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PHARMACOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF DOPAMINE RECEPTORS MEDIATING STIMULATION OF A HIGH AFFINITY GTPase IN RAT STRIATUM

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Abstract—In synaptic plasma membranes of rat striatum, activation of dopamine receptors stimulates a high affinity GTPase activity. The rank order of potency of various dopamine receptor agonists in increasing GTP hydrolysis is the following: (−)-propylnorapomorphine > (−)-apomorphine = (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene [(±)-A-6,7-DTN] > dopamine = LY 171555 > noradrenaline. The selective D-1 dopamine receptor agonist, SKF 38393, does not produce a significant increase in GTP hydrolysis. Moreover, the dopamine-stimulated GTPase activity is completely reversed by the D-2 receptor antagonists, 1-sulpiride and zetidoline, but not by the selective D-1 antagonist SCH 23390. Na^+ modulates the dopamine receptor-regulated GTP hydrolysis by increasing the percentage of stimulation and decreasing the agonist potency. Intrastriatal injection of pertussis toxin, which impairs the function of the inhibitory guanine nucleotide binding regulatory protein (N_i) of adenylate cyclase, significantly reduces the dopamine stimulation of striatal GTPase activity and the dopamine inhibition of adenylate cyclase. In contrast, cholera toxin, which blocks the stimulation of GTPase activity by hormones which increase adenylate cyclase activity, does not modify the dopamine-stimulated GTPase activity. These data indicate that the stimulation of GTPase activity elicited by dopamine results from activation of the D-2 type of dopamine receptors and is expression of the increased turnover of GTP at the level of N_i. The results are consistent with the idea that N_i is involved in the inhibitory coupling of striatal D-2 receptors to adenylate cyclase.

Activation of a membrane-bound high affinity GTPase is frequently associated with both stimulation and inhibition of adenylate cyclase by hormones and neurotransmitters [1, 2]. These opposite hormonal signals are processed to cyclase by a receptor-mediated activation of two separate regulatory proteins, one, termed N_s, mediating stimulation and another, termed N_i, mediating inhibition of adenylate cyclase [3]. Both N_s and N_i consist of three subunits named α, β and γ [4, 5]. The activation of N_s and N_i requires binding of GTP and is accompanied by the dissociation of these proteins in the α and $\beta\gamma$ subunits [4, 5]. There is evidence that the activation of both coupling systems is terminated by the hydrolysis of the bound GTP by high affinity GTPases with the consequent formation of inactive GDP–N_s and GDP–N_i complexes [6, 7]. Displacement of bound GDP by free GTP reinitiates the activation of N_s and N_i. Both N_s and N_i have been shown to possess a GTP hydrolyzing activity [8–10]. Thus, the regulatory proteins can continuously cycle from an active to an inactive state and the transmitter-induced increase of GTPase activity can be an index of the enhanced turnover of GTP at these regulatory sites. More recently, a similar mechanism involving the binding and the hydrolysis of GTP by a regulatory protein has been proposed to be operative in the coupling of hormonal receptors to phospholipase C-catalyzed breakdown of poliphosphoinositides [11]. In rat striatum, dopamine (DA)† can either stimulate or inhibit adenylate cyclase activity by acting on D-1 and D-2 receptors, respectively [12]. DA has also been reported to increase a high affinity GTPase activity (apparent K_m for GTP 0.6 μM) through a receptor-mediated mechanism in rat striatal membranes [13]. In the present study we investigate the pharmacological profile of the DA receptor mediating the stimulation of GTPase activity and the possible relationship of the increased GTP hydrolysis with the actions of the neurotransmitter on the striatal adenylate cyclase system.

