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NUCLEOTIDE INTERACTIONS WITH 5-HT_{1A} BINDING SITES DIRECTLY LABELED BY [^{3}H]-8-HYDROXY-2-(DI-*n*-PROPYLAMINO)TETRALIN ([^{3}H]-8-OH-DPAT)

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Abstract—Nucleotide interactions were examined at 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) binding sites labeled by [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). At a 10^{-4} M concentration, GTP and GDP decreased specific binding of 0.4 nM [^{3}H]-8-OH-DPAT to 47 ± 4 and 61 ± 1% of control values respectively. This nucleotide effect was significantly greater ($P < 0.005$) than observed at total 5-HT_1 binding sites labeled by 1.5 nM [^{3}H]-5-HT. GMP and adenine nucleotides had a minimal effect on [^{3}H]-8-OH-DPAT binding at concentrations less than 10^{-3} M. Saturation experiments demonstrated that 10^{-4} M GTP increased the K_D of [^{3}H]-8-OH-DPAT for 5-HT_{1A} binding sites (0.79 to 2.7 nM) without changing the number of binding sites (1.98 to 1.93 pmoles/g tissue). The K_i values of classic and novel putative 5-HT agonists were increased 2- to 4-fold in the presence of 10^{-4} M GTP. Affinities of 5-HT antagonists for the [^{3}H]-8-OH-DPAT site were not affected by the addition of 10^{-4} M GTP to the binding assay.

Guanine nucleotides interact with many hormone and neurotransmitter receptor systems. In particular, GTP is necessary for the activation of neurotransmitter-sensitive adenylate cyclase systems [1]. In radioligand binding studies, the presence of GTP decreases the affinity of agonists for dopamine [2, 3] alpha-adrenergic [4–6], beta-adrenergic [7, 8] and 5-hydropytryptamine$_2$ (5-HT_2) [9] receptors. Guanine nucleotides have also been demonstrated to affect agonist interactions with the 5-HT_1 binding site [10–13]. As a result, it has been proposed that the 5-HT_1 binding site may mediate the activation of certain 5-HT-sensitive adenylate cyclase systems in brain membranes [14–17]. However, drug interactions with other 5-HT-sensitive cyclase systems in the central nervous system are clearly unrelated to the pharmacological profile at the 5-HT_1 site [11, 18].

Recently, [^{3}H]-5-HT binding to 5-HT_1 sites has been shown to be heterogeneous. In particular, 5-HT_{1A} and 5-HT_{1B} binding site subtypes have been defined on the basis of shallow drug inhibition curves using either spiperone or 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) [19–23]. The 5-HT_{1A} site has been analyzed extensively in both radioligand [24–26] and autoradiographic [27] studies and has a pharmacological profile which differs from that observed with total 5-HT_1 binding. By contrast, minimal data exist concerning the pharmacologic characteristics of the putative 5-HT_{1B} site [28]. Furthermore, a third subtype of 5-HT_1 binding site, the 5-HT_{1C} site, has been characterized recently in choroid plexus membranes [29]. Of these three known subtypes of the 5-HT_1 binding site, the 5-HT_{1A} site is the only subtype which can be directly labeled in neuronal membranes by radioligands [24–26]. Although GTP has been reported to affect [^{3}H]-8-OH-DPAT binding [24, 25], a detailed evaluation of nucleotide effects at this binding site has not been reported. In the present study, nucleotide effects were examined at 5-HT_{1A} binding sites directly labeled with [^{3}H]-8-OH-DPAT and at total 5-HT_1 sites labeled by [^{3}H]-5-HT.

METHODS

Receptor binding assays were performed according to the methods of Peroutka and Snyder [30]. Briefly, adult rat brains were purchased from Pel-Freez Biologicals (Rogers, AK) and stored at −20° until needed. On the day of the study, the brains were defrosted and the frontal cortex was dissected. 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.

Binding assays which examined the nucleotide effects on ^{3}H-ligand binding consisted of 0.1 ml of ^{3}H-ligand (final concentrations: 0.15 to 0.20 nM [^{3}H]-8-OH-DPAT; 1.5 to 2.0 nM [^{3}H]-5-HT), 0.8 ml of tissue suspension, and 0.1 ml of nucleotide or drug. Drug competition studies were performed

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using 0.1 ml of ^{3}H-ligand, 0.1 ml of drug or buffer, 0.1 ml of either 10^{-3} M GTP or buffer, and 0.7 ml of 70 vol. tissue suspension. 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 blank taken in the presence of 10^{-5} M 5-HT. In general, 70–80% of [^{3}H]-5-HT binding and 80–90% of [^{3}H]-8-OH-DPAT binding was specific.

