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Pharmacological Characterization of Two 5-Hydroxytryptamine Receptors Coupled to Adenylate Cyclase in Guinea Pig Hippocampal Membranes

ANDREW SHENKER,[1] SAUL MAAYANI, HAREL WEINSTEIN, and JACK PETER GREEN

Department of Pharmacology, Mount Sinai School of Medicine, City University of New York, New York, New York 10029

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SUMMARY

Two 5-hydroxytryptamine (5-HT) receptors mediate stimulation of adenylate cyclase activity in membranes of adult guinea pig hippocampus. The two receptors were characterized with agonists and antagonists and with the aid of computerized curve-fitting procedures. Each receptor mediates about 50% of the maximal response to 5-HT. 5-HT is about 10-fold more potent in eliciting response through one cyclase-linked receptor (R_H) than the other (R_L). The concentrations of 5-HT that elicit half-maximal response through R_H and R_L are 43 ± 6 nM and 414 ± 53 nM, respectively. 5-Methoxytryptamine (5-MeOT) and 5-HT are approximately equipotent at each receptor. The agonists tryptamine and bufotenine are less potent than 5-HT at both receptors, and each is about 50-fold selective for R_H. The two receptors are best discriminated by the agonists 5-carboxamidotryptamine (5-$CONH_2$-T) and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), both of which are selective for R_H. 5-$CONH_2$-T is about 7-fold more potent than 5-HT at R_H. The rank order of agonist potencies at R_H (5-$CONH_2$-T > 8-OH-DPAT = 5-HT = 5-MeOT > bufotenine > tryptamine) differs from that at R_L (5-HT = 5-MeOT > bufotenine > tryptamine = 5-$CONH_2$-T > 8-OH-DPAT). Spiperone acts as a simple competitive antagonist at R_H, with a dissociation constant of 20 nM, but it is at least 100-fold less potent as an antagonist at R_L. THe relatively low affinities of the selective 5-HT antagonists ketanserin and MDL 72222 for R_H and R_L indicate that neither receptor may be classified as the 5-HT_2 or as the 5-HT_3 (i.e., peripheral neuronal) type. The characteristics of R_H suggest that it is a functional correlate of the 5-HT_{1A}-binding site in brain. R_L appears not to correspond to a known 5-HT-binding site, but it may be homologous to receptors that mediate 5-HT-stimulated adenylate cyclase activity in other systems such as infant rat colliculi. R_H and R_L may also mediate stimulation of adenylate cyclase activity by 5-HT in hippocampal membranes of adult rat.

The availability of selective antagonists has led to the classification of different types of functional 5-HT receptors in mammals, including the 5-HT_2 (1–3) and 5-HT_3 (i.e., peripheral neuronal) receptors (3–6). 5-HT receptors coupled to adenylate cyclase belong to a group of 5-HT receptors for which a specific antagonist has not yet been discovered. Some of these receptors resemble [^{3}H]5-HT-binding sites (1, 7–16). Three distinct sites labeled with high affinity by [^{3}H]5-HT have been described: the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} subtypes (12, 17, 18). 5-HT_{1A} sites are distinguished from 5-HT_{1B} and 5-HT_{1C} sites by the relatively high affinity of the 5-HT_{1A} sites for spiperone and the agonist 8-OH-DPAT (12, 17, 19). The selective agonist activity of the 5-carboxamido analog of 5-HT (5-$CONH_2$-T) has proved helpful in categorizing 5-HT_1-like receptors in the brain and periphery (3, 7, 12–16). 5-$CONH_2$-T is more potent than 5-HT at both 5-HT_{1A} and 5-HT_{1B} sites (12).

Different investigators have reached conflicting conclusions about the relationship between 5-HT_1-binding sites and cyclase-linked 5-HT receptors (1, 20–24), possibly because of the existence of multiple types of cyclase-linked 5-HT receptors and binding sites. Much of the work on 5-HT-sensitive cyclase activity was done before the heterogeneity of 5-HT_1 sites was demonstrated and before selective ligands were available. Although stimulation of cAMP accumulation by 5-HT has been reported in many membrane preparations from mammalian brain (20, 21, 23, 25–30), only the infant rat colliculi system has been characterized in some detail (27).

Conditions have been described to measure 5-HT-stimulated cyclase activity in hippocampal membranes from adult guinea pig (31) and rat (29, 32). The response to 5-HT in guinea pig membranes is concentration dependent, GTP dependent, and of sufficient magnitude to permit quantitative characterization

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[1] Present address: Department of Pediatrics, Johns Hopkins Hospital, Baltimore, MD 21205.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; 5-$CONH_2$-T, 5-carboxamidotryptamine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; EDTA, ethylenediaminetetraacetate; 5-MeOT, 5-methoxytryptamine.

of the receptors with agonists and antagonists (31–33). We reported previously that guinea pig hippocampal membranes contain two distinct 5-HT receptors coupled to adenylate cyclase, which are discriminated by the agonist 5-$CONH_2$-T and the antagonist spiperone (33). The receptor with high affinity for these two drugs was proposed to be a functional correlate of the 5-HT_{1A} site (33). In this report, additional evidence is presented for the two-receptor model. Aspects of this model were explored with the aid of computerized curve-fitting procedures and simulations.

Materials and Methods

Membrane preparation. Male Hartley-Albino guinea pigs (400–450 g) and male Sprague-Dawley rats (225–250 g) (Perfection Breeders, Douglassville, PA) were killed by decapitation and the hippocampi of each animal were removed. Tissue medium, pH 7.4 at 23°, containing 300 mM sucrose, 20 mM Tris-HCl, 1 mM EGTA, 5 mM Na_2EDTA, and 5 mM dithiothreitol, was prepared daily. The hippocampi from each guinea pig were homogenized by hand in 9 ml of ice-cold tissue medium (20 strokes, Arthur H. Thomas size C Teflon pestle tissue homogenizer). Hippocampi from each rat were homogenized in 4–4.5 ml of ice-cold medium. The homogenate was diluted 1:8 with medium and centrifuged at 39,000 × *g* for 10 min at 4°. Pellets were resuspended by vortexing in the same volume used for homogenization. This particulate fraction was stored in ice until used within the hour.

Adenylate cyclase assay. Adenylate cyclase activity was determined by measuring the conversion of [α-^{32}P]ATP to [^{32}P]cAMP. Assay medium (200 μl) was first incubated for 5 min at 30°. The reaction was initiated with 50 μl of the hippocampal preparation. The final assay mixture consisted of 80 mM Tris-HCl (pH 7.4), 0.2 mM ATP, 2 mM magnesium acetate, 10 or 20 μM GTP, 10 μM pargyline, 0.6 mM ascorbate, 4 mM theophylline, 1 mM cAMP, 125 μg of creatine phosphokinase, 5 mM creatine phosphate, 1.5 μCi of [α-^{32}P]ATP (10–50 Ci/mmol), about 100 μg of particulate protein, 60 mM sucrose, 0.2 mM EGTA, 1 mM Na_2EDTA, 1 mM dithiothreitol, and various concentrations of drugs. Determinations were done at least in triplicate. The incubation was carried out at 30° for 2 min; under these conditions, enzyme activity was linear with respect to time and protein concentration. To confirm that these conditions were adequate for drug-receptor equilibration, certain experiments were designed differently: the hippocampal membranes were first incubated with drugs and assay components (lacking [α-^{32}P]ATP) for 8 min at 30°; the assay was then initiated with [α-^{32}P]ATP and conducted for the usual 2 min. All assays were stopped by the addition of 100 μl of a solution containing 2% sodium lauryl sulfate, 45 mM ATP, and Tris, pH 7.5. After addition of [^{3}H]cAMP (10,000–30,000 cpm) to monitor recovery, the samples were placed in a boiling water bath for 3 min and cooled to room temperature, and labeled cAMP was isolated as described by Salomon (34). Recovery averaged about 70%, and reaction blanks usually represented only 5% of the lowest measured enzyme activity. Protein was determined by the method of Lowry *et al.* (35), with bovine serum albumin as the standard and tissue medium as the blank. Adenylate cyclase activity was expressed as pmol of cAMP/min/mg of protein.

