Calmodulin-Sensitive and Calmodulin-Insensitive Components of Adenylate Cyclase Activity in Rat Striatum Have Differential Responsiveness to Guanyl Nucleotides

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Abstract: The interaction between the Ca^{2+}-binding protein, calmodulin, and guanyl nucleotides was investigated in a rat striatal particulate fraction. We found that the ability of calmodulin to stimulate adenylate cyclase in the presence of guanyl nucleotides depends upon the type and concentration of the guanyl nucleotide. Adenylate cyclase activity measured in the presence of calmodulin and GTP reflected additivity at every concentration of these reactants. On the contrary, when the activating guanyl nucleotide was the nonhydrolyzable analog of GTP, guanosine-5'-(β,γ-imido)triphosphate (GppNHp), calmodulin could further activate adenylate cyclase only at concentrations less than 0.2 μM GppNHp. Kinetic analysis of adenylate cyclase by GppNHp was compatible with a model of two components of adenylate cyclase activity, with over a 100-fold difference in sensitivity for GppNHp. The component with the higher affinity for GppNHp was competitively stimulated by calmodulin. The additivity between calmodulin and GTP in the striatal particulate fraction suggests that they stim-

ulate different components of cyclase activity. The calmodulin-stimulatable component constituted 60% of the total activity. Our two-component model does not delineate, at this point, whether there are two separate catalytic subunits or one catalytic subunit with two GTP-binding proteins. The finding that GTP was unable to activate the calmodulin-sensitive component suggests that this component has either a different mode of binding to a GTP-binding protein or inherently higher GTPase activity than has the calmodulin-insensitive component. The results suggest there are two components of adenylate cyclase activity that can be differentiated by their sensitivities to calmodulin and guanyl nucleotides. **Key Words:** Adenylate cyclase—Guanyl nucleotides—Calmodulin—GTP-binding protein—Calcium—Striatum. Treisman G. J. et al. Calmodulin-sensitive and calmodulin-insensitive components of adenylate cyclase activity in rat striatum have differential responsiveness to guanyl nucleotides. *J. Neurochem.* **41**, 1398–1406 (1983).

Mammalian brain, in contrast to many peripheral tissues, contains adenylate cyclase (EC 4.6.1.1) activity that is stimulated by Ca^{2+}. The stimulation by Ca^{2+} is conferred by the endogenous Ca^{2+}-binding protein, calmodulin (CaM) (Cheung, 1980). The molecular mechanism by which CaM affects adenylate cyclase activity is not yet understood. Recent evidence suggests that CaM stimulates adenylate cyclase activity by acting on the catalytic subunit in an action independent of the GTP-binding protein (Heideman et al., 1982; Seamon and

Daly, 1982). Workers investigating the stimulation of adenylate cyclase activity in bovine cerebral cortex by CaM and guanyl nucleotides have reported either simple additivity between the two activators (Salter et al., 1981; Seamon and Daly, 1982) or synergistic effects (Brostrom et al., 1978; Heideman et al., 1982).

We have obtained data suggesting that the nature of the interaction between CaM and guanyl nucleotides in rat striatum depends on the type and concentration of the guanyl nucleotide. We have pre-

viously shown that CaM can increase the sensitivity for dopamine (DA) in a rat striatal particulate fraction (Gnegy and Treisman, 1981). Since a guanyl nucleotide was required for activation of cyclase by DA, CaM could have affected DA sensitivity by interacting with guanyl nucleotides. We further observed that CaM stimulated striatal adenylate cyclase additively with GTP, but not with its non-hydrolyzable analog, guanosine-5'-(β,γ-imido)triphosphate (GppNHp). This observation, however, was made at maximum concentrations of CaM and guanyl nucleotides (Gnegy and Treisman, 1981). In this work we further characterize the stimulation of striatal adenylate cyclase activity by CaM and guanyl nucleotides. Our data suggest the existence of two components of adenylate cyclase activity in rat striatum, one sensitive to CaM and the other insensitive to CaM. The two components could be differentiated by their sensitivities to guanyl nucleotides, as well as CaM.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200–250 g) were obtained from Charles River, Wilmington, MA. [α-^{32}P]ATP (specific activity 38 Ci/mmol) was purchased from Amersham Searle. Cyclic AMP, phosphoenolpyruvate, and ATP were obtained from Sigma Chemical Co; pyruvate kinase from Boehringer Mannheim; GTP and GppNHp (high pressure liquid chromatography-purified) from International Chemical and Nuclear Corp. Troponin C was generously donated by Dr. John Dedman, University of Texas Health Science Center at Houston.

Particulate preparation

Striatal particulate fractions depleted of Ca^{2+} and CaM were prepared as described (Gnegy and Treisman, 1981) in a modification of the method of Brostrom et al. (1978). Briefly, rats were killed by decapitation; the striata were removed and homogenized in 9 volumes of 10 mM Tris-maleate buffer, pH 7.5, containing 1 mM MgSO$_4$, 1.2 mM EGTA, and 10 μM GTP. GTP was originally included in this buffer to preserve DA stimulation of adenylate cyclase activity, but its presence was found to have no effect on the results of these experiments. The homogenate was centrifuged at 27,000 × g for 20 min, resuspended in the same buffer, and centrifuged a second time at 27,000 × g. The pellet resulting from the final centrifugation was resuspended in 10 mM Tris-maleate buffer, pH 7.5, containing 1.2 mM EGTA and 1 mM MgSO$_4$. Protein content was determined by the method of Lowry et al. (1951).

