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Serotonin Receptor Binding Sites Affected Differentially by Guanine Nucleotides

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SUMMARY

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Guanosine triphosphate (GTP) and diphosphate (GDP) decrease the binding of the agonist [^{3}H]serotonin (5-HT) to serotonin receptors in mammalian brain membranes. Binding of the antagonist [^{3}H]spiroperidol is not affected by guanine nucleotides while the mixed agonist-antagonist [^{3}H]lysergic acid diethylamide ([^{3}H]LSD) is affected in an intermediate manner. GTP lowers the apparent affinity of [^{3}H]5-HT for its receptors without changing the number of binding sites. Both the association and dissociation rates of [^{3}H]5-HT are increased by GTP. The potency of 5-HT agonists in competing for [^{3}H]5-HT but not [^{3}H]LSD or [^{3}H]spiroperidol binding sites is decreased by GTP. Competition by partial agonists and antagonists for [^{3}H]5-HT binding sites is not affected by GTP.

INTRODUCTION

The activation of many adenylate cyclase systems is regulated by guanine nucleotides (1, 2). Receptor binding sites for hormones such as glucagon (3), angiotensin (4), and prostaglandin E (5), and neurotransmitters which are associated with adenylate cyclases are also regulated by guanine nucleotides. In general, the affinity of agonists but not antagonists for receptor binding is decreased by guanine nucleotides in β-adrenergic (6), α-adrenergic (7), dopamine (8-10) and opiate (11, 12) systems. Guanine nucleotides may control the physiological response to hormones and neurotransmitters by affecting the coupling of the receptor and adenylate cyclase (1-13).

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In brain homogenates, a specific serotonin sensitive adenylate cyclase system has been described in the rat (14, 15). The serotonin-sensitive adenylate cyclase is located to synaptic membranes and its density throughout the brain parallels regional distributions of endogenous serotonin content (15). Though nucleotide influences have not been examined in brain homogenates, guanine nucleotides do regulate the serotonin-sensitive adenylate cyclase system in the liver fluke, *Fasciola hepatica* (16).

Postsynaptic serotonin receptors in the brain can be labeled by the agonist [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) (17), the mixed agonist-antagonist [^{3}H]lysergic acid diethylamide ([^{3}H]LSD) (17-20) and the antagonist [^{3}H]spiroperidol (21, 22). Differential drug affinities and regional variations in receptor number indicate that [^{3}H]5-HT and [^{3}H]spiroperidol label distinct popula-

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tions of serotonin receptors while [^{3}H]LSD labels both sites (23). In the present study we describe the regulation by guanine nucleotides of serotonin receptors labeled by the agonist [^{3}H]5-HT, while the binding of the antagonist [^{3}H]spiroperidol is not influenced by guanine nucleotides.

MATERIALS AND METHODS

Various brain regions were dissected from freshly decapitated male Sprague-Dawley rats (150–200 g) and mice. Frozen guinea pig brain (Pel-Freez) and calf brain freshly obtained from a local slaughterhouse were also utilized. All tissues were homogenized in 10 volumes of 0.32 M sucrose using a motor driven pestle. The homogenate was centrifuged at 700 × *g* for 10 min in a Sorvall RC2-B centrifuge. The supernatant fluid was decanted from the crude nuclear pellet (P1) and centrifuged at 50,000 × *g* for 10 min. The sedimented material (P2) was resuspended in 10 volumes of 50 mM Tris-HCl buffer (pH 7.5 at 25°) using a Brinkmann Polytron for 10 sec. The tissue suspension was then incubated at 37° for 10 min in the manner described by Nelson *et al.* (24) and centrifuged again at 50,000 × *g* for 10 min. The pellet was resuspended in the standard assay buffer which consisted of 50 mM Tris-HCl (pH 7.7 at 25°), 4 mM $CaCl_2$, 10 μM pargyline, and 0.1% ascorbic acid. The final tissue suspension was incubated for 15 min at 37° and then stored on ice until used.