All drugs were dissolved and diluted in the assay buffer, with the following exceptions: spiperone was dissolved first in 0.05 ml of glacial acetic acid; pirenperone was dissolved first in 1 ml ethanol; and ketanserin, buspirone and 2-(4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl)-1,2-benzisothiazol-3-(2*H*)one-1,1-dioxidehydrochloride (TVX Q 7821) were dissolved in H_2O at a concentration of 10^{-3} M. All drugs were subsequently diluted in assay buffer. Drugs were obtained from the following sources: [^{3}H]-5-HT (30 Ci/mmole; New England Nuclear, Boston, MA); [^{3}H]-8-OH-DPAT (85 Ci/mmole; Research Products International Corp., Mount Prospect, IL); 8-OH-DPAT, metatrifluoro-methylphenyl-piperizine (TFMPP), and 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeDMT) (Research Biochemicals, Inc., Waltham, MA); ketanserin, spiperone, and pirenperone (Janssen Pharmaceutical, Beerse); 5-HT, 5-methoxytryptamine (5-MT), GTP, GDP, GMP, ATP, ADP, and AMP (Sigma Chemical Co., St. Louis, MO); 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl) 1*H* indole (RU 24969) (Roussell, Paris); TVX Q 7821 (Troponwerke, Cologne); *d*-lysergic acid diethylamide (*d*-LSD) (National Institute on Drug Abuse, Bethesda, MD); and buspirone (Bristol Myers, Evansville, IN).

RESULTS

Nucleotide interactions with 5-HT_{1A} and total 5-HT_1 binding sites. Guanine and adenine nucleotide interactions with 5-HT_{1A} binding sites labeled by [^{3}H]-8-OH-DPAT and total 5-HT_1 binding sites labeled by [^{3}H]-5-HT were examined. At the 5-HT_{1A} binding site (Fig. 1), GTP and GDP produced marked displacement of [^{3}H]-8-OH-DPAT binding. Inhibition of binding was first observed at approximately 10^{-5} M nucleotide. At a concentration of 3×10^{-5} M GTP and GDP, 76 and 69% of total specific [^{3}H]-8-OH-DPAT binding was observed, respectively, compared to control values. By 10^{-4} M, both GTP and GDP reduce [^{3}H]-8-OH-DPAT binding to 43% of control values. The inflection point (determined visually) of the inhibition curve is at approximately 10^{-4} M, and the slope of the curve continually decreases between 10^{-4} M and 10^{-3} M concentrations of GTP and GDP. A similar "plateau" of GTP effects on [^{3}H]-8-OH-DPAT binding has been reported recently [25]. By 10^{-3} M nucleotide, GTP and GDP reduced [^{3}H]-8-OH-DPAT