MATERIALS AND METHODS

Materials. [γ-^{32}P]GTP (10–50 Ci/mmol), [α-^{32}P]ATP (30–50 Ci/mmol) and [2,8-^{3}H]cyclic AMP (25 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Adenyl-5′-yl-imidodiphosphate [AMPP(NH)P] was from Boehringer Mannheim. Reagents for GTPase and adenylate cyclase assays, dopamine hydrochloride, 1-noradrenaline hydrochloride, acetylcholine hydrochloride, eserine sulfate, 1-isoproterenol hydrochloride

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† Abbreviations used: DA, dopamine; EGTA, ethyleneglycolbis(β-aminoethylether)-*N*′*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; (±)-A-6,7-DTN, (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

and cholera toxin were obtained from Sigma Chemical Co. (St. Louis, MO). Pertussis toxin (islet-activating protein) was purchased from List Biological Lab. (Campbell, CA). The sources of the other drugs were as follows: (−)-apomorphine hydrochloride, (−)-propylnorapomorphine hydrochloride, (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene [(±)A-6,7-DTN] hydrobromide and (−)butaclamol, Research Biochemicals Inc. (Wayland, MA); 1-sulpiride, Ravizza (Muggiò, Italy); zetidoline, Lepetit Res. Lab. (Milano, Italy); (+)butaclamol, Ayerst (Rouses Point, NY); SCH 23390 ([R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7ol]maleate), Schering Plough (Bloomfield, NJ); spiroperidol, Janssen Pharmaceutical (Beerse, Belgium); LY 171555 ([*trans*-(−)-4aR-4,4a,5,6,7,8a,9-octahydro-5-propyl-1H(or 2H)-pyrazolo(3,4-g)-quinoline]monohydrochloride), Ely Lilly & Co. (Indianapolis, IN); SKF 38393-A, Smith Kline & French (Philadelphia, PA). Drugs were dissolved in distilled water, glacial acetic acid or ethanol just before the beginning of the enzyme assay. Control samples were incubated in the presence of equal amounts of solvent.

Methods. Male Sprague–Dawley rats (180–250 g) were used for the experiments. The animals were sacrificed by decapitation and striata quickly dissected. Synaptic plasma membranes were isolated according to the method of Jones and Matus [14] as previously described [15]. Synaptic plasma membranes were stored at −70° for no longer than ten days and thawed only once before the beginning of the assay.

GTPase assay. GTPase activity was assayed in isolated synaptic plasma membranes according to the method of Cassel and Selinger [16]. Unless otherwise indicated, the reaction mixture (final volume 100 μl) contained 50 mM HEPES/NaOH buffer (pH 7.4), 1 μM [γ-^{32}P]GTP (700–1000 cpm/pmol), 0.5 mM AMPP(NH)P, 0.5 mM ATP, 2 mM $MgCl_2$, 5 mM phosphocreatine (sodium salt), 50 U/ml of creatine phosphokinase, 0.2 mM EGTA, 0.1 mM EDTA, 0.2 mM dithiothreitol and 50 μg of bovine serum albumin. Cyclic AMP (1 mM) and 3-isobutylxanthine (0.5 mM) were also included to maintain the composition of the reaction mixture similar to that used for adenylate cyclase assay. Reaction was started by the addition of 20 μl of synaptic plasma membranes (3–5 μg of membrane protein) and carried out at 30° for 4 min. The reaction was stopped by the addition of 0.5 ml of 5% (w/v) ice-cold activated charcoal in 20 mM phosphoric acid (pH 2.5). ^{32}Pi released from [γ-^{32}P]GTP was measured in 200 μl aliquots of supernatant following centrifugation at 7000 *g* for 10 min. The high affinity GTPase was routinely calculated as described by Cassel and Selinger [16] by subtracting the amount of [γ-^{32}P]GTP hydrolyzed in the presence of 50 μM unlabeled GTP in order to correct for the activity contributed by the low affinity GTPase. In the experiment reported in Fig. 5, GTPase activity was assayed at 25° for 10 min. Moreover, the reaction mixture contained 100 mM NaCl and 80 mM Tris–HCl (pH 7.4) instead of HEPES/NaOH buffer. These assay conditions closely reproduced those found to be optimal for D-2 inhibition of adenylate cyclase [17] and therefore were used to compare the effect of pertussis toxin treatment on DA-stimulated GTPase activity and on DA-inhibited adenylate cyclase activity.