Incubation tubes received 100 μl of [^{3}H]ligand, 100 μl of various drugs, and 0.8 ml of tissue suspension during standard binding assays. Assays were performed in triplicate. The concentrations of labeled ligands were 2.0 nM [^{3}H]5-HT, 3.7 nM [^{3}H]-LSD and 0.24 nM [^{3}H]spiroperidol. The final tissue concentration was 10 mg/ml of wet weight brain tissue in all regions except the caudate where 3.5 mg/ml of wet weight brain tissue was used. The tubes were incubated at 37° (10 min for [^{3}H]5-HT and [^{3}H]LSD and 15 min for [^{3}H]spiroperidol) and then rapidly filtered under vacuum through Whatman GF/B filters with three 5 ml rinses of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°). The filters were counted by liquid scintillation spectrometry in 9 ml of Formula 947 (New England Nuclear) after 18 hr extraction at 4° at efficiencies of 38–40%.

Specific binding of the [^{3}H]ligands was defined as the excess over blanks taken in the presence of 1 μM d-LSD. Generally, 70% of total binding was specific for [^{3}H]5-HT and [^{3}H]LSD while 60% of total [^{3}H]spiroperidol binding was specific. [^{3}H]5-HT (28.2 Ci/mmole) and [^{3}H]spiroperidol (23.6 Ci/mmole) were obtained from New England Nuclear. [^{3}H]LSD (15.3 Ci/mmole) and [^{3}H]GTP (14.0 Ci/mmole) were obtained from Amersham. [^{3}H]GTP metabolism was analyzed by thin layer chromatography on PEI-cellulose F (Merck) pre-coated sheets using a 0.2 M Li Cl_2/0.2 M formic acid solvent system. The R_f values for GTP[1], GDP and GMP were 0.06, 0.14 and 0.53, respectively. All ligands were dissolved in the standard assay buffer immediately before use. Guanine and adenine nucleotides were obtained from Sigma except for Gpp(NH)p which was from P-L Biochem. All drugs were dissolved in distilled water and diluted as necessary in the standard assay buffer. The sources of other drugs were as follows: 5-HT, Sigma Chemical Co.; 5-methoxytryptamine, Aldrich Chemical Co.; d-LSD, National Institute on Drug Abuse; bufotenine, Regis Chemical Co.; spiroperidol, Janssen Pharmaceutica; clozapine, Sandoz Pharmaceuticals.

RESULTS

Differential effects of guanine nucleotides on [^{3}H]5-HT, [^{3}H]LSD and [^{3}H]spiroperidol binding associated with serotonin receptors. The drug specificity of [^{3}H]5-HT and [^{3}H]LSD binding in rat frontal cerebral cortex indicates an association with serotonin receptors (17–20, 23). The drug specificity of [^{3}H]spiroperidol in the rat frontal cortex also displays properties expected of a serotonin receptor with no evidence for binding to dopamine receptors (21–23).

At 1 mM concentration GTP reduces specific [^{3}H]5-HT binding by about 45%, while the nonmetabolizable analogue of GTP, 5′-

[1] The abbreviations used are: GTP, guanosine 5′-triphosphate; GDP, guanosine 5′-diphosphate.

guanylyl-imidodiphosphate (Gpp(NH)p), reduces the binding of the agonist [^{3}H]5-HT by about 80% (Table 1). GDP lowers [^{3}H]5-HT binding to about the same extent as GTP, while GMP has a negligible influence on [^{3}H]5-HT binding. The adenine nucleotides ATP, ADP and AMP displace only 5–10% of [^{3}H]5-HT binding while a 15% reduction is apparent with the stable ATP analogue, App(NH)p.

In contrast to the influence of guanine nucleotides on [^{3}H]5-HT binding, adenine or guanine nucleotides do not reduce [^{3}H]spiroperidol binding in the rat frontal cerebral cortex. [^{3}H]LSD is affected in a fashion intermediate to that of [^{3}H]5-HT and [^{3}H]spiroperidol. GTP and GDP reduce [^{3}H]LSD binding 21–27% while Gpp(NH)p lowers binding almost 40%. As observed with [^{3}H]5-HT binding, GMP and the adenine nucleotides do not markedly reduce the binding of [^{3}H]LSD.

Detailed displacement curves indicate that about 80% of specific [^{3}H]5-HT binding is lowered by Gpp(NH)p over the concentration range 10 μM–1 mM (Fig. 1A). The displacement curves for GTP and GDP are more shallow, with only about a 40% reduction in binding occurring over the same concentration range. No reduction in [^{3}H]5-HT binding is apparent for GMP or the adenine nucleotides over the range 1 μM–300 μM.

