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Dopamine, Acting through D-2 Receptors, Inhibits Rat Striatal Adenylate Cyclase by a GTP-Dependent Process

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Summary

This report demonstrates that the D-2 dopamine receptors that are present in rat striatum can directly inhibit the activity of adenylate cyclase in a GTP-dependent manner. *N-n*-propylnorapomorphine evoked a more pronounced inhibition than did dopamine. However, in the presence of the D-1-selective antagonist, SCH 23390, dopamine was also observed to inhibit the enzyme. Forskolin facilitated the detection of D-2 receptor-mediated inhibition by markedly stimulating striatal adenylate cyclase activity. The inhibition was antagonized in a dose-dependent manner by the D-2 receptor antagonist spiperone (K_i value = 70 pM) and was absolutely dependent on the presence of both GTP and sodium ions. Inhibition produced via D-2 receptors was additive with that produced via opiate or adenosine A1 receptors. The nonhydrolyzable GTP analogue, 5′-guanylylimidodiphosphate [Gpp(NH)p], did not substitute for GTP in promoting the D-2 receptor-mediated inhibition. It thus appears that D-2 receptors mediate adenylate cyclase inhibition by processes that have been observed for other neurotransmitters in the striatum and elsewhere. In addition, Gpp(NH)p displayed a Ca^{2+}/calmodulin dependency for its inhibitory effects that was not shared by receptor-mediated, GTP-dependent inhibition.

For almost 30 years, stimulation of adenylate cyclase has served as the archetypal second messenger signaling system for hormones and neurotransmitters. In recent years, it has become apparent that many neurotransmitters inhibit the enzyme by acting through receptor subclasses that are distinct from those which stimulate the enzyme (1, 2). This is particularly true in brain tissues, where many of the endogenous neurotransmitters have now been shown to possess a receptor subclass that is linked to inhibition of adenylate cyclase. Inhibition is mediated by a GTP-regulatory complex that is distinct from an analogous complex that promotes stimulation of the enzyme (3–5). This inhibitory complex shows distinct functional requirements for its activity; for instance, for hormonal inhibition there is a stringent requirement for hydrolyzable GTP analogues, which is not observed for stimulation (1, 4).

The striatum conforms to the general pattern of other brain regions in possessing opiate (6, 7), muscarinic cholinergic (8), and adenosine A1 (7) receptors, all of which inhibit adenylate cyclase. A paradox arises, however, in the case of the neurotransmitter that is most synonymous with the striatum, dopamine. On the basis of both pharmacological and biochemical criteria, it has been proposed that dopamine interacts with at least two receptor subtypes, termed D-1 and D-2 (9, 10). The D-1 receptor appears to modulate stimulation of adenylate cyclase in each tissue in which it is detected, including the striatum, while in the pituitary, D-2 receptors inhibit the enzyme (11). In the striatum, however, D-2 receptor-mediated inhibition of adenylate cyclase activity has been more difficult to demonstrate. Using intact striatal blocks or striatal neurons in primary culture, D-2 receptors have been shown to decrease cAMP production (12, 13). However, such findings do not preclude the possibility that dopamine may not act directly on adenylate cyclase. A precedent for such a possibility exists in the case of muscarinic cholinergic receptors, in which both M1 and M2 receptors decrease cAMP levels in intact cells, but only M1 receptors are directly linked to adenylate cyclase inhibition (14).

Attempts to determine the possible modes of interaction of dopamine acting via D-2 receptors on striatal adenylate cyclase have been confounded by the presence of D-1 receptors, which have tended to obscure the D-2 effects. The recent availability of the selective D-1 antagonist SCH 23390 (15) has made

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ABBREVIATIONS: BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*, *N*, *N′*, *N′*-tetraacetic acid; Gpp(NH)p, 5′-guanylylimidodiphosphate; NPA, *N-n*-propylnorapomorphine; PIA, *l*-phenylisopropyladenosine.

possible a determination of the effect of dopamine on D-2 receptors without the predominant D-1 effects. In fact, a recent brief report has suggested that, under such circumstances, inhibition mediated by D-2 receptors can be detected in striatal membranes (16). The present report has examined this situation and confirmed that, indeed, dopamine and related agonists can inhibit the striatal enzyme and that this process occurs with the same characteristics and GTP and Na^+ requirements as have been observed to date for all receptor subclasses that are directly linked to the inhibition of adenylate cyclase via inhibitory GTP-regulatory proteins.