Adenylate cyclase assay

Adenylate cyclase activity was measured in an assay mixture (200 μl volume) containing: 80 mM Tris-maleate buffer, pH 7.5, 5 mM MgSO$_4$, 2 mM cyclic adenosine 3',5'-monophosphate (cAMP), 4 mM phosphoenol pyruvate, 20 μg pyruvate kinase, 0.12 mM isobutylmethylxanthine, 100–150 μg of particulate membrane protein, 0.15 mM EGTA (carried over from the particulate fraction), and 1 mM [α-^{32}P]ATP (1 μCi per assay) with or without additions such as GppNHp, GTP, CaCl$_2$, and CaM. Assays were incubated for 4 min and the reaction was

stopped by heating for 1 min at 95°C. A volume of 200 μl of a solution containing 20 mM ATP and 0.7 mM [^{3}H]cAMP was then added to the tubes. The particulate material was centrifuged and the ^{32}P-labeled cAMP in the supernatant was determined by the method of Krishna et al. (1968). Recovery of the cyclic AMP was measured by using the [^{3}H]cAMP, and was usually 80–90%. Free or effective concentrations of Ca^{2+} were calculated by using a dissociation constant for calcium EGTA of 4.08 × 10^{-8} M, according to the method of Nanninga and Kempen (1971). Our calculations for free Ca^{2+} were verified on a computer program graciously provided by Dr. John Dedman, University of Texas Health Science Center, Houston.

Calmodulin preparation

CaM was purified from bovine brain by the method of Klee (1977) and demonstrated a single band on disc gel electrophoresis containing 10% polyacrylamide. CaM was prepared in the presence of millimolar concentrations of EGTA, dialyzed against 0.05 M (NH$_4$)HCO$_3$, and lyophilized. The CaM was redissolved in 10 mM Tris-maleate buffer, pH 7.5. The protein concentration was determined by ultraviolet absorption (Klee, 1977) and the method of Lowry et al. (1951). The M$_r$ determined by slab gel electrophoresis using standards of known molecular weight was 17,500, which was used to calculate the concentrations of CaM reported in this study.

RESULTS

Time dependence of activation of striatal adenylate cyclase activity by CaM, GTP, and GppNHp

Our previous study of the effect of CaM on DA-sensitive adenylate cyclase activity suggested that CaM did not interact with GppNHp in the same manner as with GTP. We performed studies with CaM and guanyl nucleotides at various time periods to ensure that our results were not affected by the lag period attributable to guanyl nucleotides in stimulation of adenylate cyclase.

The time course of these activities is shown in Fig. 1A. Since the data represent the results of three different experiments, they have been normalized for the amount of protein. Basal activity at 37°C was 106 pmol/mg protein/min; and, in these experiments, 280 nM CaM activated the enzyme to a velocity of 151 pmol/mg protein/min (Fig. 1A). Both the basal activity and CaM activation were linear with time. Adenylate cyclase activation in the presence of 10 μM GppNHp exhibited a lag period of 1.5 min, after which the activity was linear, with a velocity of 343 pmol/mg protein/min. As shown in Fig. 1A, CaM could not stimulate beyond the level attained by 10 μM GppNHp at any of the time periods, nor did it alter the lag period. On the contrary, the activation produced by 280 nM CaM and 1 μM GTP together was the sum of their individual reaction rates (Fig. 1B). GTP stimulated adenylate cyclase linearly, with a velocity of 240 pmol/mg pro-
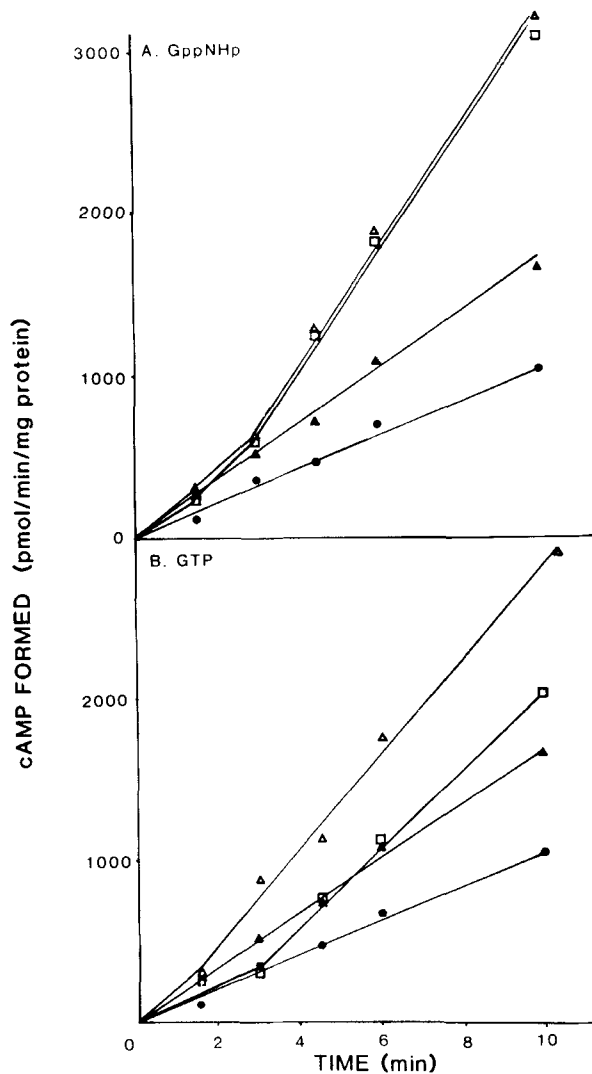
FIG. 1. Time course of adenylate cyclase activity at 37°C in a rat striatal particulate fraction activated by guanyl nucleotides, Ca^{2+}, and CaM. A rat striatal particulate fraction was prepared as described under Materials and Methods. Cyclic AMP formation was determined by standard procedures, and 200-μl aliquots were removed at specified times. The time dependence for adenylate cyclase was determined in the absence (●) and presence of 120 nM Ca^{2+} (100 μM $CaCl_2$ in the assay) and 280 nM CaM (▲), guanyl nucleotides (□), and guanyl nucleotides, CaM, and Ca^{2+} (△). **(A)** 10 μM GppNHp; **(B)** 1 μM GTP. Values represent the average of three experiments done in duplicate and are normalized for protein content. In A, slopes of the lines calculated by linear regression analysis after any apparent lag period, in pmol/mg protein/min, are: basal, 106; Ca^{2+}, CaM, 151; 10 μM GppNHp, 343; GppNHp, Ca^{2+}, and CaM, 340. Slopes in B, in pmol/min/mg protein, are: basal, 106; Ca^{2+}, CaM, 151; 1 μM GTP, 240; GTP, Ca^{2+}, and CaM, 305.

