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Dopamine Receptor Binding Regulated by Guanine Nucleotides

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SUMMARY

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Guanosine triphosphate (GTP) and diphosphate nucleotides decrease the binding of the agonist ligands [^{3}H]apomorphine and [^{3}H]ADTN to dopamine receptors in the rat corpus striatum with half maximal reduction of binding at 5 μM. These nucleotides also reduce agonist inhibition of the antagonist [^{3}H]spiroperidol binding to dopamine receptors without affecting total [^{3}H]spiroperidol binding. Guanosine monophosphate and adenine nucleotides display negligible influence on dopamine receptor binding. GTP reduces the affinity of [^{3}H]apomorphine binding with no effect on the maximal numbers of binding sites.

INTRODUCTION

Guanine nucleotides are thought to regulate adenylate cyclase systems by directly enhancing cyclase activity (1-8) and by increasing the sensitivity of the cyclase to stimulation by hormones (2-9). A role for guanine nucleotides in regulating the linkage between hormone recognition sites and adenylate cyclase is suggested by demonstrations that guanine nucleotides selectively decrease affinities of agonists for receptor binding in the case of glucagon (10, 11) and β-adrenergic (12, 13) receptors. Recent studies implicate two distinct guanine nucleotide sites in the regulation of the glucagon and β-adrenergic receptors and of adenylate cyclase activity in hepatic membranes (14) and cultured cell lines (15). The influence of guanine nucleotides upon hormone and neurotransmitter receptors may not be exclusively associated with cyclase-receptor interactions, since GTP[2] selectively decreases agonist affinities at angiotensin (16), α-noradrenergic (17) and opiate (18-20) receptors where it is unclear whether or not adenylate cyclase activation mediates their physiological effects.

Some of the synaptic effects of dopamine in the brain appear to involve stimulation of a dopamine-sensitive adenylate cyclase. In washed membranes, GTP is required for dopamine stimulation of adenylate cyclase activity (21). In preliminary studies we have reported that guanine nucleotides selectively reduce agonist binding to dopamine receptors in brain membranes (22, 23), and others have reported that GTP decreases agonist displacement of antagonist binding to dopamine receptors (24). Here we describe the detailed properties of guanine nucleotide influences upon dopamine receptor binding in brain membranes.

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[2] The abbreviation used is: GTP, guanosine triphosphate.

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MATERIALS AND METHODS

Fresh rat striata were homogenized in 100 volumes of ice-cold buffer mix (50 mM Tris, 0.1% ascorbic acid, pH 7.1) at 37° with a sonicator cell disruptor model W22OF (setting 6, 30–60 sec). The homogenate was centrifuged twice at 50,000 × *g* for 10 min (Sorvall RC2-B) with rehomogenization of the intermediate pellet in fresh buffer. The final pellet was resuspended in buffer mix at a concentration of 5 mg/ml for [^{3}H]apomorphine and [^{3}H]spiroperidol binding and 10 mg/ml for [^{3}H]ADTN binding experiments.

[1-phenyl-4-^{3}H]spiroperidol 23.6 Ci/mmole (New England Nuclear) was stored in ethanol at 0°; [^{3}H(G)]-apomorphine 10 and 7.35 Ci/mmole (New England Nuclear) was stored in 0.03 M ascorbic acid in the dark under nitrogen at 0°; ADTN (2-amino-6,7-dihydroxyl-1,2,3,4-tetrahydronapthalene) (Amersham), 3 Ci/mmole was stored in ethanol at 4°. All drugs were diluted in 0.1% ascorbic acid just before use.

