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Involvement of a High-Affinity GTPase in the Inhibitory Coupling of Striatal Muscarinic Receptors to Adenylate Cyclase

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SUMMARY

The stimulation of GTP hydrolysis has been proposed as a mechanism by which hormones inhibit receptor-coupled adenylate cyclase activity. The present study attempts to verify whether this mechanism is also operative in transmitter-mediated receptor-coupled attenuation of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] located in synaptic plasma membrane preparations. As a model, we used the inhibition of adenylate cyclase activity by muscarinic receptor activation in rat striatum. This striatal preparation contains high-affinity GTPase (EC 3.6.1-) activity which is stimulated when the recognition site for muscarinic agonists is occupied. Acetylcholine (ACh), but not nicotine, increases the V_{max} of the high-affinity GTPase, and the stimulatory effect is antagonized by atropine but not by *d*-tubocurarine. The rank order of potency of various cholinergic agonists to stimulate GTPase correlates with their ability to inhibit adenylate cyclase activity of striatal membranes. Pre-exposure of striatal membranes to guanosine-5′-*O*-(3-thiotriphosphate) causes a parallel decrease in the basal and ACh-stimulated GTPase activities and in the ACh-induced inhibition of adenylate cyclase. Treatment of the membranes with cholera toxin does not affect the ACh-stimulated GTPase activity but amplifies the extent of adenylate cyclase inhibition elicited by the cholinergic agonist. These results indicate that the stimulation of a high-affinity GTPase parallels the inhibitory coupling of central muscarinic receptors to adenylate cyclase.

INTRODUCTION

The stimulation of adenylate cyclase activity by hormones and neurotransmitters is mediated through the interaction of the recognition site for the transmitter with the guanine nucleotide-binding protein (G/F) that regulates adenylate cyclase (1). The occupancy of the recognition site by an agonist facilitates the binding of GTP to G/F and the consequent formation of active G/F-GTP complexes which stimulate the catalytic subunit of the cyclase. This activation process is under negative control by a high-affinity GTPase, which catalyzes the hydrolysis of G/F-GTP to G/F-GDP, thereby turning off the stimulation of the cyclase by the active G/F-GTP complex (2). Because of these on-off reactions, the hormonal regulation of adenylate cyclase appears as a dynamic process in which the level of enzyme activity depends on the balance between the rates of activation and deactivation of the regulatory systems. Both stimulation and inhibition of adenylate cyclase by hormones or transmitters are GTP-dependent in various cell types (3), and both are associated with stimulation of a low-K_m GTPase (2, 4, 5). The question then arises as to whether the same G/F protein that mediates the activation of adenylate cyclase also mediates the response to the inhibitory signal. Rodbell (3) has accumulated evidence indicating the existence of two separate guanine nucleotide regulatory proteins, one (N_s) mediating stimulation and the other (N_i) mediating inhibition of adenylate cyclase. More recently, Koski and Klee (4) and Aktories et al. (5) have proposed that the hormonal inhibition of adenylate cyclase occurs through a receptor-mediated stimulation of the high-affinity GTPase presumably associated with N_i. This hypothesis is based on the finding that hormonal inhibition of adenylate cyclase fails to occur in the presence of nonhydrolyzable analogues of GTP (6, 7).

We have recently shown that, in rat striatum, muscarinic receptor agonists inhibit basal adenylate cyclase activity (8) and attenuate the stimulation of this enzyme by dopamine (9). In the present study we investigate whether the muscarinic inhibition of striatal adenylate cyclase is associated with an increased hydrolysis of GTP. The results indicate that the synaptic plasma membranes of striatum contain high-affinity GTPase activity which is stimulated by cholinergic agonists with the same rank order of potencies observed for the inhibition of striatal adenylate cyclase.

EXPERIMENTAL PROCEDURES

Materials. [γ-^{32}P]GTP (10–50 Ci/mmole), [γ-^{32}P]ATP (10–40 Ci/mmole), carrier-free $H_3{}^{32}PO_4$, [2,8-^{3}H]cyclic AMP (25 Ci/mmole), and

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[γ-^{35}S]GTPγS[1] (38 Ci/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.). [α-^{32}P]ATP (30–40 Ci/mmole) was obtained from Amersham (Arlington Heights, Ill.). App(NH)p, NAD^+, GTPγS, and Gpp(NH)p were purchased from Boehringer-Mannheim (Indianapolis, Ind.). IBMX was from Calbiochem (La Jolla, Calif.). ATP (A-2383), cholera toxin, and other compounds and reagents were obtained from Sigma Chemical Company (St. Louis, Mo.).

