization-induced rclease of ^{3}H-5-HT from rat cerebellar synaptosomes and it was concluded that these autoreceptors are of the 5-HT_{1B} subtype (185). Perhaps even more convincing is the report of Engel et al (131) who showed a high correlation between the affinities of agonists or antagonists for 5-HT_{1A} and 5-HT_{1B} binding sites in rat cortex and their potency either to inhibit the electrically evoked release of 3H-5-HT from rat cortical slices (agonists) or to antagonize the inhibitory effect of 5-HT on such release (antagonists). No significant correlation was found between the affinities of these drugs at 5-HT1C or 5-HT2 binding sites and their affinities at the terminal field autoreceptor. Although a significant correlation was found between the affinity of the drugs at the 5-HT1A binding site and at the autoreceptor, several 5-HT1A selective drugs, such as DPAT, ipsapirone and spiperone, were not included in this correlation as their effects on the autoreceptor were sufficiently weak such that affinity values could not be determined. In view of this, it was concluded that the presynaptic 5-HT autoreceptor on serotonergic terminals belonged to the 5-HT1B subtype (131). Preliminary evidence has also been provided that the effect of serotonergic autoreceptors in the hippocampus may be mediated by their coupling with a G protein sensitive to pertussis toxin (186).

As mentioned previously, evidence has been provided that in the CNS of those species where the 5-HT_{1B} subtype does not seem to exist (e.g. rabbit, guinea-pig, pig, calf and human), the 5-HT_{1D} stubtype may function as the terminal field autoreceptor (31). It is interesting, then, that on serotonergic neurons the somatodendritic autoreceptor and the nerve terminal autoreceptor appear to be different subtypes of the same general class of serotonin receptor.

More recently, the 5-HT_{1B} subtype has been implicated in causing the mitogenic effect of 5-HT in fibroblasts maintained in culture through interac-

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tion with a G protein (93). It was speculated that growth factors other than 5-HT (e.g. thrombin, bradykinin) may also cause stimulation of DNA synthesis through a G protein–dependent mechanism.

In the CNS, the 5-HT_{1C} subtype is localized primarily to the choroid plexus. The choroid plexus functions as the major site of production of cerebrospinal fluid (CSF), serving not only to filter plasma but also to synthesize and secrete proteins found in CSF (187). One such protein is the iron carrier protein transferrin, which is the major constituent of the β-globulin fraction of CSF (see 188). The secretion of such proteins may be regulated by physiological agents and, very recently, 5-HT was shown to increase levels of transferrin in primary cultures of rat choroid plexus epithelial cells (148). The potency of 5-HT in causing this effect agrees well with its potency at the 5-HT_{1C} subtype, so that this may become the initial nonsecond-messenger response linked to activation of the 5-HT_{1C} subtype.

The initial suggestion that there were subtypes of receptors for 5-HT, termed D and M receptors, arose from observations made on the small intestine (7). As mentioned below, the D receptors have now been shown to belong to the 5-HT_2 class whereas M receptors have been reclassified as 5-HT_3 receptors. The D receptors are present on intestinal smooth muscle whereas the M receptors are on enteric nerves. In addition to these receptors, though, Gershon and his associates have shown in a series of investigations that the enteric nervous system has at least one additional receptor for 5-HT that does not belong to the M (5-HT_3) class of serotonergic receptor, or the 5-HT_2 class for that matter (43, 189–191). This receptor has high affinity for ^{3}H-5-HT and mediates a slow depolarization of a particular myenteric neuron (type II/AH cells) that is associated with an increase in input resistance. Effects of 5-HT at this receptor are not blocked by ICS 205-930, a selective and potent antagonist at 5-HT_3 receptors, but are specifically antagonized by either a dipeptide of 5-hydroxytryptophan (5-HTP-DP) or BRL 24924. This receptor has been termed a 5-HT_{1P} receptor as it has a high affinity for 5-HT and is a peripheral receptor. When this receptor was labeled with ^{3}H-5-hydroxyindalpine (^{3}H-5-OHIP), it was reported to be located in the lamina propria of the intestinal mucosa and the submucosal and myenteric plexuses. Specific binding of ^{3}H-5-OHIP was found also in the skin and heart, indicating that this type of peripheral receptor for 5-HT may be distributed widely in the periphery. It will be interesting to see what physiological effects of 5-HT are subserved by this type of receptor outside the enteric nervous system.

