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Characterization of Dopamine Receptors Mediating Inhibition of Adenylate Cyclase Activity in Rat Striatum

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SUMMARY

In the presence of SCH 23390, a potent blocker of D_1 dopamine receptors, dopamine inhibits adenylate cyclase activity of synaptic plasma membranes isolated from rat striatum. Maximal inhibition corresponds to a 20–25% decrease of basal enzyme activity and is reached with 100 μM dopamine. The apparent IC_{50} of dopamine is 2.5 μM. The inhibitory effect of dopamine is mimicked by various dopamine receptor agonists with the following rank order of potency: (−)-propylnorapomorphine ≥ bromocriptine > (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene = (−)-apomorphine > dopamine > LY 171555 > *l*-noradrenaline > *l*-phenylephrine. Clonidine and *l*-isoproterenol are inactive at 100 μM. Bromocriptine and LY 171555, two agents which stimulate selectively D_2 receptors, inhibit striatal adenylate cyclase activity in the absence of SCH 23390. However, bromocriptine behaves like a partial agonist. A variety of neuroleptic drugs antagonize the dopamine inhibition with a rank order of potency which qualitatively correlates with their relative affinity for D_2 receptors. *l*-Sulpiride (EC_{50} = 210 nM) and (+)-butaclamol (EC_{50} = 130 nM) are severalfold more potent than *d*-sulpiride (EC_{50} = 5 μM) and (−)-butaclamol (EC_{50} = 10 μM). The inhibitory effect of dopamine on striatal adenylate cyclase activity is dependent on the presence of GTP, with half-maximal inhibition occurring at 1 μM GTP. In the absence of SCH 23390, dopamine stimulates adenylate cyclase activity, reaching a maximum at 1 μM GTP. At higher concentrations of the nucleotide, the dopamine-stimulated enzyme activity decreases, and this decline is antagonized by the D_2 receptor blocker *l*-sulpiride. Guanyl-5′-yl imidodiphosphate, a stable analogue of GTP, has a biphasic effect on the striatal adenylate cyclase activity, inhibiting at low concentration (from 1 to 100 nM) and stimulating at higher concentrations. Selective activation of D_2 receptors by LY 171555 does not increase the extent of enzyme inhibition elicited by guanyl-5′-yl imidodiphosphate. Sodium chloride amplifies the inhibition of striatal adenylate cyclase activity by LY 171555 and reduces the potency of the D_2 agonist by a factor of 4. The dopamine-inhibited enzyme activity is lost following intrastriatal injection of kainic acid. The results indicate that in rat striatum dopamine inhibits adenylate cyclase activity by acting on postsynaptic dopamine receptors with pharmacological properties of D_2 type.

INTRODUCTION

Since two different classes of dopamine receptors, named D_1 and D_2, have been recognized (1), a great effort has been addressed towards the identification of the biochemical mechanisms mediating the transmembrane signaling at each type of receptor. While D_1 receptor activation has been shown to be linked to stimulation of adenylate cyclase in a variety of tissues, the D_2 type has been considered not to be coupled to the adenylate cyclase system (1). In the pituitary gland, however, several studies (2–5) have demonstrated that the activation of D_2 receptors is actually linked to inhibition of adenylate cyclase activity. These studies led to the hypothesis that the inhibition of cyclic AMP formation is a mechanism by which dopamine decreases the synthesis and the release of pituitary hormones like prolactin and α-melanophore-stimulating hormone.

In the brain, an inhibitory coupling of D_2 receptors to adenylate cyclase has not been yet fully documented. Stoof and Kebabian (6) reported that, in rat striatal slices, D_2 receptor agonists decreased the efflux of cyclic AMP stimulated by D_1 receptor activation and proposed that D_2 receptors could mediate inhibition of striatal cyclic AMP formation. More recently, we have shown that in the presence of SCH 23390, a putatively selective blocker of D_1 receptors (7), dopamine caused inhibition of basal adenylate cyclase activity of rat striatum (8). The inhibitory effect of dopamine was blocked by *l*-sulpiride, a specific D_2 receptor antagonist, indicating

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the presence in rat striatum of D_2 receptors coupled to inhibition of adenylate cyclase. In the present study, we further investigate the pharmacological properties of these dopamine inhibitory receptors and the modulatory effect of Na^+ and guanine nucleotides on their coupling to the adenylate cyclase system.

MATERIALS AND METHODS

Isolation of synaptic plasma membranes. Striata from 13–15 male Sprague-Dawley rats (Nossan, Italy; 200–250 g) sacrificed by decapitation were homogenized (1:10 w/v) in ice-cold 10 mM HEPES[1]/NaOH buffer (pH 7.4) containing 1 mM dithiothreitol, 1 mM EGTA, and 10% sucrose (w/v) with a motor-driven Teflon-glass tissue grinder (5-ml capacity; 0.25-mm clearance, 400 rpm). Synaptic plasma membranes were isolated according to the method of Jones and Matus (9). All steps were carried out as previously described (10), with the only exception that 10 mM HEPES/NaOH buffer substituted for 5 mM Tris/HCl buffer. Synaptic plasma membranes were slowly frozen at −70° and stored for no longer than 10 days. An aliquot was quickly thawed before the beginning of an enzyme assay; the unused portion was discarded.