Data analysis. The coefficient of variation of replicate determinations was routinely ≤5%. Stimulation of adenylate cyclase activity was calculated by subtracting mean enzyme activity in the absence of agonist (basal activity) from the mean activity in the presence of agonist. The standard error of the net stimulation was also calculated (36). Percentage stimulation was defined as (stimulation/basal) × 100. Concentration-response data were initially fit to a form of the logistic function (37):

$$E = E_{max}/[1+ (EC_{50}/[A])^N] \quad (1)$$

where E = response to agonist, E_{max} = maximal response to agonist, EC_{50} = concentration of agonist eliciting half-maximal response, $[A]$ = agonist concentration, and N = slope index.

The antagonist spiperone usually caused a 10–20% decrease in basal enzyme activity, but this effect was not clearly concentration related; the decreased basal values were used in calculating net stimulation by agonist. On three occasions 5-HT and spiperone were incubated together with the hippocampal membranes for 8 min before conducting the assay; in two of these experiments the maximal response to 5-HT was increased by 15–20% in the presence of spiperone. In those two experiments the data were expressed as a percentage of the maximal response to 5-HT for each curve before further analysis. As the fitted estimates of the affinities of spiperone did not appear to vary with time of incubation or data transformation, the results of all experiments with spiperone were combined.

Additional analysis was performed with the computerized, nonlinear least squares curve-fitting procedure FITFUN (38). Weighting was not used because the response variable (increase in adenylate cyclase activity) exhibited uniformity of variance (37). Curves from the same experiment were often fit simultaneously, a method that offers statistical advantages (39). Concentration-response data were fit to a model describing the action of drugs at two independent receptors that mediate the same response (40, 41).

A partial F test (39, 42) was used to determine whether a more complex model provided a significantly better fit to the data than a simple one. For example, the parallelism of concentration-response curves in the absence and presence of antagonist was tested by simultaneously fitting the set of curves to logistic functions where the slope indices were either allowed to vary ("variable slope model") or constrained to the same value ("common slope model"); if the more complex variable slope model provided a significantly better fit to the data, it was inferred that the curves were not parallel (37).

The natural logs of the fitted estimates of EC_{50} values and dissociation constants (K_B values) were used to calculate geometric means ± standard error (39). All other data are expressed as arithmetic means ± standard error.

Differences were tested for statistical significance with Student's t test; mean agonist slope indices were compared with one-way analysis of variance followed by Dunnett's test. For all statistical tests, the significance level was set at 0.05.

The K_B of spiperone was determined from a Schild plot (43, 44). If the slope of the plot did not significantly differ from 1.0, the intercept of a line constrained to slope = 1.0 was used.

Chemicals. The following drugs were generously donated: ketanserin tartrate and spiperone (Janssen Pharmaceutica, Beerse, Belgium); MDL 72222 methanesulphonate (Centre de Recherche Merrell International, Strasbourg, France); racemic 8-OH-DPAT hydrobromide (Lilly Research Laboratories, Indianapolis, IN); 5-$CONH_2$-T fumarate (Sandoz Ltd., Basel, Switzerland and Glaxo, Hertfordshire, England); (−)-cocaine hydrochloride, bufotenine (National Institute on Drug Abuse, Bethesda, MD). Radiochemicals were from New England Nuclear (Boston, MA).

Results

Concentration-response curves for 5-HT. Basal adenylate cyclase activity (pmol of cAMP/min/mg of protein) was 49.4 ± 1.4 for guinea pig membranes (n = 60) and 38.8 ± 2.8 for rat membranes (n = 9). Stimulation of adenylate cyclase activity by 5-HT was concentration dependent in membranes of both guinea pig (Figs. 1 and 2B) and rat (data not shown), approaching a plateau at 10 μM 5-HT. Concentrations of 5-HT greater than 100 μM evoked an additional, nonsaturable phase of stimulation which was also produced by mM concentrations of other amines, including histamine (45). Because of this apparently nonspecific effect, concentrations of serotonergic agonists greater than 100 μM were not routinely used.

The EC_{50} values for 5-HT, 140 ± 11 nM in guinea pig (n = 33) and 123 ± 24 nM in rat (n = 9), were not significantly

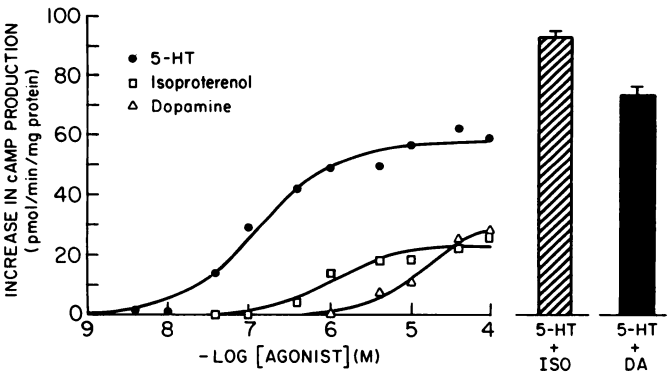

Fig. 1. Stimulation of adenylate cyclase activity by 5-HT (●), (−)-isoproterenol (□), and dopamine (△). Each point represents the mean increase in cAMP production from a single experiment in which basal enzyme activity was 66.0 ± 0.9 pmol of cAMP/min/mg of protein. The curves are best fit to Eq. 1: for 5-HT, EC_{50} = 135 nM, E_{max} = 57.9 pmol of cAMP/min/mg of protein, slope index = 0.89; for (−)-isoproterenol, EC_{50} = 1.1 μM, E_{max} = 23.2 pmol of cAMP/min/mg of protein, slope index = 1.08; for dopamine, EC_{50} = 14 μM, maximal response = 30.9 pmol of cAMP/min/mg of protein, slope index = 1.23. ▩, ■, the mean increase in cAMP production (± standard error) produced by a combination of 10 μM 5-HT and 40 μM (−)-isoproterenol (*5-HT + ISO*) and a combination of 10 μM 5-HT and 40 μM dopamine (*5-HT + DA*).

different. Concentration-response curves were equally shallow in the two species. The slope index was 0.81 ± 0.02 in guinea pig and 0.83 ± 0.08 in rat; both values were significantly less than unity. The salient difference between the two species was the greater maximal stimulation over basal activity in guinea pig, 104 ± 3% (n = 33), than in rat 42 ± 3% (n = 9). The low stimulation by 5-HT in rat hippocampal membranes discouraged further characterization of the receptors in that species.

The effect of preincubation with agonists on stimulated adenylate cyclase activity. Preincubation of guinea pig hippocampal membranes and assay components without agonists for 8 min at 30° (n = 11) caused a significant reduction in basal adenylate cyclase activity (32.7 ± 2.3 versus 49.4 ± 1.4 pmol of cAMP/min/mg of protein). Preincubation with a maximally effective concentration of histamine (400 μM) had no effect on the maximal percentage stimulation elicited by histamine (163 ± 9% versus 156 ± 2% without preincubation). In contrast, preincubation with a maximally effective concentration of 5-HT (40 μM) resulted in significantly lower stimulation by 5-HT (64 ± 4%) than that observed without preincubation.

The results of other experiments (n = 5) in which membranes were preincubated with and without agonists for 30 min before starting the assay suggested that the decreased responsiveness to 5-HT was 5-HT dependent, i.e., was not simply due to degradation of 5-HT or 5-HT receptors during the preincubation.