Synaptic plasma membranes were isolated as previously described (8). Membrane suspensions were stored at −70° for no longer than 10 days and thawed only once before the beginning of the experiment. Protein was determined by the method of Bradford (10), using bovine serum albumin as a standard.

GTPase assay. The GTPase activity was determined in triplicate according to the method described by Cassel and Selinger (11). The reaction mixture (100 µl) contained 75 mM Tris-HCl (pH 7.4), [γ-^{32}P]GTP (70–100 × 10^3 cpm), GTP (at the indicated concentrations), 2 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM App(NH)p, 5 mM phosphocreatine, creatine phosphokinase (50 units/ml), bovine serum albumin (50 µg), 0.1 mM EDTA, 0.2 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 1 mM cyclic AMP, 0.5 mM IBMX, 10 µM eserine, 100 mM NaCl, and 2–4 µg of membrane protein. The reaction was initiated by adding the membrane suspension and was continued for 4 min at 37°; it was stopped by adding 0.5 ml of 5% (w/v) ice-cold activated charcoal in 20 mM phosphoric acid (pH 2.5) (4). The samples were kept at ice-bath temperature for 10 min and then centrifuged at 7000 × *g* for 10 min. The radioactivity of 200-µl aliquots of the supernatants was measured by liquid scintillation spectrometry. The reaction product was identified as ^{32}Pi by chromatography on Whatman 3 MM paper (11). More than 85% of the radioactivity present in the charcoal supernatant co-migrated with authentic ^{32}Pi. The release of ^{32}Pi in the absence of membranes (or in the presence of membranes but incubated at ice-bath temperature for 4 min) was 0.5–2% of the total [γ-^{32}P]GTP added. This value was subtracted from the amount of ^{32}Pi released in the presence of membranes. The enzyme activity was linear with time (up to 6 min) and with the membrane protein concentration (up to 50 µg/ml). No more than 20% of the substrate was hydrolyzed under the above-mentioned conditions. When [γ-^{32}P]ATP was substituted for [γ-^{32}P]GTP in the reaction mixture containing 0.5 µM unlabeled GTP, the amount of ^{32}Pi released was approximately 7% of the total radioactivity added, but this activity was not significantly stimulated by the presence of 100 µM ACh or decreased by the addition of high concentrations (30 µM) of unlabeled GTP. Moreover, inclusion of 1 mM ouabain in the reaction mixture did not change the stimulation of ^{32}Pi release by ACh. NaCl (100 mM) was included in the reaction mixture to maintain its composition similar to that employed for the adenylate cyclase assay. The salt amplifies the inhibitory effect of ACh on striatal adenylate cyclase (8), but the presence of 100 mM NaCl was not required for the detection of the stimulatory effect of ACh on GTPase activity.

Adenylate cyclase assay. Enzyme activity was assayed in a 150-µl reaction mixture containing the same constituents as used for the GTPase assay with the exception that EDTA and App(NH)p were omitted and [α-^{32}P]ATP (30–40 cpm/pmole) was substituted for [γ-^{32}P]GTP. GTP was present at the concentrations indicated in the figure legends. [2,8-^{3}H]Cyclic AMP (~10^4 cpm) was included to monitor cyclic AMP recovery. The reaction was initiated by adding the membrane suspension (10–15 µg of membrane protein) and was carried out at 37° for 5 min. The incubation was stopped by adding 200 µl of a solution containing 2% sodium dodecyl sulfate (w/v), 45 mM ATP, and 1.3 mM cyclic AMP. Cyclic AMP was isolated according to the method of Salomon *et al.* (12).