Incubation tubes in triplicate received 100 μl of diluted [^{3}H]spiroperidol, [^{3}H]apomorphine, [^{3}H]ADTN, 100 μl of "ion mix" (final incubation concentration 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$) or H_2O for [^{3}H]spiroperidol and [^{3}H]apomorphine and 1 mM $MnCl_2$ for [^{3}H]ADTN experiments, 50 μl or 100 μl of various drugs, tissue suspension and 50 mM Tris-HCl buffer to 1 ml. For standard [^{3}H]spiroperidol assays 5000 cpm (.21 nM) spiroperidol was added to each tube of which about 2000 cpm bound to the membranes with a blank of about 400 cpm. For standard [^{3}H]apomorphine assays 25,000 cpm (2.6 nM) were added to tubes, which gave total binding of about 1500 cpm with a blank of 700 cpm. For [^{3}H]ADTN assays 24,000 cpm (8.7 nM)/tube gave total binding of 700 cpm with a blank of 400 cpm. The tubes were incubated at 37°, 10 min for [^{3}H]apomorphine and [^{3}H]ADTN, 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 buffer, pH 7.7 at 25°. The filters were counted by liquid scintillation spectrometry in 9 ml of Formula 947 (New England Nuclear) after 12 hours extraction at 4° at efficiencies of 37–44%.

Saturable or specific binding of [^{3}H]spiroperidol and [^{3}H]ADTN was measured as the excess over blanks taken in the presence of 1 μM (+)-butaclamol while 10 μM (+)-butaclamol was used for [^{3}H]apomorphine binding.

Butyrophenones and similar drugs were dissolved in a minimal volume of hot glacial acetic acid (less than 1% final volume) and brought up to 1 mM with 0.1% ascorbic acid. Other drugs were dissolved in 0.1% ascorbic acid except for nucleotides which were dissolved in distilled H_2O.

The sources of drugs were as follows: dopamine, ATP, ADP, GMP, GDP, Sigma Chemical Company; haloperidol, McNeil Laboratories; serotonin, Regis Chemical Company; chlorpromazine, Smith Kline & French; fluphenazine, E. R. Squibb and Sons; GTP, Gpp(NH)p, P & L Biochemicals; ADTN, Burroughs Wellcome; and bromocryptine and dihydroergokryptine, Sandoz.

RESULTS

^{3}H-Apomorphine binding in the presence and absence of ions: In the standard incubation mixture with "ion mix" [^{3}H]apomorphine binding is displaced stereospecifically by butaclamol as first reported by Seeman and associates (25). The (+)-isomer inhibits specific binding 50% at about 3 nM concentration being 10,000 times more potent than the (−)-isomer (Fig. 1). By contrast, in the absence of ions, both (+)- and (−)-butaclamol give negligible inhibition of binding even at 1 μM concentration with only a 10% difference in potency.

Similarly chlorpromazine, fluphenazine and spiroperidol are substantially more potent in the presence than the absence of ions (data not shown). Dopamine is almost 50 times more potent in reducing [^{3}H]-apomorphine binding in the presence than in the absence of ions. In the presence of ion mix, norepinephrine is substantially weaker than dopamine (Fig. 1). Norepinephrine effects are less sensitive to ions than dopamine effects so that in the absence of ions norepinephrine and dopamine have similar potencies. Serotonin shows very low affinity for [^{3}H]apomorphine bind-

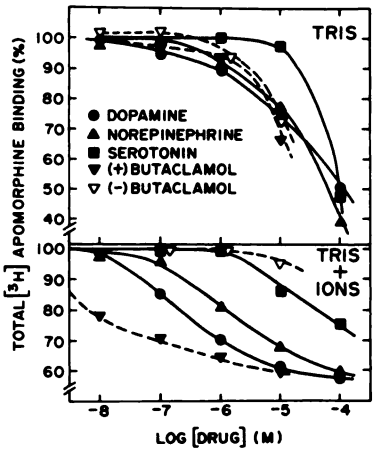

FIG. 1. *Drug competition for [^{3}H]-apomorphine binding*

Increasing concentrations of each drug were added to tubes containing 2.6 nM [^{3}H]-apomorphine and H_2O (top) or "ion mix" (120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ final concentration) and other additions described in METHODS (bottom). Results are expressed as percent total binding in the absence of drugs and are typical of 3–5 determinations (top) or the means of 3–6 experiments (bottom). Total binding/tube was typically 1500 cpm in the presence of ions and 2500 in the absence of ions with an addition of 25,000 cpm/tube.