tein/min after a lag period of less than 3 min. The lag period was decreased or obscured in the presence of CaM. Note that the velocity in the presence of both GTP and CaM was 305 pmol/mg protein/

min, nearly equal to that attained by 10 μM GppNHp.

Activation of striatal adenylate cyclase activity by GppNHp and CaM

The studies described above were performed with nearly maximum concentrations of guanyl nucleotides and CaM. We further investigated the interaction of CaM and GppNHp by examining the activation of adenylate cyclase activity at various levels of GppNHp and CaM. As illustrated in Fig. 2, the activation of adenylate cyclase by GppNHp occurred over several orders of magnitude, from 10 nM to 100 μM. Maximum adenylate cyclase activity attained in the presence of 10–100 μM GppNHp was 350 pmol/min/mg protein, similar to the specific activity attained in Fig. 1A. Stimulation of adenylate cyclase over such a wide range of concentrations is suggestive of the presence of more than one component in the system. Eadie-Hofstee analysis of adenylate cyclase activity in the presence of GppNHp gave a biphasic curve and was compatible with the presence of two components of catalytic activity, as opposed to a single activatable species.

The stimulation of adenylate cyclase in the presence of various concentrations of GppNHp with 280 nM CaM (1000 ng) and an effective concentration of 120 nM Ca^{2+} (100 μM $CaCl_2$ in the assay) is also shown in Fig. 2. Activation of adenylate cyclase by concentrations of CaM up to 600 nM gave similar results. We have previously shown that maximum stimulation by CaM occurs at an effective Ca^{2+} concentration of 120 nM (Gnegy and Treisman, 1981). As shown in Fig. 2, CaM activated basal adenylate cyclase activity from 80 to 190 pmol/min/mg protein in the absence of GppNHp. The CaM stimulation was slightly potentiated at 10 nM GppNHp, to a level of 225 pmol/min/mg protein. Despite increasing concentrations of GppNHp, the adenylate cyclase activity remained at this level in the presence of CaM, up to concentrations of 0.2–0.5 μM GppNHp. Beyond these concentrations, stimulation of adenylate cyclase activity in the presence of GppNHp and CaM was the same as that of GppNHp alone. CaM activation was not additive with that of GppNHp at any concentration of GppNHp. As can be seen in Fig. 2, CaM and low doses of GppNHp seem to competitively activate a component of adenylate cyclase activity. This component may represent an activity that plateaus at approximately 220 pmol/min/mg protein.

