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STIMULATION AND INHIBITION OF ADENYLYL CYCLASE BY DISTINCT 5-HYDROXYTRYPTAMINE RECEPTORS

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Abstract—5-Hydroxytryptamine (serotonin, 5-HT) stimulates basal adenylyl cyclase activity in membranes from guinea pig or rat hippocampi, but 5-HT inhibits forskolin-stimulated adenylyl cyclase activity in these same membranes. The opposing effects of 5-HT on adenylyl cyclase activity indicate that distinct 5-HT receptors, positively and negatively coupled to adenylyl cyclase, are present in these membranes. Stimulation of adenylyl cyclase activity is mediated by two distinct 5-HT receptors. The receptor with lower affinity for 5-HT, designated as R_L, is apparently homologous with a 5-HT receptor present in rat collicular membranes, but it is not homologous with the stimulatory receptor characterized in neuroblastoma hybrid cell (NCB-20) membranes. The receptor with higher affinity for 5-HT is homologous with the 5-HT_{1A} binding site. The magnitude of stimulation by 5-HT_{1A} receptors is variable with respect to stimulation by R_L and is sometimes completely absent. Inhibition of forskolin-stimulated adenylyl cyclase activity, in membranes from either rat or guinea pig hippocampus or rat cortex, is a functional correlate of the 5-HT_{1A} binding site. This inhibitory response was used to determine the pharmacological characteristics of drugs that reportedly have high affinity for 5-HT_{1A} binding sites, such as 1-[2-(4-aminophenyl)ethyl]-4-(3-trifluoromethylphenyl)piperazine (PAPP) and (−)pindolol. PAPP inhibited adenylyl cyclase activity in guinea pig hippocampal membranes with an EC_{50} value of 27 ± 3 nM. (−)Pindolol was a partial agonist in inhibiting adenylyl cyclase activity in guinea pig and rat hippocampal membranes. Because of the low intrinsic activity of (−)pindolol, it was tested as an antagonist of the inhibition produced by 5-HT_{1A} receptor agonists in rat hippocampal membranes. The K_b of (−)pindolol was 40 nM as measured by a Schild plot. (−)Propranolol was a simple competitive antagonist at the rat hippocampal receptor with a K_b value of 550 nM. In summary, guinea pig and rat hippocampal membranes possess two distinct populations of 5-HT receptors, a 5-HT receptor that mediates inhibition of adenylyl cyclase activity and is pharmacologically homologous with the 5-HT_{1A} binding site, and a stimulatory receptor that appears to be homologous with the 5-HT receptor first characterized in infant rat collicular membranes.

In guinea pig and rat hippocampal membranes, 5-hydroxytryptamine (serotonin, 5-HT) stimulates basal adenylyl cyclase activity [1], but inhibits forskolin-stimulated adenylyl cyclase activity [2]. *Stimulation* by 5-HT of basal adenylyl cyclase activity in either rat or guinea pig hippocampal membranes has been characterized as mediated by 5-HT_1 receptors [3]. 5-HT_{1A} receptors [4], or a mixture of 5-HT_{1A} and a second, distinct 5-HT receptor [1]. *Inhibition* by 5-HT of forskolin-stimulated adenylyl cyclase activity in guinea pig or rat hippocampal membranes is a functional correlate of the 5-HT_{1A} binding site [2]. As such, measurement of the inhibition of forskolin-stimulated adenylyl cyclase activity is a useful response for evaluating the pharmacological characteristics of ligands that bind to 5-HT_{1A} binding sites.

In this report, we have partially characterized a 5-HT receptor, distinct from the 5-HT_{1A} receptor, that mediates stimulation of basal adenylyl cyclase activity in guinea pig hippocampal membranes. We compared the pharmacological characteristics of this stimulatory receptor with 5-HT receptors that mediate stimulation of adenylyl cyclase activity in other mammalian tissues, specifically neuroblastoma hybrid cell membranes (NCB-20) and rat collicular membranes. In addition, we have further characterized the 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase activity, and have compared the pharmacology of this response to behavioral and electrophysiological responses that are reportedly mediated by 5-HT_{1A} receptors.