Involvement of receptors for other biogenic amines in the response to 5-HT. Under the present assay conditions, maximal stimulation by histamine averaged 156 ± 2% over basal activity (n = 60). Previous work showed that maximally effective concentrations of 5-HT (10 μM) and histamine (400 μM) in combination produced additive stimulation of cyclase activity, implying that the effects were mediated by different receptors (31).

5-HT and other serotonergic agonists are known in other systems to cause effects by direct or indirect activation of receptors for catecholamines (3). Because β-adrenergic and dopamine receptors positively coupled to adenylate cyclase have been found in mammalian hippocampus (29, 46), it was essential to learn whether some or all of the response to 5-HT in guinea pig membranes was due to activation of catecholamine receptors.

Stimulation of adenylate cyclase activity by the β-adrenergic agonist (−)-isoproterenol was concentration dependent (Fig. 1), with an EC_{50} of 2 ± 1 μM (n = 3). Maximal stimulation by (−)-isoproterenol averaged 31 ± 6% over basal activity, and was simply additive to the maximal stimulation by 5-HT (Fig. 1, n = 4).

Stimulation of adenylate cyclase activity by dopamine was also concentration dependent (Fig. 1), with an EC_{50} of 15 ± 2 μM (n = 3). The stimulation elicited by 100 μM dopamine averaged 44 ± 4% over basal activity (n = 6). Stimulation elicited by 10 μM dopamine was simply additive to the maximal stimulation by 5-HT (n = 3). However, stimulation produced by a combination of 40 or 100 μM dopamine and a maximally effective concentration of 5-HT was significantly less than additive (Fig. 1, n = 6). These findings suggest that stimulation by low concentrations of dopamine (i.e., ≤10 μM) is mediated by receptors distinct from those for 5-HT, but that part of the stimulation elicited by dopamine concentrations greater than 10 μM is probably due to activation of 5-HT receptors.

The response to agonists. The 5-HT analogs 5-MeOT, 5-hydroxy-*N,N*-dimethyltryptamine (bufotenine), and tryptamine stimulated adenylate cyclase activity in guinea pig membranes (Fig. 2A) with mean EC_{50} values of 193 ± 39 nM, 620 ± 158 nM, and 7.1 ± 1.8 μM, respectively (n = 4–6). The fitted estimates of maximal response to 5-HT, 5-MeOT, and tryptamine were not significantly different. It was previously shown that maximal stimulation produced by 5-MeOT or tryptamine was not additive to that produced by 5-HT (31). The E_{max} of bufotenine averaged only 88 ± 2% of the E_{max} of 5-HT; maximal stimulation produced by bufotenine (40 μM) was not additive to that produced by 5-HT (10 μM) (n = 2). 5-hydroxyindole (40 μM) exhibited neither agonist nor antagonist activity (Table 1).

The slope index of the 5-MeOT concentration-response curve (0.79 ± 0.04) was indistinguishable from that of 5-HT, but the slope indices of the curves for bufotenine (0.65 ± 0.05) and tryptamine (0.56 ± 0.02) were significantly lower. These shallow, nonparallel agonist concentration-response curves (Fig. 2A) raised the possibility that the increase in adenylate cyclase activity in this system was mediated by a heterogeneous population of 5-HT receptors, which were better discriminated by bufotenine and tryptamine than by 5-MeOT or 5-HT.

As previously reported (33), 5-$CONH_2$-T produced a distinctly biphasic concentration-response curve in the guinea pig membranes (Fig. 2B). The first component of the curve lay to the left of the 5-HT curve and reached a plateau at concentrations of 5-$CONH_2$-T from 0.1 to 4 μM. The plateau response was equal to 49 ± 2% (n = 30) of the maximal response to 5-HT. At higher concentrations, 5-$CONH_2$-T elicited a further increase in the rate of cAMP production. Fitting the initial component of 5-$CONH_2$-T curves (4 nM–4 μM; n = 13) to the logistic function yielded a mean EC_{50} of 6 ± 1 nM and a slope index (1.04 ± 0.04) not significantly different from unity, suggesting activation of only a single receptor type. Although the second component of the curve did not clearly reach a plateau, it is evident that 5-$CONH_2$-T is at least 5000 times less potent in eliciting the second part of the response than the

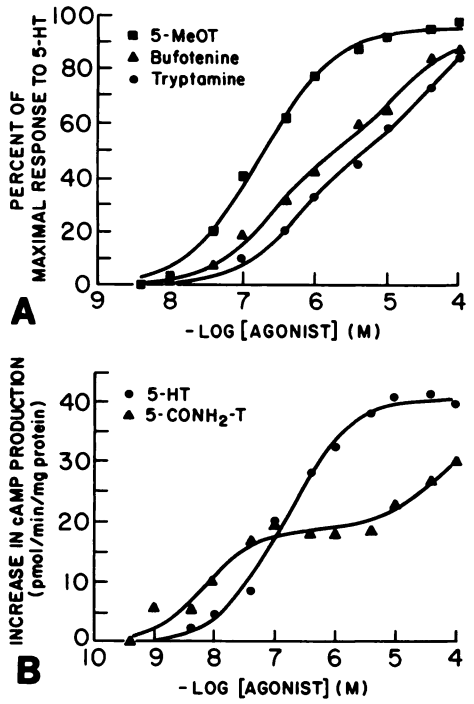

Fig. 2. Stimulation of adenylate cyclase activity by serotonergic agonists. A. Stimulation of adenylate cyclase activity by 5-MeOT (■), bufotenine (▲), and tryptamine (●). The data were compiled from two different experiments and are expressed as a percentage of the maximal stimulation by 5-HT in each experiment. The curves are best fits to Eq. 2; E_{maxH} was constrained to 50%. The fits provided the following parameter estimates: for 5-MeOT, K_H = 72 nM, K_L = 516 nM, E_{maxL} = 46%; for bufotenine, K_H = 233 nM, K_L = 14.8 μM, E_{maxL} = 44%; for tryptamine, K_H = 584 nM, K_L = 42.0 μM, E_{maxL} = 49%. B. Stimulation of adenylate cyclase activity by 5-HT (●) and 5-$CONH_2$-T (▲). Each point represents the mean increase in cAMP production from a single experiment in which basal enzyme activity was 42.5 ± 0.9 pmol of cAMP/min/mg of protein. The stimulation produced by a combination of 4 μM 5-HT and 100 μM 5-$CONH_2$-T (38.7 ± 0.8 pmol of cAMP/min/mg of protein) was not significantly different from that produced by 4 μM 5-HT alone. The curves are the best fits to Eq. 2; the data for 5-HT and 5-$CONH_2$-T were fit simultaneously with a common value for E_{maxH}. The fit provided the following parameter estimates: E_{maxH} = 18.6 pmol of cAMP/min/mg of protein; for 5-HT, K_H = 42 nM, K_L = 412 nM, E_{maxL} = 21.8 pmol of cAMP/min/mg of protein; for 5-$CONH_2$-T, K_H = 7 nM, K_L = 42.0 μM, E_{maxL} = 15.9 pmol of cAMP/min/mg of protein. In this experiment, 46% of the maximal response was due to activation of R_H.

TABLE 1

Activity of agonists and antagonists at two 5-HT receptors coupled to adenylate cyclase in guinea pig hippocampal membranes

Substance	n	K or K_B[a]	
		R_H	R_L
		nM	
Agonists			
5-HT	15	43 ± 6	414 ± 53
5-MeOT	4	51 ± 12	578 ± 107
Bufotenine	6	123 ± 25	4,790 ± 1,640[b]
Tryptamine	5	592 ± 142	33,100 ± 9,600
5-$CONH_2$-T	13	6 ± 1	31,300 ± 4,500[b]
8-OH-DPAT	4	29 ± 11[c]	>50,000
Antagonists			
Spiperone	5	24 ± 3	≥3,000
Ketanserin	3	>100	>100

Inactive agents[d]	Highest concentration tested
	μM
5-Hydroxyindole	40
Phenylbiguanide	40
MDL 72222	10
(−)-Cocaine	40

[a] Each value is the geometric mean ± standard error of parameters estimated by computer fit, as described in the text.
[b] May be a partial agonist at R_L; the K_L of 5-$CONH_2$-T is based on data from the six experiments where concentrations of 5-$CONH_2$-T as high as 100 μM were used.
[c] Partial agonist at R_H.
[d] Each substance was tested alone and in combination with 4 nM–40 μM 5-HT at least two times. Results were not different when drugs and membranes were preincubated for 8 min before assay.