To explore further the nature of the interaction between CaM and GppNHp, we determined the activation of adenylate cyclase by CaM in the presence of several concentrations of GppNHp. CaM alone stimulated adenylate cyclase activity by 100%. Eadie-Hofstee analysis of the activity revealed a single linear plot, with an apparent V_{max} of 220 pmol/min/mg protein and an apparent K_a of 70
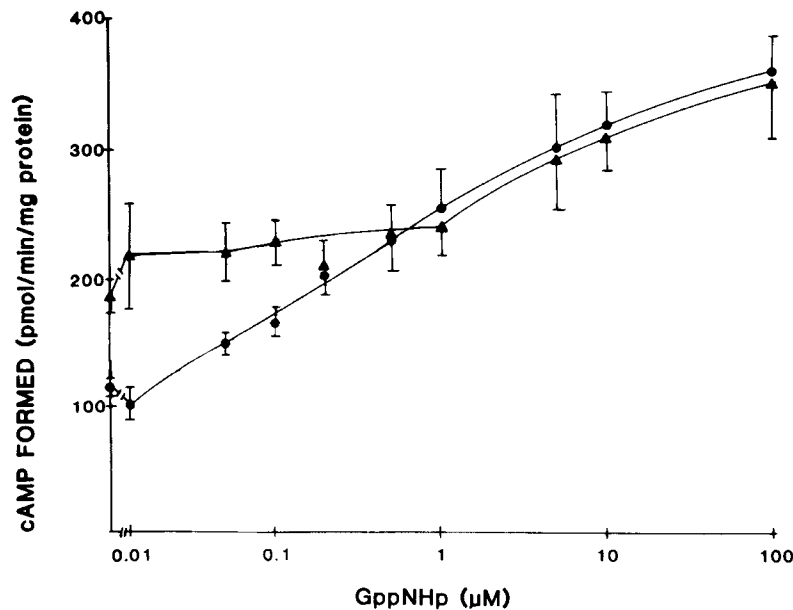
FIG. 2. Effect of Ca^{2+} and CaM on the GppNHp concentration dependence of a rat striatal particulate fraction. A rat striatal particulate fraction depleted of CaM and Ca^{2+} was prepared, and adenylate cyclase activity was determined, as described under Materials and Methods. The concentration dependence to GppNHp was measured in the absence (●) and presence of 120 nM Ca^{2+} (100 μM $CaCl_2$ added in the assay) and 280 nM CaM (▲). The results are the average of five separate experiments ± SEM.

nM, suggesting activation of one component of activity. In the absence of CaM, 0.1 μM GppNHp (Fig. 3) stimulated adenylate cyclase activity to a level of 170 pmol/min/mg protein. This activity did not change with increasing CaM until a concentration of 140 nM CaM, after which the curve followed that exhibited by CaM alone at a maximum level of 210 pmol/min/mg protein. With 0.5 μM GppNHp, the curve was flat at all concentrations of CaM, at a level of 220 pmol/min/mg protein. Similarly, in the presence of 10 μM GppNHp there was greater overall activity, but no appreciable stimulation by CaM. As demonstrated in Figs. 2 and 3, the stimulation by GppNHp was not additive with that of CaM. These data further suggest that CaM and low concentrations of GppNHp act as competitive activators of one component of activity.

This is further substantiated by an experiment performed with a particulate preparation replete with CaM that had not been subjected to EGTA extraction. In these membranes, basal activity was much higher (190 pmol/min/mg protein) than that seen in EGTA-extracted membranes. The adenylate cyclase activity in this preparation was not stimulated by the addition of exogenous CaM at any concentration tested. Under these conditions, the dose-response curve to GppNHp was superimposable on that found in EGTA-washed membranes in the presence of Ca^{2+}, CaM, and GppNHp (Fig. 2). The GppNHp dose-response curve appears to have only one component; stimulation by GppNHp did not occur until concentrations above 0.5 μM GppNHp were added (data not shown). This stimulation corresponds to that seen at higher concentrations of GppNHp in the EGTA-washed membranes. Max-

imal stimulation occurred with 100 μM GppNHp, and was 320 pmol/min/mg protein, the same maximal activity as that seen in EGTA-washed membranes with 100 μM GppNHp.
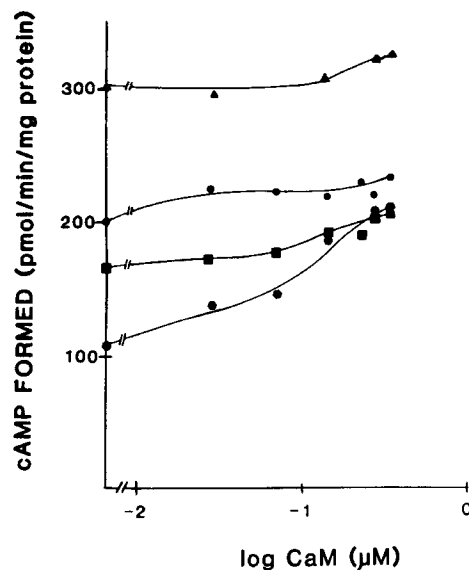

FIG. 3. Effect of GppNHp on the concentration dependence of CaM in stimulating adenylate cyclase activity in a rat striatal particulate fraction. A particulate fraction was prepared and adenylate cyclase was determined as described in the legend to Fig. 1. The concentration dependence of CaM was measured in the absence (●) and presence of the following concentrations of GppNHp: 0.1 μM (■); 0.5 μM (●); 10 μM (▲). The experiment was repeated twice for each concentration of GppNHp. The values shown varied in each experiment by approximately 10%. Assays containing CaM also contained 100 μM $CaCl_2$ (120 nM free Ca^{2+}).