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MATERIALS AND METHODS

Assay conditions. The assay conditions used to measure adenylyl cyclase activity were described previously [2]. Briefly, guinea pig or rat hippocampi were homogenized in 9 mL of a solution composed of 300 mM sucrose, 1 mM ethylene glycol bis[β-aminoethylether]-*N*,*N*'-tetraacetic acid (EGTA), 5 mM Na_2EDTA, 5 mM dithiothreitol and 20 mM Tris–HCl (pH = 7.4 at 23°). The homogenate was diluted 8-fold in the same medium and centrifuged at 500 *g* for 5 min at 0°. The supernatant fraction was centrifuged at 39,000 *g* for 10 min and the pellet from this centrifugation was resuspended in 9 mL of the homogenization buffer. These resuspended membranes were kept on ice until used; aliquots (50 μL) of the resuspended membranes were added to the incubation buffer to start the assay. The final components of the assay were: 10 μM forskolin, 100 mM

NaCl, 10 μM GTP, 2 mM magnesium acetate, 0.2 mM ATP, 1 mM cAMP, 80 mM Tris–HCl, 60 mM sucrose, 0.2 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 1–2 μCi of [α-^{32}P]ATP (New England Nuclear, Boston, MA), 20–60 μg of membrane protein, 4 mM theophylline, 10 μg creatine phosphokinase, 5 mM creatine phosphate, and various concentrations of agonists and antagonists. The assay was incubated at 30° for 315 sec and stopped by the addition of stopping solution [5]. The conversion of [α-^{32}P]ATP to [^{32}P]cAMP was measured as described by Salomon [5]. [^{3}H]cAMP (15,000 cpm) (New England Nuclear) was added to monitor recovery of [^{32}P]cAMP. Protein concentrations were measured by the method of Lowry *et al.* [6]. 1-[2-(4-Aminophenyl)ethyl]-4-(3-trifluoromethylphenyl)piperazine (PAPP)* was provided by Dr. Jean Shih and (−)pindolol was the gift of Sandoz Pharmaceuticals (Basel, Switzerland). *m*-Trifluoromethylphenylpiperazine (TFMPP) was obtained from the Aldrich Chemical Co. (Milwaukee, WI); (−)propranolol was obtained from Ayerst Laboratories (New York, NY). Sources of all other drugs have been reported previously [2].

Data analysis. Concentration–response data obtained from inhibition of adenylyl cyclase activity were analysed by fitting a three-parameter logistic equation to the data. The equation used was:

$$R = R_i - (R_i - R_f)/[([A]/EC_{50}) + 1] \quad (1)$$

where R is the rate of adenylyl cyclase activity (picomoles of cAMP/min/mg protein) in the presence of a specified concentration of agonist, $[A]$; R_f is the rate in the absence of an inhibitory agonist (i.e. the rate of forskolin-stimulated activity alone); R_i is the rate after maximal inhibition by the agonist; and EC_{50} is the concentration of the agonist that produces a half-maximal inhibition, or $(R_f + R_i)/2$.

Concentration–response data obtained from stimulation of adenylyl cyclase activity were analysed by fitting the following equation to the data:

$$R = R_s + (R_o - R_s)/(1 + [A]/EC_{50}) \quad (2)$$

Where R_o is the rate of adenylyl cyclase activity in the absence of a stimulatory drug (i.e. basal activity); R_s is the maximal rate after stimulation by a drug (E_{max}); EC_{50} is the concentration of drug that produces a half-maximal response; and $[A]$ is the concentration of drug that elicits a rate of R.

The K_b values of antagonists were determined by the method of Arunlakshana and Schild [7]. The equation used was:

$$\log [dr - 1] = n \cdot \log [B] - \log K_b \quad (3)$$

where dr is the ratio of the EC_{50} values of an agonist obtained in the presence and absence of a specified concentration of an antagonist, $[B]$; n is the slope of the regression line; and K_b is the dissociation constant of the antagonist. If the fit of Eqn 3 to the antagonist data was not improved significantly by letting n vary ($P = 0.05$), n was set equal to one before evaluating dissociation constants.

When single concentrations of antagonists were used to determine approximate dissociation constants of antagonists, the antagonist dissociation constant was estimated using the following equation [8]:

$$p = \frac{1}{1 + (K_a/[A])(1 + [B]/K_b)} \quad (4)$$

where p is the fractional receptor occupancy at a given concentration of an agonist, $[A]$; $[B]$ is the concentration of antagonist; and K_a and K_b represent the dissociation constants of the agonist and antagonist respectively. Assuming that the fractional response is proportional to receptor occupancy, Eqn 4 can be used to estimate the dissociation constant of an antagonist.

All experiments were repeated at least three times, and representative experiments are shown in the figures. Data points are the means of three measurements. Equations were fitted to the concentration–response data with a non-linear regression analysis program using FITFUN, a computer modeling program available on the PROPHET Computer System.