Treatment of striatal plasma membranes with cholera toxin. Cholera toxin (210 µg/ml) was activated by incubation at 30° for 10 min in a medium containing 50 mM sodium phosphate buffer (pH 7.1), 100 mM NaCl, 5 mM $MgCl_2$, 20 mM dithiothreitol, and bovine serum albumin (0.2 mg/ml). This solution was added in equal volume (600 µl) to a membrane suspension (400–700 µg/ml) containing 1 mM ATP, 1 mM NAD, 200 µM GTP, 50 mM sodium phosphate buffer (pH 7.1), creatine kinase (100 units/ml), 29 mM phosphocreatine, and 100 K striatal supernatant (0.8 mg/ml). Control membranes were suspended in the same solution without toxin. Membranes were incubated for 20 min at 30°. The incubation was terminated by adding 8 volumes of ice-cold buffer containing 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, and 0.25 M sucrose. The samples were centrifuged for 1 hr at 150,000 × *g* at 4°. The pellet was washed once with 2 ml of ice-cold Tris-EDTA buffer with no sucrose and centrifuged as above for 30 min. The final pellet was resuspended in ice-cold 10 mM Tris-HCl (pH 7.4)/1 mM EDTA/1 mM dithiothreitol to a protein concentration of 200–300 µg/ml and used for the enzyme assay. The activation of striatal adenylate cyclase activity was dependent on the concentration of the toxin (maximal activation was obtained with 50 µg/ml of cholera toxin), on the time of exposure to the toxin (a plateau was reached after 10 min), and on the presence of 100 K supernatant. The latter was prepared by two sequential centrifugations at 100,000 × *g* for 60 min of rat striatum homogenate [1:2 (w/v) in 5 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol]. No adenylate cyclase activity was detected in this supernatant fraction.

Treatment of membranes with GTPγS. Membranes (500 µg/ml) were incubated at 30° for 5 min without and with various concentrations of GTPγS in a buffer containing 10 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol, and 1 mM $MgCl_2$. At the end of the incubation period, the membrane suspension was diluted with 7 volumes of ice-cold Tris buffer and centrifuged at 45,000 × *g* for 15 min. Pellets were resuspended in the same Tris buffer and used for enzyme assay. The amount of GTPγS remaining bound to striatal membranes under these conditions was determined by incubating the membranes in the presence of [γ-^{35}S]GTPγS (1.2 × 10^5 cpm/tube) and various concentrations of unlabeled GTPγS. After correcting for the specific activity of [γ-^{35}S]GTPγS at each concentration, the following values (expressed as picomoles of GTPγS bound per milligram of protein were obtained: 0.01 µM GTPγS, 10.4; 0.1 µM GTPγS, 108; 1.0 µM GTPγS, 290; 5.0 µM GTPγS, 414; 10 µM GTPγS, 412. Since only 4 µg of membrane protein were used per tube in the GTPase assay, even if all of the GTPγS had dissociated and was available to compete with GTP as substrate for the GTPase, the highest concentrations would be 0.8 nM at 1 µM and 8 nM at 10 µM. Essentially complete inhibition of GTPase was obtained at concentrations of both 1 and 10 µM GTPγS (Fig. 5). The relative potencies for effects of GTPγS versus Gpp(NH)p on adenylate cyclase and GTPase range from 3-fold to 13-fold (13–16): in rat striatum, GTPγS is 4-fold more potent on cyclase than is Gpp(NH)p (EC_{50}; 0.2 µM versus 0.8 µM), and the IC_{50} for Gpp(NH)p inhibition of striatal GTPase is 0.66 µM. Therefore, the carryover of GTPγS will be in the range of 20-fold below the K_i for the GTPase.

RESULTS

Isotope dilution curves of GTP hydrolysis by striatal synaptic plasma membranes revealed the presence of at least two GTPase activities. The release of ^{32}Pi from [γ-^{32}P]GTP was markedly decreased by the addition of low concentrations of unlabeled GTP. The curve reached a plateau at concentrations of GTP higher than 10 µM, indicating the presence of high-affinity GTPase activity with a K_m lower than 1 µM. This activity accounted for approximately 60% of the total [γ-^{32}P]GTP hydrolysis. Low-affinity GTPase activity (approximate K_m of 140 µM and V_{max} of about 65 nmoles/min/mg of protein) was also detected. ACh (100 µM) increased the hydrolysis of GTP only at concentrations of unlabeled GTP lower than 30 µM and did not affect the low-affinity GTPase activity. The high-affinity GTPase was routinely calculated as

[1] The abbreviations used are: GTPγS, guanosine-5'-*O*-(3-thiotriphosphate); App(NH)p, adenylyl-5'-imidodiphosphate; Gpp(NH)p, guanylyl-imidodiphosphate; IBMX, 1-methyl-3-isobutylxanthine; ACh, acetylcholine.