Activation of striatal adenylate cyclase activity by GTP and CaM

The activation of striatal adenylate cyclase activity by GTP was determined in the presence and absence of 280 nM CaM and 120 nM Ca^{2+}. As shown in Fig. 4, stimulation of striatal adenylate cyclase activity by GTP reached a maximum value at 1 μM GTP. Contrary to the results with GppNHp, the concentration dependence and Eadie-Hofstee analysis of GTP stimulation were consistent with action at a single kinetic component. Eadie-Hofstee analysis of the stimulatory phase revealed a linear kinetic plot, with an apparent K_a of 90 nM and an apparent V_{max} of 230 pmol/min/mg protein. The maximum activity that was stimulated by GTP above basal activity was 130 pmol/min/mg protein. CaM and Ca^{2+} stimulated adenylated cyclase activity in an additive manner at every concentration of GTP (Fig. 4). Maximum adenylate cyclase activity detected in the presence of CaM and GTP was 310 pmol/min/mg protein, similar to the activity found in the presence of 100 μM GppNHp.

The data in Fig. 5 further establish the additive nature of the activation by GTP and CaM. We examined the activation of adenylate cyclase activity in the striatal particulate preparation by various concentrations of CaM in the presence and absence of 1 μM GTP. It can be readily seen in Fig. 5 that adenylate cyclase activity was susceptible to further stimulation by GTP at all concentrations of CaM. These experiments suggested that the CaM activation was independent of that of GTP.

Analysis of the stimulation of striatal adenylate cyclase by GppNHp

As discussed above, Eadie-Hofstee analysis of the data of Fig. 2 was compatible with the presence of two components of adenylate cyclase activity. Approximate kinetic constants were calculated with
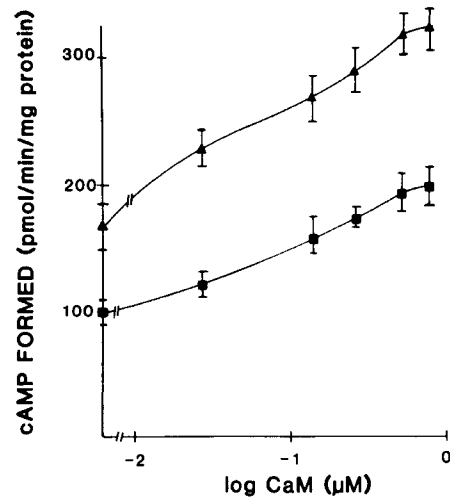

FIG. 5. Activation of striatal adenylate cyclase activity by CaM in the presence and absence of GTP. A particulate fraction was prepared and adenylate cyclase activity was determined as described in the legend to Fig. 1. The concentration dependence of CaM was measured in the absence (■) and presence (▲) of 1 μM GTP. All assays contained 100 μM $CaCl_2$ (120 nM free Ca^{2+}). The results are the average of four separate experiments carried out in duplicate ± SEM.

the assumption of two catalytic components, using the successive correction cycle of Spears et al. (1971). One component had an apparent K_a of 30 nM and an apparent V_{max} of 223 pmol/min/mg protein. The second component was less sensitive to GppNHp, and had an apparent K_a of 3.4 μM and an apparent V_{max} of 131 pmol/min/mg protein. The assumption of two components of GppNHp-stimulated adenylate cyclase activity with these kinetic constants was supported by computer analysis. When the derived kinetic constants were applied to a program assuming two independent enzyme ac-

FIG. 4. Activation of striatal adenylate cyclase activity by GTP in the absence and presence of Ca^{2+} and CaM. A rat striatal particulate fraction depleted of CaM and Ca^{2+} was prepared, as described under Materials and Methods. Adenylate cyclase activity was measured (see Materials and Methods) as a function of GTP in the absence (●) and presence of 120 nM Ca^{2+} (100 μM $CaCl_2$ added in the assay) and 140 nM CaM (■). The results are the average of four separate experiments carried out in duplicate ± SEM.
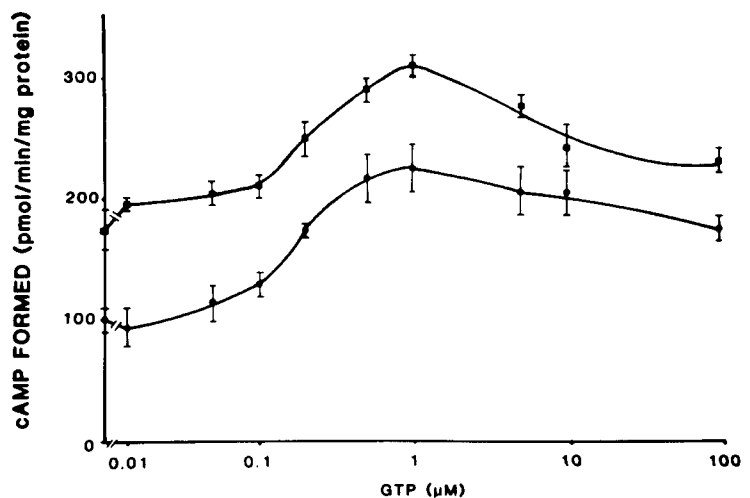
tivities with Michaelis-Menten kinetics reactive to the same substrate (in this case, activator) the solid line shown in Fig. 6 was generated. As illustrated in Fig. 6, there was an excellent fit between the theoretical curve shown by the solid line and experimental points, and they appeared to be superimposed. The data, however, did not fit a model assuming one catalytic activity with a single apparent K_a for GppNHp. The dashed line in Fig. 6 was generated assuming a single catalytic activity with a K_a of 0.3 μM. It is obvious that no matter what the value for the K_a, or position of the curve along the abcissa, a model of a single catalytic component does not fit the experimental data.