Materials and Methods

Preparation of Striatal Plasma Membranes

Various membrane preparation methodologies were evaluated, including crude "P2" preparations, individual fractions from sucrose gradients, variations on the composition of the homogenization buffer, etc. The conditions finally decided upon provided the most reproducible method for detecting inhibitory regulation of the striatal enzyme. Of particular importance was inclusion of protease inhibitors to protect the endogenous protrease-susceptible GTP-inhibitory system, which was monitored by both pertussis toxin labeling and inhibition by other inhibitory effectors of the striatal system, i.e., opiate, muscarinic cholinergic, and adenosine receptor agonists. Various preincubation conditions, that aimed to eliminate inhibition by endogenous neurotransmitters, were also evaluated as a prelude to performing adenylate cyclase assays. The procedure outlined below, with its washing steps, was found to be optimal.

Freshly dissected striata from male Sprague-Dawley rats (170–250 g) were placed in a buffer containing 50 mM Tris (pH 7.4), 1 mM dithiothreitol, 0.1 mM, diethylenetriaminepentaacetic acid, 0.1 mM benzamidine, and 0.1 mM phenylmethylsulfonyl fluoride, to which 10% sucrose (w/w) was added. The tissue was homogenized by hand in a glass homogenizer with 20 ml of buffer/g of wet weight. The suspension was centrifuged at 121 × *g* for 10 min; then, the pellet was discarded. Subsequently, the supernatant was centrifuged at 12,000 × *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in a glass homogenizer with the original homogenizing volume of preparation buffer (without sucrose), to which 0.5 M NaCl was added. This suspension was put on ice for 10 min before centrifugation at 12,000 × *g* for 10 min. The pellet was resuspended in the same volume, as before, of preparation buffer without sucrose and centrifuged at 12,000 × *g* for 10 min. This pellet was suspended in a small volume of preparation buffer containing sucrose (10%). Aliquots (2–2.5 ml) of this suspension were layered on continuous 28–36% sucrose gradients in 38-ml centrifuge tubes. The gradients were centrifuged at 82,500 × *g*, 1 hr, 4°. The main turbid band (at ca. 33%) in each tube was collected with a Pasteur pipette, and these fractions were combined. This suspension was diluted approximately 4-fold with preparation buffer without sucrose and centrifuged at 82,500 × *g*, 30 min, 4°. The pellet was resuspended in the same buffer at 0.5–1 mg of protein/ml. The aliquots were frozen under liquid nitrogen for storage. Basal activity varied somewhat between membrane preparations, ranging from a low value of ca. 40 to a high value of ca. 140 pmol/min/mg. Despite the variability in basal activity, receptor-mediated inhibition was consistently 15–20% and forskolin consistently stimulated basal activity by ca. 4-fold.

Adenylate Cyclase Assays

The adenylate cyclase assay conditions outlined are the results of considerable attempts at amplifying what began as a variably detectable inhibition by the neurotransmitter receptor agonists. The stability afforded by the inclusion of chelators and sulfhydryl reagents was critical, as were the other conditions of the assay, which had been previously optimized for the detection of inhibitory regulation of the fat cell adenylate cyclase (17).

Adenylate cyclase activity was measured by the method of Salomon *et al.* (18). The assay mixture contained: α-^{32}P-ATP (1 μCi), 0.1 mM cAMP, 15 mM Tris-HCl (pH 7.4), 1.25 mM $MgCl_2$, 0.025 mM ATP, 150 mM NaCl, and 0.01 mM GTP (as indicated), 1 unit/ml of adenosine deaminase, 4 mM creatine phosphate, 25 units/ml of creatine phosphokinase, and 0.25 mg/ml of BSA. The final assay volume was 100 μl. Frozen aliquots of membranes were thawed and diluted to 0.3 mg/ml in 50 mM Tris-HCl (pH 7.4) containing 1 mM EDTA and 1 mg/ml of BSA, so that 7.5 μg of membrane protein, as determined by the method of Bradford (19), was assayed. Reactions were incubated for 10 min at 24° and stopped by the addition of 0.1 ml of 2% sodium lauryl sulfate, 1.3 mM cAMP, and 49 mM ATP. Recovery was monitored with 15,000 cpm of ^{3}H-cAMP. Under these conditions, activity was linear both with time (up to 12 min in the presence of forskolin) and with protein concentration (up to 10 μg/assay). The performance of the regenerating system was also checked occasionally by subjecting an aliquot of reaction mixture to thin layer chromatographic analysis in 0.3 M LiCl. Typically, 90% of the radioactivity remained as ATP following a normal incubation.