Guanine nucleotides have a lesser effect on [^{3}H]LSD than on [^{3}H]5-HT binding (Fig. 1B). An approximately 40% reduction in [^{3}H]LSD binding occurs with Gpp(NH)p over the concentration range 50 μM-1 mM. GTP and GDP display somewhat lesser influences on [^{3}H]LSD binding while GMP and the adenine nucleotides are inactive at any concentration between 1 μM and 1 mM. With [^{3}H]spiroperidol binding, neither guanine nor adenine nucleotides lower specific binding at any concentration examined over the range of 1 μM–1 mM (Fig. 1C).

The activity of the serotonin-sensitive adenylate cyclase differs in various brain regions (14, 15) as does the binding of several ligands labeling serotonin receptors (18, 19). Accordingly, we have explored the influence of nucleotides on [^{3}H]5-HT binding in various regions of calf, rat, mouse, and guinea pig brain (Table 2). In all regions of all species examined, guanine nucleotides exert essentially the same effect at 1 mM concentrations. GTP reduces binding 40–50%, similar to the effect of GDP. Gpp(NH)p lowers binding 80–90% while GMP has negligible effects on binding. Adenine nucleotides displace 0–20% of [^{3}H]5-HT binding.

Stability of GTP under assay conditions. The potency of guanine nucleotides in reducing [^{3}H]5-HT binding is substantially less than has been reported at other neurotransmitter receptor binding sites (7–12). This lesser potency does not appear to be due merely to the metabolic degradation of the added guanine nucleotides for several reasons. First, the reduction by guanine nucleotides of [^{3}H]5-HT binding in rat frontal cerebral cortex is the same at 4 ° as at 37° (Table 3). Second, to directly evaluate GTP disposition under our present incubation conditions, we examined the identity of radioactivity in the media after incubating membranes with [^{3}H]GTP under conditions identical to those used in the binding studies. Thin layer chromatographic analysis (n = 3) of [^{3}H]GTP after incubating with tissue membranes for 10 min at 37° reveals that 98 ± 2% of the radioactivity remains as GTP. Third, Gpp(NH)p, the nonmetabolized analogue of GTP, is also

TABLE 1

Effect of nucleotides on [^{3}H]ligand binding to serotonin receptors in rat frontal cerebral cortex

The standard [^{3}H]ligand binding assays were conducted as described in MATERIALS AND METHODS. Nucleotides were added at 1 mM final concentration. Values are the percent specific [^{3}H]ligand binding determined in the absence of nucleotides. The results are the means ± standard errors of four to nine experiments, each performed in triplicate.

	[^{3}H]5-HT	[^{3}H]LSD	[^{3}H]spiroperidol
GTP	55 ± 1	73 ± 2	102 ± 4
GDP	64 ± 2	79 ± 2	102 ± 3
GMP	98 ± 3	92 ± 2	98 ± 4
Gpp(NH)p	21 ± 2	62 ± 5	93 ± 7
ATP	90 ± 3	90 ± 6	95 ± 5
ADP	90 ± 3	98 ± 3	95 ± 6
AMP	94 ± 1	99 ± 2	92 ± 5
App(NH)p	84 ± 1	96 ± 2	102 ± 5