ing sites in the presence or absence of ions. Thus in the absence of ions [^{3}H]apomorphine binding does not display the characteristic drug specificity of dopamine receptors while in the presence of "ion mix," the previously reported (22) homogeneous displacement by agonists and biphasic displacement by antagonists is observed. This is thought to reflect labeling, in part, of different populations of receptors by agonists and antagonists, respectively (26). In the present study, for routine experiments [^{3}H]apomorphine and [^{3}H]spiroperidol binding has been examined in the presence of "ion mix" since this incubation medium has been utilized routinely in this laboratory in an extensive number of investigations of dopamine receptor binding (26). However, because NaCl decreases [^{3}H]-ADTN binding, these experiments were conducted in the presence of 1 mM $MnCl_2$ which gives maximal binding with a characteristic dopaminergic profile (23) (Usdin, Creese and Snyder, in preparation).

Guanine nucleotide effects on [^{3}H]-apomorphine and [^{3}H]-spiroperidol binding: Guanine nucleotides selectively reduce [^{3}H]-apomorphine binding to dopamine receptors (Fig. 2, Table 1). Half maximal reduction of binding occurs at about 5 μM GTP (Fig. 2). Gpp(NH)p (5′-guanylylimidodiphosphate) and GTP inhibit [^{3}H]-apomorphine binding with similar potency and with displacement curves which have similar slopes. By contrast GMP, ATP, ADP and AMP have negligible effects at concentrations up to 50 μM (Table 1). In contrast

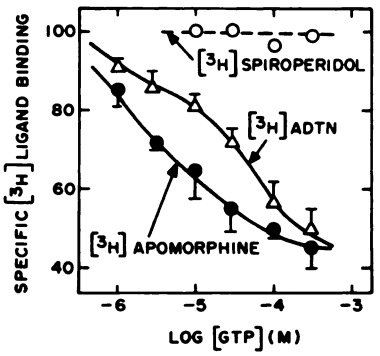

FIG. 2. *Effect of GTP on [^{3}H]-ligand binding*

Increasing concentrations of GTP were added to tubes containing standard concentrations of [^{3}H]-apomorphine, [^{3}H]-ADTN and [^{3}H]-spiroperidol. Specific binding of each ligand was determined under standard assay conditions and is the mean of three experiments for [^{3}H]-apomorphine and [^{3}H]-ADTN while the [^{3}H]spiroperidol points are from one experiment repeated at least 25 times at a single (50 μM) concentration. Bars indicate S.E.M.

TABLE 1

Nucleotide regulation of specific ^{3}H-apomorphine binding

Various concentrations of nucleotides were added to tubes containing 2.6 nM [^{3}H]apomorphine. Specific binding was determined as previously described and expressed as the percentage of specific binding in the absence of nucleotides. The results are the means ± S.E.M. of 3–8 experiments.

	1 μM	10 μM	50 μM
	(% Control)	*(% Control)*	*(% Control)*
GTP	77 ± 7	52 ± 6	46.7 ± 6
GppNHp	60 ± 5	59 ± 6	45.7 ± 6
GDP	72 ± 3	58 ± 9	51 ± 10
GMP			96 ± 2
ATP			89 ± 5
ADP			86 ± 4

to the potent influences of GTP upon [^{3}H]apomorphine binding, as much as 100 μM GTP fails to affect stereospecific [^{3}H]-spiroperidol binding (Fig. 2). The differential effect of GTP on [^{3}H]-apomorphine and [^{3}H]-spiroperidol binding appears to depend on the agonist versus antagonist properties of the respective [^{3}H]-ligands since influences of GTP upon binding of the agonist [^{3}H]-ADTN are quite similar to effects on [^{3}H]-apomorphine binding (Fig. 2). The smaller effect of GTP on [^{3}H]-ADTN binding may be explained by the use of $MnCl_2$ in the incubation mixture, since 1 mM $MnCl_2$ reduces the inhibition of [^{3}H]-apomorphine binding by GTP from 50% to 26% at 100 μM GTP concentration.