Modifications in the membrane preparation and adenylate cyclase assay conditions for the determination of the effects of Ca^{2+}/calmodulin. For experiments designed to examine the role of calcium and calmodulin in the regulation of adenylate cyclase, the following additions to the standard procedure were employed. Frozen membranes were thawed and diluted 3-fold with a buffer consisting of 50 mM Tris-HCl (pH 7.4), 1 mM EGTA, and 1 mg/ml of BSA. This suspension was centrifuged at 12,000 × *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in 3 ml of the above buffer. This process was repeated twice (for a total of three washes). Following the third centrifugation, the pellet was suspended in 40 mM Tris-HCl (pH 7.4), 0.8 mM EGTA, and 0.8 mg/ml of BSA, to give a final EGTA concentration of 200 μM in the assay. The adenylate cyclase assay mixture contained the same components described above with the following modifications: 0.2 mM ATP, 80 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 20 mM NaCl, and either 0.3 μM Gpp(NH)p or 0.01 mM GTP as indicated. All other parameters of the assay were as described above.

Determination of free Ca^{2+} Concentrations

In the experiments in which free Ca^{2+} concentrations were varied, free concentrations were calculated as previously described (20). This involved an iterative computing procedure for the solution of the equations describing the complexes formed in a mixture comprising all of the ingredients involved in the assay of adenylate cyclase, i.e., ATP, GTP, EGTA, H^+, Mg^{2+}, Ca^{2+}, and Na^+. The association constants used were those quoted by Sillen and Martell (21).

Materials

SCH 23390 was provided by the Schering-Plough Corp. (Bloomfield, NJ), spiperone by Janssen Pharmaceutical (Beerse, Belgium), and *S*-sulpiride by Ravissa S.p.A. (Milan, Italy). NPA was obtained from Research Biochemicals Inc. (Wayland, MA). All other drugs and chemicals used were of the best quality commercially available.

Statistics

Experiments were repeated two to five times, except where noted, using one to three batches of membranes pooled from a minimum of 20 rats. Although, as indicated in the figure legends, in all cases representative data are shown, statistical analyses were carried out using mean values from all the experiments. Data were analyzed using a three-way analysis of variance using the presence of drug, the drug dosage, and the triplicate determinations as the within-group variables. At no times were significant main effects or interactions found for the triplicate determinations (replicates). When significant differences were observed involving the effect of drugs or drug doses, the significance between these variables was further assessed using either Duncan's test (22) or Dunnett's multiple comparison test (23), respectively.