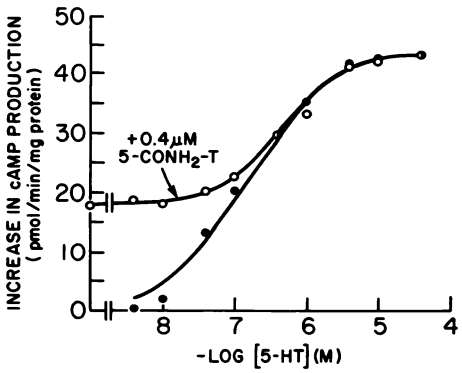

Fig. 3. Stimulation of adenylate cyclase activity by 5-HT alone (●) and by 5-HT in the presence of 0.4 μM 5-$CONH_2$-T (○). Each point represents the mean increase in cAMP production from a single experiment in which basal enzyme activity was 37.2 ± 0.5 pmol of cAMP/min/mg of protein. The curves are the result of simultaneously fitting the response data for 5-HT alone and for 5-HT plus 5-$CONH_2$-T to Eqs. 2 and 3, respectively. It was assumed that 5-$CONH_2$-T was a full agonist on R_H (β_H = 1.0). The apparent affinities of 5-$CONH_2$-T for R_H and R_L were fixed, based on average results; the effect of 0.4 μM 5-$CONH_2$-T on R_L is negligible. The fit provided the following parameter estimates: for 5-HT, K_H = 32 nM, K_L = 447 nM; E_{maxH} = 18.4 pmol of cAMP/min/mg of protein; E_{maxL} = 24.9 pmol of cAMP/min/mg of protein. Each curve was also fit individually to Eq. 1 (curves not shown). The curve for 5-HT alone had an EC_{50} of 137 nM and a slope index of 0.82. The curve for 5-HT in the presence of 5-$CONH_2$-T had an EC_{50} of 554 nM and a slope index of 0.92. Similar results were obtained in three additional experiments. Preincubating the agonists and membranes together for 8 min did not affect the shape or relative position of the curves.

first part. Stimulation produced by 100 μM 5-$CONH_2$-T was not additive to the maximal stimulation produced by 5-HT (n = 4; see legend to Fig. 2B), indicating that the entire response to 5-$CONH_2$-T was mediated by the same receptors that mediated the response to 5-HT.

These observations led to the hypothesis that guinea pig hippocampal membranes contain two distinct 5-HT receptors coupled to adenylate cyclase, each contributing about 50% to the total response. The predictions of this two-receptor model were then tested.

Agonists and the two-receptor model. If 0.4 μM 5-$CONH_2$-T elicits maximal stimulation through one receptor population without significantly affecting the second (see Fig. 2B), then stimulation of adenylate cyclase activity by another agonist in the presence of 0.4 μM 5-$CONH_2$-T should appear monophasic and reflect activation only of the second receptor population. The results of a representative experiment of this design are shown in Fig. 3. The concentration-response curve for 5-HT in the presence of 0.4 μM 5-$CONH_2$-T is nearly superimposable on the upper portion of the 5-HT curve. As expected, the mean slope index of such curves (0.88 ± 0.08, n = 4) was not significantly different from 1.0. From these results it was deduced that the initial, high affinity components of the

curves for 5-$CONH_2$-T and 5-HT are mediated by the same receptor. In the following discussion, this receptor is referred to as R_H, and the receptor that mediates the second, low affinity components of the curves is referred to as R_L. Experiments analogous to the one shown in Fig. 3 were performed with 5-MeOT, tryptamine, and bufotenine and revealed that these three agonists were also selective for R_H; Fig. 4 shows the results of an experiment performed with bufotenine.

Concentration-response data were fit to a model describing the action of an agonist at two independent receptors that mediate the same response (41):

$$E = E_{maxH}[A]/(K_H + [A]) = + E_{maxL}[A]/(K_L + [A]) \quad (2)$$

where E represents agonist-stimulated adenylate cyclase activity; E_{maxH} and E_{maxL} represent the maximal stimulation due to R_H and R_L, respectively; $[A]$ represents agonist concentration; and K_H and K_L represent the EC_{50} values of the agonist at R_H and R_L, respectively. It was assumed that responses due to the activation of R_H and R_L were simply additive, as additivity of responses mediated by different receptors has been demonstrated in this system (Fig. 1; Ref. 31). E_{maxH} was estimated by measuring the stimulation produced by 0.4 μM 5-$CONH_2$-T in each experiment. Data from earlier experiments were analyzed by constraining the percentage of maximal response to 5-HT mediated by R_H to be 50%, based on the average results with 5-$CONH_2$-T (i.e., 49 ± 2%). Estimates of K_H and K_L generated by a computerized, nonlinear least squares curved-fitting procedure (38) are summarized in Table 1. Fitted curves are illustrated in Fig. 2. As expected from the two-receptor model, the mean K_L of each agonist estimated by computer fit was comparable to the EC_{50} of that agonist in the presence of 0.4 μM 5-$CONH_2$-T (for example, see legend to Fig. 3).

Based on the fitted estimates of E_{maxL} for 5-HT and 5-$CONH_2$-T, the calculated intrinsic activity of 5-$CONH_2$-T at R_L is 0.8 ± 0.1. Because the second component of the 5-$CONH_2$-T curve did not reach a plateau within the concentration range studied, however, it is unclear whether 5-$CONH_2$-T is actually a partial agonist at R_L.

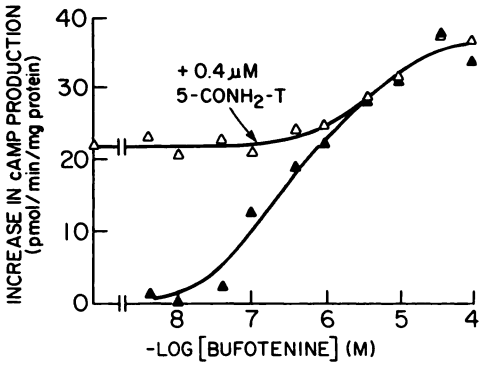

Fig. 4. Stimulation of adenylate cyclase activity by bufotenine alone (▲) and by bufotenine in the presence of 0.4 μM 5-$CONH_2$-T (△). Each point represents the mean increase in cAMP production from a single experiment in which basal enzyme activity was 49.2 ± 0.9 pmol of cAMP/min/mg of protein and the maximal response to 5-HT was 43.5 ± 1.7. Results with 5-HT and 5-$CONH_2$-T showed that $E_{maxH} = E_{maxL} = 22$ pmol of cAMP/min/mg of protein. The apparent affinities of 5-$CONH_2$-T for R_H and R_L were fixed, based on average results; the effect of 0.4 μM 5-$CONH_2$-T on R_L was negligible. Data on stimulation by bufotenine alone ($[B] = 0$) and by bufotenine in the presence of 5-$CONH_2$-T ($[B] = 0.4$ μM) were fit simultaneously to two functions in the form of Eq. 3. Bufotenine was assumed to be a full agonist at R_H ($\alpha_H = 1.0$) and a partial agonist at R_L. The fit provided the following estimates for bufotenine: $K_H = 126$ nM, $K_L = 4.5$ μM, $\alpha_L = 0.69$. Residual sum of squares was 44. Fitting the same data to a model in which bufotenine was assumed to be a partial agonist at R_H and a full agonist at R_L resulted in a worse fit (residual sum of squares = 70).