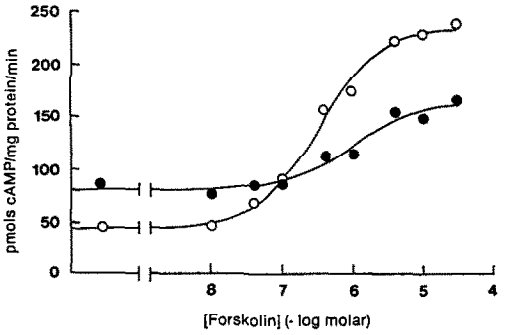

Fig. 1. Forskolin-stimulated adenylyl cyclase activity. Guinea pig hippocampal membranes were incubated with various concentrations of forskolin in the presence (●) or absence (○) of 10 μM 5-HT. Data points are means of three measurements. Data points preceding the break in the curves represent activity in the absence of forskolin and were included in the fit of Eqn 2 to the data.

RESULTS

Forskolin, with an EC_{50} value of 0.4 μM, stimulated adenylyl cyclase activity in guinea pig hippocampal membranes by 4- to 5-fold (Fig. 1). In the absence of forskolin, 5-HT stimulated basal adenylyl cyclase activity by an average of approximately 100%. Conversely, in the presence of concentrations of forskolin greater than 0.1 μM, 5-HT had a net inhibitory effect, with a maximal percentage of inhibition by 5-HT that averaged 30%. Histamine stimulated adenylyl cyclase activity in these membranes in the presence and absence of forskolin (data not shown).

Guinea pig hippocampal membranes were used to examine stimulation of adenylyl cyclase by 5-HT because they are more responsive to stimulation by

* Abbreviations: PAPP, 1-[2-(4-aminophenyl)ethyl]-4-(3-trifluoromethylphenyl)piperazine; TFMPP, *m*-trifluoromethylphenylpiperazine; PAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; 5-CT, 5-carboxamidotryptamine; cAMP, cyclic AMP; and Hepes, *N*-a-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

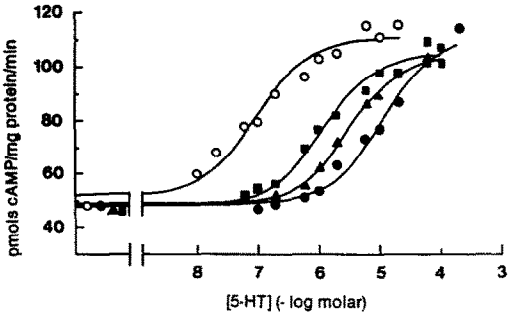

Fig. 2. Antagonism of 5-HT-stimulated adenylyl cyclase activity by spiperone, fluphenazine and mianserin. Guinea pig hippocampal membranes were incubated with various concentrations of 5-HT (○) in the presence of a 10 μM concentration of spiperone (●), fluphenazine (▲) or mianserin (■). Data points are means of three measurements. Data points preceding the breaks in the curves represent activity in the absence of 5-HT and were included in the fit of Eqn 2 to the data.

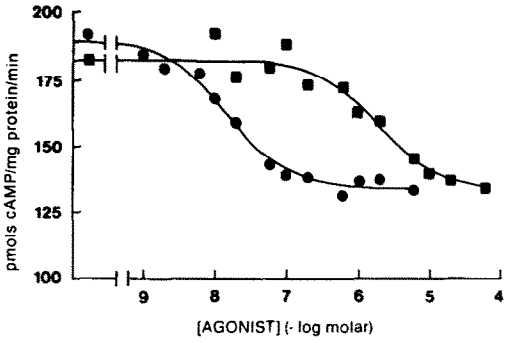

Fig. 3. Inhibition of adenylyl cyclase activity by PAPP and TFMPP. Guinea pig hippocampal membranes were incubated with 10 μM forskolin and various concentrations of PAPP (●) or TFMPP (■). The points preceding the break in the curve represent activity measured in the absence of agonist and were included in the fit of Eqn 1 to the data.