Adenylate cyclase assay. Enzyme activity was routinely assayed in a 150-μl reaction mixture containing 80 mM Tris/HCl (pH 7.4), 0.5 mM [α^{32}-P]ATP (30–50 cpm/pmol), 2mM $MgCl_2$, 1 mM cyclic AMP, 0.5 mM 3-isobutyl-1-methylxantine, 5 mM phosphocreatine (sodium salt), 50 units/ml creatine phosphokinase, 0.33 mM EGTA, 1.33 mM dithiothreitol, 50 μg of bovine serum albumin, 50 μM GTP, and 100 mM NaCL. Cyclic [8-^{3}H]AMP (approximately 10^4 cpm) was included to monitor cyclic AMP recovery. The reaction was initiated by adding the membrane suspension (10–15 μg of protein) and was carried out at 25° for 20 min. The incubation was stopped by adding 200 μl of a solution containing 2% sodium dodecyl sulfate (w/v), 45 mM ATP, 1.3 mM cyclic AMP (pH 7.5). The samples were placed in a boiling water bath for 3 min, and cyclic AMP was isolated by sequential chromatography on Dowex 50W-X4 and on neutral alumina as described by Salomon *et al.* (11). The enzyme activity appeared linear with time of incubation for up to 30 min. Assays were run in triplicate with an intra-assay variation (deviation of replicate from the mean) of less than 2%.

When present in the reaction mixture, SCH 23390 was used at a concentration of 0.1 μM. This concentration completely blocked the activation of adenylate cyclase by 100 μM dopamine and was 500-fold lower[2] than the EC_{50} value of SCH 23390 in antagonizing the inhibition of striatal adenylate cyclase elicited by 100 μM LY 17 1555, a selective D_2 agonist (see "Results"). Moreover, at this concentration, SCH 23390 did not affect basal adenylate cyclase activity.

Striatal lesions with kainic acid. Male rats (250–300 g) were anesthetized with chloral hydrate (400 mg/kg intraperitoneal) and placed in a stereotaxic frame. Kainic acid, dissolved in 10 mM sodium phosphate buffer (pH 7.4), was injected (1 μg/0.5 μl over a 5-min period) into the head (3 mm anterior; 2.8 mm lateral; −5.0 mm vertical) and body (1 mm anterior; 3.5 mm lateral; −4.5 mm vertical) of the right caudate nucleus, according to the atlas of Pellegrino and Cushman (12). The contralateral caudate nucleus was injected with an equal volume of vehicle. Ten days after surgery, animals were decapitated and synaptic plasma membranes were isolated from the control and lesioned caudate nucleus. Protein content was determined by the method of Bradford (13), using bovine serum albumin as a standard.

Materials. [α-^{32}P] ATP (20–50 Ci/mmol) was obtained from New England Nuclear. Cyclic [8-^{3}H]AMP (20–30 Ci/mmol) was purchased from Amersham Corp. Reagents for adenylate cyclase assay, kainic acid, dopamine hydrochloride, *l*-noradrenaline hydrochloride, *l*-phenylephrine hydrochloride, *l*-isoproterenol hydrochloride, *dl*-propranolol hydrochloride, and chlorpromazine hydrochloride were obtained from Sigma. Guanyl-5′-yl imidodiphosphate was from Boehringer Mannheim. The other compounds were obtained from the following sources: SCH 23390 ([R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol]maleate), Schering Plough (Bloomfield, NJ); spiroperidol, domperidone, and pimozide, Janssen Pharmaceutical (Beerse, Belgium); haloperidol, McNeil Laboratories (Fort Washington, PA); bromocriptine and clozapine, Sandoz (Basel, Switzerland); clebopride methanesulfonate, *l*- and *d*-sulpiride, Ravizza (Muggiò, Italy); trifluoperazine dihydrochloride, Smith, Kline & French Laboratories (Philadelphia, PA); phentolamine hydrochloride, Ciba-Geigy (Summit, NJ); zetidoline, Lepetit Research Laboratories (Milan, Italy); (+)-butaclamol, Ayerst (Rouses Point, NY); (−)-butaclamol, (−)-apomorphine, (±)-2-amino-6,7-dihydroxy-1,2,3,4,-tetrahydronaphthalene hydrobromide, (−)-propylnorapomorphine hydrochloride, Research Biochemicals Inc. (Wayland, MA); LY 171555 ([*trans*-(−)-4a*R*-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H* (or 2*H*)-pyrazolo(3,4-*g*)-quinoline]monohydrochloride), Ely Lilly & Co. (Indianapolis, IN); clonidine, Boehringer Ingelheim, Ltd.(Ridgefield, CT); promethazine hydrochloride, Farmitalia (Milan, Italy); and *cis*-flupenthixol, Lundbeck Co. (Copenhagen, Denmark).

Drugs were dissolved in distilled water, diluted hydrochloric acid, glacial acetic acid, or ethanol just before the beginning of the enzyme assay. When glacial acetic acid or ethanol was used, the final concentration in the reaction mixture was not higher than 1 mM and 0.05%, respectively. Control samples were incubated in the presence of equal amounts of solvent.

RESULTS

When striatal adenylate cyclase activity was assayed in the presence of 0.1 μM SCH 23390 to prevent D_1 receptor activation, dopamine and other agonists of both D_1 and D_2 receptors inhibited the enzyme activity in a concentration-dependent manner (Fig. 1A). The maximal inhibition elicited by dopamine, (−)-propylnorapomorphine, and A-6,7-DTN corresponded to a 20–25% decrease of basal enzyme activity. (−)-Apomorphine was slightly less effective than dopamine, its maximal effect being equal to 80% of that obtainable with dopamine. However, this difference was not statistically significant. Among the α- and β-adrenergic receptor agonists tested, only *l*-noradrenaline inhibited striatal adenylate cyclase activity as effectively as dopamine, whereas *l*-isoproterenol and clonidine had no significant effect (Fig. 1B). The inhibition of striatal adenylate cyclase produced by a combination of dopamine and noradrenaline, each at maximally effective concentrations, was no greater than with either agonist alone (Table 1). Furthermore, the noradrenaline inhibition was antagonized more potently by spiroperidol (EC_{50} = 2.3 nM), a dopaminergic blocker, than by phentolamine (EC_{50} = 3 μM), and α-adrenergic antagonist.