Activation of striatal adenylate cyclase activity by CaM and guanyl nucleotides at 25°C

The data in Fig. 2 suggested that there were two components of adenylate cyclase activity, one of which could be stimulated by CaM, as well as by GppNHp. Experiments performed at 25°C show more directly the nature of the interaction between GppNHp and CaM. At lower temperatures the stimulation by guanyl nucleotides is considerably reduced, while CaM stimulation is not appreciably affected. It is, therefore, possible to see more clearly the qualitative difference between the interaction of CaM with GppNHp compared with GTP. GppNHp and CaM appear to behave more like competitive activators than in the additive fashion seen with GTP.

As illustrated in Fig. 7A, basal adenylate cyclase activity was not altered by lowering the temperature to 25°C. Similarly, activation by CaM was unaltered at 25°C, although it exhibited a short lag

period. Ca^{2+} and CaM stimulated the enzyme by 100%, resulting in a velocity of 233 pmol/mg protein/min. The stimulation produced by 10 μM GppNHp, however, was sharply reduced from that at 37°C. After a lag period of 3 min, GppNHp stimulated the adenylate cyclase activity linearly, at a rate of 185 pmol/mg protein/min, slightly less than that of CaM. Even though the rate with GppNHp alone was less than that with CaM, stimulation in the presence of CaM and GppNHp resembled that of GppNHp alone. Further, CaM did not alter the lag phase elicited by GppNHp. This indicates that at high concentrations GppNHp can interfere with activation by Ca^{2+} and CaM.

In contrast to its actions at 37°C, 1 μM GTP did not activate striatal adenylate cyclase at 25°C (Fig. 7B). The velocity with GTP and CaM resembled that with CaM alone. The maximum velocity attained in the presence of CaM and GTP at 25°C was only 200 pmol/mg protein/min, as opposed to 343 pmol/mg protein/min at 37°C. In contrast to the blocking action of GppNHp, CaM activation was not affected by the presence of GTP.

DISCUSSION

The stimulation of rat striatal adenylate cyclase activity by CaM and guanyl nucleotides has been examined. We found that the ability of CaM to stimulate adenylate cyclase in the presence of guanyl nucleotides depends upon the type and concentration of the guanyl nucleotide. Adenylate cyclase activity measured in the presence of CaM and GTP reflected additivity at every concentration of these
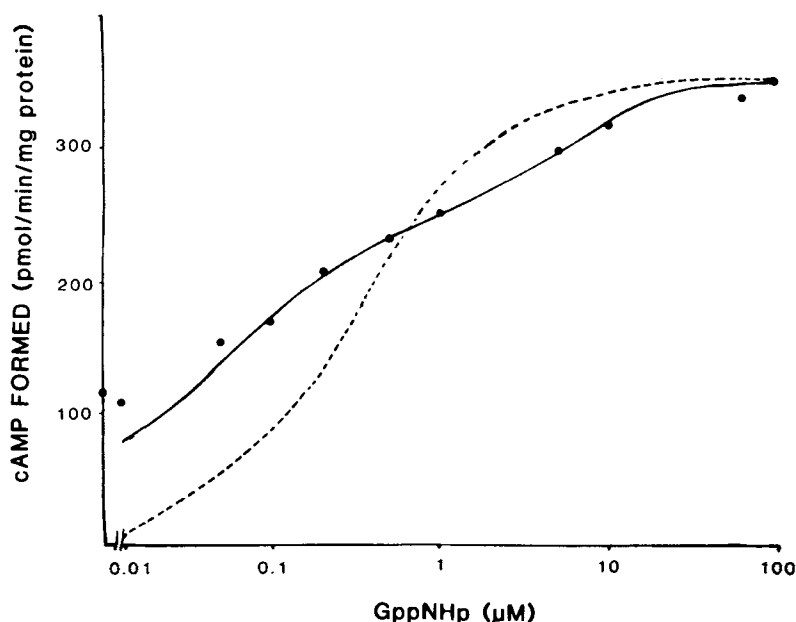