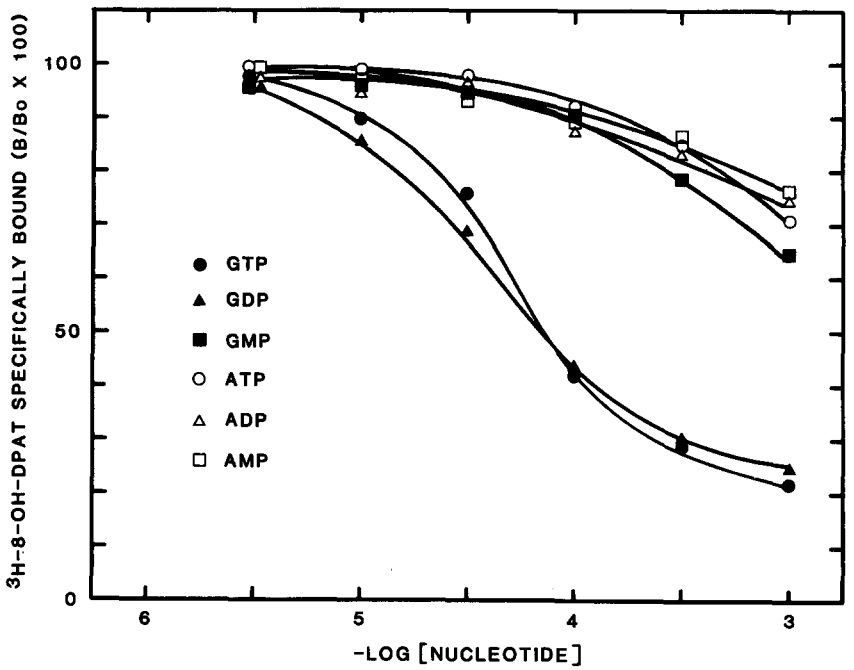

Fig. 1. Effects of increasing concentrations of nucleotide on the specific binding of [^{3}H]-8-OH-DPAT to rat cortical homogenates. Increasing concentrations of GTP (●), GDP (▲), GMP (■), ATP (○), ADP (△), and AMP (□) were added to tissue suspensions containing 0.2 nM [^{3}H]-8-OH-DPAT as described in Methods. Specific binding was defined as the excess over blanks taken in the presence of 10^{-5} M 5-HT. Values are expressed as the percentage of the specific binding of [^{3}H]-8-OH-DPAT determined in the absence of nucleotides. Data are the means of triplicate assays performed in a single experiment. Each experiment was repeated six to ten times with values which varied less than 20%.

binding to 22 and 25% of control values respectively. By contrast, no effect on the specific binding of [^{3}H]-8-OH-DPAT was observed with either GMP or the adenine nucleotides until a concentration of 10^{-4} M, at which point approximately 87–92% of control values was observed. At a concentration of 3×10^{-4} M, GMP reduced specific binding to the 5-HT_{1A} site to 79% of control values, whereas the adenine nucleotides reduced [^{3}H]-8-OH-DPAT binding to approximately 85% of control levels. At 10^{-3} M nucleotide, GMP reduced [^{3}H]-8-OH-DPAT binding to 65% of control valves whereas the adenine nucleotides only reduced binding to 70–77% of control values.

At a concentration of 10^{-4} M, nucleotide interactions were examined at 5-HT_{1A} sites labeled by 0.4 nM [^{3}H]-8-OH-DPAT and at total 5-HT_1 sites labeled by 1.5 nM [^{3}H]-5-HT in rat cortical homogenates. As shown in Table 1, the effect of GTP in reducing [^{3}H]-8-OH-DPAT binding to the 5-HT_{1A} site (47 ± 4% of control values) was significantly greater than its effect at total 5-HT_1 sites (74 ± 6% of control values) $P < 0.001$). Similarly, GDP decreased the binding of [^{3}H]-8-OH-DPAT (61 ± 1% of control values) significantly more ($P < 0.05$) than it decreased [^{3}H]-5-HT binding to 5-HT_1 sites (80 ± 6% of control values). GMP and the adenine nucleotides had minimal and similar effects (92–100% of control values) at a concentration of 10^{-4} M on both 5-HT_{1A} sites labeled by [^{3}H]-8-OH-DPAT and total 5-HT_1 sites labeled by [^{3}H]-5-HT.

Saturation curve analysis of GTP interactions with 5-HT_{1A} binding sites labeled by [^{3}H]-8-*OH-DPAT.* The binding of increasing concentrations of [^{3}H]-8-OH-DPAT was analyzed in the absence or presence of 10^{-4} M GTP. The concentration of [^{3}H]-8-OH-DPAT ranged from 0.1 to 9.0 nM. Specific binding at a concentration of 0.1 nM [^{3}H]-8-OH-DPAT represents approximately 80% of total binding. The amount of specific [^{3}H]-8-OH-DPAT binding plateaus at approximately 4 nM where specific binding represented approximately 60% of total binding. In the presence of 10^{-4} M GTP, the amount of specific binding was reduced to approximately 40–50% of control values at a concentration range of 0.1 to 1 nM. As the concentration of [^{3}H]-8-OH-DPAT was increased, the amount of specific binding began to approach the values obtained in the control conditions. Half-maximal binding was observed at approximately 2.5 nM. As shown in Fig. 2, Scatchard analysis of the [^{3}H]-8-OH-DPAT saturation data was monophasic in both the absence and presence of 10^{-4} M GTP. In the absence of GTP, [^{3}H]-8-OH-DPAT had a B_{max} value of 1.98 pmoles/g tissue and a K_D value of 0.79 nM. In the presence of 10^{-4} M GTP, the B_{max} value was unchanged (1.93 pmoles/g tissue) but the slope of the Scatchard plot was decreased markedly, resulting in a K_D value of 2.7 nM.