In the absence of SCH 23390, adenylate cyclase activity was inhibited by LY 171555, the active enantiomer of the selective D_2 agonist LY 141865 (14), and by bromocriptine, an agonist of D_2 and an antagonist of D_1 receptors (15) (Fig. 2). The maximal enzyme inhibition by LY 171555 was achieved at a concentration of 100 μM and corresponded to a 15–18% of basal enzyme activity ($n = 8$, $p < 0.001$; paired *t*-test). This effect was antagonized by spiroperidol (EC_{50} = 3 nM) more potently than by phentolamine (EC_{50} = 100 μM), indicating that LY 171555 was acting on D_2 rather than α_2-adrenergic recep-

[1]The abbreviations used are: HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; A-6,7-DTN, (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; Gpp(NH)p, guanyl-5′-yl imidodiphosphate.

[2] P. Onali, M. C. Olianas, G. L. Gessa, unpublished observations.

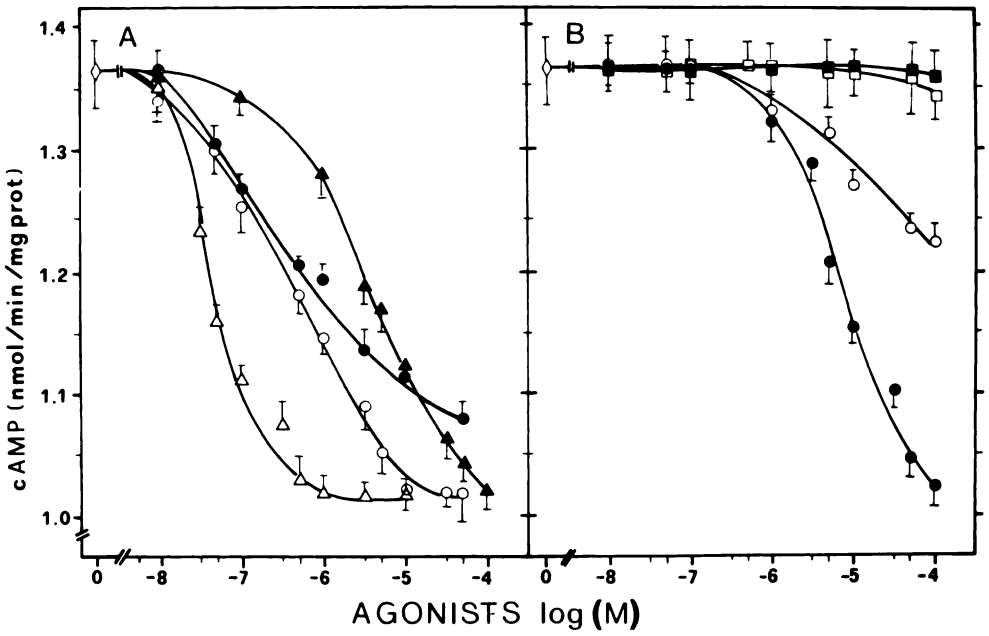

FIG. 1. *Effect of various dopaminergic (A) and adrenergic (B) agonists on striatal adenylate cyclase activity assayed in the presence of 0.1 μM SCH 23390*

A: ◇, control activity; △, (−)-propylnorapomorphine; ○ (±)-A-6,7-DTN; ● (−)-apomorphine; ▲, dopamine. B: ◇, control activity; ●, *l*-noradrenaline; ○, *l*-phenylephrine; ■, clonidine; □, *l*-isoproterenol. The values are the mean ± SEM of three determinations performed in four different membrane preparations.

TABLE 1

Lack of additivity of dopamine- and noradrenaline-inhibited adenylate cyclase activities

Enzyme activity was assayed in the presence of 0.1 μM SCH 23390. Values are the mean ± SE of the number of determinations reported in parentheses.

Experimental conditions	Adenylate cyclase activity
	pmol/min/mg protein
Control	1510 ± 20 (7)
Dopamine, 100 μM	1140 ± 25[a] (10)
Noradrenaline, 100 μM	1160 ± 30[a] (5)
Dopamine + noradrenaline	1170 ± 15[b] (5)

[a] $p < 0.001$ versus control.

[b] Not significantly different from either noradrenaline or dopamine alone (Student's *t*-test).

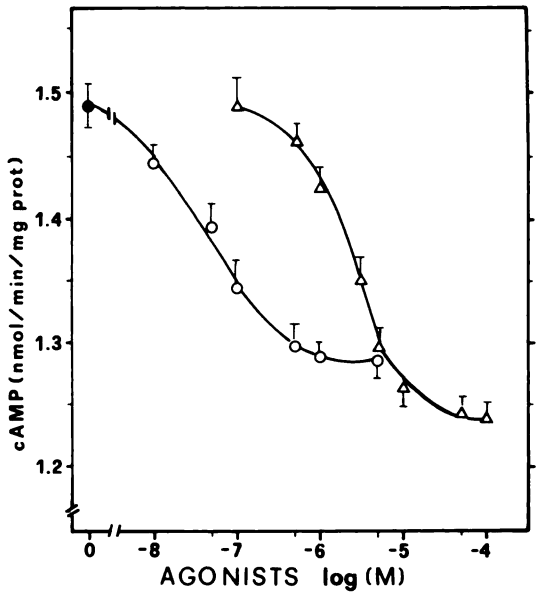

FIG. 2. *Concentration-dependent inhibition of striatal adenylate cyclase by bromocriptine (○) and LY 171555 (△).*

●, control activity. The enzyme activity was assayed in the absence of SCH 23390. Values are the mean ± SEM of six determinations performed in three different membrane preparations.

tors. Bromocriptine was maximally effective at 5 μM, but it elicited only a 13–15% inhibition ($n = 8$, $p < 0.001$; paired *t*-test). Moreover, bromocriptine reduced the inhibition elicited by 100 μM dopamine to the same level as that obtainable with bromocriptine alone at maximal effective concentrations (Fig. 3). The low efficacy of bromocriptine was not due to a slow time course for binding to the dopamine receptors, since prolonging the incubation time to 30 min did not increase the maximal effect of the ergot.