To probe further the validity of the two-receptor model, data from experiments in which the response to an agonist, A, was measured in the presence of a single concentration of another drug, B, were fit to a general model describing the competitive interaction of two drugs (40) at two independent receptors mediating the same response:

$$E = \frac{\alpha_H E_{maxH}[A]}{K_H(1 + [B]/K_{BH}) + [A]} + \frac{\beta_H E_{maxH}[B]}{K_{BH}(1 + [A]/K_H) + [B]} + \frac{\alpha_L E_{maxL}[A]}{K_L(1 + [B]/K_{BL}) + [A]} + \frac{\beta_L E_{maxL}[B]}{K_{BL}(1 + [A]/K_L) + [B]} \quad (3)$$

where E represents agonist-stimulated adenylate cyclase activity; E_{maxH} and E_{maxL} represent the maximal stimulation due to R_H and R_L, respectively; K_H and K_L represent the dissociation constants of A for R_H and R_L, respectively; α_H and α_L represent the intrinsic activities of A at R_H and R_L, respectively; K_{BH} and K_{BL} represent the dissociation constants of B for R_H and R_L, respectively; and β_H and β_L represent the intrinsic activities of B at R_H and R_L, respectively.

5-HT and 5-$CONH_2$-T. Data on the response to 5-HT in the presence of 0.4 μM 5-$CONH_2$-T were adequately fit by Eq. 3 when 5-$CONH_2$-T was assumed to be a full agonist at R_H (Fig. 3A). Converse experiments in which the response to 5-$CONH_2$-T was measured in the presence of 0.1 μM 5-HT (data not shown) were also satisfactorily fit to the two-receptor model.

5-HT and bufotenine. When the data for bufotenine were first analyzed, it was assumed that the slightly lower E_{max} (88% of the maximal response to 5-HT) was due to partial agonism at R_L (i.e., $\alpha_L \sim 0.8$). The alternative explanation, that bufotenine was a partial agonist at R_H ($\alpha_H \sim 0.8$), was also evaluated. Simulations based on Eq. 3 indicated that the two models were theoretically distinguishable by measuring the responses to bufotenine alone and to bufotenine in the presence of a concentration of 5-$CONH_2$-T that elicits maximal stimulation through R_H. In practice, the differences were difficult to detect because of scatter in the data and because of the relatively high intrinsic activity of bufotenine. The results shown in Fig. 4 produced the least ambiguous data; the model in which bufotenine is a partial agonist at R_L provides a more satisfactory explanation of the data than the model in which bufotenine is a partial agonist at R_H.

Response to 8-OH-DPAT. 8-OH-DPAT, a 5-HT_{1A}-selective agonist, stimulated adenylate cyclase activity in this preparation (Fig. 5) with an EC_{50} of 29 ± 11 nM and a slope index (0.88 ± 0.08) that was not significantly different from unity ($n = 4$). Maximal stimulation by 8-OH-DPAT represented 0.8 ± 0.1 of the stimulation elicited by 0.4 μM 5-$CONH_2$-T. A maximally effective concentration of 8-OH-DPAT inhibited the stimulation by 0.4 μM 5-$CONH_2$-T (Fig. 5). Unlike the other agonists tested, concentrations of 8-OH-DPAT as high as 400 μM did not elicit a second component of stimulation.

Antagonism by spiperone and the two-receptor model. Spiperone (30 nM–40 μM) shifted the 5-HT concentration-response curve to the right in a manner that was surmountable and concentration dependent, but nonparallel (Fig. 6). In five of six experiments the results of a partial F test indicated that

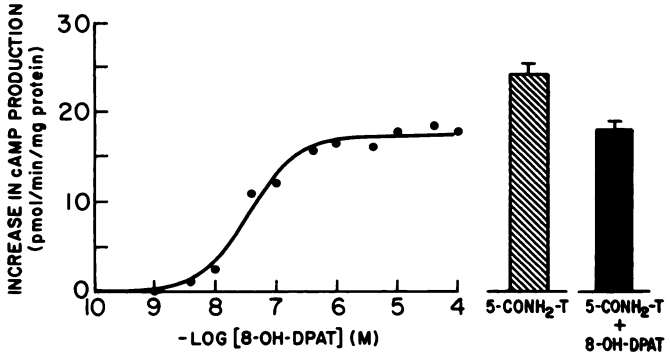

Fig. 5. Stimulation of adenylate cyclase activity by 8-OH-DPAT. Each point represents the mean increase in cAMP production from a single experiment in which basal activity was 48.0 ± 0.5 pmol of cAMP/min/mg of protein. The curve is the best fit to Eq. 1: EC_{50} = 36 nM; E_{max} = 17.2 pmol of cAMP/min/mg of protein; slope index = 1.09. In this experiment the maximal response to 8-OH-DPAT was 71% of the response to 0.4 μM 5-$CONH_2$-T. The *bars* (mean stimulation ± standard error) show response to 0.4 μM 5-$CONH_2$-T in the absence and presence of 4 μM 8-OH-DPAT.

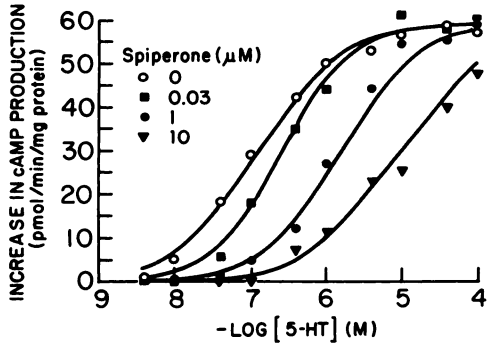

Fig. 6. Antagonism of 5-HT by spiperone. Stimulation of adenylate cyclase activity by 5-HT alone (○) and by 5-HT in the presence of spiperone at 30 nM (■), 1 μM (●), and 10 μM (▼). Each point represents the mean increase in cAMP production from a single experiment in which basal enzyme activity was 56.4 ± 1.5 pmol of cAMP/min/mg of protein. Spiperone alone caused small decreases (≤10%) in basal activity; these decreased values were used in calculating the net stimulation by 5-HT. Spiperone had no effect on maximal stimulation by 5-HT in this experiment. Parallelism of the concentration-response curves was assessed as described in Materials and Methods. The estimated slope indices provided by the variable slope fit (curves not shown) were 0.82, 0.99, 1.06, and 0.63 for the curves in the presence of 0, 30 nM, 1 μM and 10 μM spiperone, respectively. The more complex variable slope model fit these data significantly better than the common slope model [$F(3,31)$ = 11.5]. The data were then fit simultaneously to four functions in the form of Eq. 3 (curves shown in figure). The mean stimulation by 0.4 μM 5-$CONH_2$-T in this experiment (28.7 pmol of cAMP/min/mg of protein) was used as an estimate of E_{maxH}. The fit provided the following estimates: E_{maxL} = 29.7 pmol of cAMP/min/mg of protein; for 5-HT, K_H = 38 nM, K_L = 392 nM; for spiperone: K_{BH} = 9 nM, K_{BL} = 2.1 μM.

the data were fit significantly better by the more complex "variable slope" model than by the "common slope" model (e.g., see legend to Fig. 6). Concentration-response curves for 5-HT obtained in the presence of ≤1 μM spiperone were consistently steeper than the control curve, whereas those obtained in the presence of ≥10 μM spiperone were shallower. If the nonparallelism of the 5-HT curves in the presence of spiperone is ignored and a Schild plot is constructed from fits to the common slope model, the slope of the plot (0.78 ± 0.07) is significantly less than 1.0. The conditions of the assay (nonphysiological buffer, inclusion of pargyline, 2 min incubation at 30°) made it very unlikely that the atypical antagonism was due to uptake or metabolism of 5-HT (47).

