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ANTAGONISM OF 5-HYDROXYTRYPTAMINE$_{1A}$ (5-HT$_{1A}$) RECEPTOR-MEDIATED MODULATION OF ADENYLATE CYCLASE ACTIVITY BY PINDOLOL AND PROPRANOLOL ISOMERS

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Abstract—The interactions of the steroisomers of pindolol and propranolol with 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) binding sites and adenylate cyclase activity were examined in rat hippocampus. (−)Pindolol and (−)propranolol displayed high affinity for 5-HT$_{1A}$ binding sites, and their affinities were not affected significantly by the addition of 10^{-4} M GTP to the radioligand assay. The selective 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) decreased forskolin-stimulated adenylate cyclase activity. The (−)isomers of pindolol and propranolol did not affect basal or forskolin-stimulated activity but, at a concentration of 10^{-5} M, they reversed the 8-OH-DPAT inhibition of the forskolin-stimulated cyclase activity. The (+)isomers were less potent in producing this effect. These data suggest that (−)pindolol and (−)propranolol are potent antagonists at 5-HT$_{1A}$ receptors in rat hippocampus.

Multiple 5-hydroxytryptamine (5-HT) receptors have been identified in the central nervous system [1, 2]. To a large degree, the determination of the functional significance of 5-HT receptor subtypes has been facilitated by the availability of potent agonists and antagonists. For example, 5-HT has been observed to modulate adenylate cyclase activity in brain. Stimulation of cyclic AMP (cAMP) by 5-HT has been reported [3–5], but only the infant rat colliculi system has been characterized extensively [6]. More recently, De Vivo and Maayani [7] and Bockaert *et al.* [8] reported an inhibition of forskolin-stimulated adenylate cyclase by 5-HT in guinea pig hippocampal membranes, and they suggested that this inhibitory response may be mediated by 5-HT$_{1A}$ receptors. This effect is mimicked by tryptamine analogs as well as by a variety of 5-HT$_{1A}$-selective agonists such as 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), buspirone and ipsapirone. Spiperone was reported to antagonize this response competitively [7, 8].

However, spiperone displays an affinity of 120 nM for 5-HT$_{1A}$ binding sites [9]. A more potent antagonist of 5-HT$_{1A}$ receptors has not yet been identified although such an agent might be a useful pharmacologic tool for the further analysis of 5-HT$_{1A}$ receptor function [10]. A variety of evidence suggests that certain beta-adrenergic drugs may interact with 5-HT receptors. For example, Middlemiss [11] showed that propranolol displaces [^{3}H]5-HT from its binding sites in rat frontal cortex and that the (−)stereoisomer has a 25-fold greater affinity than (+)propranolol for these sites. In addition, (−)propranolol and (−)pindolol block some of the behavioral effects elicited by serotonergic agonists [12, 13]. Physiological studies indicate that (−)propranolol blocks the inhibition of dorsal raphe cell firing induced by 8-OH-DPAT, whereas (+)propranolol is ineffective in antagonizing this effect [14]. Moreover, (−)pindolol and (−)propranolol exhibit K_i values of 4.5 and 160 nM, respectively, versus 5-HT$_{1A}$ binding sites in human frontal cortex [15]. These findings led us to analyze the abilities of two putative-adrenergic agents, pindolol and propranolol, to interact with both 5-HT$_{1A}$ binding sites and 5-HT modulated adenylate cyclase activity in rat hippocampal membranes.

MATERIALS AND METHODS

Radioligand binding assay. Receptor binding assays were performed according to the method of Peroutka [9]. Briefly, adult rat brains were purchased from Pel-Freez Biologicals (Rogers, AK) and were stored at −20° until needed. On the day of the study, the brains were thawed and the hippocampi were removed. Tissues were homogenized in 20 vol. of 50 mM Tris–HCl buffer (pH 7.7 at 25°) using a Brinkmann polytron and then centrifuged in an IEC B20A centrifuge at 49,000 *g* for 10 min. The supernatant fraction was discarded, and the pellet was resuspended in the same volume of Tris–HCl buffer and incubated at 37° for 10 min prior to a second centrifugation at 49,000 *g* for 10 min. The final pellet was resuspended in 80 vol. of Tris–HCl buffer containing 10 μM pargyline, 4 mM calcium chloride, and 0.1% ascorbic acid. The suspensions were immediately used in the binding assay. Radioligand binding studies consisted of 0.1 ml of [^{3}H]8-OH-DPAT, 0.1 ml buffer or displacing drug, and 0.8 ml tissue suspension. Binding assays which examined GTP effects on [^{3}H]8-OH-DPAT binding consisted of 0.1 ml [^{3}H]8-OH-DPAT, 0.1 ml of drug or buffer, 0.1 ml of either 10^{-3} M GTP or buffer, and 0.7 ml of tissue suspension diluted to 70 vol. A final concentration of 0.3 to 0.4 nM [^{3}H]8-OH-DPAT was