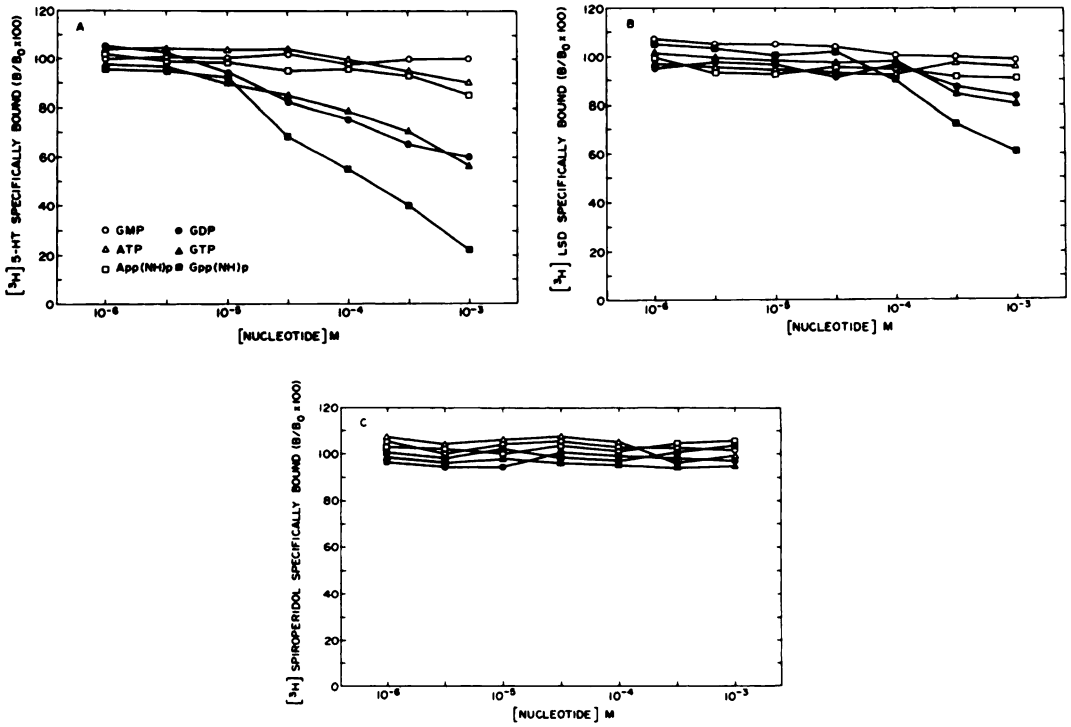

FIG. 1. *Effects of increasing concentrations of nucleotides on the specific binding of [³H]ligands to rat frontal cerebral cortex tissue*

Increasing concentrations of (○) GMP, (●) GDP, (▲) GTP, (■) Gpp(NH)p, (△) ATP and (□) App(NH)p were added to a P2 fraction prepared from the frontal cerebral cortex of adult male rats. Specific binding for each ligand, in the absence or presence of nucleotides was measured as described in the MATERIALS AND METHODS. Values are expressed as a percentage of the specific binding determined in the absence of nucleotides. (B_o). Each point is the average of triplicate determinations. Values in three separate experiments varied less than 20%. (A) [³H]5-HT (B) [³H]LSD (C) [³H]spiroperidol.

much weaker in inhibiting [³H]5-HT binding than it is in displacing agonist binding to the α-(7), dopamine (8) and opiate (11, 12) receptors in brain homogenates.

Saturation properties and kinetics of [³H]5-HT binding as influenced by GTP. To ascertain the mechanism of the GTP influence on [³H]5-HT binding, we examined the saturation and kinetics of binding in rat frontal cerebral cortex (Fig. 2). In the absence of GTP specific [³H]5-HT binding is saturable with maximal binding attained at about 20 nM and half maximal binding apparent at about 3 nM. Scatchard analysis indicates a single population of [³H]5-HT binding sites with a dissociation constant (K_D) of 2.7 nM and a maximal number of binding sites (B_{max}) of 9.0 pmoles/g tissue. This K_D value is about one third of the K_D we previously reported for [³H]5-HT binding (17) but resembles values described by Nelson *et al.* (24). The discrepancy is related to the fact that in the study of Nelson *et al.* (24) and in the present study we have preincubated the membranes to remove residual endogenous 5-HT that would otherwise diminish the apparent affinity of [³H]5-HT for receptor binding sites.

The three concentrations of GTP examined, 10 μM, 0.1 mM, and 1 mM progressively increase the K_D value up to 5.1 nM at 1 mM GTP. None of the GTP concentrations affect the B_{max} value. Thus, it appears that GTP reduces [³H]5-HT binding predominantly by decreasing the apparent affinity of binding sites for 5-HT.

To evaluate how GTP alters the affinity of 5-HT for receptor sites, we examined the influence of 1 mM GTP on the association and dissociation of [³H]5-HT (Fig. 3). In

TABLE 2

Effect of nucleotides on [^{3}H]5-HT binding in various brain regions of different species

Brain membranes were prepared as described in MATERIALS AND METHODS. Calf tissue was obtained fresh and dissected before being placed in storage at −70° for up to two weeks. Rat brain regions and mouse cortex were obtained from freshly decapitated animals. Whole guinea pig brains stored at −70° for 1–3 days were thawed before the frontal cortex was removed. Binding assays were performed as described in MATERIALS AND METHODS. Nucleotides were added at 1 mM final concentration. Values are the percent of specific [^{3}H]5-HT binding in the absence of nucleotides. The results are the means ± standard errors of three experiments, each performed in triplicate.