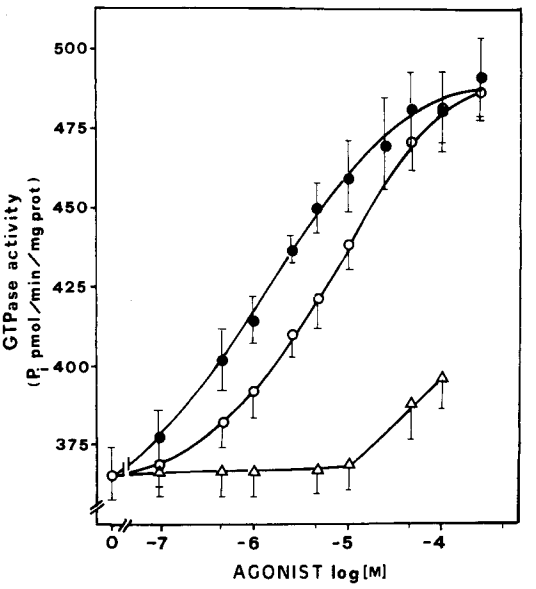

Fig. 1. Response of stratial GTPase activity to increasing concentrations of dopamine, 1-noradrenaline and 1-isoproterenol. Enzyme activity was assayed at 1 μM GTP in the presence of the indicated concentrations of dopamine (●), 1-noradrenaline (○) and 1-isoproterenol (△). Values are the mean ± SEM of five experiments, using three different membrane preparations. Each agonist was tested in each membrane preparation and was present at all the indicated concentrations in every experiment.

Adenylate cyclase assay. The enzyme activity was assayed in a reaction mixture containing the same constituents present in the GTPase assay with the exception that EDTA and AMPP(NH)P were omitted and [α-^{32}P]ATP (0.5 mM, 30–40 cpm/pmol) was substituted for [γ-^{32}P]GTP. The assay was carried out at 30° for 5–10 min. In the experiment reported in Table 4 the assay was carried out at 25° for 20 min. Cyclic AMP was isolated according to the method of Salomon *et al.* [18].

Intrastriatal injections. Pertussis toxin was dissolved in 50 mM sodium phosphate buffer and 250 mM NaCl (pH 7.0). Cholera toxin was dissolved in 50 mM Tris–HCl, 200 mM NaCl, 3 mM NaN_3 and 1 mM EDTA (pH 7.5). Male Sprague–Dawley rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame. Bacterial toxins (3.75 μg) were injected into the right caudate nucleus at three positions (1.25 μg in 2.5 μl over a 9 min period): A + 3.5, L 2.8, V − 4.0 and V − 5.0; A + 1.5, L 3.5, V − 4.0, according to the atlas of Pellegrino and Cushman [19] with bregma as zero. Control animals were injected with an equal volume of vehicle containing 3.75 μg of bovine serum albumin. Thirty to forty hours after surgery the animals were sacrificed by decapitation and synaptic plasma membranes were isolated from vehicle- and toxin-treated striata. Each membrane preparation

Table 1. Effect of various neuroreceptor stimulants on high affinity GTPase activity of rat striatum

	GTPase activity (pmol Pi/min/mg protein)	Δ activity
Basal	318 ± 10	
Dopamine 100 μM	438 ± 13*	120
1-Noradrenaline 100 μM	435 ± 17*	117
Dopamine + 1-noradrenaline	442 ± 15*	124
Acetylcholine 100 μM	483 ± 10*	165
Dopamine + acetylcholine	608 ± 14*	290

Values are the mean ± SEM of three experiments performed on a single membrane preparation. Δ activity indicates the net enzyme stimulation elicited by each agonist, alone or in combination. When the effect of acetylcholine was tested the reaction mixture contained 10 μM eserine.

* $P < 0.001$ vs basal. Student's *t*-test.

was obtained from pooled striata of four rats. Three different membrane preparations were tested.

Protein content was determined by the method of Bradford [20] using bovine serum albumin as a standard.

Statistical analysis. The enzyme assays were carried out in triplicate. The intra-assay variation was less than 2%. Statistical significance between the concentration–response curves was determined by analysis of variance [21]. In the other experiments Student's *t*-test was used. Changes in the EC_{50} value of DA due to either Na^+ or pertussis toxin treatment were assessed by comparison with the EC_{50} value of DA obtained in the same experiment under control conditions.

K_i values of DA receptor antagonists were calculated according to the equation [22]: $IC_{50}/[1 + (C/EC_{50})]$, where IC_{50} is the concentration of antagonist which reverses by 50% the DA stimulation; C is the concentration of DA and EC_{50} is the concentration of DA producing half-maximal stimulation.