Effects of 10^{-4} M GTP on drug competition for 5-HT_{1A} binding sites. A series of drug competition studies was performed in the presence or absence of 10^{-4} M GTP. This concentration of GTP was selected on the basis of its ability to significantly decrease [^{3}H]-8-OH-DPAT binding while allowing a measurable amount of residual [^{3}H]-8-OH-DPAT binding to the 5-HT_{1A} site. At the 5-HT_{1A} site labeled by [^{3}H]-8-OH-DPAT, all agents produced monophasic displacement with Hill values of approximately unity (Fig. 3). For example, 5-HT displaced specific [^{3}H]-8-OH-DPAT binding at concentrations ranging from 10^{-10} M to 3×10^{-8} M. In the presence of 10^{-4} M GTP, the displacement curve was shifted to the right, corresponding to a 3- to 4-fold increase in the IC_{50} value, with no change in the slope of the displacement curve (Fig. 3A). Similarly, 8-OH-DPAT (Fig. 3B) produced monophasic displacement of [^{3}H]-8-OH-DPAT with an IC_{50} value of approximately 0.7 nM. Again, an approximately 4- to 5-fold shift in the IC_{50} value was observed, with 8-OH-DPAT displacement of [^{3}H]-8-OH-DPAT in the presence of 10^{-4} M GTP. By contrast (Fig. 3C), 10^{-4} M GTP had no effect on the displacement of [^{3}H]-8-OH-DPAT by metergoline.

Table 1. Effect of 10^{-4} M nucleotide on ligand binding to 5-HT_{1A} and total 5-HT_1 binding sites

	% Specific binding of control values		
Nucleotide	[^{3}H]-8-OH-DPAT (5-HT_{1A})	[^{3}H]-5-HT (5-HT_1)	P value
GTP	47 ± 4	74 ± 6	<0.001
GDP	61 ± 1	80 ± 6	<0.05
GMP	96 ± 2	97 ± 4	NS
ATP	92 ± 2	97 ± 3	NS
ADP	93 ± 2	99 ± 1	NS
AMP	93 ± 5	100 ± 2	NS

Binding assays were performed as described in Methods. Specific binding was defined as the excess over blanks taken in the presence of 10^{-5} M 5-HT. Values are given as the percentage of specific ^{3}H-ligand binding determined in the absence of nucleotide (260 ± 7 cpm). Studies were performed using 0.4 nM [^{3}H]-8-OH-DPAT or 1.5 nM [^{3}H]-5-HT (each concentration representing approximately 50% of the radioligand K_D value under control conditions). Each result is the mean ±S.E. of three to ten experiments. The significance level (P value) between nucleotide effects at 5-HT_{1A} and total 5-HT_1 sites was determined using the two-tailed *t*-test. NS = not significant.