The two-receptor model explained the atypical antagonism of 5-HT by spiperone. Curves in the absence and presence of different concentrations of spiperone were fit simultaneously to a model describing the competition between 5-HT and spiperone for two independent receptors (Fig. 6):

$$E = \frac{E_{maxH}[A]}{K_H(1 + [B]/K_{BH}) + [A]} + \frac{E_{maxL}[A]}{K_L(1 + [B]/K_{BL}) + [A]} \quad (4)$$

where E represents agonist-stimulated adenylate cyclase activity; E_{maxH} and E_{maxL} represent the maximal stimulation due to R_H and R_L, respectively; $[A]$ represents agonist concentration; K_H and K_L represent the EC_{50} values of agonist A for R_H and R_L, respectively; $[B]$ represents antagonist concentration; and K_{BH} and K_{BL} represent the dissociation constants of the antagonist B at R_H and R_L, respectively. Eq. 4 is a simplified form of Eq. 3 where drug B has zero intrinsic activity at R_H and R_L, i.e., it is an antagonist at both receptors. This analysis yielded estimates of the apparent dissociation constants of spiperone for R_H and R_L, referred to as K_{BH} and K_{BL}, respectively.

Spiperone appeared to have considerably higher affinity for R_H (K_{BH} = 17 ± 4 nM) than for R_L (K_{BL} = 3.0 ± 1.6 μM). The higher affinity of spiperone for the receptor mediating the initial component of the 5-HT response curve is manifest in the steepening of the curve that occurs in the presence of low concentrations of spiperone. The component of the curve mediated by R_L is not initially affected, but the EC_{50} of 5-HT at R_H increases with increasing concentrations of spiperone (i.e., $K_H' = K_H(1 + [\text{spiperone}]/K_{BH})$, so that the relative positions of the two components are *reversed* ($K_H' > K_L$) as spiperone concentration is increased. In the presence of high concentrations of spiperone the slope of the 5-HT curve decreases because the upper component of the curve (now mediated by R_H) is shifted proportionately more than the lower component (see Ref. 41).

The effect of 100 nM spiperone on the concentration-response curve for bufotenine was measured in one experiment. As was found with 5-HT as the agonist, the curve in the presence of spiperone was significantly steeper and shifted to the right of the control curve; these data were adequately fit by the two-receptor model. The estimate of the K_{BH} of spiperone was 30 nM, which is comparable to the value obtained when 5-HT was the agonist (i.e., 17 nM).

In contrast to its complex antagonism of 5-HT (Fig. 6), spiperone (30 nM–10 μM) was a simple competitive antagonist of the first component of the 5-$CONH_2$-T curve (Fig. 7; Ref. 33). No deviation from parallelism was observed in any of five experiments. The slope of the Schild plot constructed from these experiments (1.10 ± 0.08) was not significantly different from 1.0. The intercept of a line constrained to slope = 1.0 was therefore used to estimate the dissociation constant of spiperone at the receptor (R_H) activated by low concentrations of 5-$CONH_2$-T (Fig. 8). The intercept (pA_2 = 7.62 ± 0.06) corresponds to a K_B of 24 nM.

As the stimulation by 8-OH-DPAT appeared to be mediated only by R_H, the effect of spiperone on the response to 8-OH-DPAT was tested. Spiperone (0.1–10 μM) was a simple competitive antagonist of 8-OH-DPAT, with pA_2 = 7.69 ± 0.13, or K_B = 21 nM.

The direct estimates of the affinity of spiperone for R_H

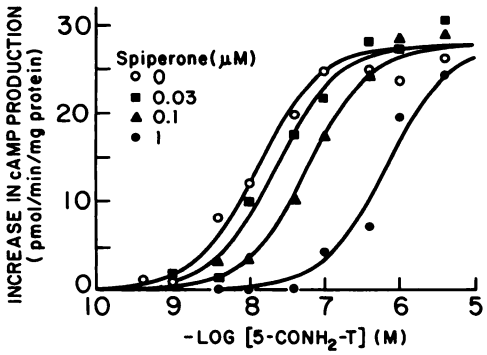

Fig. 7. Antagonism of 5-$CONH_2$-T by spiperone. Stimulation of adenylate cyclase activity by 5-$CONH_2$-T alone (○) and by 5-$CONH_2$-T in the presence of spiperone at 30 nM (■), 100 nM (▲), and 1 μM (●). Each point represents the mean increase in cAMP production from a single experiment in which basal enzyme activity was 45.3 ± 0.8 pmol of cAMP/min/mg of protein. The curves are the best fit to the common slope model described in Materials and Methods (slope index = 1.05). The data were not fit significantly better by the variable slope model [$F(3,25) = 1.48$]. The degree of shift produced by each concentration of spiperone was expressed as the ratio of EC_{50} values in the presence and absence of spiperone (concentration ratio, *CR*). The data from this experiment and four others were used to construct a Schild plot (Fig. 8).

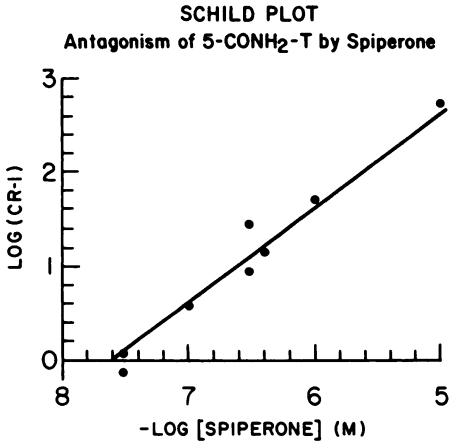

Fig. 8. Schild plot of antagonism of 5-$CONH_2$-T by spiperone. Schild plot of the data on antagonism of 5-$CONH_2$-T (0.4 nM–4 μM) by spiperone. The data are from five experiments, including results shown in Fig. 7 and Ref. 33. The regression of log (*CR*−1) vs. log [spiperone] resulted in a straight line with a slope (1.10 ± 0.08) not significantly different from 1.0. The intercept of a line constrained to slope = 1.0 (shown here) was used to estimate the K_B of spiperone at the receptor selectively activated by low concentrations of 5-$CONH_2$-T, i.e., R_H. The intercept ($pA_2 = 7.62 \pm 0.06$) corresponds to a K_B of 24 nM.

obtained with 5-$CONH_2$-T and 8-OH-DPAT are very close to the values obtained indirectly by computer-fitting of the data on antagonism of 5-HT or bufotenine by spiperone.

The low potency of 5-$CONH_2$-T in eliciting the second component of stimulation makes it very difficult to verify the K_B of spiperone for R_L that was inferred from fitting, i.e., 3 μM. Concentrations of spiperone as high as 10 μM did not shift the second component of the concentration-response curve for 5-$CONH_2$-T (33), and results with 40 μM spiperone were inconsistent. Thus, the K_{BL} of spiperone may be even greater than 3 μM.

Antagonism by ketanserin. Ketanserin, at a concentration of 100 nM, which is at least 50 times its K_B value for the 5-HT_2 receptor (2, 3), had negligible effect (<2-fold shift) on the concentration-response curves of 5-HT and 5-$CONH_2$-T (Table 1). Higher concentrations of ketanserin (1–50 μM) shifted the curve of 5-$CONH_2$-T to the right in a surmountable manner, but also caused a marked decrease in the slope of the curve ($n = 4$). High concentrations of ketanserin (4–100 μM) also shifted the 5-HT curve to the right, but the shifts appeared parallel; it is unclear whether this effect is produced by a complex inhibition of response mediated by R_H. Although preincubation of drugs and membranes for 8 min did not increase the magnitude of the shifts produced by ketanserin, under these conditions the maximal response to 5-HT was potentiated ($n = 2$; +30%, +76%) in the presence of 20 μM ketanserin.