5-HT than are rat hippocampal membranes, although the two tissues appear to be qualitatively similar in their responsiveness to 5-HT [1]. 5-HT stimulated basal adenylyl cyclase activity in guinea pig hippocampal membranes with an EC_{50} of 200 ± 40 nM (mean ± SEM of three experiments). The concentration–response data were fit with equations derived from the interaction of receptors with a single, homogeneous population of receptors (Eqn 2). Stimulation by 5-HT was also measured in the presence of 10 μM spiperone, fluphenazine and mianserin. In the presence of each of these antagonists, the concentration–response curves to 5-HT were shifted to the right in a parallel manner (Fig. 2). As shown in Fig. 2, the concentration–response data fit the equations with a high coefficient of variation. The percentage of stimulation of adenylyl cyclase by 5-HT was the same in the absence and presence of the antagonists (approximately 100%). However, the EC_{50} value of 5-HT was increased in the presence of each of the antagonists. By determining the increase in the EC_{50} value of 5-HT in the presence of a 10 μM concentration of each of the antagonists, and using Eqn 4, an estimate was made of the dissociation constants of each of the antagonists. The K_b values of these three antagonists were estimated to be: 170 ± 8 nM (spiperone), 400 ± 45 nM (fluphenazine) and 1.8 ± 0.09 μM (mianserin) (values are geometric means of results from three experiments ± SEM). Stimulation by 5-HT_{1B} selective agonists, such as TFMPP, or 5-HT_{1A} selective agonists, such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (PAT), was small (<20% of the total stimulation produced by 5-HT in all of these experiments), and sometimes completely absent.

PAPP and TFMPP inhibited forskolin-stimulated adenylyl cyclase activity in cell-free preparations from guinea pig and rat hippocampi (Fig. 3). The concentration–response data were described by a simple logistic equation (Eqn 1). The EC_{50} values of PAPP and TFMPP were 27 ± 3 and 900 ± 160 nM respectively (values are geometric means from three experiments ± SEM). The average maximal percentages of inhibition of forskolin-stimulated activity were 33 ± 3 (PAPP) and 25 ± 5 (TFMPP) (values are means ± SD).

In membrane preparations from both guinea pig and rat hippocampi, (−)pindolol inhibited forskolin-stimulated adenylyl cyclase activity. The magnitude of inhibition by (−)pindolol was greater in guinea pig membranes than in rat membranes, and averaged about 9% of the total stimulation by forskolin. The magnitude of this response was too low to determine accurately the EC_{50} of (−)pindolol. Therefore, (−)pindolol was tested as an antagonist of the inhibition elicited by 5-HT. Concentration–response curves to 5-HT were shifted parallel and to the right in the presence of (−)pindolol (Fig. 4). In the presence of high concentrations of (−)pindolol, the magnitude of the maximal inhibition elicited by 5-HT was reduced from an average of 30% to an average of 22% of forskolin-stimulated activity, consistent with the partial agonism observed when (−)pindolol was tested in the absence of 5-HT. A Schild plot of the antagonism by (−)pindolol yielded a K_b value of 40 ± 4 nM (Fig. 4) in rat hippocampal membranes. A partial F test of the Schild plot indicated that letting the slope vary did not improve significantly the fit of the line to the data; therefore, the line was fitted with a slope of one to determine the pA_2 value of (−)pindolol.

Concentration–response curves to 5-carboxyamidotryptamine (5-CT) were shifted to the right in the presence of increasing concentrations of (−)propranolol (Fig. 5). The average inhibition by 5-CT in the presence of (−)propranolol was 29%, nearly identical to the average inhibition by 5-CT in the absence of (−)propranolol (30%) [2]. Similar results were obtained with 5-HT. A Schild plot of the antagonism by (−)propranolol yielded a K_b value of 550 ± 75 nM in rat hippocampal membranes. Letting the slope vary from one did not improve the fit of the line to the data; therefore, a unit slope was used to determine the pA_2 of (−)propranolol. The standard deviation was calculated as the standard