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used in all experiments. Assays were incubated at 25° for 30 min and then were rapidly filtered under vacuum through Whatman GF/B filters with two 5-ml washes using 50 mM Tris–HCl buffer. Radioactivity was measured by liquid scintillation spectroscopy in 5 ml of Aquasol (New England Nuclear, Boston, MA) at 54% efficiency. Specific binding was defined as the excess over blanks taken in the presence of 10^{-5} M 5-HT. In general, 80–90% of [^{3}H]8-OH-DPAT binding was specific. The IC_{50} values were determined by log-logit analysis of drug competition studies. K_i values were determined by the equation $K_i = IC_{50}/(1 + [L]/K_D)$ where $[L]$ is the concentration of radioligand. The K_D of [^{3}H]8-OH-DPAT was 1.0 nM in rat hippocampus.

Preparation of membranes for adenylate cyclase studies. Male Sprague–Dawley rats (180–200 g) were killed by decapitation, and the hippocampi were removed. Tissue medium was prepared daily (pH 7.4 at 23°) and contained 300 mM sucrose, 20 mM Tris–HCl, 1 mM ethylene glycol bis-(β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA), 5 mM Na_2EDTA, and 5 mM dithiothreitol. The hippocampi from each rat were homogenized by hand in 4 ml of ice-cold tissue medium. The homogenate was diluted 1:8 with medium and centrifuged at 39,000 *g* for 10 min at 4°. Pellets were resuspended in the same volume used for homogenization. This particulate fraction was maintained at 0° and assayed within an hour [7].

Measurement of adenylate cyclase activity. 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 (50–80 μg) of the hippocampal preparation. The final assay mixture consisted of 25 mM Tris–HCl (pH 7.4), 0.2 mM ATP, 5 mM magnesium acetate, 10 μM GTP, 10 μM pargyline, 0.6 mM ascorbate, 4 mM theophylline, 50 μM cAMP, 50 μg of creatine phosphokinase, 5 mM creatine phosphate, 1.5 μCi of [α-^{32}P]ATP, 60 mM sucrose, 0.2 mM EGTA, 1 mM Na_2 EDTA, 1 mM dithiothreitol and various concentrations of drugs. The incubation was carried out at 30° for 10 min. Assays were stopped by the addition of 100 μl of a solution containing 2% sodium lauryl sulfate, 45 mM ATP, 1.3 mM cAMP in Tris–HCl buffer (pH 7.5). After addition of [^{3}H]cAMP (15,000 cpm) to monitor recovery, the samples were boiled for 3 min and cooled to room temperature. Labeled cAMP was isolated by sequential chromatography on a Dowex 50 cation exchanger and on a neutral alumina as described by Salomon [16]. Adenylate cyclase activity was expressed as picomoles of cAMP per minute per milligram of protein. Protein was determined by the method of Lowry *et al.* [17] with bovine serum albumin as the standard and tissue medium as the blank.

Chemicals. Drug sources were as follows: 5-HT, GTP, ATP (disodium salt), cyclic AMP, creatine phosphate, creatine phosphokinase, EGTA, theophylline and pargyline (Sigma Chemical Co., St. Louis, MO); (−)- and (+)pindolol (Sandoz, Ltd., East Hanover, NJ); (−)- and (+)propranolol (Ayerst Laboratories, New York, NY); and [^{3}H]8-OH-DPAT (100 Ci/mmol) and [α-^{32}P]ATP (54 Ci/mmol) (Dupont-New England Nuclear, Boston, MA). Propranolol isomers were dissolved in assay buffer. Pindolol isomers were dissolved in ethanol at 10^{-3} M and then diluted in assay buffer.