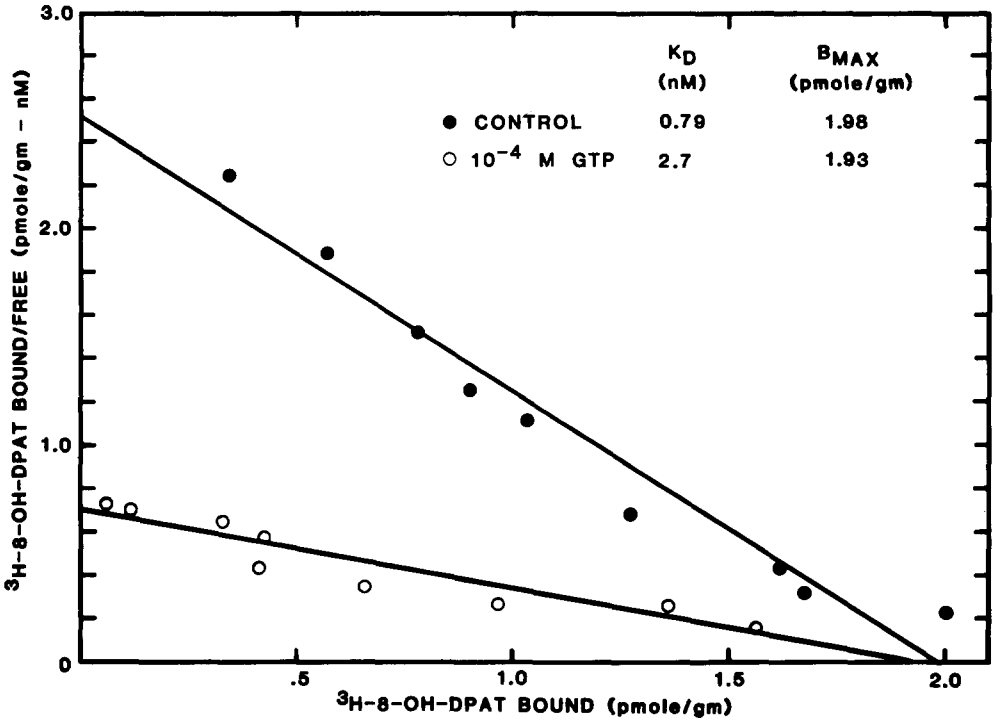

Fig. 2. Scatchard analysis of [^{3}H]-8-OH-DPAT binding in the absence or presence of 10^{-4} M GTP. Saturation experiments were performed using increasing concentrations of [^{3}H]-8-OH-DPAT ranging from 0.1 to 9 nM in the absence (●) or presence (○) of 10^{-4} M GTP. Binding assays were performed as described in Methods. Specific binding was defined as the excess over blanks taken in the presence of 10^{-5} M 5-HT. Data are the means of triplicate assays performed in a single experiment. Linear regression analysis was used to determine the axis intercept points. A significant correlation coefficient ($P < 0.01$) was obtained for a linear fit for both conditions. The experiment was repeated three times with results which varied by less than 20%.

K_i values for [^{3}H]-8-OH-DPAT binding sites were determined for a series of serotonergic drugs in the absence or presence of 10^{-4} M GTP. As shown in Table 2, the K_i values of classical and novel serotonergic agonists increased significantly by the addition of GTP. For example, the K_i of 5-HT for

Table 2. Drug affinities for 5-HT$_{1A}$ binding sites in the absence or presence of 10^{-4} M GTP

	K_i (nM)		
Drug	[^{3}H]-8-OH-DPAT	[^{3}H]-8-OH-DPAT + 10^{-4} M GTP	P value
8-OH-DPAT	0.56 ± 0.06	1.6 ± 0.3	<0.05
d-LSD	1.2 ± 0.05	3.6 ± 0.5	<0.01
5-HT	1.2 ± 0.05	3.5 ± 0.7	<0.01
5-MT	1.8 ± 0.3	8.2 ± 2	<0.05
5-MeDMT	1.9 ± 0.2	3.5 ± 0.2	<0.01
TVX Q 7821	2.3 ± 0.08	4.0 ± 0.5	<0.05
RU 24969	2.9 ± 0.5	5.0 ± 0.8	<0.05
Buspirone	7.6 ± 2	19 ± 4	<0.05
TFMPP	75 ± 5	210 ± 50	<0.05
Metergoline	3.7 ± 0.2	4.7 ± 0.7	NS
Spiperone	140 ± 10	190 ± 30	NS
Pirenperone	1100 ± 300	1200 ± 300	NS

Binding studies were performed as described in Methods. Data are the means ±S.E. of three to eight experiments, each performed in triplicate. IC_{50} values were determined by log-logit analysis and apparent K_i values were calculated by $K_i = IC_{50}/(1 + [^3H]\text{-}8\text{-}OH\text{-}DPAT/K_D)$. K_D values in the absence (0.79 nM) and presence (2.7 nM) of 10^{-4} M GTP were obtained from the experiment shown in Fig. 2. The mean K_i values were statistically compared using the two-tailed *t*-test. NS = not significant.