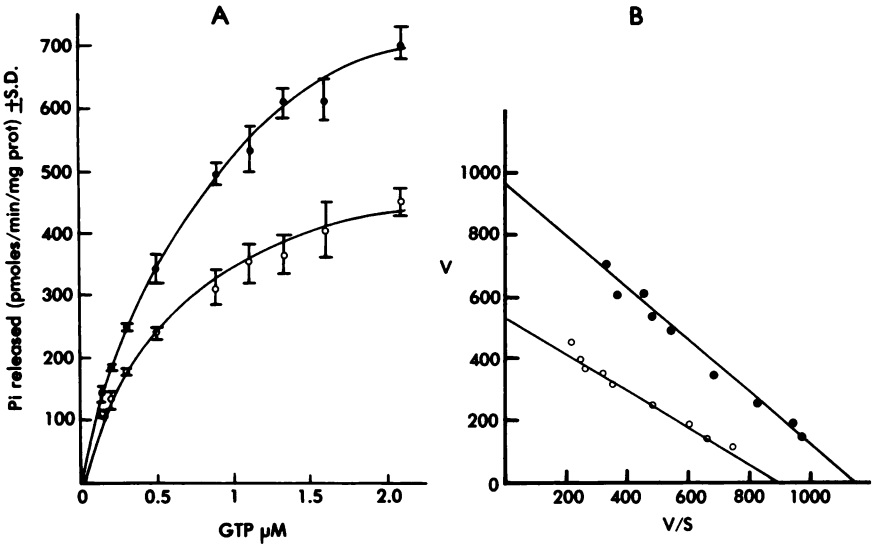

FIG. 1. *Stimulation by ACh of striatal GTPase activity*

A. Stimulation by ACh of striatal GTPase as a function of GTP concentration. Values for the high-affinity GTPase were calculated by subtracting the hydrolysis due to low-affinity GTPase (see Results) and correcting for the specific activity of [γ-^{32}P]GTP at each concentration of GTP. Assays were carried out in the absence (○) and in the presence (●) of 100 μM ACh. The values reported are means ± standard deviation of triplicate determinations from one experiment representative of five experiments performed on four different membrane preparations. The range of variability was <8%.

B. Hofstee plot of the data reported in A. ○ Basal GTPase activity; ●, GTPase stimulated by 100 μM ACh. Correlation coefficients were 0.99 for both lines.

described by others (4, 5, 11), by subtracting the amount of [γ-^{32}P]GTP hydrolyzed in the presence of 30 μM unlabeled GTP in order to correct for the activity contributed by the low-affinity GTPase.

The kinetic properties of the stimulatory effect of ACh on the high-affinity GTPase of rat striatum are illustrated in Fig. 1A. The basal enzyme activity increased as a function of the GTP concentration and reached saturation at approximately 2 μM GTP. ACh increased the rate of GTP hydrolysis at each concentration of GTP used. Hofstee analysis of these data (Fig. 1B) showed that the basal high-affinity GTPase had an apparent K_m of 0.72 ± 0.10 μM and a V_{max} of 626 ± 72 pmoles/min/mg of protein. In the presence of ACh, the V_{max} increased to 1066 ± 113 pmoles/min/mg of protein, whereas the K_m (0.90 ± 0.10 μM) was not significantly different from that displayed by the basal enzyme activity (mean ± standard error of the mean; $n = 4$). Thus, the stimulatory effect of ACh was due to an increase in the maximal rate of hydrolysis.

The increase in high-affinity GTPase activity was dependent on the concentration of ACh (Fig. 2). The concentrations of the agonist which caused half-maximal

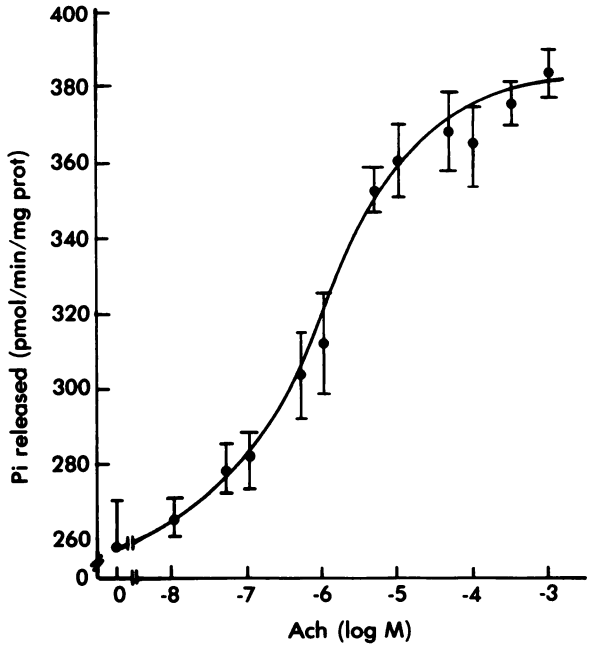

FIG. 2. *Concentration-dependent stimulation of GTPase activity by ACh in striatal synaptic plasma membranes*

GTPase activity was assayed at 0.5 μM GTP in the presence of the indicated concentrations of acetylcholine. Values are means ± standard error of the mean of four experiments performed on three different membrane preparations.