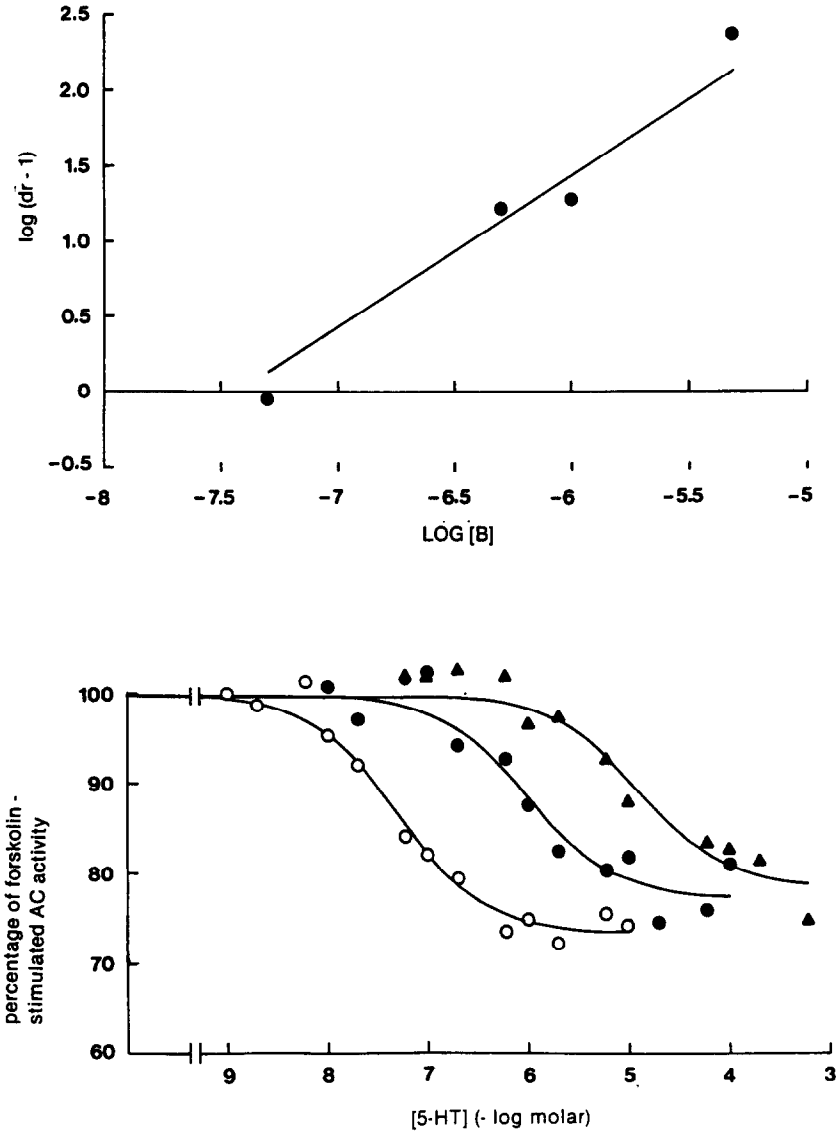

Fig. 4. Antagonism by (−)pindolol of 5-HT-inhibited adenylyl cyclase activity. Forskolin-stimulated activity in each experiment was normalized to 100 for comparison among experiments. Rat hippocampal membranes were incubated with 10 μM forskolin and various concentrations of 5-HT (○), in the presence of 0.5 (●) or 5 μM (▲) (−)pindolol. Forskolin-stimulated activity in the absence of 5-HT or (−)pindolol was 157 (●) or 151 (▲) pmol/mg protein/min. Equation 3 (Schild plot) was fitted to the dose-ratios obtained in the presence of four different concentrations of (−)pindolol. The equation was fitted with a unit slope ($r^2 = 0.96$).

deviation of the linear regression multiplied by the K_b value.

In membranes from rat cerebral cortex, no stimulation of adenylyl cyclase activity by 5-HT was measured at concentrations of 5-HT up to 100 μM (data not shown). However, inhibition of adenylyl cyclase activity by 5-HT was measured. The inhibition of forskolin-stimulated adenylyl cyclase activity by the 5-HT_1 selective ligand 5-CT and the 5-HT_{1A} selective ligand buspirone is shown in Fig. 6. The mean EC_{50} value of 5-CT was 11 ± 2 nM and the mean maximal percentage of inhibition was 17 ± 3% [data (mean ± SEM) from four experiments]. Buspirone was a partial agonist in this tissue, with a mean percentage of inhibition that was less than 10% of total forskolin-stimulated activity, and an EC_{50} of approximately 0.5 μM. Because of the low percentage of inhibition by buspirone, it was also tested as an antagonist in rat cerebral cortical membranes. Buspirone, at a concentration of 10 μM, shifted the concentration–response curve to 5-CT to the right by 10-fold, indicating that its K_b value was approximately 1 μM. Both PAT and 5-HT also potently inhibited forskolin-stimulated adenylyl