To determine whether guanine nucleotides alter the affinity or the number of binding sites for [^{3}H]-apomorphine, we measured binding of progressively increasing concentrations of [^{3}H]-apomorphine (Fig. 3). In the absence of GTP only one population of [^{3}H]-apomorphine binding sites are apparent with a dissociation constant (K_D) of 4.8 ± 0.5 nM and a maximal number of binding sites (B_{max}) of 27.1 ± 0.9 pmole/g. GTP (5 μM) reduces the apparent affinity of the binding sites by 59 ± 6% with no change in the number of binding sites.

Inhibition of specific [^{3}H]-spiroperidol binding by drugs in the presence and absence of guanine nucleotides: Lesion studies indicate that some dopamine receptors labeled by the antagonists [^{3}H]-haloperidol and [^{3}H]-spiroperidol are localized to different neuronal populations than dopamine receptors monitored by assays of the dopamine-sensitive adenylate cyclase or by [^{3}H]-apomorphine binding (27). However, at least some dopamine receptors are thought to exist in interconvertible states labeled by agonists such as [^{3}H]-apomorphine or [^{3}H]-dopamine and antagonists such as [^{3}H]-haloperidol or [^{3}H]-spiroperidol (26). If [^{3}H]-spiroperidol does label some of the same receptors which bind dopamine and apomorphine, then one might expect guanine nucleotides to affect dopamine induced inhibition of [^{3}H]-spiroperidol binding. Accordingly we examined the potencies of dopamine, apomorphine, ADTN and several neuroleptics as inhibitors of [^{3}H]-spiroperidol binding in the presence and absence of GTP (Fig. 4 and 5). Addition of GTP (50 μM) reduces the potency of dopamine in inhibiting [^{3}H]-spiroperidol by about 3 fold. Though the displacement curve of dopamine is shifted to the right in the presence of GTP, its slope appears the same in the presence or absence of GTP. GDP and Gpp(NH)p reduce the potency of dopamine to the same extent as GTP, while ATP, ADP, AMP and GMP are without effect at 50 μM concentration. GTP reduces ADTN and apomorphine inhibition of [^{3}H]-spiroperidol binding to the same extent as it reduces dopamine effects. Unlike the behavior of the agonists, several neuroleptic dopamine antagonists display the same potency in inhibiting [^{3}H]-spiroperidol in the presence and absence of GTP (Fig. 4). Two ergots, dihydroergokryptine and bromocryptine, are dopamine agonists in the pituitary (28) while bromocryptine exhibits central dopaminergic activity in rats and man (29). However, neither of these drugs exhibits agonist activity at the striatal dopamine-sensitive adenylate cyclase where they act as antagonists at high concentrations (29). GTP has no effect on the inhibition of [^{3}H]-spiroperidol binding by these ergots (Fig. 5).

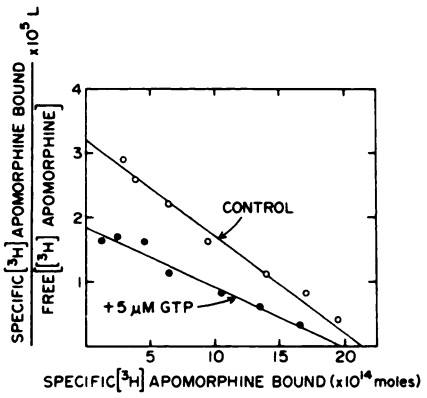

FIG. 3. *Scatchard plot of [^{3}H]-apomorphine saturation as altered by GTP*

Increasing concentrations of [^{3}H]-apomorphine were added to tubes with and without 5 μM GTP. Specific binding was determined as previously described. The experiment was replicated five times.