TABLE 1

Potency of cholinergic agonists in stimulating high-affinity GTPase activity of striatal synaptic membranes

GTPase activity was assayed at 0.5 μM GTP. EC_{50} values were obtained from log-probit plots of the stimulation of enzyme activity, expressed as percentage of the maximal effect, at different concentrations (at least 10) of each agonist.

Cholinergic agonist	EC_{50}[a]	Relative potency[b]
	μM	
Oxotremorine	0.33 ± 0.04 (3)	570
ACh	1.9 ± 0.3 (5)	100
Arecoline	4.1 ± 0.5 (3)	46
Carbachol	3.8 ± 0.3 (3)	50
Bethanechol	20.0 ± 3.5 (3)	9
Nicotine	—[c]	

[a] Values are means ± standard error of the mean of the number of determinations indicated in parentheses.

[b] Relative to ACh.

[c] Inactive at 100 μM (two determinations).

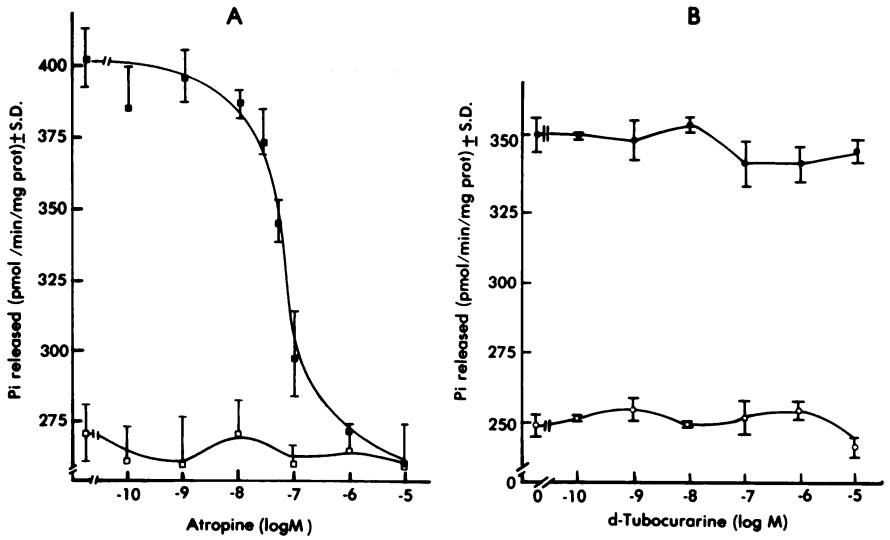

FIG. 3. *Effect of atropine and d-tubocurarine on ACh-stimulated GTPase activity*

A. Concentration-dependent blockade of ACh-stimulated GTPase by atropine. GTPase activity was assayed at 0.5 μM GTP either in the absence (□) or in the presence (■) of 100 μM ACh and the indicated concentrations of atropine sulfate. Values are means ± standard deviation of three experiments.

B. Failure of *d*-tubocurarine to inhibit the ACh-stimulated GTPase activity. Enzyme activity was assayed as in A in the absence (○) and in the presence (●) of 100 μM ACh at the indicated concentrations of *d*-tubocurarine. Values are means ± standard deviation of three experiments.

(EC_{50}) and maximal stimulation (40–50% of the basal enzyme activity) were approximately 1.5 μM and 100 μM, respectively. These values are similar to those obtained for the ACh-mediated inhibition of the adenylate cyclase activity in rat striatum (8). As reported in Table 1, various cholinergic agonists mimicked the stimulatory effect of ACh on the high-affinity GTPase activity. The rank order of potency (oxotremorine > ACh > carbachol > bethanechol) was the same as that observed when these compounds were tested as inhibitors of striatal adenylate cyclase (8). Their efficiency was similar to that of ACh, with the exception of bethanechol, which maximally stimulated the enzyme by only 20%. This compound was also the least effective in decreasing the striatal adenylate cyclase activity. Nicotine, at a concentration of 100 μM, was ineffective, indicating that the increase in GTP hydrolysis was mediated through occupancy of muscarinic receptors. Furthermore, the enzyme stimulation elicited by ACh was antagonized in a concentration-dependent fashion by atropine (IC_{50} = 50 nM) but not by *d*-tubocurarine, a blocker of the nicotinic receptor (Fig. 3A and B). Neither cholinergic antagonist affected the basal rate of GTP hydrolysis.