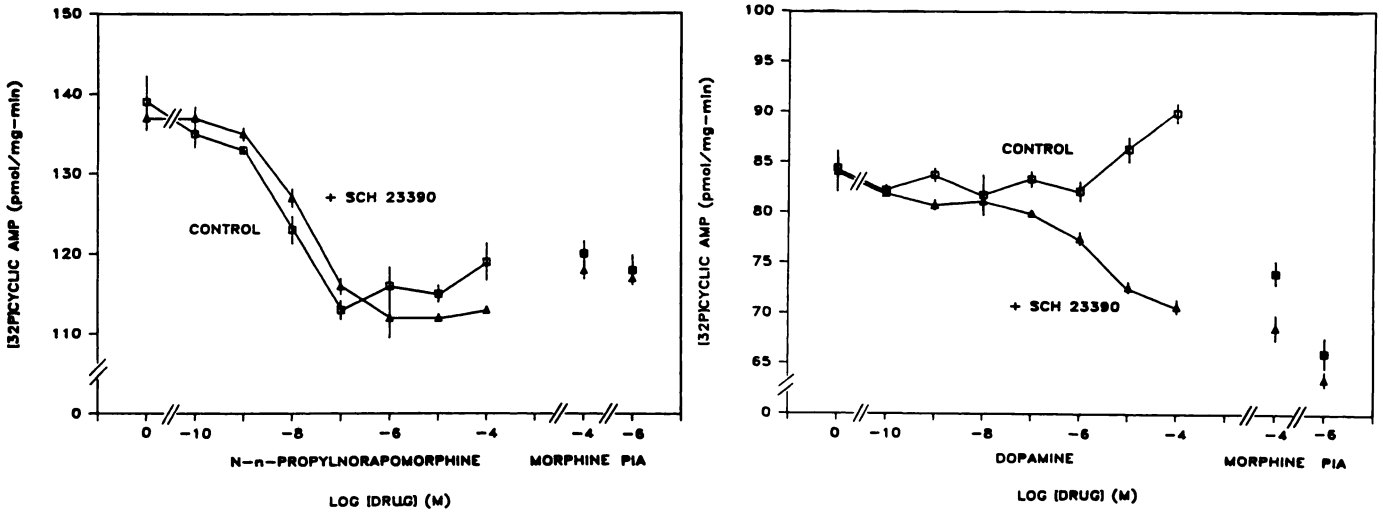

Fig. 1. Addition of the D-1 receptor antagonist SCH 23390 produces little change in the inhibition of rat striatal adenylate cyclase activity induced by (−)-NPA but reveals that induced by dopamine. The activity of adenylate cyclase was measured as a function of the dose of (−)-NPA (*left panel*) or dopamine (*right panel*) in the absence (□) or presence (△) of 0.1 μM SCH 23390. In each experiment, the inhibitions produced by 100 μM morphine (control, ■; +SCH 23390, ▲) and 1 μM PIA (control, ■; +SCH 23390, ▲) were measured to quantify the extent of maximal receptor-mediated inhibition. Values shown are representative data from one experiment with means ± SE for triplicate determinations.

Results

NPA has been reported to be more selective for D-2 than for D-1 receptors (24, 25). This is illustrated in Fig. 1 in which it is apparent that NPA alone caused a statistically significant inhibition of the striatal enzyme, whereas dopamine did not. That this difference is due to the predominance of D-1 effects produced by dopamine is shown by the inclusion of the recently described D-1-selective antagonist, SCH 23390 (15). In the presence of SCH 23390, inhibition by dopamine was clearly apparent at 100 nM. Whether or not SCH 23390 was included in the assay, NPA caused significant inhibition from doses of 10 nM onwards. At a concentration of 0.1 μM, SCH 23390 also blocked any stimulation of adenylate cyclase activity produced by the higher doses of NPA or dopamine (Fig. 1).

The ability of NPA to inhibit the forskolin-activated state of the striatal enzyme was examined (Fig. 2). The absolute magnitude of the inhibition was increased in the presence of forskolin (from 18 to ca. 45 pmol/mg-min), which rendered the inhibition easier to investigate. The inhibition expressed as a percentage of control activity, however, was not enhanced by the addition of forskolin. It can also be seen that, whether in the absence or presence of forskolin, the apparent K_i value for NPA occurred at approximately 10 nM (Fig. 2).

To determine whether the inhibition of adenylate cyclase activity was mediated by D-2 dopamine receptors, inhibition by NPA was examined in the absence or presence of the D-2-selective antagonist spiperone. The NPA inhibition curve was shifted 6-fold to the right in the presence of a submaximal concentration of spiperone (0.4 nM) and almost 50-fold by a higher concentration (4 nM; Fig. 3). Schild plots of such data from three experiments yielded a K_i value for spiperone of 71 ± 4.3 pM, which agrees with the apparent K_d for this compound at rat striatal D-2 receptors, when determined by direct binding studies (26). Another D-2 receptor antagonist, *S*-sulpiride, antagonized the NPA-induced inhibition of adenylate cyclase activity; however, it also displayed anomalous kinetic behavior that rendered the data not amenable to Schild analysis (not shown).

To determine whether this D-2 receptor-mediated inhibition occurs via familiar GTP-mediated pathways, inhibition by 10 μM NPA was examined in the absence or presence of GTP and its nonhydrolyzable congener, Gpp(NH)p (Table 1). In the absence of GTP, no significant inhibition by NPA was measurable whether or not activity was stimulated by forskolin. In the presence of GTP, but in the absence of sodium ions, there was no effect of NPA (Table 2). However, NPA inhibited the activity of adenylate cyclase if both GTP and NaCl were present (Table 2). Thus, an absolute requirement for both GTP and Na^+ was demonstrated.

Gpp(NH)p evokes a marked biphasic response (inhibition followed by stimulation) from dually regulated adenylate cyclase systems, such as the striatum (4). Under the assay conditions used in the present studies, which were optimal for the observation of inhibition, the inhibitory effect of Gpp(NH)p predominated (Table 1). At no point in the dose response to Gpp(NH)p, however, was there any facilitation of the inhibition by Gpp(NH)p by NPA or any of the other inhibitory neurotransmitters that are effective in the striatum (not shown).[1] This lack of effectiveness of Gpp(NH)p at promoting neurotransmitter inhibition has been universally observed in dually regulated systems (1–4).