Estimation of the concentrations of the agonists which elicited half-maximal effect (IC_{50}) (Table 2) revealed the following rank order of potency: (−)-propylnorapomorphine ≥ bromocriptine > (+)-A-6,7-DTN = (−)-apomorphine > dopamine > LY 171555 > *l*-noradrenaline > *l*-phenylephrine. The Hill coefficient value (nH) was approximately 1 for most of the agonists, with the exception of (−)-apomorphine (nH = 0.64) and A-6,7-DTN (nH = 0.73).

Two dopamine receptor antagonists, sulpiride and butaclamol, reversed adenylate cyclase inhibition elicited by dopamine stereospecifically (Fig. 4). Thus, (+)-butaclamol antagonized the dopamine inhibition with an EC_{50} of 130 nM, whereas (−)-butaclamol, the inactive enanti-

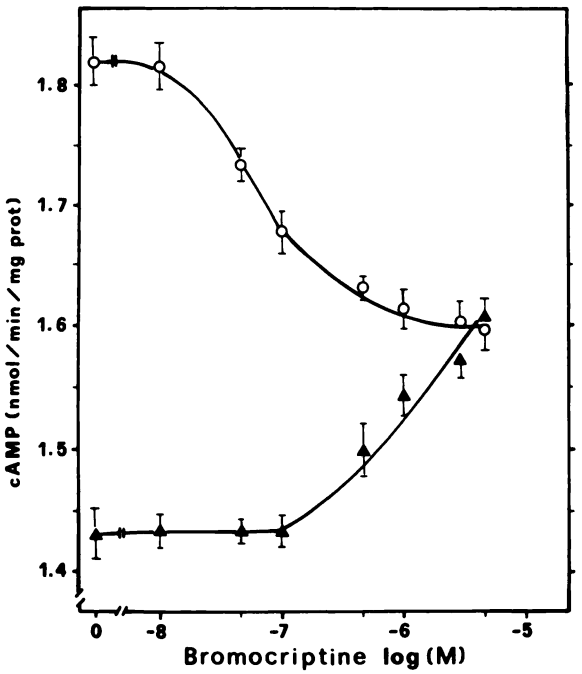

FIG. 3. *Antagonism of dopamine-inhibited adenylate cyclase activity by increasing concentrations of bromocriptine*

The enzyme activity was assayed at the indicated concentrations of bromocriptine in the presence (▲) and in the absence (○) of 100 μM dopamine. The reaction mixture contained 0.1 μM SCH 23390. The D_1 blocker did not significantly affect either the efficacy or the potency of bromocriptine in inhibiting striatal adenylate cyclase activity. Values are the mean ± SEM of three determinations.

TABLE 2

Potency of various dopaminergic and adrenergic agonists in inhibiting striatal adenylate cyclase activity

The IC_{50} values represent the concentration of agonist required for half-maximal inhibition of striatal adenylate cyclase activity. With the exception of LY 171555 and bromocriptine, each agonist was tested in the presence of 0.1 μM SCH 23390.

Agonist	IC_{50}[a]
	μM
Dopamine	2.5 ± 0.2 (8)
(−)-Propylnorapomorphine	0.045 ± 0.003 (5)
Bromocriptine	0.050 ± 0.002 (6)
(±)-A-6, 7-DTN	0.350 ± 0.01 (3)
(−)-Apomorphine	0.400 ± 0.04 (4)
LY 171555	3.51 ± 0.3 (6)
l-Noradrenaline	8.1 ± 0.4 (3)
l-Phenylephrine	>100 (3)
l-Isoproterenol	Inactive at 100 μM (3)
Clonidine	Inactive at 100 μM (3)

[a] Values are the mean ± SE of the number of determinations reported in parentheses.

omer, was ineffective at concentrations as high as 10 μM. *l*-Sulpiride was approximately 24 times more active than *d*-sulpiride. As reported in Table 3, a number of neuroleptic drugs antagonized the dopamine-induced inhibition of striatal adenylate cyclase activity. Among these agents, spiroperidol was the most potent, whereas non-neuroleptic drugs, like promethazine, phentolamine and *dl*-propranolol showed the lowest antagonistic activity. At the concentrations used, none of these receptor blockers significantly affected basal adenylate cyclase activity by themselves.

The inhibitory effect of dopamine on striatal adenylate cyclase required the presence of GTP (Fig. 5). In the absence of added nucleotide, dopamine did not affect adenylate cyclase activity. Only at concentrations of GTP higher than 0.1 μM could the dopamine inhibition be detected. The concentration of GTP required by dopamine for half-maximal inhibition was approximately 1 μM. Previous studies have shown that also stimulation of striatal adenylate cyclase by dopamine is dependent on the presence of GTP (16). We have estimated that the concentration of GTP required for half-maximal stimulation of the striatal enzyme by dopamine was 50 nM. At the concentrations of GTP higher than 1 μM, the dopamine-stimulated adenylate cyclase activity declined markedly (Fig. 6). This decline was antagonized by *l*-sulpiride, indicating that dopamine activation of D_2 receptors was involved in the fall-off of the dopamine-stimulated adenylate cyclase activity.