A common feature of adenylate cyclase inhibition by hormones and neurotransmitters is a GTP requirement which is expressed also by the inability of less hydrolyzable analogues of GTP, such as Gpp(NH)p and GTPγS, to support the inhibition (6, 7). This observation suggests that the hydrolysis of GTP is essential for the hormonal inhibition of adenylate cyclase. In rat striatum, ACh failed to reduce the rate of cyclic AMP formation in the absence of GTP (8) or when Gpp(NH)p was substituted for GTP.[2] Figure 4 shows that the addition of Gpp(NH)p to the GTPase assay inhibited the hydrolysis of GTP by the high affinity enzyme. Kinetic analysis of the data according to Dixon (17) revealed that the Gpp(NH)p inhibition was competitive, with an apparent K_i of 0.66 μM. To ascertain whether the inhibition of GTP hydrolysis by stable GTP analogues correlates with their ability to activate adenylate cyclase irreversibly, and to prevent the enzyme inhibition, striatal membranes were pretreated with different concentrations of GTPγS. The effect of this treatment on the ACh inhibition of adenylate cyclase and on the ACh stimulation of the high-affinity GTPase was then determined. As shown in Fig. 5A, following the binding of GTPγS to striatal membranes, basal adenylate cyclase activity increased and the activity of high-affinity GTPase decreased as a func-

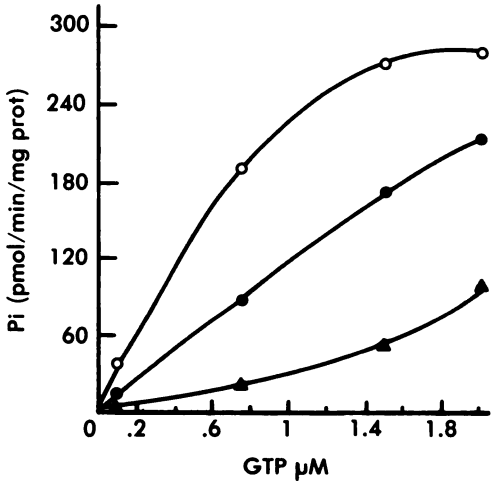

FIG. 4. *Inhibition of GTP hydrolysis by Gpp(NH)p in striatal synaptic plasma membranes*

GTP hydrolysis was assayed at the indicated concentrations of GTP in the absence (○) and in the presence of 1 μM (●) and 10 μM (▲) Gpp(NH)p. For estimation of the inhibitory constant of Gpp(NH)p (see text), GTP hydrolysis was measured in another experiment at the same GTP concentrations and in the presence of six different concentrations of Gpp(NH)p ranging from 0.1 to 5 μM.

[2] P. Onali, M. C. Olianas, J. P. Schwartz, and E. Costa, unpublished observations.

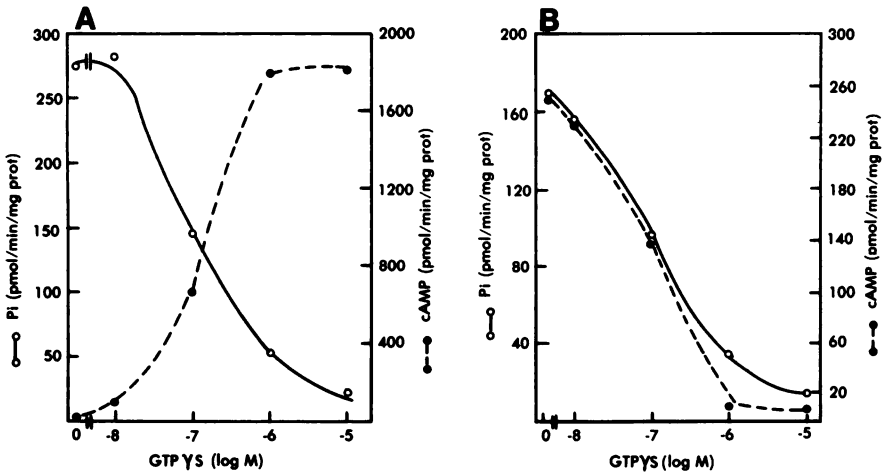

FIG. 5. *Effect of preincubation of striatal synaptic plasma membranes with GTPγS on adenylate cyclase and GTPase activities*