Since the affinity of various antagonists for the 5-HT_2 binding site correlates well with their affinities for D receptors measured in functional studies (192, 193), the D receptor was considered to be the 5-HT_2 receptor. Criteria suggested by Bradley et al (33) for classification of 5-HT_2 receptors were that responses elicited by their activation: (*a*) be potently antagonized by com-

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pounds with high affinity for the 5-HT_2 binding site, particularly ketanserin and methysergide; (*b*) not be potently inhibited by antagonists with selectivity for other types of receptors for 5-HT (e.g. MDL 72222, ICS 205-930). A selective agonist criterion should be also met for the 5-HT_2 categorization, but no selective agonists were available then. Now, however, drugs such as DOB or DOI (190) can be used to fulfill this criterion.

The development of potent and selective antagonists of the 5-HT_2 receptor, such as ketanserin (44), has facilitated greatly the assignment of certain effects mediated by 5-HT to its activation of the 5-HT_2 receptor. As selective 5-HT_2 agonists have become available (194), it will be important to ascertain their effects on the responses that have been attributed to 5-HT_2 receptor activation. As reviewed by Cohen (195), 5-HT contracts vascular smooth muscle in many species, including humans, by its activation, at least in part, of 5-HT_2 receptors. It seems clear, though, that other types of receptors for 5-HT also can mediate contractile effects of this indolealkylamine on vascular smooth muscle. In addition to its ability to contract directly vascular smooth muscle, 5-HT can also amplify the contractile effects of other vasoconstrictors (196) and it has been claimed that this effect, because it can be blocked by ketanserin, is mediated by 5-HT_2 receptors (197). Just how much these effects of 5-HT contribute normally to the maintence of vascular tone is still uncertain, as is the question of whether the hypotensive effect of ketanserin is due primarily to its blockade of 5-HT_2 receptors or its other pharmacological properties (e.g. it is also an α_1 adrenoceptor antagonist) (198). The hypotensive effect of ketanserin in hypertensive patients does not seem to be mimicked by ritanserin, a 5-HT_2 antagonist that does not block α_1 adrenoceptors (199).

Vascular permeability can be increased by 5-HT, perhaps due to its increasing endothelial cell gap width and microcirculatory blood flow (200). The increase in vascular permeability can be antagonized by blockade of 5-HT_2 receptors (200). Given this, it would be of interest to study the effects of 5-HT_2 antagonists in microcirculatory and cutaneous edema especially as Ortmann et al (201) found a high correlation between the ability of antagonists to block 5-HT-induced forepaw edema in the rat with their affinity for the 5-HT_2 receptor.

Serotonin has effects on platelet function, ranging from eliciting a reversible shape change to causing irreversible aggregation. Ketanserin blocks the 5-HT-induced aggregation (202), from which it was inferred that this effect is mediated by 5-HT_2 receptors. Such receptors exist on the platelets of several species (203, 204). Other data also support the idea that the effect of 5-HT on platelet function is mediated by 5-HT_2 receptors (see 205).

In the CNS, activation of 5-HT_2 receptors can cause head shakes or twitches in rodents (206; see 176). This pharmacologically mediated head

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shaking response occurs by a mechanism different from that elicited by mechanical stimulation of the aural pinnae (253). Certain electrophysiological effects of 5-HT are also mediated by 5-HT_2 receptors. In the prefrontal cortex of the rat, 5-HT elicits inhibitory responses; interestingly, such inhibition can be enhanced by the administration of ketanserin (208). 5-HT_2 and 5-HT_1 receptors may mediate opposite electrophysiological effects such that blockade of the 5-HT_2 receptor allows the effect of 5-HT on 5-HT_1 receptors to occur unimpeded. In the facial motor nucleus in the brainstem, 5-HT does not by itself induce firing of quiescent cells but does facilitate the excitatory effects of transmitters such as glutamate (209). This effect has been ascribed to activation of 5-HT_2 receptors (see 210). Similarly, activation of 5-HT_2 receptors facilitates the activation of locus coerulus cells by tactile stimulation (211). Consistent with these electrophysiological effects, it has recently been reported that the iontophoretic application of 5-HT onto locus coeruleus cells had no consistent effect on the spontaneous discharge of these cells but did consistently attenuate the excitatory effect of glutamate (212). The type of 5-HT receptor that mediates this effect is unclear although Bobker & Williams (213) presented some evidence that the ability of 5-HT to inhibit depolarizing synaptic potentials recorded from rat locus coeruleus neurons in a slice preparation was mediated by activation of both the 5-HT_{1A} and 5-HT_{1B} subtype.