Effects of substances active at 5-HT_3 receptors. (−)-Cocaine, which has a K_B of 1 μM for excitatory peripheral neuronal receptors (6), did not stimulate adenylate cyclase activity or affect the concentration-response curve of 5-HT at concentrations as high as 40 μM. MDL 72222, which was shown to have an IC_{50} of 1 nM for excitatory peripheral neuronal receptors (3, 4), was similarly inactive on the cyclase-coupled receptors at a concentration of 10 μM. Phenylbiguanide, a selective agonist approximately equipotent with 5-HT at certain peripheral neuronal 5-HT receptors (48), was also without effect on the cyclase-coupled receptors when tested at a concentration of 40 μM.

Is 8-OH-DPAT an antagonist at R_L? Although 8-OH-DPAT did not appear to be an agonist at R_L, the possibility that it was an antagonist at R_L was assessed. The effect of a selective partial agonist at R_H on the concentration-response curve of a drug that is a full agonist at R_H and R_L was computer simulated; it was first assumed that the partial agonist (drug *B*) had no affinity for R_L (Fig. 9). For purposes of illustration, the intrinsic activity of drug B was set at 0.5 ($\beta_H = 0.5$). The complex shift of the concentration-response curve that is produced by the selective partial agonist is due to its effect on the component of response mediated by R_H and its lack of effect on the component of response mediated by R_L. In the presence of increasing concentrations of *B*, the component of the response curve mediated by R_L does not shift. In the presence of 10,000 K_{BH} of *B*, the first and the final components of the response (25% stimulation each) are due to activation of R_H; the "middle" component (50% stimulation) is due to activation of R_L (Fig. 9). It is evident that, if drug *B* was also an antagonist

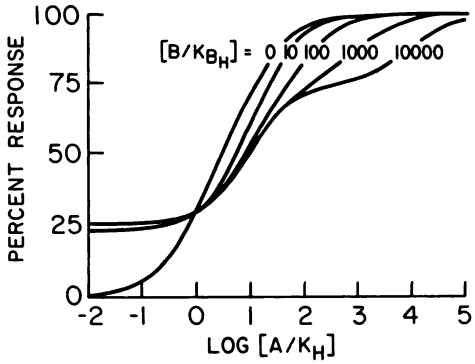

Fig. 9. Simulated effect of a partial agonist at R_H on the concentration-response curve to a drug that is a full agonist at R_H and R_L. Simulated interaction of an agonist, *A*, possessing 10-fold selectivity for R_H ($K_H = 1$, $K_L = 10$) with a drug, *B*, that is a partial agonist at R_H ($K_{BH} = 1$, $\alpha_H = 0.5$) and that has no affinity for R_L ($K_{BL} = \infty$). Maximal stimulation mediated by each receptor is 50% of the total response. The figure shows the response to *A* alone and *A* in the presence of increasing concentrations of *B* ([B/K_{BH}] = 0, 10, 100, 1,000, 10,000).

at R_L, its effect would be seen as a shift of the "middle" component of the curve to the right.

Fig. 10 shows that the upper part of the concentration-response curve to 5-HT was shifted slightly to the right in the presence of 50 μM 8-OH-DPAT (~1700 K_{BH}), a result that was expected from simulations similar to the one shown in Fig. 9. The data shown in Fig. 10 were adequately fit by a two-receptor model in which 8-OH-DPAT was assumed to have no affinity for R_L, i.e., the small shift is explained solely by the partial agonism of 8-OH-DPAT at R_H. A more complex model, which included the antagonist dissociation constant of 8-OH-DPAT at R_L as a variable parameter, did not provide a significantly better fit of the data. Similar results were obtained in two other experiments. At the concentrations tested, there was no evidence that 8-OH-DPAT is recognized at R_L.

Correlations between agonist potency and affinity for 5-HT$_1$-binding sites. The pharmacological characteristics of R_H suggested that it corresponds to the 5-HT$_{1A}$-binding site (33). This hypothesis was explored by comparing the negative logs of K_H values for the six agonists in Table 1 with the pK_D values of these agonists at 5-HT$_{1A}$-, 5-HT$_{1B}$-, and 5-HT$_{1C}$-binding sites in brain (12). There was a highly significant correlation between $-\log(K_H)$ and pK_D for 5-HT$_{1A}$ sites (r = 0.99, p = 0.005), but not for 5-HT$_{1B}$ (r = 0.57, p = 0.24) or 5-HT$_{1C}$ (r = −0.53, p = 0.28) sites. The exact potency of 8-OH-DPAT at R_L is not known, but the potencies of the remaining five agonists at R_L were not significantly correlated with their affinities for any of the 5-HT$_1$ subtypes.

Discussion

Stimulation of adenylate cyclase activity by 5-HT in membranes of adult guinea pig hippocampus was shown to be concentration dependent, GTP dependent, and additive to responses elicited by histamine (31), dopamine, and (−)-isoproterenol (Fig. 1), indicating that distinct 5-HT receptors mediate the effect. The finding that high concentrations of dopamine, greater than 10 μM, cross-react with 5-HT receptors in this preparation is not unprecedented: similarity in the recognition sites of some dopamine and 5-HT receptors has previously been suggested (27, 49). Tryptamine and 5-MeOT produced responses that were additive to the response to histamine, but not additive to the response produced by 5-HT (31), implying that these agonists act on the same receptors as does 5-HT. Analogous experiments showed that bufotenine and 5-CONH$_2$-T also act through these 5-HT receptors.

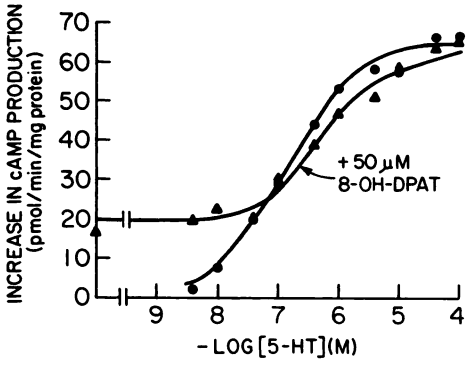

Fig. 10. Stimulation of adenylate cyclase activity by 5-HT alone (●) and by 5-HT in the presence of 50 μM 8-OH-DPAT (▲). Each point represents the mean increase in cAMP production from a single experiment in which basal enzyme activity was 53.2 ± 0.7 pmol of cAMP/min/mg of protein. The curves are the result of simultaneously fitting the response data for 5-HT alone and for 5-HT plus 8-OH-DPAT to Eqs. 2 and 3, respectively. Stimulation by 0.4 μM 5-CONH$_2$-T (26.6 pmol of cAMP/min/mg of protein) was used as an estimate of E_{max_H}. In the fit shown here, the K_{BH} of 8-OH-DPAT was set to 30 nM and it was assumed that 50 μM 8-OH-DPAT had no antagonist activity at R_L. The fit provided the following estimates: E_{max_L} = 38.0 pmol of cAMP/min/mg of protein; for 5-HT, K_H = 25 nM, K_L = 415 nM; for 8-OH-DPAT, β_H = 0.7. A more complex model in which K_{BL} was included as a variable parameter did not provide a significantly better fit to the data.

The two-receptor model. A series of observations showed that the 5-HT receptors linked to stimulation of adenylate cyclase activity were not homogeneous. The slopes of the concentration-response curves for 5-HT, 5-MeOT, bufotenine, and tryptamine were shallow and nonparallel, and the concentration-response curve for the 5-HT$_1$-selective agonist 5-CONH$_2$-T was biphasic (Fig. 2; Ref. 33). Additional evidence for the two-receptor model was obtained from experiments in which the response to one agonist was measured in the presence of a selected concentration of another agonist (Figs. 3 and 4). These data allowed the definition of a high affinity receptor, R_H, and a low affinity receptor, R_L (Table 1). 5-HT and 5-MeOT were essentially equipotent and exhibited 10-fold selectivity for R_H. Bufotenine and tryptamine were less potent at both receptors, and each was about 50-fold selective for R_H. 5-CONH$_2$-T was about 7-fold more potent than 5-HT at R_H, and was at least 5000-fold more potent at R_H than at R_L. The 5-HT$_{1A}$-selective agonist 8-OH-DPAT was a potent partial agonist at R_H, but showed no affinity for R_L (Figs. 5 and 10).