DISCUSSION

The major finding of the present study is

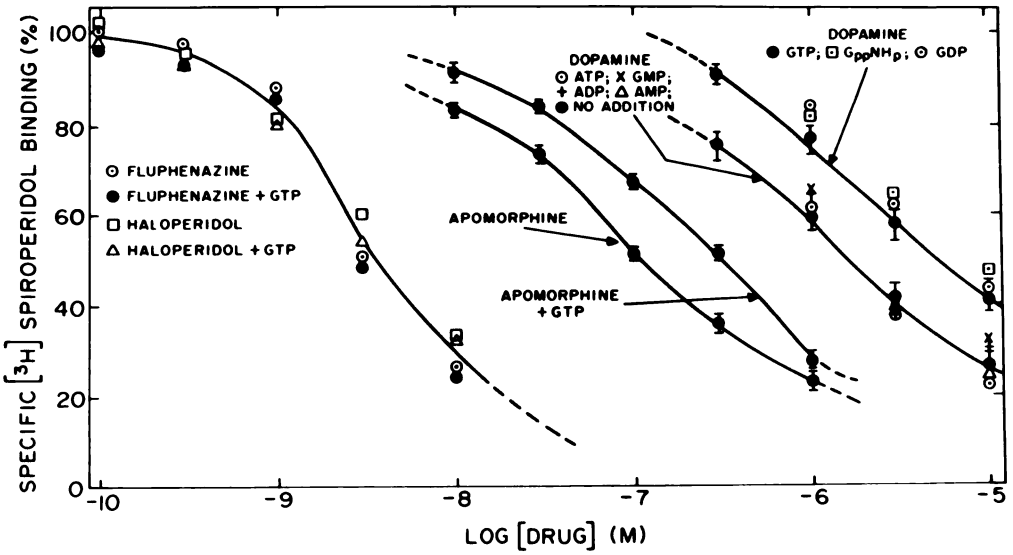

FIG. 4. *Drug competition for [^{3}H]-spiroperidol binding in the presence and absence of nucleotides*
Increasing concentrations of each drug in the presence or absence of 50 μM nucleotide were added to tubes containing [^{3}H]-spiroperidol. Specific binding with added drugs is expressed as a percentage of the specific binding in the absence of drugs. Data are means of 3–7 experiments. Bars indicate S.E.M.

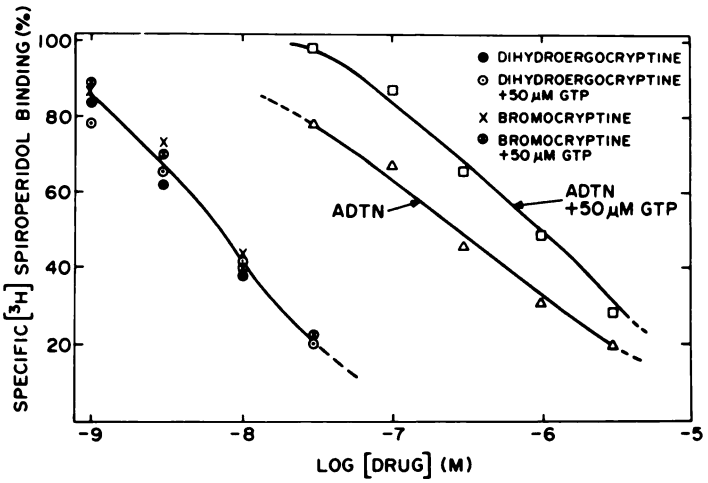

FIG. 5. *Drug competition for [^{3}H]-spiroperidol binding in the presence and absence of nucleotides*
Increasing concentrations of each drug in the presence or absence of 50 μM nucleotide were added to tubes containing [^{3}H]-spiroperidol. Specific binding with added drugs is expressed as a percentage of the specific binding in the absence of drugs. Data are means of 3–7 experiments. Bars indicate S.E.M.