RESULTS

Concentration–response curves of striatal GTPase activity to increasing concentrations of DA, noradrenaline (NA) and 1-isoproterenol showed that DA was the most potent catecholamine to stimulate the enzyme activity (Fig. 1). The concentration of DA which elicited half-maximal stimulation (EC_{50}) was 1.6 ± 0.2 μM (mean ± SEM, N = 10). NA was as effective as DA in increasing GTPase activity, but it was less potent (EC_{50} = 7.1 ± 0.4 μM; mean ± SEM, N = 5). 1-Isoproterenol did not affect the enzyme activity at concentrations up to 10 μM while higher concentrations produced a small and not significant increase of GTP hydrolysis (at 100 μM isoproterenol $P > 0.05$ vs basal). The activation of GTPase produced by a combination of DA and NA, each at maximally effective concentration was no greater than with either agent alone (Table 1), indicating that NA stimulated GTPase activity by acting on the same receptor activated by DA. On the contrary, the stimulatory effect of DA was additive with that elicited by acetylcholine acting on muscarinic receptors [23]. Like DA, non-selective DA receptors agonists, such as (−)-propylnorapomorphine, (−)-apomorphine and (±)A-6,7-DTN and the selective

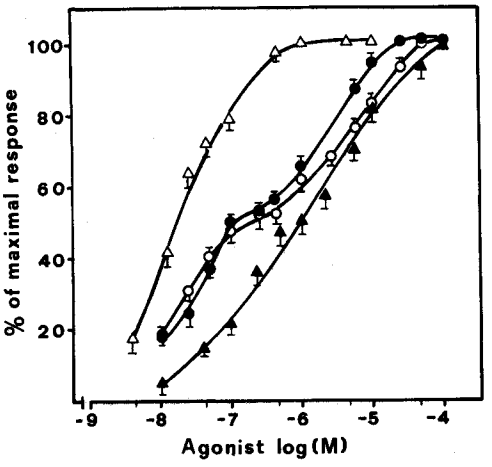

Fig. 2. Concentration-dependent stimulation of striatal GTPase activity by (−)-propylnorapomorphine (△), (−)-apomorphine (●), (±)-A-6,7-DTN (○) and LY 171555 (▲). The stimulation of GTPase activity by the agonists is reported as a percentage of the maximal effect at the indicated concentrations of each agonist. Values are the mean ± SEM of 5–6 experiments performed using four different membrane preparations. (−)-Propylnorapomorphine (1 μM), (−)-apomorphine (50 μM), (±)-A-6,7-DTN (50 μM) and LY 171555 (100 μM) stimulated the GTPase activity by 32.0% ± 2 ($P < 0.001$), 29.5% ± 1.5 ($P < 0.001$), 29.0% ± 3 ($P < 0.001$) and 19.5% ± 2 ($P < 0.005$) of basal enzyme activity, respectively (mean ± SEM). Basal enzyme activity was 330 ± 13 pmol Pi/min/mg protein ± SEM.

D-2 DA receptor agonist LY 171555 [24] stimulated striatal GTPase activity in a concentration-dependent manner (Fig. 2). The concentration–response curves of (−)-apomorphine and (±)-A-6,7-DTN were markedly biphasic, displaying high- and low-affinity components separated by an evident plateau. The maximal stimulation of GTPase activity by (−)-propylnorapomorphine, (−)-apomorphine and (±)-A-6,7-DTN corresponded to approximately 85% of that obtainable with DA. On the other hand, LY 171555 was less effective than DA, the maximal stimulation being 60% of that elicited by DA. Estimation of the concentration of the agonists which elicited half-maximal effect (Table 2) yielded the

Table 2. Potency of various dopaminergic and adrenergic agonists in stimulating striatal GTPase activity

Agonist	EC_{50} (μM)
(−)-propylnorapomorphine	0.02 ± 0.003 (5)
(±)-A-6,7-DTN	0.20 ± 0.02 (6)
(−)-apomorphine	0.10 ± 0.04 (6)
LY 171555	1.00 ± 0.3 (5)
Dopamine	1.60 ± 0.2 (10)
1-Noradrenaline	7.10 ± 0.4 (5)
1-Isoproterenol	Inactive at 10 μM (5)
SKF 38393	Inactive at 10 μM (3)

The EC_{50} value refers to the concentration of the agonist required for half-maximal stimulation of striatal GTPase activity calculated in relation to the maximal effect obtainable with that agonist. Values are the mean ± SEM of the number of determinations reported in parentheses.