RESULTS

Drug interactions with 5-HT_{1A} binding sites in rat hippocampal membranes. The apparent affinity (K_i; nM) of the isomers of pindolol and propranolol for 5-HT_{1A} receptors labeled by [^{3}H]8-OH-DPAT in rat hippocampal membranes was determined. As shown

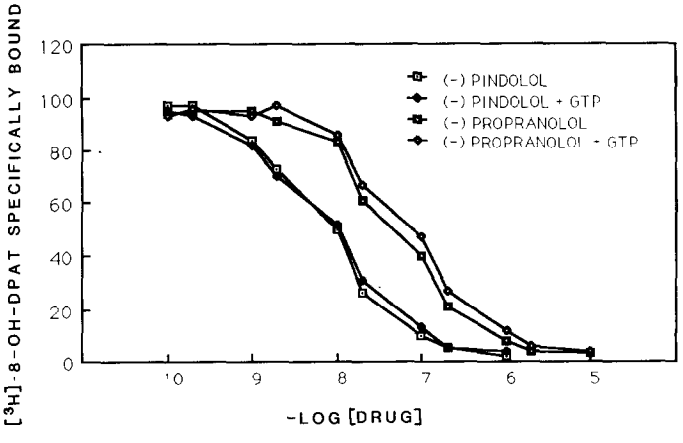

Fig. 1. Effect of 10^{-4} M GTP on (−)pindolol and (−)propranolol interactions with 5-HT_{1A} binding sites in rat hippocampus. Increasing concentrations of (−)pindolol and (−)propranolol were added to tissue suspensions containing 0.3 to 0.4 nM [^{3}H]8-OH-DPAT as described in Materials and Methods. Specific binding was defined as the excess over blanks taken in the presence of 10^{-5} M 5-HT. Specific binding in the absence and presence of 10^{-4} M GTP was 2214 and 974 cpm respectively. Data are expressed as the percentage of the specific binding of [^{3}H]8-OH-DPAT determined in the absence of the radioligand and represent the means of triplicate assays performed in a single experiment. Each experiment was repeated at least three times.

Table 1. Drug interactions with 5-HT_{1A} binding sites labeled by [^{3}H]8-OH-DPAT in rat hippocampus

Drug	K_i (nM) vs [^{3}H]8-OH-DPAT binding
(−)Pindolol	8.3 ± 1
(−)Propranolol	68 ± 10
(+)Pindolol	1400 ± 400
(+)Propranolol	3100 ± 400

Radioligand binding studies were performed as described in Materials and Methods. Data given are the means ± SEM of at least three experiments, each performed in triplicate. The IC_{50} values were determined by log-logit analysis, and apparent K_i values were calculated according to the equation $K_i = IC_{50}/(1 + [^3H]8\text{-OH-DPAT}/K_D)$.

in Table 1, (−)pindolol was the most potent agent with a K_i value of 8.3 ± 1 nM. (−)Propranolol was approximately an order of magnitude less potent than (−)pindolol. By contrast, (+)pindolol and (+)propranolol displayed only a moderate affinity for this binding site with K_i values in the micromolar range. Hill slope analysis of the competition curves for these drugs gave the following results: (−)pindolol = 0.90 ± 0.03; (−)propranolol = 1.0 ± 0.08; (+)pindolol = 1.0 ± 0.05; and (+)propranolol = 0.94 ± 0.03.

Effects of 10^{-4} M GTP on (−)pindolol and (−)propranolol interactions with 5-HT_{1A} binding sites. Drug competition studies were performed in the presence or absence of 10^{-4} M GTP. This nucleotide concentration was selected because it has been shown to reduce significantly [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors while allowing for an analysis of residual [^{3}H]8-OH-DPAT binding [18]. As shown in Fig. 1, a concentration of 10^{-8} M (−)pindolol competed for approximately 50% of specific [^{3}H]8-OH-DPAT binding. In the presence of 10^{-4} M GTP, the ability of (−)pindolol to compete for [^{3}H]8-OH-DPAT binding sites was essentially identical to its potency in the absence of GTP (Fig. 1). Similar data were observed with (−)propranolol. The K_i values for both (−)pindolol (9.8 ± 2 nM) and (−)propranolol (71 ± 5 nM) in the presence of 10^{-4} M GTP were not significantly different ($P > 0.05$; t-test) than the K_i values observed in the absence of GTP (Table 1).

Drug effects on basal adenylate cyclase activity. Basal adenylate cyclase activity in the rat hippocampal membrane preparation was approximately 15 pmol/mg protein/min. This basal level of adenylate cyclase activity was not stimulated by 5-HT or 8-OH-DPAT at concentrations ranging from 10^{-9} M to 10^{-3} M. The inability of 5-HT to alter basal cyclase activity in membranes from adult rats has been reported previously [19, 20].