When Gpp(NH)p, a less hydrolyzable analogue of GTP, substituted for GTP, adenylate cyclase activity showed a biphasic response with inhibition occurring at nanomolar concentrations and activation at micromolar concentrations of the nucleotide (Fig. 7). The inhibitory response (about 30–40% decrease of the enzyme activity assayed in the absence of any added nucleotide) was maximal and half-maximal with 100 and 12 nM Gpp(NH)p, respectively. The stimulatory response ensued promptly at concentrations above 100 nM, reaching a 2.5-fold increase at 10 μM Gpp(NH)p. Neither the inhibitory or the stimulatory responses of adenylate cyclase to Gpp(NH)p were significantly affected by 100 μM LY 171555. Time course analysis of striatal enzyme activity revealed that the inhibition elicited by Gpp(NH)p (100 nM) occurred with a time lag of about 75 sec; thereafter, the cyclic AMP formation was linear for at least 20 min. In the presence of LY 171555 (100 μM), the time lag for Gpp(NH)p-induced inhibition was slightly, but not significantly, reduced to 65 sec (results not shown).

The D_2-mediated inhibition of striatal adenylate cyclase activity was affected by Na^+. In the absence of added Na^+, LY 171555 (100 μM) could significantly inhibit the enzyme activity by 12.5% ($n = 6$, $p < 0.01$ paired *t*-test). However, the addition of 150 mM NaCl increased the percentage of maximal inhibition elicited by LY 171555 to 18.1% ($n = 6$, $p < 0.005$ versus no NaCl added; paired *t* test). Moreover, NaCl, but not KCl, reduced the IC_{50} value of LY 171555 from 0.81 to 3.6 μM (Fig. 8) and that of dopamine from 0.72 to 2.9 μM (results not shown).

D_2 dopamine receptors are located on different cellular structures within the striatum. A portion of the receptor population resides on neurons intrinsic to the striatum or originating in this brain area (17). D_2 receptors are also present on neuronal afferents to the striatum (17, 18) and on glial cells (19). To gain information on the cellular localization of the D_2 receptors mediating inhibition of adenylate cyclase, kainic acid was injected intrastriatally, and the inhibitory response to dopamine was examined in the control and lesioned striatum.

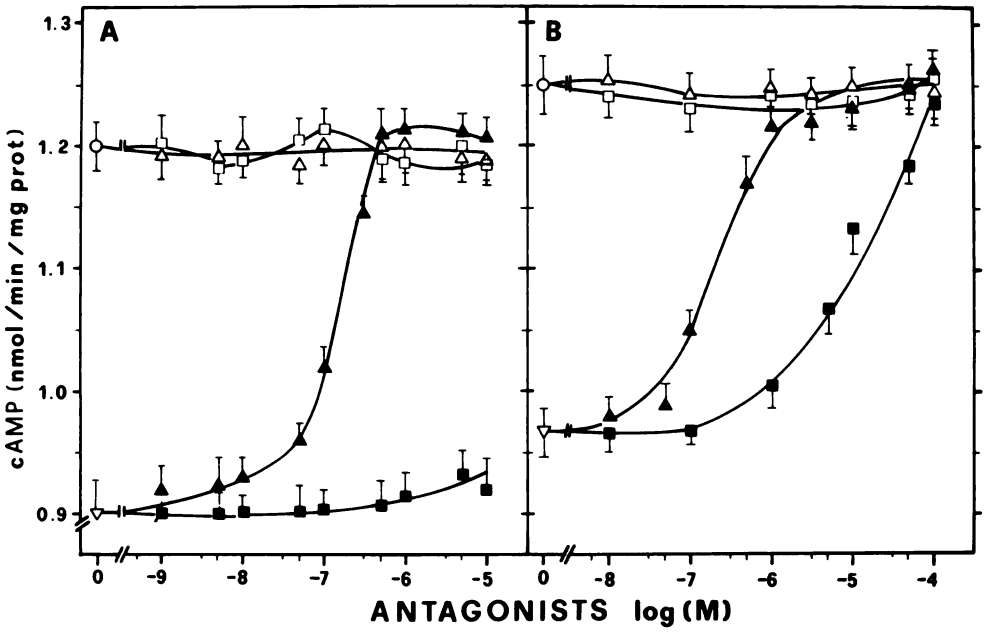

FIG. 4. *Antagonism of dopamine-induced inhibition of striatal adenylate cyclase by (+)- and (−)-butaclamol (A) and by l- and d-sulpiride (B)*
The effect of increasing concentrations of each antagonist was evaluated on the enzyme activity assayed in the absence (*open symbols*) and in the presence (*closed symbols*) of 100 μM dopamine. The reaction mixture contained 0.1 μM SCH 23390. A: ○, control activity; ▽, dopamine; □ and ■, (−)-butaclamol; △ and ▲ (+)-butaclamol. B: ○, control activity, ▽, dopamine; □ and ■, *d*-sulpiride; △ and ▲, *l*-sulpiride. Values are the mean ± SEM of three determinations performed on two membrane preparations.

TABLE 3

Relative potency of various dopaminergic and adrenergic antagonists in reversing the dopamine-induced inhibition of striatal adenylate cyclase activity

The EC_{50} values represent the concentration of antagonist required for half-maximal reversal of the inhibitory effect elicited by dopamine (100 μM) in the presence of 0.1 μM SCH 23390. Each antagonist was tested at concentrations ranging from 1 nM to 100 μM. EC_{50} values were determined from log-probit plots of the reversal (expressed as percentage of the maximal effect) at different concentrations of each antagonist.