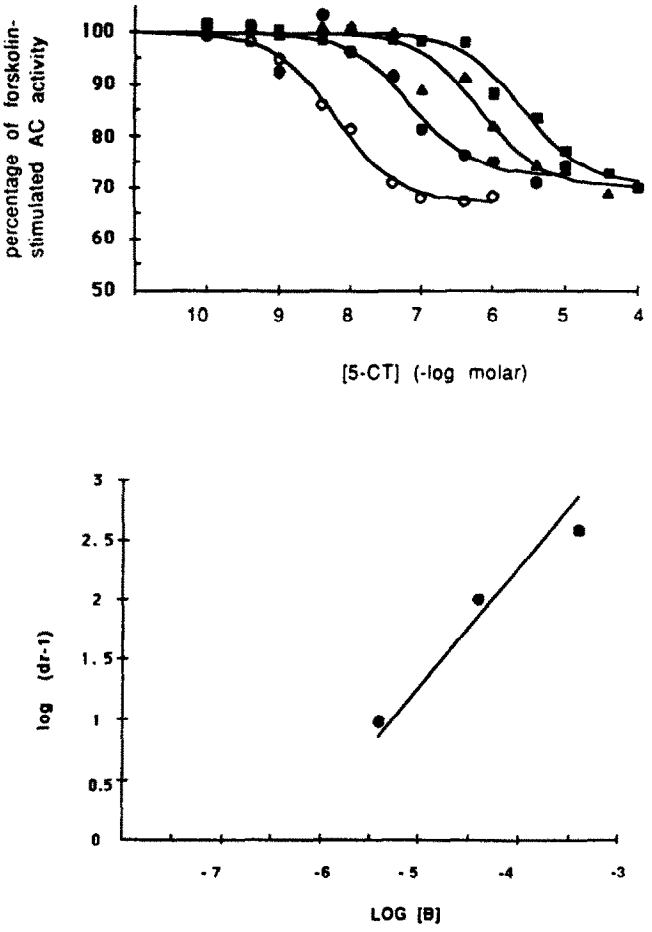

Fig. 5. Antagonism by (−)propranolol of 5-CT-inhibited adenylyl cyclase activity. Forskolin-stimulated activity in each experiment was normalized to 100 for comparison. Rat hippocampal membranes were incubated with various concentrations of 5-CT (○) in the presence of 4 (●), 40 (▲) or 400 (■) μM (−)propranolol. Forskolin-stimulated activity in each experiment was 348 (●), 323 (▲) or 297 (■) pmol/mg protein/min. Equation 3 (Schild plot) was fit to the dose-ratios obtained in the presence of different concentrations of propranolol.

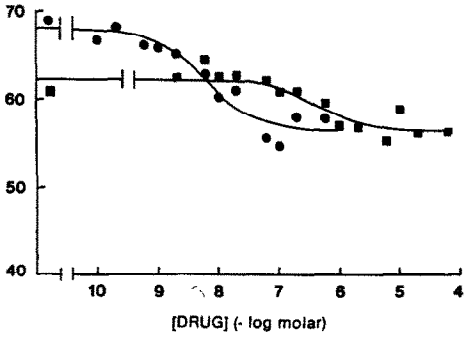

Fig. 6. Inhibition of adenylyl cyclase activity by 5-CT and buspirone in membranes from rat cerebral cortex. Rat cerebral cortical membranes were incubated with various concentrations of 5-CT (●) or buspirone (■). Data points preceding the break in the curve represent activity measured in the absence of agonist and were included in the fit of Eqn 1 to the data.

cyclase activity in rat cerebral cortical membranes, with EC_{50} values < 100 nM (data not shown).

DISCUSSION

Rat and guinea pig hippocampi have been used extensively to investigate the effects of 5-HT. In experiments using hippocampal tissue, 5-HT elicits different, and sometimes opposing, effects. For example, 5-HT increases and decreases the evoked population spike amplitude in the rat hippocampal slice preparation [9], 5-HT modulates three distinct potassium channels in rat hippocampal neurons [10], and 5-HT stimulates basal but inhibits forskolin-stimulated adenylyl cyclase activity in rat and guinea pig hippocampal membranes [1, 2]. These diverse effects of 5-HT suggest that multiple types of 5-HT receptors, each coupled to different effector systems, are present in the rodent hippocampus. By characterizing the receptors coupled to adenylyl cyclase, the relationship between these receptors and those

that are coupled to other effector systems (such as ion channels) may become more apparent.