	GTP	GDP	GMP	Gpp(NH)p	ATP	ADP	AMP	App(NH)p
Calf cerebral cortex	58 ± 4	64 ± 2	90 ± 6	14 ± 4	81 ± 1	86 ± 7	93 ± 4	98 ± 2
Calf caudate	66 ± 5	51 ± 8	96 ± 3	20 ± 8	91 ± 6	93 ± 6	94 ± 0	94 ± 4
Calf inferior colliculi	59 ± 5	44 ± 2	96 ± 5	13 ± 5	89 ± 3	83 ± 7	97 ± 2	87 ± 5
Calf superior colliculi	53 ± 6	46 ± 2	98 ± 2	9 ± 4	83 ± 2	95 ± 2	98 ± 1	91 ± 5
Rat hippocampus	66 ± 2	61 ± 3	99 ± 2	9 ± 2	80 ± 3	87 ± 2	99 ± 5	87 ± 4
Rat caudate	51 ± 1	59 ± 2	105 ± 4	17 ± 4	83 ± 5	91 ± 4	92 ± 3	93 ± 2
Mouse cerebral cortex	64 ± 6	61 ± 6	100 ± 6	17 ± 5	81 ± 6	88 ± 3	100 ± 1	95 ± 3
Guinea pig cerebral cortex	63 ± 6	59 ± 3	104 ± 9	12 ± 4	80 ± 6	88 ± 5	91 ± 5	99 ± 3

TABLE 3

Comparison of guanine nucleotide effects on [^{3}H]5-HT binding in rat frontal cerebral cortex at 37° and 0°

The standard [^{3}H]5-HT assay was performed at 37° as described in MATERIALS AND METHODS. For assays at 0°, guanine nucleotides, [^{3}H]5-HT and tissue suspension were added at 0°. The assay tubes were then incubated in the dark at 0° for 2 hr before filtration. Nucleotides were added at 1 mM final concentration. The results are the percent of specific [^{3}H]5-HT binding as determined in the absence of nucleotides. Values are the means ± standard errors of three experiments, each performed in triplicate.

	37°	0°
GTP	57 ± 3	57 ± 3
GDP	66 ± 2	62 ± 1
GMP	96 ± 6	94 ± 3
Gpp(NH)p	22 ± 2	25 ± 1

the absence of GTP, [^{3}H]5-HT associates very rapidly, reaching maximal levels at about 5 min and attaining 75% of maximal values by 1 min. In the presence of GTP the association of [^{3}H]5-HT is even more rapid, attaining 87% of maximal level at 1 min. Because of the extremely rapid association rates, it is difficult to calculate the change in the rate constant of association in the presence of 1 mM GTP.

The dissociation of specifically bound [^{3}H]5-HT was examined by first labeling the receptors with [^{3}H]5-HT during a 10 min incubation at 37° whereupon 1 μM *d*-LSD was added and binding examined at varying intervals. In experiments evaluating the influence of GTP, 1 mM GTP was added simultaneously with the *d*-LSD. In the absence of GTP, [^{3}H]5-HT dissociates rapidly so that 50% of specific binding is dissociated by approximately 15 sec. GTP accelerates this dissociation rate so that 50% dissociation occurs in about 5 sec. Both in the absence and presence of GTP, [^{3}H]5-HT dissociation is virtually complete by 1 min. This pattern for association and dissociation of [^{3}H]5-HT at 37° in the absence of GTP resembles our earlier findings (17).

Influence of GTP on the potency of competitors for serotonin receptors. At guanine nucleotide regulated neurotransmitter receptors in which [^{3}H]agonists and [^{3}H]antagonists label the same receptor, GTP reduces the binding of [^{3}H]agonists only. However, at these receptors, GTP decreases the potency of agonists in competing for [^{3}H]antagonist binding. Such patterns have been described for guanine nucleotide regulation of β-adrenergic (6), dopamine (9, 10) and opiate (11, 12) receptors. By contrast, at α-adrenergic receptors the antagonist [^{3}H]WB-4101 labels a physically distinct receptor from that labeled by the agonists [^{3}H]clonidine, [^{3}H]epinephrine and [^{3}H]norepinephrine (25, 26). GTP decreases the binding of [^{3}H]clonidine, [^{3}H]-epinephrine and [^{3}H]norepinephrine but has no effect on [^{3}H]WB-4101 binding.