a selective effect of guanine nucleotides upon agonist interactions with dopamine receptors. These effects are quite similar to those described at glucagon, β-noradrenergic, opiate and angiotensin receptors (1–20). Thus the effect is elicited by GTP, its non-metabolized analogue, Gpp(NH)p, and by GDP but not by GMP, ATP, ADP and AMP. Also the effect is agonist-specific since it is apparent with [^{3}H]-apomorphine and [^{3}H]-ADTN but not with [^{3}H]-haloperidol or [^{3}H]-spiroperidol. GTP reduces the affinity of [^{3}H]-apomorphine for binding sites with negligible effects on the number of sites. Guanine nucleotides also decrease the affinity of α-noradrenergic receptors

without changing the numbers of sites (17). Similar selective effects on affinity are elicited by GTP at opiate (18–20), angiotensin (16), and glucagon binding sites (6).

Interestingly, guanine nucleotides also selectively reduce agonist potency in inhibiting [^{3}H]-spiroperidol binding. Considerable differences in drug specificity have suggested that [^{3}H]-haloperidol in part labels distinct receptors from those associated with the dopamine-sensitive adenylate cyclase and labeled by [^{3}H]-dopamine (30, 31). However the nucleotide specificity of guanine nucleotides in reducing agonist potency in inhibiting [^{3}H]-spiroperidol binding is similar to their specificity on [^{3}H]-apomorphine binding. This suggests that in altering agonist inhibition of [^{3}H]-spiroperidol binding, guanine nucleotides may act at the site labeled by [^{3}H]-apomorphine. Lesion studies have indicated that in part [^{3}H]-spiroperidol and [^{3}H]-haloperidol do label dopamine receptors which are located on different neuronal populations than those labeled by [^{3}H]-apomorphine (27). Thus, kainic acid microinjection in the corpus striatum, a treatment which selectively destroys neurons intrinsic to the corpus striatum, reduces dopamine-sensitive adenylate cyclase activity 85% and specific [^{3}H]apomorphine binding 65% while [^{3}H]-haloperidol and [^{3}H]-spiroperidol binding are reduced only about 45–50%. The portion of [^{3}H]-haloperidol binding not contained on intrinsic neurons lesioned by kainic acid appears to be localized in major part to neurons projecting to the corpus striatum from the cerebral cortex, since [^{3}H]-haloperidol binding not eliminated by kainic acid treatment is abolished by cerebral cortex ablation. The fact that the majority of [^{3}H]-apomorphine binding and almost all the dopamine-sensitive adenylate cyclase are eliminated by kainic acid suggests that [^{3}H]-apomorphine may label in large part dopamine receptors associated with the dopamine-sensitive adenylate cyclase.

Whether or not the [^{3}H]haloperidol or [^{3}H]spiroperidol binding abolished by kainic acid treatment involves the cyclase linked receptors labeled by [^{3}H]apomorphine has not been clear. This possibility is favored by our data indicating that GTP alters agonist inhibition of spiroperidol binding. It is also supported by the observation that GTP fails to affect the potency of dopamine in displacing [^{3}H]spiroperidol binding in kainate lesioned animals (Creese, Usdin and Snyder, in preparation, 32). That this effect is specific for only agonists which act at the recognition site of the dopamine-sensitive adenylate cyclase is argued by the lack of effect of GTP in changing the potency of bromocryptine in inhibiting [^{3}H]spiroperidol binding. Bromocryptine is an agonist at pituitary dopamine receptors presumably not linked to adenylate cyclase but an antagonist at the dopamine-sensitive adenylate cyclase (28, 29).

In conclusion, GTP inhibits dopamine [^{3}H]agonist binding by lowering receptor-ligand affinity. This effect appears specific to agonists of the dopamine-sensitive adenylate cyclase. The fact that GTP reduces agonist potency in inhibiting [^{3}H]-spiroperidol binding argues that, in spite of different drug specificities, [^{3}H]spiroperidol and [^{3}H]apomorphine may in part both label states of the dopamine receptor associated with the striatal dopamine-sensitive adenylate cyclase.

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