Antagonist	EC_{50}
	nM[a]
Spiroperidol	8 ± 1.1
Clebopride	39 ± 11
Domperidone	61 ± 21
Haloperidol	65 ± 5.8
Chlorpromazine	65 ± 8.8
Trifluoperazine	90 ± 20
Pimozide	100 ± 20
(+)-Butaclamol	130 ± 17
cis-Flupenthixol	135 ± 10
Zetidoline	140 ± 8.0
l-Sulpiride	210 ± 30
Clozapine	1450 ± 290
d-Sulpiride	5000 ± 1000
Promethazine	8000 ± 900
Phentolamine	8000 ± 1500
(−)-Butaclamol	>10,000
dl-Propranolol	>100,000

[a] Values presented are the mean ± SE obtained from three experiments performed in triplicate.

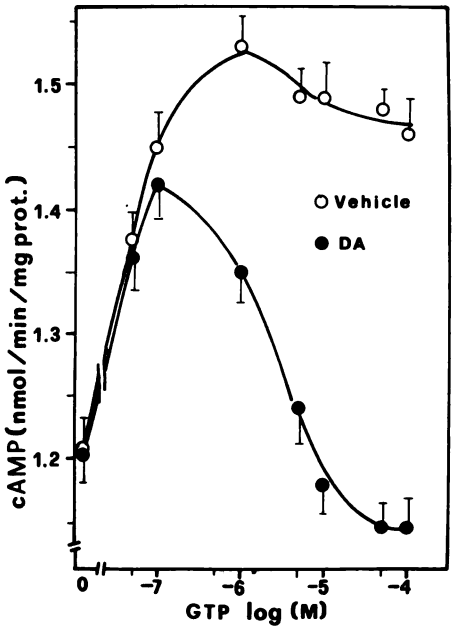

FIG. 5. *GTP dependence of the inhibition of adenylate cyclase by dopamine*
The enzyme activity was assayed in the absence (○) and in the presence (●) of 100 μM dopamine (*DA*). The reaction mixture contained 0.1 μM SCH 23390. Values are the mean ± SEM of three determinations.

Kainic acid has been shown to destroy neuronal cell bodies located in the striatum with minimal damage to axons and nerve endings projecting to this region (20). As shown in Table 4, 10 days after the injection of the neurotoxin, basal adenylate cyclase activity was de-

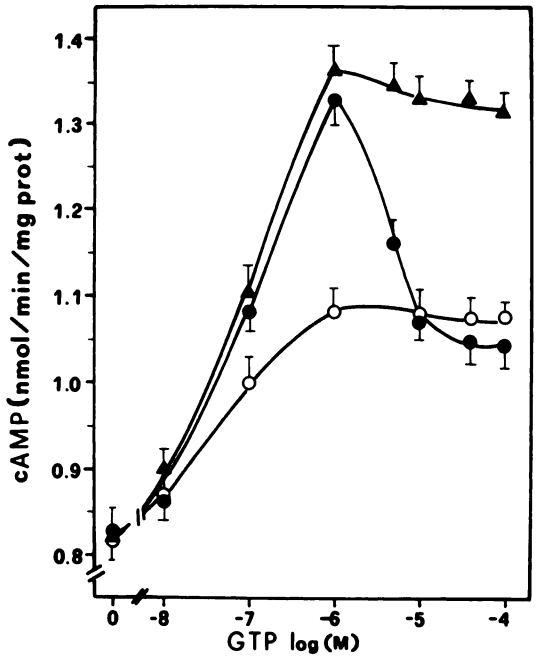

FIG. 6. *Effect of increasing concentrations of GTP on the stimulation of striatal adenylate cyclase by dopamine*

Enzyme activity was assayed in the presence of vehicle (○) 100 μM dopamine (●), and dopamine plus 5 μM 1-sulpiride (▲). When assayed alone, *l*-sulpiride had no effect on the GTP-dependent enzyme activity. Values are the mean ± SEM of four experiments.

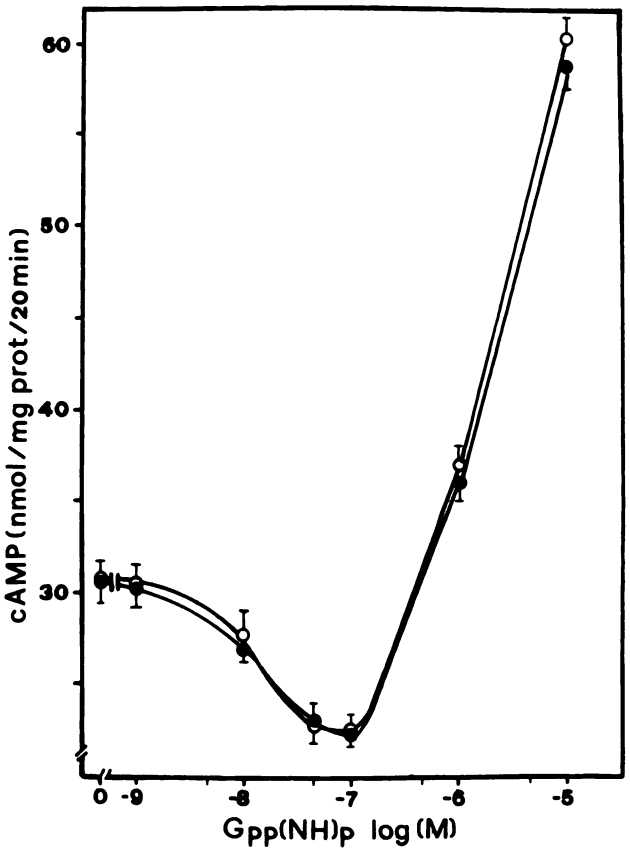

FIG. 7. *Failure of LY 171555 to affect the Gpp(NH)p-dependent adenylate cyclase activity in rat striatum*

The enzyme activity was assayed at the indicated concentrations of Gpp(NH)p in the absence (○) and in the presence (●) of LY 171555. Because under these conditions the enzyme activity was not linear with time (see text), the total amount of cyclic AMP formed during the incubation period is reported. The values are the mean ± SEM of the experiments performed in triplicate.

creased by approximately 70% in the lesioned striatum as compared to the contralateral control striatum. In the presence of SCH 23390, dopamine inhibited the enzyme activity in the intact but not in the lesioned striatum.