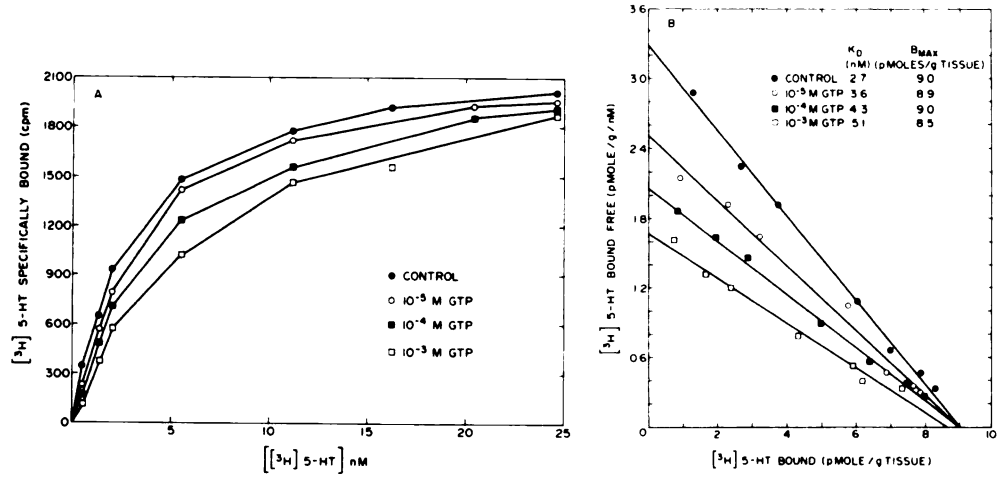

FIG. 2. *The effect of GTP on [³H]5-HT binding to serotonin receptors as a function of increasing concentrations of [³H]5-HT*

Rat frontal cerebral cortex homogenates were prepared from a P2 fraction as described in MATERIALS AND METHODS. Specific binding was determined with various concentrations of [³H]5-HT with no GTP (●), 10 μM GTP (○), 0.1 mM GTP (■), or 1 mM GTP (□). Nonspecific binding was determined by the addition of 1 μM LSD. Points shown are those obtained in a single experiment performed in triplicate. The experiment was performed three times with values that varied less than 25%. (A) Specific [³H]5-HT binding (B) Scatchard plots of the data in A.

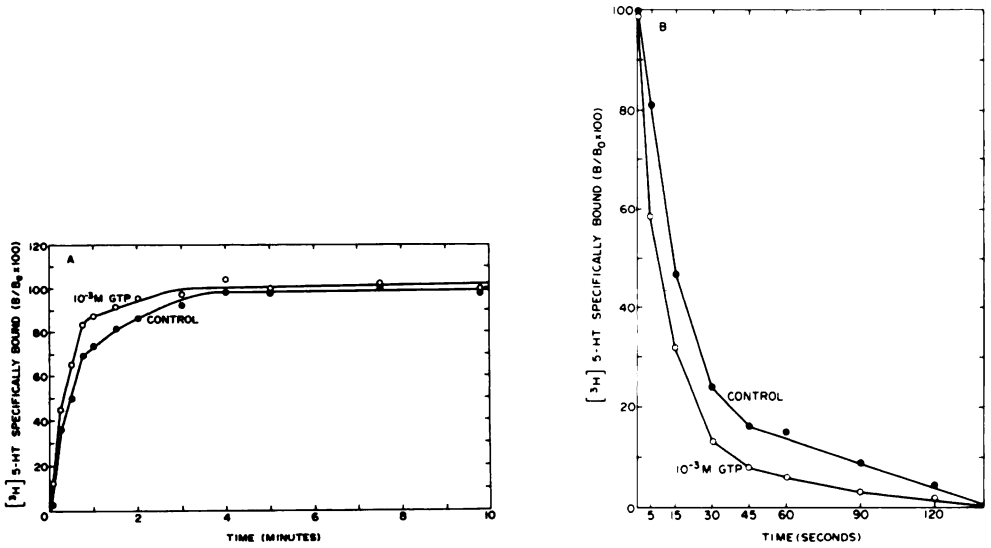

FIG. 3. *The effects of GTP on the time course of association and dissociation of [³H]5-HT binding to serotonin receptors*

A. The time course of association of [³H]5-HT binding to serotonin receptors was examined in the presence and absence of 1 mM GTP under standard assay conditions at 37°. Rat frontal cerebral cortex tissue was prepared as described in MATERIALS AND METHODS. Specific binding was determined for each point from the amount of radioactivity displaced by 1 μM LSD. The experiment was replicated three times.