Table 3. Effect of NaCl on basal and dopamine-stimulated GTPase activities in rat striatum

	GTPase activity (pmol Pi/min/mg protein)	
	−NaCl	+NaCl (100 mM)
Basal	306 ± 8	169 ± 2*
Dopamine 100 μM	410 ± 10† (33.9%)	270 ± 4† (59.7%)

The reaction mixture contained phosphocreatine Tris salt instead of phosphocreatine sodium salt. Values are the mean ± SEM of 3 experiments performed with a single membrane preparation.

* $P < 0.001$ vs no NaCl added.

† $P < 0.001$ vs basal. Student's t-test. In parentheses is reported the percentage of enzyme stimulation elicited by dopamine.

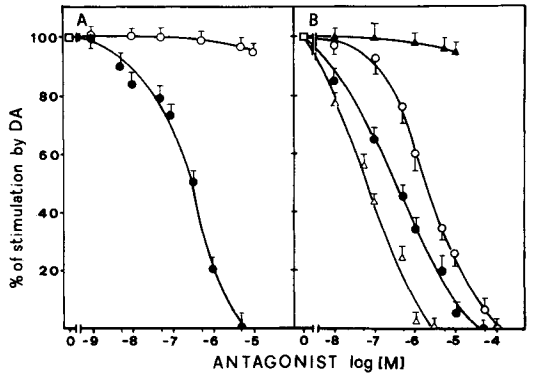

Fig. 3. Antagonism of dopamine stimulation of striatal GTPase by various dopamine receptor blockers. A. The effect of increasing concentration of (+)butaclamol (●) and (−)butaclamol (○) was evaluated on the enzyme activity stimulated by 100 μM dopamine. Values are the mean ± SEM of 5 experiments. B. The effect of increasing concentrations of spiroperidol (△), 1-sulpiride (●), zetidoline (○) and SCH 23390 (▲) was evaluated on the enzyme activity stimulated by 50 μM dopamine. For each antagonist values are the mean ± SEM of 3 experiments.

Data are reported as percentage of the net effect of dopamine, considered as 100, at the indicated concentrations of each antagonist. Enzyme activities (pmol Pi/min/mg protein ± SEM) were: in A basal 350 ± 17, DA 475 ± 19, N = 5; in B: basal 390 ± 20, DA 518 ± 21, N = 9. At the concentrations used none of the antagonists significantly affected basal GTPase activity by themselves.

following rank order of potency: (−)-propylnorapomorphine > (−)-apomorphine = (±)-A-6,7-DTN > dopamine = LY 171555. The putatively selective D-1 receptor agonist, SKF 38393 [24], tested at concentrations ranging from 0.01 to 10 μM, maximally stimulated the enzyme activity by 6–7%. This change was not statistically significant. Under assay conditions similar to those used for GTPase, SKF 38393 increased striatal adenylate cyclase activity by 66% with an EC_{50} value of 30 nM. (+)Butaclamol, a non-selective blocker of D-1 and D-2 sites, antagonized the DA stimulation of GTPase, whereas (−)butaclamol, the inactive enantiomer, was ineffective (Fig. 3A). The apparent K_i value of (+)butaclamol was 5 nM. Spiroperidol, a potent D-2 receptor antagonist [24], blocked the DA response with a K_i value of 1.8 nM (Fig. 3B). The selective D-2 receptor antagonist 1-sulpiride [24] completely reversed the DA stimulation of GTP hydrolysis with a K_i value of 15 nM. In contrast, the selective D-1 receptor antagonist, SCH 23390 [25], was unable to counteract the DA effect at concentrations as high as 10 μM which were supramaximal in antagonizing the stimulation of striatal adenylate cyclase by 100 μM DA. We also tested the effect of another D-2 receptor antagonist, zetidoline [26], which completely reversed the DA stimulation of GTPase with a K_i value of 60 nM (Fig. 3B), but it failed to significantly block the stimulation of striatal adenylate cyclase activity elicited by 50 μM DA at concentrations up to 50 μM.