DISCUSSION

The common response of striatal adenylate cyclase to dopamine and other agonists of both D_1 and D_2 receptors consists of an increased formation of cyclic AMP. This response is considered to be mediated by agonist occupancy of D_1 receptors (1). However, when dopamine activation of D_1 sites is impaired by SCH 23390, a specific D_1 blocker, dopamine causes inhibition of basal adenylate cyclase activity (8), thereby providing evidence for the occurrence in striatum of a bimodal regulation of the enzyme by dopamine. The present study demonstrates that the dopaminergic inhibition of rat striatal adenylate cyclase is mediated by activation of dopamine receptors with pharmacological properties of D_2 type. Thus, the rank order of potency of various dopamine agonists in inhibiting adenylate cyclase activity ((−)-propylnorapomorphine > A-6,7-DTN = apomorphine > dopamine) is typical of an interaction with the D_2 dopamine receptor (21). Moreover, in the absence of D_1 receptor blockade, adenylate cyclase inhibition is elicited by LY 171555, a selective D_2 receptor agonist, and by bromocriptine. However, bromocriptine behaves like a partial agonist, as it is less effective than dopamine in decreasing the enzyme activity and, when combined with dopamine, antagonizes the inhibitory response elicited by a maximal effective concentration of dopamine. Noradrenaline inhibits the enzyme activity as effectively as dopamine, although with a lower potency. It is likely that the noradrenaline effect is due to an activation of D_2 receptors since it is more sensitive to blockade by spiroperidol, a dopaminergic antagonist with a weak activity on α-adrenergic receptors (22), than it is by phentolamine, an α-adrenergic antagonist. Moreover, the noradrenaline and dopamine effects are not additive, suggesting that the catecholamines act on the same receptor.

Several neuroleptic drugs antagonize the dopamine-induced inhibition of striatal adenylate cyclase activity. Their potency correlates with their relative affinity for D_2 dopamine receptors (21). Thus, spiroperidol, haloperidol and chlorpromazine are effective at nanomolar concentrations, whereas clozapine is active only at micromolar concentrations. The sterospecificity of the dopamine inhibition of the striatal adenylate cyclase activity is demonstrated by the different sensitivity to the isomers of butaclamol and sulpiride. The inactivity of (−)-butaclamol and the 24-fold difference in the potency of *l*-and *d*-sulpiride in reversing dopamine inhibition are similar to the values observed in D_2 dopamine receptor radioligand binding studies (21).

It has been postulated (23–25) that D_2 dopamine receptors exist in two interconvertible affinity states, one

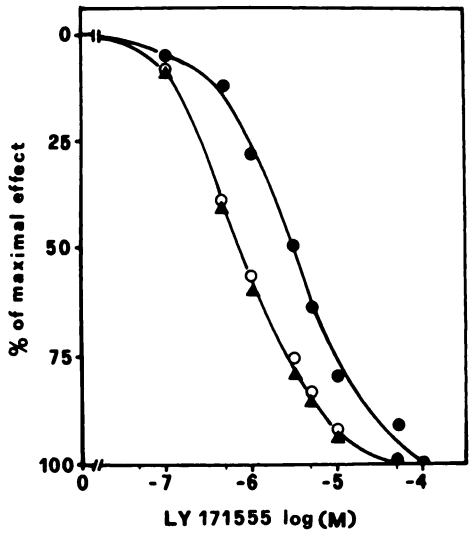

FIG. 8. *Effect of NaCl and KCl on the concentration-response curve of LY 171555 in inhibiting striatal adenylate cyclase*

Enzyme activity was assayed in the absence of added salt (○) and in the presence of either 150 mM NaCl (●) or 150 mM KCl (▲). In these experiments, the reaction mixture contained phosphocreatine Tris salt instead of phosphocreatine sodium salt. Enzyme activities (expressed as pmol of cyclic AMP/min/mg of protein ± SEM) were: 688 ± 35 with no salt added (n = 7), 1270 ± 50 with NaCl (n = 4), and 1250 ± 60 with KCl (n = 3).

TABLE 4

Intrastriatal injection of kainic acid abolishes the ability of dopamine to inhibit adenylate cyclase

Values are the mean ± SE of three experiments performed on two membranes preparations.