The M receptor has no established binding site equivalent and, so as to follow the 5-HTx classification scheme, Bradley et al (33) renamed the M receptor as the 5-HT_3 receptor. The development of potent selective antagonists (see Table 1) and an agonist, 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) (115) have provided useful tools for the characterization of the 5-HT_3 receptor. Accordingly, Bradley et al (33) suggested that responses mediated by 5-HT_3 receptors: (*a*) be potently reduced by selective 5-HT_3 antagonists; (*b*) not be inhibited potently by selective antagonists of other types of receptors for 5-HT; and (*c*) be mimicked by 2-methyl 5-HT at concentrations comparable to that of 5-HT. It appears that there may even be subtypes of the 5-HT_3 receptor (115, 214).

The 5-HT_3 receptor initially appeared to be confined to peripheral neurons where they mediate depolarizing actions of 5-HT (see 215, 216). For example, activation of 5-HT_3 receptors in postganglionic autonomic neurons and the enteric nerves of the small intestine causes depolarization and release of neurotransmitter. Their activation in sensory neurons can produce neuronal depolarization as well as pain, wheal, and flare (see 216). Voltage-clamp studies have shown that the depolarization mediated by 5-HT_3 receptors is caused by a transient inward current (217); furthermore, these responses densensitize rapidly (217). The increase in membrane conductance due to 5-HT_3 receptor activation may result from the opening of a channel that

conducts monovalent cations nonselectively (see 218). The rapidity of the 5-HT_3-mediated electrophysiological response in NG 108-15 cells and cultured mouse hippocampal neurons caused Yakel & Jackson (219) to speculate that this receptor is directly coupled to an ion channel such that the response is not mediated by a second messenger. Derkach et al (220) have now provided direct evidence for this by recording the currents though single ion channels activated by 5-HT_3 receptors in excised membrane patches from neurons of the guinea pig submucous plexus.

An exciting recent finding has been the demonstration of specific binding of 5-HT_3 radioligands in the brains of several species including humans (51, 221). Data have begun to emerge from which it has been inferred that the 5-HT_3 receptor may mediate behavioral functions in the central nervous system (222).

The scheme proposed by Bradley et al (33) was very useful in that it was sufficiently flexible to incorporate new information, particularly as new selective agonists and antagonists became available. Also, the classification primarily used functional responses elicited in isolated peripheral tissues where definitive characterization of the effects of agonists and antagonists is more easily obtainable than in organs such as brain. Leff & Martin (13), in a recent scholarly review article, also praised Bradley et al (33) for emphasizing functional correlates of radioligand binding data in the classification of receptors for 5-HT. However, they questioned the pharmacological properties of several of the antagonists used to classify 5-HT receptors, in particular, antagonists such as methiothepin (223) whose effects, at least in some tissues, deviate from simple competition. Also, the use of ketanserin in functional assays may be problematic as its affinity for the 5-HT_2 receptor, as indicated by pA2 values, varies by about two orders of magnitude, e.g. from 8.4 in rat aorta (225) to 10.4 in rat femoral vein (226). Since ketanserin does appear to act competitively and good evidence is not yet available for subtypes of 5-HT_2 receptors (but see 227), Leff & Martin (13) were particularly concerned about this apparent quantitative difference in its affinity for 5-HT_2 receptors in different tissues. Similar problems existed with the antagonists used in the classification of 5-HT_3 receptors (115, 228, 229). These considerations caused Leff & Martin (13) to conclude that whereas the relative inactivity of drugs like methiothepin, ketanserin, and ICS 205-930 at certain types of 5-HT receptors makes them useful qualitatively to distinguish receptor types, they are of limited use quantitatively in the classification of 5-HT receptors.

Although we agree with a number of the issues raised by Leff & Martin (13), some of their concerns about the problems with antagonists could be due to tissue-specific factors. For example, the tissues being studied may express multiple subtypes of 5-HT receptors, each involved in the response being measured; this would lead to differences in potency from tissue to tissue for a

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given drug (e.g. ketanserin). Additionally, it is possible that tissue specific processes such as degradation, uptake, etc, could vary for a given drug (e.g. 230). Use of cell lines could clarify these issues. Furthermore, the use of functional potencies of agonists to classify receptors can be confounded by different degrees of "spare receptors" in various tissues. Nevertheless, it may be productive to use some of the tryptamine analogs used by Leff & Martin (13) on responses in the CNS that can be measured in vitro (e.g. second-messenger responses; ^{3}H-5-HT release, etc) to better categorize their linkage to a subtype of 5-HT receptor.