The DA stimulation of striatal GTPase activity was affected by Na^+ (Table 3). The addition of 100 mM NaCl inhibited basal GTPase activity by 45%, whereas it did not significantly affect the net stimulation produced by DA. As a consequence, the percentage of maximal enzyme stimulation elicited by DA was markedly enhanced by the addition of NaCl. Moreover, analysis of the concentration–response curves of DA revealed that Na^+ increased the EC_{50} value of DA from 0.8 ± 0.1 μM to 4.1 ± 0.2 μM (mean ± SEM, N = 3) (Fig. 4).

Bacterial toxins, like cholera toxin and pertussis toxin, are known to differentially affect the function of N_s and N_i proteins. Thus, cholera toxin ADP-ribosylates N_s, enhances hormonal stimulation of adenylate cyclase and blocks the increased GTP hydrolysis produced by activators of adenylate cyclase [27, 28]. On the other hand, pertussis toxin ADP-ribosylates N_i and attenuates hormal inhibition of adenylate cyclase as well as the stimulation of GTPase by agents that inhibit adenylate cyclase [2, 29]. Figure 5 shows that the stimulation of striatal GTPase by DA was reduced by approximately 50% by pertussis toxin treatment. Moreover, the EC_{50} value of DA was increased by threefold (from 1.9 to 6.1 μM) in membranes isolated from toxin injected striata. In contrast, the intrastriatal injection of an equal amount of cholera toxin did not significantly affect the stimulatory response of striatal GTPase to DA. When adenylate cyclase activity was assayed in these membrane preparations, it was found that

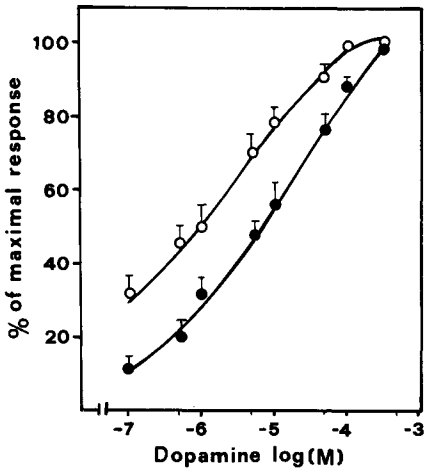

Fig. 4. Effect of NaCl on the concentration-response curve of striatal GTPase activity to dopamine. Enzyme activity was assayed in the absence of added salt (○) and in the presence of 100 mM NaCl (●) at the indicated concentrations of DA. In this experiment the reaction mixture contained 50 mM HEPES–Tris buffer (pH 7.4) and phosphocreatine Tris salt instead of HEPES–NaOH buffer and phosphocreatine sodium salt. Values are the mean ± SEM of three experiments. Basal enzyme activities were as reported in Table 3.

pertussis toxin treatment attenuated the DA-inhibited enzyme activity by 51%, whereas cholera toxin treatment increased striatal adenylate cyclase activity by threefold and amplified the DA response by 40% (Table 4).

DISCUSSION

A number of evidence indicate that GTP exerts a modulatory action on striatal DA receptor function. Thus both D-1 mediated stimulation and D-2 mediated inhibition of striatal adenylate cyclase activity requires the presence of GTP [17, 30, 31]. Moreover, guanine nucleotides have been shown to regulate the binding of agonists to striatal D-1 and D-2 sites, apparently by promoting the conversion of the recognition site from a high- to a low-affinity state for the agonist [32, 33]. These effects are considered to be mediated by the binding of GTP to guanine nucleotide-dependent regulatory protein(s), likely N_s and N_i, and the consequent dissociation of these regulatory proteins from the recognition sites [34]. Consistent with the hypothesis that striatal DA receptors are coupled to N proteins is the observation that DA increases a high affinity GTPase activity in rat striatal membranes [13]. In the present study we investigate the pharmacological profile of this stimulatory response and examine whether the increased GTP hydrolysis could reflect an enhanced N_s and/or N_i activity. The data obtained by using non-selective and selective agonists and antagonists of D-1 and D-2 DA receptors indicate that striatal GTPase is activated via stimulation of D-2 type of DA receptors. Thus, the rank order of potency of various DA agonists in stimulating GTPase activity (−)-propylnorapomorphine > (−)-apomorphine = (±)-A-6,7-DTN > DA is typical of an interaction with D-2 DA receptors [35]. The selective D-2 receptor agonist LY 171555 is capable of stimulating the enzyme activity, whereas the compound SKF 38393, a selective D-1 agonist, fails to produce a significant change. However, LY 171555 is less effective than DA indicating a partial agonist property of the compound. A similar response was previously observed