In a separate series of experiments the inhibition that is evoked by Gpp(NH)p was compared to that brought about by receptors in concert with GTP. Earlier studies by one of us (20) of the hippocampal adenylate cyclase indicated that both GTP-dependent receptor-mediated and Gpp(NH)p-mediated inhibition required the presence of Ca^{2+}/calmodulin. This parameter was also examined in the striatum in the present studies. In membranes that had been depleted of calcium/calmodulin by repeated washing, the readdition of calmodulin with steadily increasing free Ca^{2+} concentrations stimulated activity by ca. 35% (Fig. 4A). Addition of Gpp(NH)p (0.3 μM) elicited a stimulation at low free Ca^{2+} concentrations, which developed into inhibition at Ca^{2+} concentrations around 1 μM. In contrast

[1] M. K. Ahlijanian, M. K. Halford, and D. M. F. Cooper, manuscript in preparation.

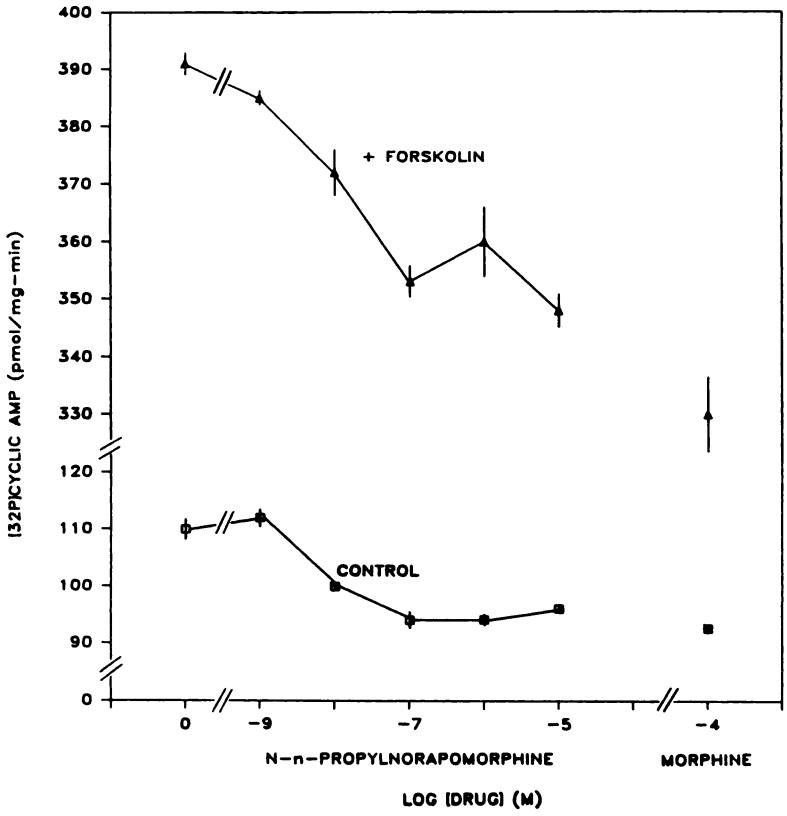

Fig. 2. The inhibition of rat striatal adenylate cyclase activity produced by (−)-NPA is more apparent when the activity is stimulated by forskolin. Values shown are representative data from a single experiment expressed as the means ± SE for triplicate determinations in the absence (□) or presence (△) of 1 μM forskolin. Morphine (100 μM; control, ■; +forskolin, ▲) was included to quantify the maximal extent of receptor-mediated inhibition.