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Molecular Biological Classification Schemes

Within the past two years, the genes encoding three (5-HT_{1A}, 5-HT_{1C}, and 5-HT_2) serotonin receptors have been cloned. This fact makes it possible to consider whether or not knowledge of the gene sequence encoding a specific receptor should be required before accepting a putative subtype as "real." For the receptors noted above, pharmacological evidence obtained prior to the molecular biological data had allowed the suggestion that these three receptors were distinct. On the other hand, it is entirely possible that the gene for a given subtype of serotonin receptor will be discovered prior to any suggestion of its existence based on pharmacological data. For example, in a recent provocative study (231), the investigators used the technique of the polymerase chain reaction to obtain a number of unique clones that appear to code for members of the G-protein–coupled receptor family. In particular, one clone has a very high degree of homology to the 5-HT_{1A} receptor and may be a novel subtype of serotonin receptor.

Experience over the past few years has demonstrated that the existence of subtypes of receptors can be proposed on the basis of molecular biological data. For example, although the pharmacological and functional data generated to date support the existence of only three subtypes of muscarinic cholinergic receptors (232), molecular biological experiments have demonstrated five distinct but related genes encoding proteins that have the properties of muscarinic receptors in both the human and rat genome (233, 234). Messenger RNA encoding four of these receptors is expressed in rat tissues (235). Thus, four or five muscarinic receptors may possibly be expressed in these species. However, which cloned receptor corresponds to which pharmacologically defined receptor is still somewhat unclear, especially with regard to m3, m4, and m5 receptors. This then raises the question of whether or not a criterion required for defining a receptor subtype should include cloning and expressing the gene for a putative receptor. The fact that a number of subtypes of serotonin receptors have already been cloned and expressed makes this suggestion possible for at least some serotonin receptors.

Indeed, such information is already being used to suggest alternative classifications for subtypes of 5-HT receptors. Thus, the fact that both the

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5-HT_{1C} and 5-HT_2 receptors are linked to activation of the phospholipase C cascade (and a similar pharmacological profile of these two receptors) and that the primary sequences of these proteins demonstrate a high (51%) degree of identity led Hoyer (236) to suggest that the 5-HT_{1C} receptor more properly belongs in the 5-HT_2 receptor classification. Similarly, after cloning the 5-HT_2 receptor, Pritchett et al (82) noted a greater structural similarity between it and the 5-HT_{1C} subtype than that which occurred between the 5-HT_{1A} and 5-HT_{1C} subtype. They proposed also that the 5-HT_{1C} receptor be moved into the 5-HT_2 classification. These views were formally championed recently in a short review by Hartig (224). He attempted a more molecular definition of subtypes of receptors for 5-HT to deemphasize radioligand affinity states. (For the reasons stated above, we agree wholly with this de-emphasis). He divided the 5-HT receptors according to their linkage to the G-protein superfamily (5-HT_1 and 5-HT_2 receptors) or the ligand-gated ion channel superfamily (5-HT_3 receptor). Second, he used the structural data of the cloned 5-HT receptors to move the 5-HT_{1C} receptor into the 5-HT_2 class of receptors. Thus, the Hartig (224) scheme for receptor classification is based upon membership in classes of signal-transducing processes with subclassifications on the degree of homology among subtypes within each class. For comparative purposes, Table 2 shows the classification and the criterion

Table 2 SCHEMES OF RECEPTOR CLASSIFICATION

Nomenclature	Selective agonist	Selective antagonist
I. Bradley et al (33)		
"5-HT_1-like"	5-Carboxamidotryptamine	Methysergide
5-HT_{1A}		Methiothepin
5-HT_{1B}		
5-HT_{1C}		
5-HT_{1D}		
5-HT_2	- - - -	Ketanserin
		Methysergide
5-HT_3	2-Methyl-5-hydroxytryptamine	MDL 72222
		ICS 205-930

Nomenclature	Signal transducing process	Classification of Bradley et al (33)
II. Hartig (224)		
5-HT_{1_α}	G Protein	5-HT_{1A}
5-HT_{2_α}	G Protein	5-HT_2
5-HT_{2_β}	G Protein	5-HT_{1C}
5-HT_3	Ligand-gated ion channel	5-HT_3

on which Bradley et al (33) and Hartig (224) based their proposals. An interesting aspect of Hartig's scheme (224) is that both his 5-HT_2 type receptors are coupled to the phospholipase C cascade whereas both the 5-HT_{1A} and 5-HT_{1B} subtypes are linked to adenylyl cyclase (see above).