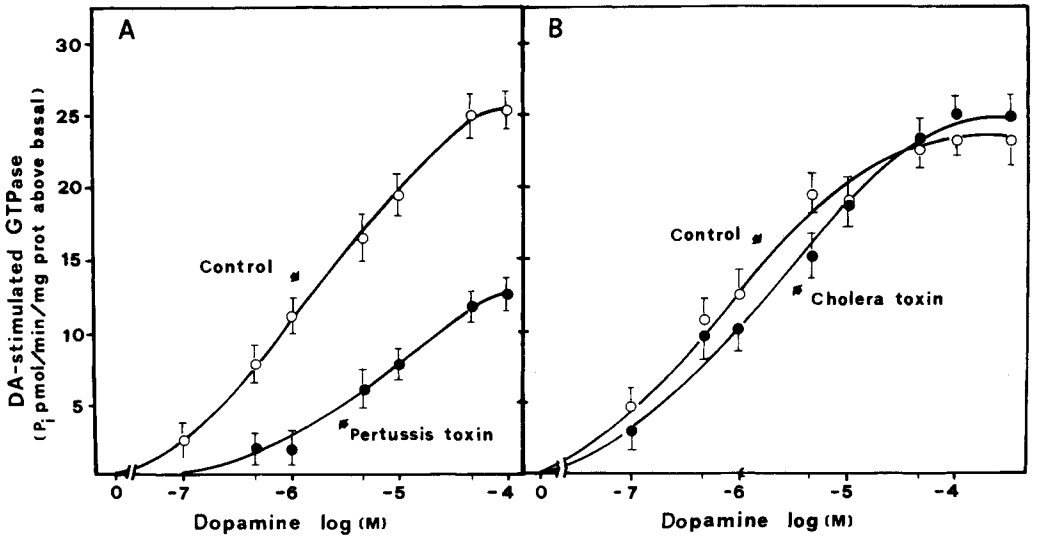

Fig. 5. Effect of intrastriatal injection of pertussis toxin (A) and of cholera toxin (B) on dopamine-stimulated GTPase activity. Enzyme activities were assayed in membranes isolated from pooled striata injected with either vehicles (open symbols) or toxins (closed symbols) at the indicated concentrations of DA. Reaction mixtures contained 100 mM NaCl and 80 mM Tris–HCl (pH 7.4) instead of 50 mM HEPES–NaOH. Incubations were carried out at 25° for 10 min. Basal enzyme activities (pmol Pi/min/mg protein ± SEM) were: in A vehicle 56.7 ± 2, pertussis toxin 48.0 ± 4, N = 3; in B vehicle 62.0 ± 3, cholera toxin 69.5 ± 3, N = 3. In A $P < 0.05$ for the difference between the two curves by analysis of variance.

Table 4. Effect of intrastriatal injection of pertussis toxin and of cholera toxin on dopamine-inhibited adenylate cyclase activity

Treatment	DA-inhibited adenylate cyclase (pmol cyclic AMP/min/mg protein ± SEM)	
	Vehicle	Toxin
A: Cholera toxin	105 ± 7	150 ± 10*
B: Pertussis toxin	112 ± 9	55 ± 6†

Values indicate the enzyme activity inhibited by DA (100 μM) below basal values. Basal enzyme activities (pmol cyclic AMP/min/mg protein ± SEM) were: in A, vehicle 464 ± 13; cholera toxin 1300 ± 25; in B, vehicle 490 ± 15, pertussis toxin 480 ± 18. For each treatment, data were obtained from three experiments using three separate membrane preparations.

* $P < 0.05$; † $P < 0.02$ vs vehicle. Student's *t*-test.

when LY 171555 was tested as inhibitor of striatal adenylate cyclase activity [17]. Moreover, the obtained EC_{50} values of agonists in stimulating GTP hydrolysis correlate quantitatively with their potency in inhibiting striatal adenylate cyclase.