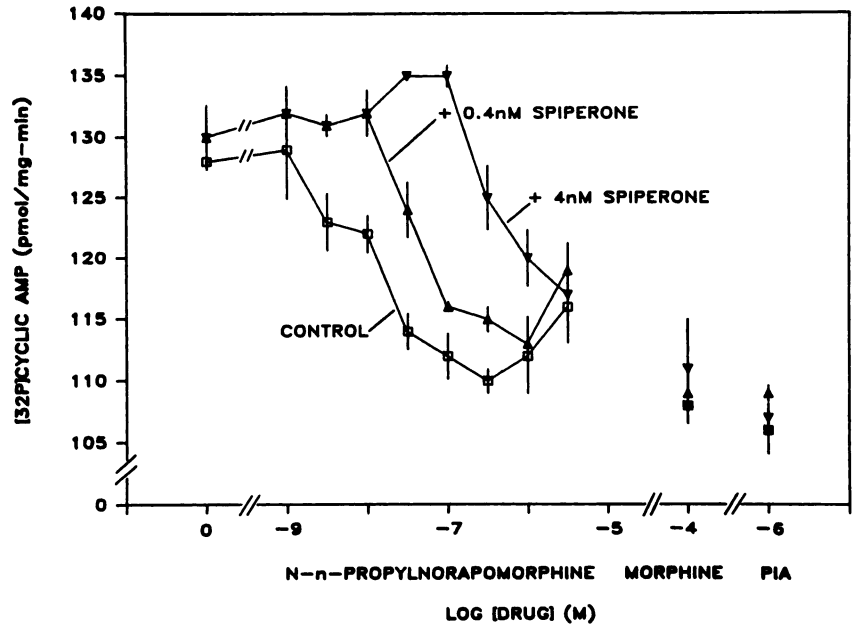

Fig. 3. The apparent affinity of (−)-NPA to inhibit rat striatal adenylate cyclase activity is reduced by the addition of submaximal concentrations of the D-2 receptor antagonist spiperone. Adenylate cyclase activity was measured in the presence of varying concentrations of (−)-NPA, 100 μM morphine, or 1 μM PIA in the absence of inhibitor (NPA, □; morphine or PIA, ■), in the presence of 0.4 nM spiperone (NPA, △; morphine or PIA, ▲) and in the presence of 4 nM spiperone (NPA, ▽; morphine or PIA, ▼). The values shown are representative data from a single experiment with mean values ± SE for triplicate determinations.

with the above results, when the calcium ion dependence for NPA (or morphine; not shown) was examined, no such interaction was observed. Receptor-mediated, GTP-dependent inhibition remained relatively constant and independent of Ca^{2+}, throughout the Ca^{2+} titration curve.

The issue of whether the D-2 receptor-mediated inhibition described thus far overlaps with that mediated by opiates and adenosine was examined in the experiment shown in Table 3. In the absence of forskolin, maximally effective concentrations of morphine, PIA, and NPA each evoked approximately a 20-pmol/mg-min decrement in activity. When either morphine or PIA was used in combination with NPA, an additive decrement of 20 pmol was observed. It is also apparent from Table 3 that the morphine inhibition was reversed by naloxone either in the presence or absence of NPA, but that naloxone did not antagonize the effect of NPA. A similar situation pertains in the presence of forskolin (Table 3). Here NPA did not appear to inhibit as effectively as either morphine or PIA, but when combined with either compound an increased inhibition was observed.

TABLE 1
GTP is required for inhibition of rat striatal adenylate cyclase activity by (−)-NPA both in the absence and presence of forskolin

Forskolin	Guanine nucleotide	Adenylate cyclase activity		
		NPA[a]		
		0	10 μM	100 μM
μM	μM	pmol/mg protein-min		
0	0	69 ± 2.5	67 ± 2.7	72 ± 3.4
0	10, Gpp(NH)p	46 ± 1.4	47 ± 1.6	60 ± 2.1[b]
0	10, GTP	56 ± 3.2	50 ± 2.5[b]	54 ± 2.9
1	0	250 ± 5.4	250 ± 5.8	250 ± 6.3
1	10, Gpp(NH)p	99 ± 4.1	100 ± 4.3	110 ± 4.4[b]
1	10, GTP	210 ± 3.9	180 ± 3.7[b]	180 ± 4.0[b]

[a] Values are means ± SE for $N = 6$; membranes pooled from 20 rat striata were assayed in triplicate in two separate experiments.
[b] Statistical significance was determined using analysis of variance followed by Dunnett's multiple comparison test (22); $p < 0.01$ when compared with appropriate control (absence of NPA).

TABLE 2
Sodium ions are required for inhibition of rat striatal adenylate cyclase by (−)-NPA

	Adenylate Cyclase Activity	
	NPA[a]	
	0	10 μM
GTP 10 μm, 0 NaCl	81.5 ± 3.05	85.1 ± 1.84
GTP 10 μm, 150 mM NaCl	136 ± 1.70	119 ± 1.03[b]

[a] Values are means ± SE; membranes pooled from 20 rat striata were assayed in triplicate.
[b] Statistical significance was determined using a two-tailed Student's t test; $p < 0.001$ when compared with appropriate control (absence of NPA).

Discussion

The present report demonstrates that dopamine and the more potent agonist NPA, acting through D-2 receptors, inhibit the striatal adenylate cyclase in a GTP- and sodium-dependent manner. The modest inhibition is dose dependent and correlates with the anticipated greater potency of NPA relative to dopamine, at binding to D-2 receptors in the anterior pituitary (27). Forskolin markedly stimulates the activity of striatal adenylate cyclase and amplifies the absolute degree of D-2 receptor-mediated inhibition. That the inhibition is mediated by D-2 receptors is demonstrated by the spiperone reversal of the effect, which is achieved with a K_i value similar to that derived from Scatchard analysis of ^{3}H-spiperone binding to rat striatal membranes (26). This value is slightly lower than that reported to reverse dopamine-induced inhibition of adenylate cyclase in rat anterior pituitary homogenates (28). These observations confirm and extend recent reports of similar findings (16, 29, 30). They are also in agreement with observations of a D-2 receptor-mediated decrease in cAMP accumulation in cultured rat striatal neurons (13). The GTP requirement for the inhibition is absolute and the observation that the nonhydrolyzable analogue, Gpp(NH)p, will not substitute for GTP confirms that the inhibition is mediated by conventional inhibitory GTP-regulatory complex pathways (2, 4) rather than those through which, for instance, progesterone inhibits *Xenopus laevis* adenylate cyclase (31).