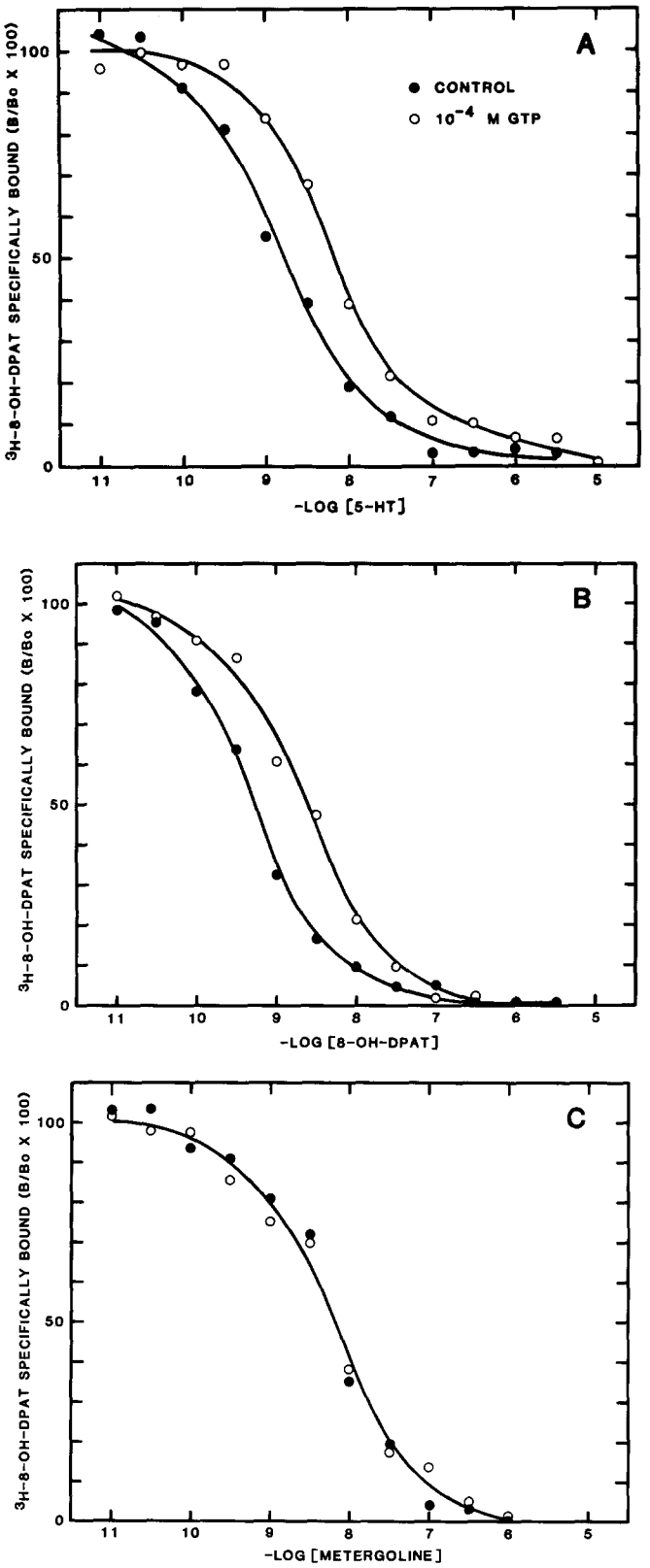

Fig. 3. Effect of 10^{-4} M GTP on drug inhibition of [^{3}H]-8-OH-DPAT binding in rat cortical membranes. Binding experiments were performed as described in Methods using 0.2 nM [^{3}H]-8-OH-DPAT and increasing concentrations of 5-HT (A), 8-OH-DPAT (B) and metergoline (C) in the absence (●) or presence (○) of 10^{-4} M GTP. The data shown are the results of a single experiment, performed in triplicate. Experiments were repeated three to four times.

5-HT_{1A} sites was increased significantly from 1.2 to 3.5 nM ($P < 0.01$) by the addition of 10^{-4} M GTP. Similarly, the K_i values for 5-MT, 5-MeDMT, and *d*-LSD were increased significantly by a factor of 2- to 4-fold in the presence of 10^{-4} M GTP. The affinities of the RU 24969, TFMPP, buspirone, and TVX Q 7821 for the [^{3}H]-8-OH-DPAT binding site were also affected significantly by 10^{-4} M GTP. The K_i values for these novel putative agonists were shifted 2- to 3-fold. These shifts are in agreement with the 3-fold increase in the K_D value of [^{3}H]-8-OH-DPAT for the 5-HT_{1A} site induced by 10^{-4} M GTP. By contrast, the affinities of serotonergic antagonists (metergoline, spiperone, and pirenperone) for 5-HT_{1A} sites were not affected by the addition of GTP.