Drug effects on forskolin-stimulated adenylate cyclase activity. Forskolin, at a concentration of 10^{-5} M, caused an approximately 8-fold stimulation of basal cyclase activity in our system. As shown in Fig. 2, the forskolin-induced stimulation was inhibited by 8-OH-DPAT at concentrations above 10^{-8} M. At a concentration of 10^{-6} M 8-OH-DAPT, the amount of forskolin-stimulated cyclase activity was reduced to 60% of initial levels, with only a minimal additional effect observed with 8-OH-DPAT concentrations as high at 10^{-4} M. By contrast, neither the pindolol nor propranolol isomers significantly altered the forskolin-stimulated cyclase activity in rat hippocampal membranes (Fig. 2).

Drug effects on 8-OH-DPAT modulation of forskolin-stimulated cyclase activity. The abilities of (−)pindolol, (−)propranolol, (+)pindolol and (+)propranolol to modulate 8-OH-DPAT inhibition of forskolin-stimulated adenylate cyclase activity were determined. As shown in Fig. 3, both (−)isomers were able to reverse the inhibition of

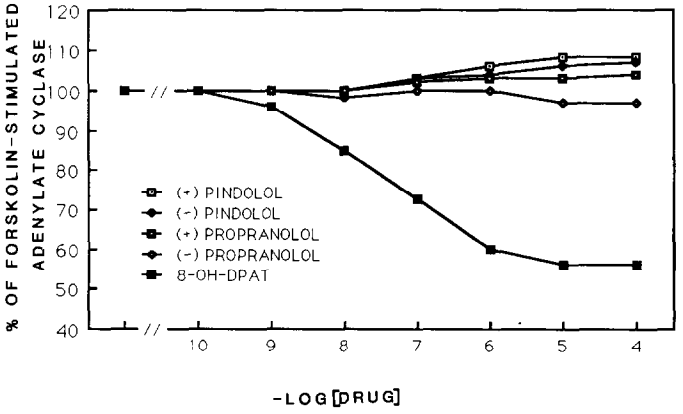

Fig. 2. Drug effects on forskolin-stimulated adenylate cyclase activity. Adenylate cyclase activity was determined as described in Materials and Methods. Various concentrations of 8-OH-DPAT, (−)pindolol, (+)pindolol, (−)propranolol and (+)propranolol were used to determine drug effects on forskolin-stimulated adenylate cyclase activity. The curves were normalized to 100% for each experiment. The data points represent the mean of three assays performed in a single experiment. The point preceding the break in the curve represents the mean of three measurements of forskolin-stimulated activity in the absence of drug. Each experiment was repeated three times.

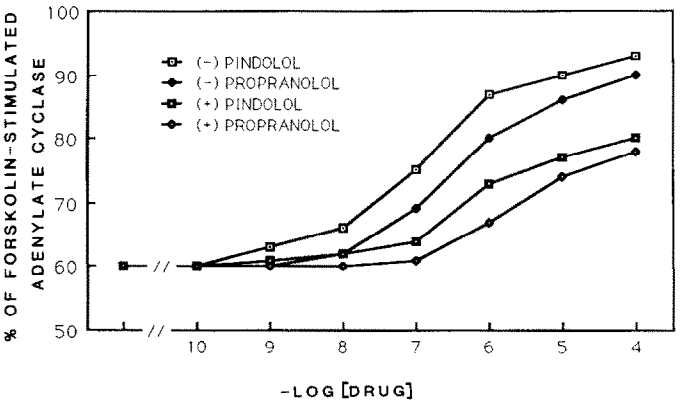

Fig. 3. Reversal of the effects of 8-OH-DPAT by (−)pindolol and (−)propranolol. The experimental conditions were the same as described in the legend of Fig. 2, with the exception that a concentration of 10^{-6} M 8-OH-DPAT was present in all assays. Various concentrations of (−)pindolol, (+)pindolol, (−)propranolol and (+)propranolol were analyzed. The data points represent the mean of three assays performed in a single experiment. Each experiment was repeated three times.