	Adenylate cyclase	
	Basal	Dopamine (100 μM)
	cAMP pmol/mg protein/min	
Control side	1500 ± 30	1190 ± 15[a]
Lesioned side	435 ± 10	430 ± 12[b]

[a] $p < 0.01$.
[b] Not significant (paired t-test).

with high and the other with low affinity for agonists. Guanine nucleotides have been proposed to modulate the equilibrium between these two states by promoting the conversion of the receptors from the high to the low affinity state. In the pituitary gland, it has been shown that the dopaminergic inhibition of adenylate cyclase activity correlates with high affinity agonist binding to the D_2 dopamine receptor (5). In the striatum, analysis of agonists/^{3}H-antagonist competition curves for binding to the D_2 receptor indicates that dopamine and other dopaminergic agonists bind to the high affinity state at nanomolar concentrations and to the low affinity state at micromolar concentrations (24, 25). A comparison of the IC_{50} values of agonists in inhibiting striatal adenylate cyclase with the agonist dissociation constants for the two states of the D_2 receptor reported in these radioligand binding studies (24, 25) indicates that in rat striatum the agonist potencies for adenylate cyclase inhibition correlate better with the apparent low affinity state of the D_2 receptor. This parameter, together with the observation of Hill coefficient values close to unity for most of the agonists tested, may suggest that occupancy of a homogeneous population of low affinity sites mediates the D_2 inhibition of striatal adenylate cyclase. However, the concentration-response curves of (−)-apomorphine and A-6,7-DTN display Hill coefficient values significantly lower than 1, indicating that these dopamine agonists can elicit inhibition of adenylate cyclase by interaction with more than one affinity form of the striatal D_2 receptor. Moreover, it should be pointed out that the values of agonists binding to the high and low affinity states of the striatal D_2 receptors were obtained under experimental conditions different from those used in the present study. Therefore, the correlation of the dopaminergic inhibition of striatal adenylate cyclase with the low affinity state of the D_2 receptor should be considered with caution.

Na^+ appears to modulate the D_2 inhibition of striatal adenylate cyclase. As reported for other adenylate cyclase systems (26, 27), Na^+ amplifies the inhibition of the enzyme activity elicited by D_2 receptor stimulation. Moreover, the ion produces a 4-fold decrease of the IC_{50} values of LY 171555 and of dopamine. This effect appears to be specific for Na^+ since an equimolar concentration of KCl fails to affect the D_2 receptor-mediated response. This finding is in agreement with the results obtained in radioligand binding studies showing that Na^+ effectively decreases the agonist affinity of the receptor for several neurotransmitters (28–30), including the striatal D_2 receptor (31, 32).

The dependence of the dopamine inhibition on the presence of GTP suggests that D_2 receptors control striatal adenylate cyclase via an interaction with a guanine nucleotide-dependent inhibitory protein (N_i) (33). This interaction may activate N_i which then inhibits the enzyme activity. The concentration of GTP required by dopamine to elicit inhibition of adenylate cyclase via D_2 receptors is severalfold higher than that required by dopamine to stimulate the enzyme activity via D_1 receptors. These results are in agreement with previous observations in other tissues where adenylate cyclase is subject to bimodal regulation by hormones and neurotransmitters (26, 27). The different GTP requirement suggests that D_1 activation and D_2 inhibition of adenylate cyclase are mediated via different coupling proteins, most likely N_s and N_i, respectively (33). The results shown in this paper indicate that at micromolar concentrations of GTP, which are close to those present intracellularly, dopamine activation of D_2 receptors curtails the dopamine stimulation of the enzyme. This would suggest that D_1 stimulatory and D_2 inhibitory receptors may co-exist on the same striatal neurons and interact by modulating the response of a common adenylate cyclase system. Interestingly, the results obtained by kainic acid lesioning of rat striatum indicate that like D_1 receptors linked to stimulation of cyclase (18), D_2 inhibitory sites are located on neurons intrinsic to the striatum, postsynaptically to the nigrostriatal dopaminergic afferents. However, because of the heterogeneity of the neuronal population of rat striatum and the possible presence of multiple pools of adenylate cyclase, the observed changes

in cyclic AMP formation in response to dopamine may as well result from the algebraic sum of D_1 stimulation and D_2 inhibition of separate enzyme activities. Further studies employing selective D_1 receptor agonists are required to ascertain whether D_2 receptors inhibit the same pool of striatal enzyme activity stimulated by D_1 receptors.

It is noteworthy that the striatal enzyme activity assayed in the presence of Gpp(NH)p, a nonhydrolyzable analogue of GTP, is not inhibited by D_2 receptor activation. LY 171555, at concentrations (100 μM) maximally effective in inhibiting adenylate cyclase when GTP is present, has no effect on both the inhibition and stimulation of the enzyme activity elicited by low and high concentrations of Gpp(NH)p, respectively. Moreover, LY 171555 does not significantly shorten the time lag required by Gpp(NH)p to cause the enzyme inhibition. This finding is in agreement with previous observations indicating that hormonal inhibition of adenylate cyclase cannot be detected in the presence of stable analogues of GTP (27). More recently, however, Jackobs *et al.* (26) have shown that inhibitory hormones potentiate and accelerate the inhibition of adenylate cyclase elicited by nanomolar concentrations of stable analogues of GTP. The lack of a synergistic effect of D_2 agonist and Gpp(NH)p on the enzyme inhibition could be explained by assuming that in our experimental conditions the binding of Gpp(NH)p to N_i may occur independently from agonist occupancy of D_2 receptors or that in the striatum the fraction of N_i proteins regulated by D_2 receptors is small compared to the whole pool of N_i which can be activated by Gpp(NH)p.

Although the enzyme inhibited by dopamine is only 20–25% of the overall basal enzyme activity, the physiological consequence of the dopamine effect could be of greater significance if one considers that only the GTP-sensitive form of the enzyme is susceptible to inhibition by dopamine and that not all this enzyme activity is under inhibitory control by D_2 receptors exclusively. Moreover, as reported for other inhibitory transmitters (27), in the intact tissue a modest decrease in adenylate cyclase activity may result in a greater decrease in cyclic AMP levels due to the activity of phosphodiesterases. The demonstration that also in rat striatum, like in the pituitary gland, D_2 receptor activation decreases adenylate cyclase activity indicates that the inhibition of cyclic AMP formation may represent a common mechanism for transmembrane signaling by dopamine via at least a certain subtype of D_2 receptors.

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