In striatal membranes, radioligand-binding studies have proposed that the D-2 recognition site can exist in two affinity states, one with high and the other with low affinity for agonists [36, 37]. The biphasic concentration-response curves of (−)-apomorphine and (±)-A-6,7-DTN observed in the present study suggest that these agonists recognize an heterogeneous population of high and low affinity sites both linked to stimulation of GTPase activity in rat striatum. Whether this heterogeneity reflects the binding to two different affinity states of a single D-2 recognition site or to D-2 receptors differing in affinity for the agonists has still to be defined. It is noteworthy that the concentration-response curves of (−)-apomorphine and (±)-A-6,7-DTN in inhibiting striatal adenylate cyclase activity displayed Hill coefficient values lower than 1 [17].

The DA stimulation of striatal GTPase activity is blocked by (+)butaclamol, but not by (−)butaclamol indicating that the effect is stereospecific. Moreover, the potent D-2 receptor blocker spiroperidol and the selective D-2 receptor antagonists 1-sulpiride and zetidoline completely antagonize the stimulatory effect of DA with a potency consistent with an action on D-2 sites. In contrast, the selective D-1 receptor antagonist SCH 23390 is ineffective at concentrations 50–100 fold higher than those required to completely block DA activity on D-1 sites.

As shown for other receptor-regulated GTPase activities [38, 39], Na^+ markedly amplifies the DA-stimulated GTP hydrolysis. Moreover, in agreement with radioligand-binding studies showing that Na^+ reduces the agonists affinity for the striatal D-2 recognition sites [40], the ion decreases the potency of DA in stimulating the enzyme activity. Similar changes have been found to be induced by Na^+ on D-2-mediated inhibition of striatal adenylate cyclase [17].

The intrastriatal injection of pertussis toxin, but not of cholera toxin, markedly reduces the DA-stimulated GTPase activity and decreases the potency of DA. Moreover, the pertussis toxin treatment decreases the DA-inhibited adenylate cyclase activity by the same extent as the DA-stimulated GTPase activity. In rat striatum, pertussis toxin has been reported to decrease the affinity of D-2 DA receptors for agonists apparently by ADP-ribosylating the N_i protein [41]. These results suggest that the striatal GTPase activity sensitive to DA results from D-2 receptor-induced activation of N_i coupled to inhibition of adenylate cyclase.

Recent studies have shown that in rat anterior pituitary D-2 DA receptors are linked not only to inhibition of adenylate cyclase, but also to inhibition of inositol phospholipids hydrolysis [42, 43]. In some tissues receptor-mediated regulation of inositol phospholipids hydrolysis is impaired by pertussis toxin treatment, indicating the involvement of a GTP-binding regulatory protein which may be different from N_i [11]. Although the occurrence of D-2 DA receptors regulating inositol phospholipids hydrolysis has not been yet described in rat striatum, we cannot rule out the possibility that the D-2-stimulated GTPase activity may also result from activation of *N* protein(s) coupled to signal transduction systems different from adenylate cyclase.

The present data are, however, in contrast with the observation of Tirone *et al.* [44] reporting that DA stimulates striatal GTPase by acting on D-1 sites linked to stimulation of adenylate cyclase. We do not have a definite explanation for this discrepancy. Despite we carried out the GTPase assay under conditions which allowed DA and other DA agonists to efficiently stimulate adenylate cyclase activity via D-1 sites, we were unable to detect a significant D-1-mediated stimulation of GTPase activity. In different tissues activation of GTPase by hormones that stimulate adenylate cyclase either has not been observed [38] or has been found to be very low when compared to the effect elicited by hormones that inhibit adenylate cyclase [39]. These contradictory observations are yet not fully explained but they may be indicative of crucial differences in the mode of activation of the GTPase activities expressed by N_s and N_i.

The present study demonstrates the occurrence in the rat striatum of a functional coupling of D-2 receptors to a high affinity GTPase activity likely associated with N_i. The increase of GTP hydrolysis induced by DA agonists may therefore constitute a useful index of the efficiency of the transduction mechanism that couples the D-2 recognition site to the effector system(s) such as adenylate cyclase.

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