Classification Based on Analogy With the Enzyme Code

We agree with the spirit of Hartig's suggestion (224) and wish to extend and amplify it by proposing a nosology for receptors based on the nomenclature devised for enzymes by the International Union of Biochemistry (I.U.B.), commonly known as the Enzyme Commission (EC) system (239). For example, in the EC system an enzyme is classified by four numbers, the first indicating where in the six main divisions (e.g. oxidoreductases, transferases, hydroxylases, etc) the enzyme belongs. Below, we propose a similar nomenclature for receptors to generate what may be termed "RC" numbers. Thus, receptors can be classified by four numbers with the first referring to the activating ligand (e.g. 5-HT), the second to the superfamily (e.g. G-protein-linked), the third to the (predominant) second-messenger system modulated by the receptor (e.g. adenylyl cyclase), and the fourth referring to the primary sequence (from cloning and sequencing the cDNA or gene), with the number assignment determined by the chronological order in which the sequences were reported. If these criteria are used to determine RC numbers, many receptors cannot yet be assigned a complete number. For example, those receptors not yet cloned and sequenced cannot be assigned in the fourth position. Nevertheless, as time goes by, more and more numbers will be assigned.

To begin such a cataloging scheme one must arbitrarily assign numbers to activating ligands, such as is suggested in Table 3. Similarly, one must assign numbers to the known superfamilies as well as second messengers (Table 3). The assignments in this table are random and the lists are, obviously, incomplete. At this juncture we are simply putting forward a relatively novel idea regarding nosology. Under such a scheme the 5-HT_{1A} receptor, for example, would receive a number of 3.1.1.1, whereas the 5-HT_{1C} receptor would be 3.1.2.1, the 5-HT_2 receptor would be 3.1.2.2, and the 5-HT_3 receptor would be 3.2.X.X. This last number contains "Xs" to indicate incomplete current knowledge. Similarly, for example, the insulin receptor would be 5.3.3.1, the muscle nicotinic receptor would be 1.2.4.1 and the m_1 muscarinic receptor would be 1.1.2.1, and so forth. This method of classification brings all the advantages of Hartig's proposed scheme (224) in that it is clear which receptors belong to the same superfamily and which share the same second-messenger systems. This catalogue could result in a volume which organizes all known receptors and where one could amplify on each. Thus, a list of references, pharmacological preferences, and alternate second-

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Table 3 Proposed scheme for classification of receptors analogous to the enzyme code

Activating ligand	Superfamily	Second messenger
1 = Acetylcholine	1 = G Protein-linked	1 = Cyclic AMP
2 = Norepinephrine	2 = Ion channels	2 = Phosphoinositides
3 = Serotonin	3 = Growth factors	3 = Tyrosine Kinase
4 = Estrogen	4 = Steroid receptors	4 = Na^+
5 = Insulin		5 = Ca^{++}
Etc		6 = K^+
		Etc

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messenger pathways utilized by the receptor could all be compiled and become associated with each entry.

The advantages of this scheme include a clearer indication of the similarities and differences between receptors; however, it would introduce some potential difficulties. For example, binding sites for allosteric modulators, such as glycine on the NMDA-type glutamate receptor, in most senses fit the definition of receptor (14). However, they may be part of another receptor, i.e. a receptor may have more than one endogenous ligand that binds to distinct binding sites. Alternatively, the choice of endogenous ligand is not always clear or necessarily constant from tissue to tissue. For example, the β_2-adrenergic receptor is likely to be a receptor for epinephrine in the periphery since norepinephrine is approximately ten times less potent than epinephrine at this receptor (238). On the other hand, although the administration of 6-hydroxydopamine causes an increase in β_1-adrenergic receptors in at least a few areas in the CNS the density of β_2-adrenergic receptors increases; this indicates that norepinephrine is likely to be the transmitter at least at some β_2-adrenergic receptors (239). Another potential problem lies with the possibility that multiple second messengers may be modulated by the same receptor. Thus, for example, the m_2 muscarinic receptor not only inhibits adenylyl cyclase but also stimulates phosphoinositide hydrolysis (240). Whether this or any other receptor expresses a preference for a given second-messenger system is unclear since with the m_2 muscarinic receptor, the inhibition of adenylyl cyclase activity evidently occurs at much lower agonist concentrations than does the stimulation of phosphoinositide hydrolysis; this indicates a stronger coupling to the former than to the latter signaling system (240).

We feel that this classification scheme actually would not replace but rather complement existing nomenclatures. Thus, the 5-HT_{1A} receptor is unlikely to be referred to as the 3.1.1.1 receptor in common terminology; rather this receptor would retain its trivial name (5-HT_{1A}) and reference to its RC

number would be made in manuscripts. This RC number and the publication of a source volume with precise definitions would provide a reference resource just as the EC system has been for enzyme nomenclature.

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