FIG. 6. Comparison of fits, assuming two independent adenylate cyclase activities activated by GppNHp. The shape of a curve representing two independent components of adenylate cyclase activated by GppNHp (solid line) was fitted with our experimental data for activation by GppNHp. The apparent kinetic constants for GppNHp activation derived as described in the text are: Component 1: K_{act}(app) = 30 nM; V_{max}(app) = 223 pmol/min/mg protein; Component 2: K_{act}(app) = 3.4 μM; V_{max} = 131 pmol/min/mg protein. The dashed line represents a model based on the assumption of one site of adenylate cyclase stimulation by GppNHp having an apparent K_a for GppNHp of 0.3 μM and an apparent V_{max} of 350 pmol/min/mg protein.
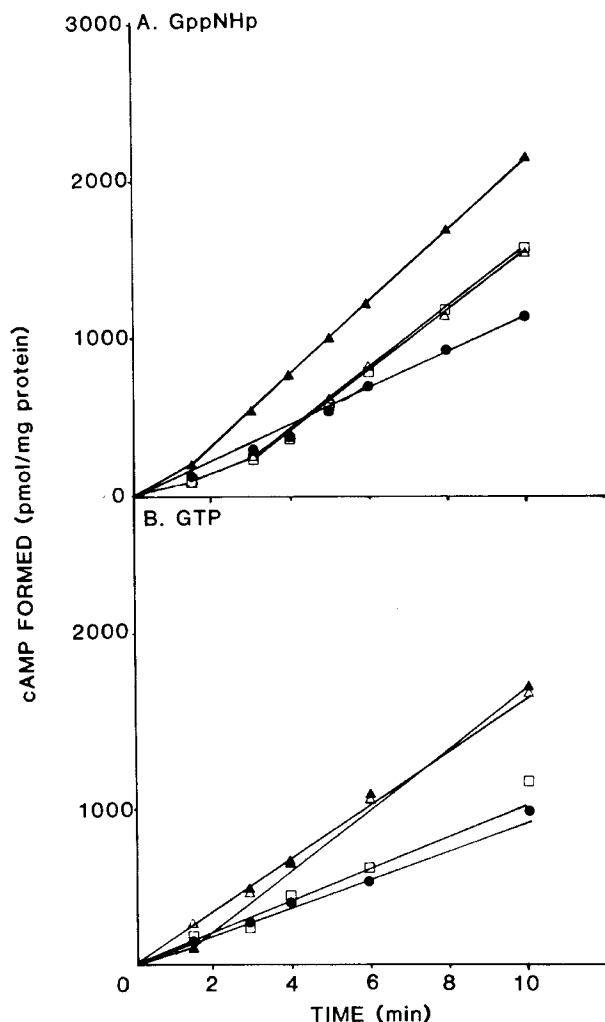
FIG. 7. Time course of adenylate cyclase activity at 25°C in a rat striatal particulate fraction activated by guanyl nucleotides, Ca^{2+}, and CaM. A rat striatal particulate fraction was prepared as described in the legend to Fig. 1. Cyclic AMP formation was determined by standard procedures, and 200-μl aliquots were removed at stated times. The time dependence for adenylate cyclase was determined in the absence (●) and presence of 120 nM Ca^{2+} (100 μM $CaCl_2$ in the assay) and 140 nM CaM (▲), guanyl nucleotides (□), and guanyl nucleotides, CaM and Ca^{2+} (△). **(A)** GppNHp; **(B)** GTP. Values represent the averages of three experiments done in duplicate and normalized for protein content. In A, slopes of the lines, calculated by linear regression analysis after any apparent lag period, in pmol/mg protein/min, are: basal, 124; Ca^{2+}, CaM, 233; 10 μM GppNHp, 185; GppNHp, Ca^{2+}, and CaM, 185. Slopes for B, in pmol/mg protein/min, are: basal, 100; Ca^{2+}, CaM, 195; 1 μM GTP, 120; GTP, Ca^{2+}, and CaM, 190.

reactants. On the contrary, when the activating guanyl nucleotide was the nonhydrolyzable analog GppNHp, CaM could further activate adenylate cyclase only at concentrations less than 0.2–0.5 μM GppNHp. Kinetic analysis of our data suggests that there are two different components of adenylate cyclase activity in rat striatal membranes, which can

be distinguished by their responses to CaM and guanyl nucleotides.

Analysis of the activation of adenylate cyclase by GppNHp kinetically fitted a model assuming two independent components activated by the same ligand. The apparent activation constants for GppNHp had a 100-fold difference in sensitivity: a high-affinity component had an apparent K_a of 30 nM, and a lower-affinity component had an apparent K_a of 3.4 μM. Both CaM and GTP, however, appeared to stimulate a single component of adenylate cyclase activity. CaM and GTP stimulated adenylate cyclase activity in an additive, noninteractive manner, suggesting action at different components. In addition, stimulation of adenylate cyclase activity attained in the presence of maximum concentrations of GTP and CaM was the same as that reached by a maximum concentration of GppNHp.

Contrary to the additive effects attained with CaM and GTP, CaM could not stimulate adenylate cyclase activity at concentrations of GppNHp above 0.2 μM. This was the case even in the presence of high CaM concentrations, and at all time points tested. At GppNHp concentrations above 0.2 μM, the stimulation with GppNHp and CaM resembled that of GppNHp itself. This suggests that CaM can activate the component having the greater sensitivity to GppNHp. The data further suggest that CaM itself can maximally stimulate this component, but that its ability to stimulate cyclase cannot be expressed in the presence of increasing concentrations of GppNHp. This might be expected, because of the irreversible nature of GppNHp action. The data in Figs. 2 and 3, however, suggest that CaM and GppNHp behave in the manner of competitive activators of this component, thus indicating that the membranes contain two components of adenylate cyclase activity, both of which can be activated by GppNHp. Only one component is stimulated by Ca^{2+} and CaM; the CaM-insensitive component, on the other hand, appears to respond to GTP.