Membranes were preincubated as described under Experimental Procedures in the absence or in the presence of the indicated concentrations of GTPγS. After washing, the membranes were assayed for GTPase and adenylate cyclase activities in the absence and in the presence of 100 μM ACh. In A, the changes of basal enzyme activities at each concentration of GTPγS present in the preincubation step are reported. Adenylate cyclase was assayed at 100 μM GTP, and the increase elicited by the GTPγS pretreatment (reported on the right ordinate) was calculated by subtracting the adenylate cyclase activity of membranes not exposed to GTPγS (at 100 μM GTP, this activity was 922 ± 10 pmoles of cyclic AMP per minute per milligram of protein). Basal GTPase activity (left ordinate) was assayed at 1 μM GTP. In B, the activity of the ACh-stimulated GTPase (left ordinate) and ACh-inhibited adenylate cyclase activity (right ordinate) are reported as a function of the concentrations of GTPγS in the preincubation medium. ACh-stimulated GTPase refers to the net enzyme activity stimulated by 100 μM ACh at 1 μM GTP. ACh-inhibited adenylate cyclase is expressed as the difference between the enzyme activity assayed in the absence and in the presence of 100 μM ACh and 100 μM GTP. Values are means of three experiments with a range of variability <10%.

tion of the concentration of the nucleotide. Figure 5B shows that concentrations of GTPγS which caused a decrease in the extent of adenylate cyclase inhibition elicited by ACh were equally effective in inhibiting the ACh-stimulated GTPase activity. These results indicate that the inhibition of GTPase activity by GTPγS goes hand in hand with the reduction of the inhibitory effect of ACh on adenylate cyclase.

A stable activation of adenylate cyclase can also be achieved by exposure of cell membranes to cholera toxin (18). The toxin catalyzes the ADP ribosylation of the G/F protein (19, 20) and suppresses the stimulation of GTPase following the activation of receptors which stimulate adenylate cyclase (21–23). As shown in Fig. 6A, pretreatment of striatal synaptic plasma membranes with a maximally effective concentration of cholera toxin (100

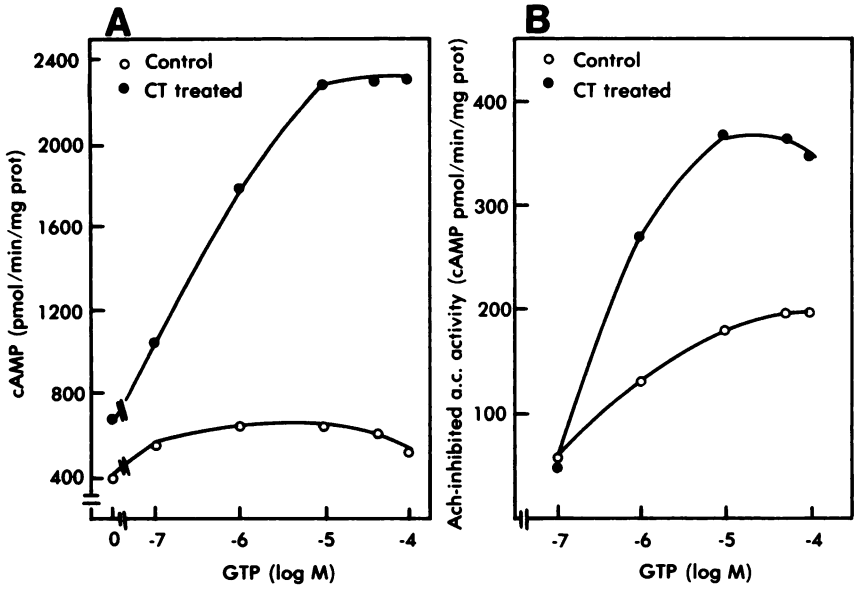

FIG. 6. *Effect of pretreatment of striatal synaptic membranes with cholera toxin on the basal (A) and ACh-inhibited (B) adenylate cyclase activities*

Membranes were preincubated with and without cholera toxin (100 μg/ml) and washed as described under Experimental Procedures. Aliquots of the final membrane suspensions were assayed for adenylate cyclase activity at the indicated concentrations of GTP. ACh-inhibited adenylate cyclase (B) was calculated as the difference between the enzyme activity assayed in the absence and in the presence of 100 μM ACh at each concentration of GTP. Values are means of three experiments. The standard deviation for each point was <6%.