The two-receptor model explained the atypical antagonism of 5-HT by spiperone. Spiperone produced a nonparallel shift of the concentration-response curve of 5-HT (Fig. 6). The K_B values of spiperone at R_H and R_L were estimated from computer fits of the 5-HT data according to the two-receptor model. The K_B of spiperone for one of the receptors, R_H, was also directly and confidently determined with agonists that were highly selective for R_H. The K_B values of spiperone obtained with 5-CONH$_2$-T and 8-OH-DPAT as agonists were almost identical, namely, 24 nM and 20 nM. These values are similar to those obtained by computer fitting of the complex antagonism of 5-HT (K_{BH} = 17 nM) and bufotenine (K_{BH} = 30 nM).

Other systems have been described in which two populations of receptors mediating the same responses are revealed by nonparallel concentration-response curves for agonists and/or by the complex behavior of antagonists; changes in the slope of an agonist curve in the presence of a selective antagonist have been stimulated (41) and observed (53–57). Differing affinities of an antagonist for two receptors activated by a single agonist have been shown to result in a Schild plot with a shallow slope (47), as was found here with spiperone and 5-HT. Computerized simulations based on the two-receptor model reveal characteristics of the system that are not intuitively obvious (e.g., Fig. 9, Ref. 41). Even when computer-modeling methods are used, deviations from a one-receptor

model may be difficult to detect experimentally unless highly selective agonists or antagonists are available (41, 56, 57).

The lack of a selective agonist for R_L prevented direct measurements of the K_B of spiperone for R_L. Computer fitting of the antagonism of 5-HT and 5-$CONH_2$-T suggested a K_{BL} of at least 3 μM. Definitive characterization of R_L awaits the identification of selective agonists or preparations that contain only R_L.

Classification of the cyclase-linked 5-HT receptors. The relatively low affinity of ketanserin for R_H and R_L (Table 1) shows that neither receptor may be classified as a 5-HT_2 receptor, an inference consistent with previous reports that 5-HT receptors coupled to adenylate cyclase are not 5-HT_2-like (1, 29). Spiperone also has much lower affinity for R_H (K_{BH} = 20 nM) and R_L ($K_{BL} \geq 3$ μM) than for the 5-HT_2 receptor (K_B = 1 nM; Ref. 3). The complex actions of high concentrations of ketanserin (1–50 μM) observed in the cyclase system remain unexplained.

Neither R_H nor R_L resemble 5-HT_3 receptors. 5-MeOT is equipotent to 5-HT at both cyclase-linked receptors, but much less potent than 5-HT at 5-HT_3 receptors (4). (−)-Cocaine, MDL 72222, and phenylbiguanide, which are known to act at 5-HT_3 receptors in the periphery (4, 6, 48), were without effect on the cyclase-coupled 5-HT receptors.

Evidence suggests that R_H is a functional correlate of the 5-HT_{1A}-binding site in brain. R_H has been defined in guinea pig hippocampus, a region known to contain a high density of 5-HT_{1A}-binding sites (17). The dissociation constants of spiperone for R_H (20 nM) and for the 5-HT_{1A}-binding site in guinea pig hippocampus (13–18 nM; Ref. 17) are similar. Furthermore, the potencies of six agonists that activate R_H (Table 1) are highly correlated (r = 0.99) with their potencies in competing for 5-HT_{1A}-binding sites in brain (12). The 5-HT_{1A}-selective agonist 8-OH-DPAT is approximately equipotent with 5-HT in activating R_H and in binding to the 5-HT_{1A} site; 5-$CONH_2$-T is several times more potent than 5-HT in both systems (Table 1; Ref. 12). In addition, the atypical anxiolytic buspirone, recently shown to be selective for 5-HT_{1A}-binding sites (58), is also a potent partial agonist at R_H (K_H = 130 nM; Ref. 59).

The K_H values of agonists for stimulation of adenylate cyclase (Table 1) are 3- to 30-fold greater than their dissociation constants at 5-HT_{1A} sites (12). This difference may be a reflection of the different conditions used for the adenylate cyclase assay, notably the presence of guanine nucleotide, and would be consistent with the known effects of GTP in decreasing agonist affinity for 5-HT_{1A} sites in hippocampus (24, 60).

Membranes of adult rat hippocampus also exhibit 5-HT-stimulated adenylate cyclase activity (29, 32, 61), but maximal stimulation by 5-HT is only about 40%. Maximal response to 5-HT in hippocampal membranes of both guinea pig and rat is selectively increased by pretreatments known to deplete endogenous brain 5-HT (29, 31). Under our assay conditions, the EC_{50} value and the slope index of the concentration-response curve to 5-HT in adult rat hippocampal membranes did not differ from those in guinea pig membranes. In addition, the concentration-response curve to 5-$CONH_2$-T in rat resembled that in guinea pig, with 0.4 μM 5-$CONH_2$-T eliciting a response equal to approximately one-half the E_{max} produced by 5-HT.[2]

These findings suggest that a heterogeneous population of stimulatory 5-HT receptors might also be found in the rat. Under assay conditions different from ours, however, only evidence of the high affinity 5-HT_{1A} site was found (61). The existence of two cyclase-linked 5-HT receptors, one neuronal and one glial, was reported by Fillion (23) but was not corroborated (21, 24, 26, 30). Insufficient pharmacological data precludes relating the two receptors described by Fillion (23) to the two in guinea pig hippocampus.

There is no evidence to associate R_L with a known 5-HT_1-binding site subtype; this is in agreement with previous work that showed that low affinity 5-HT receptors linked to stimulation of cyclase activity do not correspond to 5-HT_1 sites (20, 21, 24, 62). The EC_{50} of 5-HT for R_L (0.4 μM) is similar to the EC_{50} of 5-HT found in several other cyclase systems (23, 26, 30, 62, 63). Furthermore, spiperone is a weak antagonist of R_L ($K_B \geq 3$ μM) and of 5-HT receptors associated with adenylate cyclase in cultured NCB-20 cells (63), in liver fluke (64), and in infant rat colliculi (20). 8-OH-DPAT, which was inactive at R_L (Figs. 5 and 10), is also inactive at a low affinity 5-HT receptor coupled to cAMP production in cultured murine neurons (62), and it has very low potency (EC_{50} = 9 μM) at the infant rat colliculi receptors (19). Additional studies are needed to establish whether R_L and the low affinity 5-HT receptors linked to adenylate cyclase in infant rat colliculi and in the other systems are homologous.

5-HT_{1A} receptor-mediated inhibition of adenylate cyclase activity. Under different assay conditions, the 5-HT_{1A} receptor in both guinea pig and rat hippocampal membranes mediates not stimulation, but inhibition of forskolin-stimulated adenylate cyclase activity (51, 52). Neurons cultured from fetal mouse brain also exhibit a high affinity 5-HT_{1A}-like receptor that inhibits vasoactive intestinal polypeptide-stimulated cAMP formation (62). In the experiments described here, 5-HT-mediated inhibition was not observed, nor was there pharmacological evidence of a mixed stimulatory/inhibitory effect by 5-HT or the other agonists. Additional work is needed to explore the finding that the 5-HT_{1A} receptor may mediate both stimulation and inhibition of adenylate cyclase activity *in vitro*, a finding that has precedents for other receptors (65). For example, the β-adrenergic receptor has similarly been shown to react not only with the guanine nucleotide-binding protein that stimulates cyclase activity, but also with the inhibitory protein that inhibits cyclase activity (66, 67).

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Send reprint requests to: Dr. Jack Peter Green, Chairman, Department of Pharmacology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029.