Shenker *et al.* [1] characterized the stimulation of guinea pig hippocampal adenylyl cyclase by 5-HT and reported that stimulation is mediated by a mixture of two receptor populations: a receptor with high affinity for 5-HT that is congruent with the 5-HT_{1A} binding site, and a second receptor, with lower affinity for 5-HT, that was designated by them as R_L. The conditions used to measure stimulation of adenylyl cyclase activity in this report differ from those of Shenker *et al.* primarily in that we used a lower concentration of membrane protein (approximately 30 μg per assay as compared to 100 μg), and included 150 mM NaCl. Using the assay conditions described in this paper, the contribution of 5-HT_{1A} receptors to the total stimulation produced by 5-HT was reduced, and sometimes completely absent. The absence of a 5-HT_{1A} receptor component to stimulation was evidenced by the fact that, in some experiments, 5-HT_{1A} receptor-selective agonists such as PAT, PAPP and 5-CT at concentrations of 1 μM did not stimulate adenylyl cyclase activity, although 5-HT was still effective in stimulating adenylyl cyclase activity. Presumably, the 5-HT receptor that mediated stimulation of adenylyl cyclase activity in the absence of 5-HT_{1A} receptor-stimulated activity was the receptor with lower affinity for 5-HT (R_L) that was measured by Shenker *et al.* [1]. The fact that a pharmacological assay can be manipulated to produce different effects for a given receptor (i.e. 5-HT_{1A} receptors can mediate both stimulation and inhibition of adenylyl cyclase) supports the proposal that effector systems should not be used for the classification of receptor subtypes [11].

5-HT receptor-mediated stimulation of adenylyl cyclase has been described in membrane preparations other than guinea pig and rat hipocampus: in infant rat collicular membranes [12, 13] and in membranes from NCB-20 cells [14]. These preparations contain 5-HT receptors which are distinguished by differences in the apparent affinities of spiperone, fluphenazine and mianserin. In NCB-20 cell membranes, mianserin and fluphenazine are potent competitive antagonists at the NCB-20 5-HT receptor, with dissociation constants in the range of 10 to 100 nM, but spiperone is essentially ineffective in antagonizing the stimulation of cAMP production elicited by 5-HT in intact NCB-20 cells [15]. Nelson *et al.* [13] reported IC_{50} values for spiperone, fluphenazine and mianserin in antagonizing the stimulation of adenylyl cyclase by 5-HT at the rat collicular receptor. These IC_{50} values can be used to estimate dissociation constants of these antagonists by the Cheng–Prusoff equation [16]. Spiperone has an approximate K_i value of 200 nM, whereas fluphenazine and mianserin are less potent, with K_i values of 500 nM and 1 μM respectively. The K_b values of spiperone, fluphenazine and mianserin at the guinea pig hippocampal 5-HT receptor (see Results) closely matched the values obtained with rat collicular membranes, supporting the contention [1] that the guinea pig hippocampal 5-HT receptor is homologous with the infant rat collicular receptor, but it is not homologous with the NCB-20 receptor. This classification can only be tentative because of the lack of potent and selective antagonists for the stimulatory receptor. Although several authors have commented on the possibility that the collicular and hippocampal receptors may be congruous, little pharmacological evidence has been reported to support such a claim. The hippocampal 5-HT receptor does not appear to resemble any known binding site for 5-HT, nor can it be classified within any of the classification schemes established for functional 5-HT receptors [11, 17, 18]. Recently, however, Dumuis *et al.* [19] suggested that the collicular receptor may be of the 5-HT_3 class because ICS 205-930 (a selective antagonist of 5-HT_3 receptors) antagonizes the collicular receptor with a K_i of nearly 1 μM.

Inhibition of adenylyl cyclase activity by 5-HT in guinea pig and rat hippocampal membranes is mediated by 5-HT_{1A} receptors [2]. The classification of the inhibitory receptor as 5-HT_{1A} is strengthened by the following findings: PAPP was at least 20-fold more potent than TFMPP in inhibiting adenylyl cyclase activity in guinea pig hippocampal membranes (Fig. 3), close to the ratio of the affinities of these two drugs for 5-HT_{1A} binding sites in rat cortex [20]; the K_b values of (−)pindolol and (−)propranolol obtained in the adenylyl cyclase assay (Figs. 4 and 5) are close to the K_d values of these drugs for 5-HT_{1A} binding sites, 20 and 100 nM respectively [21]; in membranes from rat cerebral cortex, a tissue with a high density of 5-HT_{1A} binding sites [22], 5-HT_{1A} receptor-selective ligands inhibited forskolin-stimulated activity (Fig. 6).

The homology among 5-HT_{1A} receptors in rat hippocampal membranes and those 5-HT receptors that mediate other central effects of 5-HT in the rat was examined. Inhibition of the firing rate of raphe neurons may be mediated, at least in part, by 5-HT_{1A} receptors at the dorsal raphe nucleus [23, 24]. PAPP, administered intravenously to rats, inhibits the spontaneous firing activity of the raphe [25], whereas TFMPP is much less effective, similar to the ratio of the effective concentrations of these drugs in inhibiting adenylyl cclase activity (Fig. 3). (−)Propranolol competitively antagonizes the inhibition of raphe firing induced by ipsapirone in rat brain slices [24], and competitively antagonized the inhibition of adenylyl cyclase elicited by 5-HT_{1A} receptor agonists in rat hippocampal membranes (Fig. 5). Many other drugs that inhibit adenylyl cyclase activity in rat hippocampal membranes [2] also inhibit the firing of the raphe in rat brain slices [26–28], including lysergic acid diethylamide (LSD), buspirone and PAT. PAT is approximately twice as potent as LSD in inhibiting raphe firing, as it is in inhibiting adenylyl cyclase activity in rat and guinea pig hippocampal membranes.