Another finding to support this conclusion was obtained by using a particulate fraction prepared without EGTA extraction. This fraction is replete with CaM, and shows no further activation by CaM. GppNHp stimulated these membranes only at concentrations above 0.5 μM, consistent with activation of the CaM-insensitive component of activity, which had a high apparent K_a for GppNHp. This is also consistent with the suggestion that CaM and low GppNHp concentrations act competitively to activate adenylate cyclase.

There have appeared several reports of the existence of two components of adenylate cyclase in brain, one stimulated by CaM and Ca^{2+}, the other independent of CaM. Brostrom et al. (1977) analyzed these components by their differing sensi-

tivity to fluoride and CaM. Heideman et al. (1982) have partially purified a CaM-sensitive adenylate cyclase activity from bovine cerebral cortex. It is interesting to note that this activity was stimulated by GppNHp, as well as by CaM, but was not activated by GTP.

The difference between GppNHp and GTP interaction with CaM was not due to depletion of GTP during the assay, since a regenerating system was present. To demonstrate this further, we carried out an assay containing [^{3}H]GTP and identified the labeled nucleotides by thin-layer chromatography. There was no depletion of [^{3}H]GTP at the end of the assay period. Similarly, the lack of additivity between CaM and GppNHp was not due to a property unique to GppNHp. The interaction between CaM and another nonhydrolyzable analog of GTP, guanosine-5'-O-(3-thiotriphosphate) (GTPγS), was identical to that of CaM and GppNHp. Although our assay buffer did not routinely contain calcium, we evaluated the effect of calcium on GTP- and GppNHp-mediated activation of adenylate cyclase. The low concentration of calcium used in the assay ($10^{-7}M$ free Ca^{2+}) had an inhibitory effect on stimulation by GppNHp, especially low concentrations of GppNHp. Even considering the decreased value in the stimulation by GppNHp in the presence of Ca^{2+} alone, however, there was not an additive relationship between CaM and GppNHp. In fact, our studies demonstrated that calcium could selectively inhibit one of the components of GppNHp activation (Treisman and Gnegy, unpublished results).

The experiments conducted at 25°C (Fig. 7) demonstrated more clearly the fact that GppNHp will block activation by CaM, and that the two do not additively stimulate adenylate cyclase. Although Ca^{2+} and CaM alone activated adenylate cyclase to a velocity of 233 pmol/mg protein/min, when 10 μM GppNHp was present, the activation by Ca^{2+} and CaM was not greater than that of GppNHp alone. Similarly, CaM could not stimulate adenylate cyclase activity during the GppNHp-induced lag phase. This blockade of CaM activation by GppNHp was not found by some investigators working with cerebral cortex (Brostrom et al., 1978; Heideman et al., 1982). This could be due to the Ca^{2+} effect, since Ca^{2+} was routinely included in their buffers. It is also possible that the cerebral cortical enzyme is not regulated in the same way as that in the striatum.

Since GTP rather than GppNHp is the physiologically relevant guanyl nucleotide, it is very likely that GTP can activate the CaM-sensitive component *in vivo*. There could be a higher GTPase activity at the CaM-sensitive component or an alteration in the coupling of the GTP-binding protein to this component. Preliminary results have shown that CaM inhibits GTPase activity in a Ca^{2+}-dependent manner in striatal membranes (unpublished ob-

servations). Our two-component model does not delineate, at this point, whether there are two separate catalytic subunits or one catalytic subunit with two GTP-binding proteins. Our model is compatible, however, with the finding that GTP is not required for CaM activation and that CaM probably acts at a catalytic subunit of adenylate cyclase (Salter et al., 1981; Heideman et al., 1982; Seamon and Daly, 1982).

In summary, we suggest the existence of two components of adenylate cyclase activity in rat striatum. One component can be activated by Ca^{2+} and CaM and has a higher affinity for GppNHp. A CaM-insensitive component can be activated by higher concentrations of GppNHp, as well as of GTP. The CaM-sensitive component represents approximately 60% of the total activity stimulated by GppNHp. Although GppNHp and CaM appear to be competitive activators at one component, increasing GppNHp can block the activation by CaM. It is possible that tissues containing adenylate cyclase activity that does not respond to calmodulin lack the calmodulin-sensitive component found in brain. The inability of GTP to activate the CaM-sensitive component suggests that this component has either a different mode of binding to a GTP-binding protein or inherently higher GTPase activity than has the CaM-insensitive component. We have previously shown that CaM can affect DA sensitivity in the presence of either GTP or GppNHp (Gnegy and Treisman, 1981). This suggests that DA might stimulate adenylate cyclase through both components, with GTP as the guanyl nucleotide.

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