μg/ml) caused a 7-fold enhancement of the GTP-sensitive adenylate cyclase activity (calculated as the net picomoles of cyclic AMP stimulated by GTP). This increase was associated with an amplification of the GTP-dependent inhibition of the enzyme by ACh (Fig. 6B). Pretreatment with cholera toxin, however, caused only a slight decrease (5–15%) of either the ACh-stimulated or the basal high-affinity GTPase activity. The enzyme activities assayed at 0.5 μM GTP (expressed as picomoles of P_i per minute per milligram of protein were: in control membranes, basal, 266 ± 1; 100 μM ACh, 393 ± 1; in cholera toxin-treated membranes, basal, 234 ± 2; 100 μM ACh, 338 ± 1 (values are the means ± standard error of triplicate determinations from one experiment representative of seven experiments performed on five separate membrane preparations). The same result was obtained regardless of the GTP concentration at which the GTPase activity was assayed, over a range from 0.05 to 2μM.

DISCUSSION

Striatal synaptic plasma membranes contain a high-affinity GTPase which is activated by muscarinic receptor agonists (Fig. 1), and this stimualtion is associated with the inhibitory coupling of muscarinic receptors to striatal adenylate cyclase. The GTPase K_m for GTP (0.9 μM) is close to the GTP concentration required by ACh to elicit half-maximal inhibition of adenylate cyclase (0.6 μM). The concentration-response curve of GTPase stimulation by ACh (Fig. 2) is almost superimposable on that obtained for the attenuation of adenylate cyclase (8). The rank order of potency and the efficiency of various cholinergic agonists in stimulating GTP hydrolysis (Table 1) correlate with their ability to inhibit the adenylate cyclase (8). Moreover, the parallel declines of the ACh-induced inhibition of adenylate cyclase and of the ACh-stimulated GTPase observed in GTPγS-pretreated membranes (Fig. 5) suggest that some of the guanine nucleotide-binding sites occupied by GTPγS are those involved in the hydrolysis of GTP and those which in turn are required for the inhibition of adenylate cyclase by ACh.

Cassel *et al.* (2) originally proposed that the hydrolysis of GTP bound to G/F by a specific high-affinity GTPase terminates the activation of adenylate cyclase and demonstrated that a stimulatory hormone increases the activity of the GTPase (2). Since then, several laboratories have confirmed the finding that hormones which activate adenylate cyclase increase GTPase activity (4, 5, 11, 23) but in addition have shown that hormones which inhibit adenylate cyclase cause an even larger increase in GTPase activity (4, 5). In line with these results, Michel and Lefkowitz (24) have shown in platelet membranes that both a stimulatory and an inhibitory hormone for the adenylate cyclase can increase the release of Gpp(NH)p from these membranes and further that these effects are additive. They propose that the apparent GTPase activity is actually a manifestation of guanine nucleotide turnover stimulated by receptor occupation, although there are several guanine nucleotide-binding sites in membranes (6) and the binding which they measure could be occurring at another site. These results have led to the idea that stimulatory and inhibitory hormones regulate adenylate cyclase via comparable mechanisms which require guanine nucleotides and GTPase activity, but which utilize distinct guanine nucleotide-binding proteins. More recent work has confirmed the existence of two separate adenylate cyclase regulatory proteins, one stimulatory (N_s), and the other inhibitory (N_i), both of which are capable of binding guanine nucleotides and are associated with high-affinity GTPase activity (3, 25, 26). One GTPase is stimulated in the process of activation of adenylate cyclase and is inhibited by cholera toxin, whereas the other is linked to the inhibitory control of the enzyme and is insensitive to cholera toxin (21–23, 27). A 41,000 M_r guanine nucleotide-binding protein (N_i) different from N_s has been identified recently in solubilized preparations of human erythrocytes and rabbit liver membranes (25, 26). This protein is ADP-ribosylated by pertussis toxin (25, 26, 28, 29), but not by cholera toxin (25). In neuroblastoma-glioma hybrid cells, pertussis toxin prevents both the opioid-induced attenuation of adenylate cyclase and the stimulation of GTPase activity (29), suggesting that GTP hydrolysis or exchange is required for inhibition of cyclase.

Our results are in agreement with this general scheme. Muscarinic agonists inhibited striatal adenylate cyclase and stimulated a high-affinity GTPase. Both effects required a guanine nucleotide. Treatment of striatal membranes with cholera toxin activated the cyclase but had no effect on the muscarinic stimulation of GTPase. Therefore, we propose that the muscarinic receptors in rat striatum control adenylate cyclase via interaction with an N_i-coupling protein, resulting in cyclase inhibition. Thus the stimulation of GTP hydrolysis by ACh reflects an increased turnover of guanine nucleotides at the N_i regulatory site and appears to be a crucial step in the sequence of events which processes the inhibitory signal to adenylate cyclase.

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