The fact that the inhibition of NPA is additive with that evoked by either opiates or PIA may suggest that separate pools of adenylate cyclase activity respond to dopamine receptors and to either opiate or adenosine receptors. The simplest explanation for the observation would be that separate cells mediate the effects of these neurotransmitters. Autoradiographic studies are consistent with this proposal; they show a distinct lateral to medial gradient for D-2 receptors in striatum (32) but a uniform distribution of receptor clusters for opioid receptors at similar levels in the striatum (33). However, this proposal will require electrophysiological or more detailed anatomical studies for its resolution.

These observations, then, would seem to resolve the long-standing paradox of the existence of D-2 receptors in the striatum, which had been linked to inhibition of adenylate cyclase in the pituitary, but which could not be demonstrated to be so linked in the striatum. It would appear that the reason for the previous difficulties resided in the dominating presence of D-1 receptors, the use of assay conditions that favored stimulation over inhibition, and the modest degree of the effect. Detection of this effect was assisted by the current availability

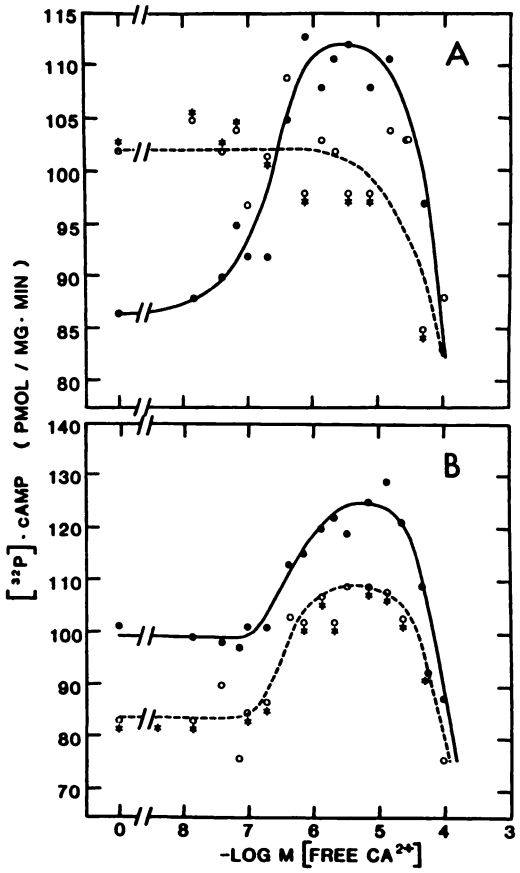

Fig. 4. Calcium/calmodulin role in Gpp(NH)p- and receptor-mediated inhibition. A. Calcium/calmodulin dependence of Gpp(NH)p-induced inhibition of striatal adenylate cyclase. Activity was measured as outlined under Materials and Methods in the absence (●) or presence (○) of 0.2 μM Gpp(NH)p, in the presence of the indicated concentrations of free Ca^{2+}. B. Calcium/calmodulin independence of NPA-induced inhibition of striatal adenylate cyclase. Activity was measured as outlined under Materials and Methods in the absence (●) or presence (○) of 1 μM NPA at the concentrations of free Ca^{2+} indicated. In both experiments the membranes were washed free of calmodulin as described under Materials and Methods; 1 μM calmodulin was then added back to the membranes. *Data points* represent the mean values from three experiments that were carried out in triplicate. Significant differences from control [i.e., the absence of either Gpp(NH)p or NPA] are indicated by • ($p < 0.05$, using analysis of variance followed by Duncan's test).