DISCUSSION

The major finding of the present study is that GTP and GDP were potent and selective inhibitors of [^{3}H]-8-OH-DPAT binding to 5-HT_{1A} receptors. These nucleotides decreased the affinity of [^{3}H]-8-OH-DPAT for its binding site without changing the number of recognition sites. In addition, the inclusion of 10^{-4} M GTP resulted in a significant increase in the apparent K_i of putative agonists but not antagonists at the 5-HT_{1A} site. By contrast, GMP and the adenine nucleotides were inactive at this specific subtype of 5-HT_1 receptor. Therefore, these findings are similar to previous studies of nucleotide effects at total 5-HT_1 sites labeled by [^{3}H]-5-HT [10–13]. However, the effects of GTP and GDP at 5-HT_{1A} sites observed in the present study were more potent than found at total 5-HT_1 binding sites.

In certain systems, a functional relationship has been proposed between high affinity [^{3}H]-5-HT binding sites and a 5-HT-sensitive adenylate cyclase [14–17, 31]. For example, cyclase activity in horse striatal synaptosomal membranes is stimulated by nanomolar concentrations of 5-HT and inhibited by serotonergic antagonists in the same rank order as is high affinity [^{3}H]-5-HT binding [32, 33]. Similarly, kainic acid destroys both 5-HT-sensitive cyclase activity and high affinity [^{3}H]-5-HT binding in rat striatum [14]. In adult rat hippocampal membranes, 5-HT-stimulated cyclase activity is inhibited by metergoline and spiperone but not by ketanserin or mianserin [16], a pattern consistent with mediation by 5-HT_1 receptors. In other systems, however, no correlation exists between 5-HT-sensitive cyclase activity and [^{3}H]-5-HT binding sites [11, 18, 34]. In particular, in newborn rat, drug interactions with a 5-HT-sensitive cyclase do not correlate with affinities for total [^{3}H]-5-HT binding sites [11].

The inability to correlate certain 5-HT-sensitive cyclase systems with drug affinities for total 5-HT_1 binding may be secondary to the heterogeneity of these binding sites. For example, 8-OH-DPAT has nanomolar affinity for the 5-HT_{1A} site, yet its IC_{50} value at total 5-HT_1 binding sites is in the micromolar range [22–24]. In guinea pig hippocampal membranes, 8-OH-DPAT is a potent partial agonist (EC_{50} = 30 nM) of the 5-HT-sensitive cyclase [35]. The ability of 8-OH-DPAT to stimulate this cyclase might have been considered evidence that the cyclase was not related to total 5-HT_1 sites. By contrast, the concentration of [^{3}H]-8-OH-DPAT producing half-maximal stimulation of adenylate cyclase activity in newborn rat colliculi is 8600 nM [23]. Thus, depending on the selectivity of the agents studied, the pharmacological profile at the 5-HT_{1A} site may not correlate with drug effects at total 5-HT_1 binding sites. The pharmacologic differentiation of 5-HT_{1A} from total 5-HT_1 sites may facilitate correlations between radioligand data and 5-HT-sensitive adenylate cyclase systems. Future studies are needed to more clearly delineate nucleotide effects at other 5-HT_1 subtype sites.

The effect of GTP on 5-HT_{1A} sites is approximately an order of magnitude more potent than previously documented for heterogeneous 5-HT_1 sites. For example, Peroutka *et al.* [10] showed that 10^{-3} M GTP reduced [^{3}H]-5-HT binding to 50–60% of control values. In the present study, by contrast, 10^{-4} M GTP and GDP produced an even greater decrease in the binding of [^{3}H]-8-OH-DPAT to the 5-HT_{1A} binding site. In many 5-HT-sensitive adenylate cyclase systems, the potency of GTP in facilitating 5-HT-induced stimulation of cyclic AMP production is observed at concentrations of 10^{-6} M to 10^{-4} M [11, 12, 16, 33, 36]. Thus, the GTP effect observed at 5-HT_{1A} sites is nearly equipotent with the effects observed on GTP facilitation of the 5-HT-sensitive cyclase in the rat and guinea pig hippocampus and newborn rat colliculi [11, 12, 16, 36]. Theoretically, these GTP effects involve the binding of GTP to a G protein, which simultaneously stimulates cyclase activity and decreases agonist affinities for the receptor [37–39]. The ability of GTP to affect both 5-HT-sensitive cyclase stimulation and the binding of [^{3}H]-8-OH-DPAT at similar concentrations suggests that the 5-HT_{1A} site may be linked to an adenylate cyclase in the central nervous system.

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