B. The time course of dissociation of [³H]5-HT binding to serotonin receptors in the rat frontal cerebral cortex was examined in the presence and absence of 1 mM GTP. Standard assay conditions as described in MATERIALS AND METHODS were used. The sample volumes were incubated at 37° for 10 min until equilibrium had been achieved. At this time, 1 μM LSD ± 1 mM GTP was added to initiate dissociation, and the samples were filtered at increasing time intervals. The experiment was replicated three times.

Moreover, GTP fails to affect the potency of agonists in competing for [^{3}H]WB-4101 binding (7).

In order to delineate the effects of GTP on drug competition for serotonin receptors, we evaluated a group of serotonin agonists, partial agonists, and antagonists as defined by their activity on the serotonin-sensitive adenylate cyclase in rat brain (14, 27). In competing for the binding of [^{3}H]5-HT, 1 mM GTP reduces the potency of the agonists 5-HT and 5-methoxytryptamine by 65–115% (Table 4). Bufotenine and d-LSD behave as partial agonists of the serotonin-sensitive adenylate cyclase in both rat brain homogenates (27) and *Fasciola hepatica* (28). The potency of these partial agonists is not significantly affected by the presence of GTP. Similarly, the antagonists spiroperidol and clozapine display the same potency in competing for [^{3}H]5-HT binding in the absence or presence of 1 mM GTP. By contrast, GTP fails to alter the potency of all drugs tested in competing for [^{3}H]LSD and [^{3}H]spiroperidol binding (Table 5).

TABLE 4

Effect of GTP on affinities of competitors for [^{3}H]5-HT binding sites on rat frontal cerebral cortex membranes

Rat frontal cerebral cortex membranes were incubated with 2.0 nM [^{3}H]5-HT for 10 min at 37° together with four concentrations of unlabeled drugs, in the presence or absence of 1 mM GTP, under standard assay conditions as described in MATERIALS AND METHODS. IC_{50} values were determined by log-probit analysis and apparent K_I calculated by $K_I = IC_{50}/(1 + [^3H]5\text{-HT}/K_D)$. The values used for the [^{3}H]5-HT K_D value were 2.7 nM in the absence of GTP and 5.1 nM in the presence of 1 mM GTP. Values given are the means ± standard errors for three experiments, each performed in triplicate. Values of p were determined by the Student's t-test (two-tailed).

Drug	K_I	
	Control	1 mM GTP
	(nM)	
Agonists		
5-HT	2.1 ± 0.1	4.5 ± 0.2[a]
5-Methoxytryptamine	5.6 ± 0.1	9.1 ± 0.3[a]
Partial agonists		
d-LSD	8.8 ± 0.2	12 ± 2
Bufotenine	34 ± 6	47 ± 4
Antagonists		
Spiroperidol	650 ± 100	690 ± 200
Clozapine	1100 ± 200	1200 ± 200

[a] Significantly different from controls, $p < 0.05$

TABLE 5

Effect of GTP on affinities of competitors for [^{3}H]-LSD and [^{3}H]spiroperidol binding sites in rat frontal cerebral cortex membranes

Rat frontal cerebral cortex membranes were incubated with 3.7 nM [^{3}H]LSD or [^{3}H]spiroperidol for 10 and 15 min, respectively, together with four concentrations of unlabeled drugs, in the presence or absence of 1 mM GTP. Standard assay conditions were used as described in MATERIALS AND METHODS. IC_{50} values were determined from log-probit analysis and are the means ± standard errors from three experiments, each performed in triplicate.

Drug	IC_{50}	
	Control	1 mM GTP
	(nM)	
[^{3}H]LSD		
5-HT	300 ± 20	260 ± 30
5-Methoxytryptamine	790 ± 100	970 ± 200
d-LSD	11 ± 1	13 ± 0.5
Bufotenine	460 ± 100	420 ± 10
Spiroperidol	33 ± 3	26 ± 6
Clozapine	150 ± 20	140 ± 20
[^{3}H]Spiroperidol		
5-HT	4300 ± 700	4500 ± 300
5-Methoxytryptamine	3900 ± 900	4300 ± 80
d-LSD	12 ± 1	12 ± 2
Bufotenine	1100 ± 200	900 ± 100
Spiroperidol	0.72 ± 0.1	0.73 ± 0.1
Clozapine	18 ± 1	17 ± 1