the forskolin-induced stimulation caused by 8-OH-DPAT. At a concentration of 10^{-5} M, both (−)pindolol and (−)propranolol reversed the effect of 10^{-6} M 8-OH-DPAT on forskolin-stimulated adenylate cyclase activity. The apparent K_i values of the antagonist effects of (−)pindolol and (−)propranolol were 8.4 ± 1 and 50 ± 4 nM respectively. The (+)isomers of pindolol and propranolol were considerably less potent in producing this effect (apparent K_i values = 63,000 ± 30,000 and 89,000 ± 48,000 nM respectively). Therefore, the rank order and absolute potencies of these drugs in the adenylate cyclase system are nearly identical to their affinities for the 5-HT_{1A} binding site (Table 1). We also tested the effect of spiperone in this system. As has been reported by other investigators [7, 8], spiperone (10^{-8} to 10^{-5} M) had no effect on basal cyclase activity but did block the effects of 5-HT over this same concentration range.

DISCUSSION

The major finding of the present study was that (−)pindolol and (−)propranolol were potent antagonists of 5-HT_{1A} receptor-mediated effects on adenylate cyclase in rat hippocampal membranes. Several authors have reported a 5-HT-stimulated adenylate cyclase [3–5]. However, most of these studies were performed before the heterogeneity of 5-HT_1 binding sites was elucidated. More recent studies have focused on the forskolin-stimulated adenylate cyclase system in hippocampus which has been shown to be inhibited by 5-HT_{1A} receptor agonists [7, 8]. Similarly, Weiss *et al.* [21] reported that 5-HT_{1A} receptors mediate inhibition of vasoactive intestinal polypeptide-stimulated cAMP production in purified striatal and cortical neurons in culture.

[^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors can be modulated by guanine nucleotides [18, 22, 23]. These GTP effects theoretically involve the binding of GTP to a G protein, which simultaneously modulates adenylate cyclase activity and decreases agonist affinities for the receptor without affecting the binding of antagonists [24–26]. Our results show that GTP does not shift the affinity of (−)pindolol or (−)propranolol, suggesting that these drugs are antagonists at 5-HT_{1A} receptors.

This conclusion is directly demonstrated in the present study since (−)pindolol and (−)propranolol potently antagonized 5-HT_{1A}-mediated adenylate cyclase inhibition in rat hippocampus. As shown above, (−)pindolol and (−)propranolol blocked the inhibitory effect of 10^{-6} M 8-OH-DPAT on forskolin-stimulated cyclase activity. These drugs appear to be pure antagonists at 5-HT_{1A} receptors since, when tested alone, the drugs had no effect on forskolin-stimulated cyclase activity. By contrast, it has been reported recently that (−)propranolol is a mixed agonist-antagonist at 5-HT autoreceptors in the rat hippocampus [27].

The data in the present study are also relevant for the functional analysis of 5-HT_{1A} receptors. For example, it has been reported that 5-HT_{1A} selective compounds like 8-OH-DPAT and ipsapirone inhibit raphe cell firing, and this effect can be blocked by (−)propranolol [14]. Our data confirm this hypothesis and indicate that (−)pindolol is an even more potent antagonist at the 5-HT_{1A} receptor.

The ability of the selective 5-HT_{1A} agonist 8-OH-DPAT to inhibit the effect of forskolin on adenylate cyclase activity also provides evidence that 5-HT_{1A} receptors inhibit adenylate cyclase activity via the inhibitory guanine nucleotide subunit, designated G_i protein. The diterpene forskolin stimulates adenylate cyclase in membranes from many rat tissues in a rapid and reversible manner [28, 29]. The forskolin-elicited increase in cAMP can be inhibited by a number of drugs including alpha$_2$-adrenergic antagonists, A_1-adenosine receptor agonists and opiates [30]. Inhibition of adenylate cyclase by these agents requires GTP and is mediated by G_i protein. Similarly, the ability of 5-HT_{1A} receptors to modulate forskolin-stimulated adenylate cyclase activity suggests a linkage to G_i in the rat hippocampus.

Andrade *et al.* [31] suggested that 5-HT_{1A} receptors in the rat hippocampus are linked to a G protein which directly couples 5-HT_{1A} receptors to potassium channels. The combination of our adenylate cyclase studies and their data suggests that 5-HT_{1A} receptors in rat hippocampus may be directly linked to two independent effector systems: a G protein which modulates potassium channels and a G_i protein which inhibits adenylate cyclase. Alternatively, the same G protein may be coupled to two independent effector systems. Studies are in progress to attempt to differentiate these possibilities.

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