(−)Pindolol blocks some aspects of the 5-HT behavioral syndrome that are induced by 5-HT_{1A} receptor agonists [29], suggesting that it is an antagonist at central 5-HT receptors. However, (−)pindolol also depresses the turnover of 5-HT *in vivo*, a characteristic of 5-HT receptor agonists [30]. The mixed agonist and antagonist actions of (−)pindolol can be explained by measuring its effect of adenylyl cyclase activity (Fig. 4). (−)Pindolol depressed the maximal percentage of inhibition by 5-HT, demonstrating that it is a partial agonist at the

5-HT_{1A} receptor. The partial agonism of (−)pindolol was more apparent when tested as an agonist in guinea pig hippocampal membranes, where it produced a small but measurable decrease in forskolin-stimulated adenylyl cyclase activity (data not shown). However, the low percentage of inhibition by (−)pindolol indicates that it has a low intrinsic efficacy at the 5-HT_{1A} receptor, and therefore (−)pindolol will block the effects of a full agonist such as 5-HT. Because intrinsic activity is a tissue-dependent phenomenon, the intrinsic activity of a partial agonist may be different depending on the concentration or sensitivity of receptors in a particular tissue [30, 31]. The results obtained with (−)pindolol are compatible with its having both agonist and antagonist properties in different pharmacological test systems, i.e. (−)pindolol blocks the 5-HT behavioral syndrome induced by 5-HT_{1A} receptor agonists, yet it depresses 5-HT turnover *in vivo*.

In addition to coupling to the adenylyl cyclase effector system, 5-HT_{1A} receptors are also directly coupled to potassium channels [32]. It is interesting to speculate on why 5-HT_{1A} receptors would mediate both activation of a potassium conductance and, concomitantly, inhibition of adenylyl cyclase activity. One possibility is that the reduction in cAMP contributes to an overall increase in potassium conductivity by activating potassium conductances that are regulated by cAMP. Another possibility is that the reduction in cAMP acts to coordinate other aspects of cell physiology with the hyperpolarized state of the neuron. For example, many of the steps involved in the synthesis and release of 5-HT from neurons are regulated by cAMP or calcium or both, including tryptophan transport into neurons, tryptophan hydroxylase activity, and the depolarization-induced release of 5-HT from terminals [33–36]. Therefore, 5-HT_{1A} receptor-activation, by decreasing intracellular cAMP, and by activating a potassium conductance and thereby reducing the influx of Ca^{2+}, may exert a concerted inhibitory effect on the synthesis and release of 5-HT (or of any other neurotransmitter whose synthesis and release are also regulated by cAMP and calcium). Inhibition of the synthesis and release of neurotransmitters may be a desirable goal of a neuron that is hyperpolarized and not firing.

In summary, guinea pig and rat hippocampal membranes possess two distinct populations of 5-HT receptors, a 5-HT receptor that mediates inhibition of adenylyl cyclase activity and is pharmacologically homologous with the 5-HT_{1A} binding site, and a stimulatory receptor that appears to be homologous with the 5-HT receptor first characterized in infant rat collicular membranes. The inhibitory 5-HT_{1A} receptor is apparently homologous with 5-HT receptors that mediate other central effects of 5-HT, including inhibition of raphe firing, depression of 5-HT turnover *in vivo*, and some aspects of the 5-HT behavioral syndrome. Measurement of adenylyl cyclase activity is an *in vitro* correlate of these *in vivo* effects of 5-HT_{1A} receptor activation.

Note. Dumuis *et al.* [37] and Oskenberg and Peroutka [38] have reported recently that (−)pindolol and (−)propranolol are competitive antagonists at the murine hippocampal neuron and rat hippocampal membrane 5-HT receptor that is negatively coupled to adenylyl cyclase. Their values for the affinity of these drugs agree with ours. However, we conclude from the concentration–response data with (−)pindolol that it is a partial agonist with low intrinsic efficacy at the 5-HT_{1A} receptor (Fig. 4), and not a simple, competitive antagonist.

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