DISCUSSION

The major finding of this study is that guanine nucleotides regulate at least one population of serotonin receptors in brain membranes. The characteristics of the guanine nucleotide effects closely resemble the nucleotide effects on other hormone and neurotransmitter receptors (6–12). Thus, guanine nucleotides affect agonist interactions with the receptors but not antagonist interactions. GTP lowers the affinity of 5-HT for receptors without changing the number of [^{3}H]5-HT binding sites. Also, the decrease in 5-HT affinity appears to be mediated in a large part by an acceleration of the dissociation rate from receptor sites. One implication of these findings is that the guanine nucleotide regulation of agonist binding to serotonin receptors may indicate a receptor-cyclase coupled system. Al-

though a serotonin-sensitive cyclase exists in brain membranes (14, 15), a regulatory role for guanine nucleotides has heretofore been demonstrated only in the liver fluke *Fasciola hepatica* (16). Though most receptor binding systems regulated by guanine nucleotides are linked to adenylate cyclase, GTP does influence angiotensin receptor binding for which no adenylate cyclase association has been directly demonstrated (4).

The second major finding of the present study is the differential influence of guanine nucleotides on two populations of serotonin receptor binding sites. In an accompanying paper, we show that the serotonin receptors labeled by [^{3}H]5-HT are physically distinct entities from those labeled by [^{3}H]spiroperidol, while those sites labeled by [^{3}H]LSD include both populations of receptors (23). Guanine nucleotides fail to influence [^{3}H]-spiroperidol binding and have only a modest influence on [^{3}H]LSD binding consistent with the partial binding of [^{3}H]LSD to the same receptors labeled by [^{3}H]5-HT. Guanine nucleotides have no influence on the ability of agonists or antagonists to compete for [^{3}H]spiroperidol or [^{3}H]LSD binding sites. This pattern of guanine nucleotide effects at the two serotonin receptors closely resembles the differential effects of guanine nucleotides at the two distinct α-adrenergic receptors labeled respectively by the agonists [^{3}H]clonidine, [^{3}H]-epinephrine and [^{3}H]norepinephrine and antagonist [^{3}H]WB-4101 (25, 26). Guanine nucleotides reduce the binding of agonists to α-adrenergic receptors but do not alter the potency of either agonists or antagonists in competing for the binding of [^{3}H]-WB-4101 to a distinct population of α-receptors (7).

One way in which the guanine nucleotide effects observed here differ mrkedly from previous studies is that GTP is 20–100 fold weaker in influencing receptor interactions of [^{3}H]5-HT than of several other hormones and neurotransmitters. GTP is also approximately 10-fold weaker in inhibiting [^{3}H]5-HT binding than in facilitating the serotonin-sensitive cyclase in the liver fluke (16). The decreased potency in our system is not due to metabolic degradation of GTP during the assay procedure. Similar discrepancies between guanine nucleotide effects on receptor binding and associated cyclases have been reported for glucagon (2) and opiate (11) receptors. From the numerous studies on guanine nucleotide regulation of adenylate cyclase (1, 2, 13, 29) a generalized model has emerged: guanine nucleotides influence both receptors and adenylate cyclase through functionally and possibly physically distinct regulatory sites. As a result, the influence of guanine nucleotides on cyclase activation need not parallel the effects on receptor ligand binding. The potency of guanine nucleotides in regulating receptor binding may be a measure of the "coupling" of the receptor to the cyclase. Maguire *et al.* (30) have noted nearly 100,000 fold variations in the degree of "coupling" between β-adrenergic receptors and adenylate cyclase in different species and organs. The K_D of [^{3}H]agonist binding was compared to the K_{ACT} of the β-sensitive cyclase (30). This ratio varied from 100 in cholera-treated S49 cells to 0.001 in rat heart and liver. Rat brain 5-HT receptors may simply reflect one end of this continuum, thus suggesting poor "coupling" of 5-HT receptors with an associated adenylate cyclase. 5-HT receptors in other tissues may respond to lower concentrations of guanine nucleotides, although no such organ or system has yet been described. It is, therefore, conceivable that the relatively weak effect of guanine nucleotides on serotonin receptors in rat brain reflects a lesser "coupling" of receptor and cyclase than in systems in which GTP is more potent.

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