TABLE 3
Interaction of (−)-NPA with opiate receptors inhibiting adenylate cyclase activity in rat striatal membranes

Inhibition	NPA (0)		+NPA (10 μM)	
	^{32}P-cAMP produced	% Inhibition	^{32}P-cAMP produced	% Inhibition
	pmol/mg-min		*pmol/mg-min*	
Without forskolin[a]				
Control	147 ± 0.85		124 ± 4.65	
PIA (1 μM)	128 ± 2.82[b]	13	109 ± 4.51[b]	12
Morphine (100 μM)	120 ± 2.23[b]	18	109 ± 1.13[b]	12
Naloxone (10 μM) + morphine (100 μM)	143 ± 2.05	3	121 ± 2.08	2
Naloxone (10 μM)	148 ± 1.94	0	118 ± 1.50	5
With forskolin (1 μM)[a]				
Control	480 ± 1.48		417 ± 1.21	
PIA (1 μM)	378 ± 1.19[b]	21	341 ± 2.85[b]	18
Morphine (100 μM)	410 ± 0.58[b]	15	369 ± 1.24[b]	11
Naloxone (10 μM) + morphine (100 μM)	460 ± 0.40[b]	4	412 ± 4.27	1
Naloxone (10 μM)	471 ± 1.41	2	404 ± 3.62	3

[a] Values are means ± SE; membranes pooled from 20 rat striata were assayed in triplicate.
[b] Statistical significance was determined using analysis of variance followed by Dunnett's multiple comparison test (22); $p < 0.01$ when compared with appropriate control.

of the D-1-selective antagonist SCH 23390 and awareness of conditions that are optimal for detection of inhibitory regulation, coupled with optimization of membrane preparation procedures.

An alternative explanation for the persistent difficulties in detecting D-2 receptor-mediated inhibition and for the modest degree of the effect is that, in intact striatal neurons, D-2 receptors are not directly linked to the inhibition of adenylate cyclase. It may be only as a result of selectively disruptive membrane preparative techniques that this apparent association is observed. It is possible that D-2 receptors are indirectly linked to the inhibition of adenylate cyclase in intact striata, in a manner analogous to that by which muscarinic cholinergic receptors inhibit cAMP production in 1321N1 astrocytoma cells (14), and also in a manner analogous to that by which insulin is linked to the inhibition of cAMP production by intact hepatocytes and fat cells. A recent report indicates that insulin can reduce cAMP levels in hepatocytes as a consequence of activating a cAMP phosphodiesterase with a requirement for guanine nucleotides (34). Insulin also inhibits adenylate cyclase in a broken liver cell preparation in a GTP-dependent manner (35). Another set of observations is also pertinent to this issue. In the liver, angiotensin and α-adrenergic receptors do not normally inhibit adenylate cyclase. However, if the membranes are prepared in the presence of high concentrations of EDTA, this effect can be demonstrated (36). One of us previously proposed (4) that this latter effect might be due to a rearrangement of regulatory components that could be brought about as a result of selective membrane preparation methodologies. However, the possibility that D-2 receptors decrease cAMP levels in intact striata by the activation of a phosphodiesterase is rendered less likely based on earlier studies. Stoof and Kebabian (12) demonstrated that D-2 receptors could reduce cAMP levels in striatal slices, whether or not a phosphodiesterase inhibitor was present.

These considerations, together with the present observations, raise the possibility that some of the modest inhibitions of adenylate cyclase activity that can be demonstrated as a result of invasive membrane perturbations may reflect compatibility between different regulatory components rather than the true situation *in vivo*. The possibility of linkage of D-2 receptors to other transmembrane-signaling systems, such as those involving phosphatidylinositol metabolism (37), is not eliminated by these studies. However, for the present, the observation is presented that D-2 receptors can be demonstrated to inhibit striatal adenylate cyclase in a GTP-dependent fashion, presumably through inhibitory GTP-regulatory proteins.

A surprising finding in the present studies was that receptor-mediated inhibition of adenylate cyclase activity was not dependent on whether the basal activity was stimulated by calcium/calmodulin. This result was surprising in the light of earlier studies of the hippocampal enzyme in which it was shown that receptor-mediated inhibition was absolutely dependent on calmodulin-maintained activity (20). However, perhaps this result in the striatum might have been anticipated in the light of the very recent demonstration (30) of D-2 receptor-mediated inhibition of the striatal enzyme in membranes that had been prepared in the presence of EGTA. However, the observation that, in both striatum and hippocampus, Gpp(NH)p-mediated inhibition was dependent on the presence of ca. 1 μM calcium/calmodulin was quite unpredictable. The differences in the regulatory properties of the adenylate cyclase from these two brain areas may lie in differences in their catalytic subunits. The hippocampal enzyme is quite responsive to calcium/calmodulin, which can evoke a 4-fold stimulation of activity (20). The striatal enzyme is only moderately stimulated (ca. 35%) by this regulator, as seen in the present and earlier studies (38, 39). Whether this difference in stimulation by calcium/calmodulin will explain the different regulatory properties of the two systems remains to be determined.

Acknowledgments

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