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Differential Regulation by Calmodulin of Basal, GTP-, and Dopamine-Stimulated Adenylate Cyclase Activities in Bovine Striatum

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Abstract: The concentration requirements of calmodulin in altering basal, GTP-, and dopamine-stimulated adenylate cyclase activities in an EGTA-washed particulate fraction from bovine striatum were examined. In the bovine striatal particulate fraction, calmodulin activated basal adenylate cyclase activity 3.5-fold, with an EC_{50} of 110 n*M*. Calmodulin also potentiated the activation of adenylate cyclase by GTP by decreasing the EC_{50} for GTP from 303 ± 56 n*M* to 60 ± 10 n*M*. Calmodulin did not alter the maximal response to GTP. The EC_{50} for calmodulin in potentiating the GTP response was only 11 n*M* as compared to 110 n*M* for activation of basal activity. Similarly, calmodulin increased the maximal stimulation of adenylate cyclase by dopamine by 50–60%. The EC_{50} for calmodulin in eliciting this response was 35 n*M*. These data demonstrate that calmodulin can both activate basal adenylate cyclase and potentiate adenylate cyclase activities that involve the activating GTP-binding protein, N_s. Mechanisms that involve potentiation of N_s-mediated effects are much more sensitive to calmodulin than is the activation of basal adenylate cyclase activity. Potentiation of GTP-stimulated adenylate cyclase activity by calmodulin was apparent at 3 and 5 m*M* $MgCl_2$, but not at 1 or 10 m*M* $MgCl_2$. These data further support a role for calmodulin in hormonal signalling and suggest that calmodulin can regulate cyclic AMP formation by more than one mechanism. **Key Words:** Basal ganglia—Guanyl nucleotides—Calcium—Dopamine—Adenylate cyclase. **Harrison J. K. et al.** Differential regulation by calmodulin of basal, GTP-, and dopamine-stimulated adenylate cyclase activities in bovine striatum. *J. Neurochem.* **51,** 345–352 (1988).

A limited number of mammalian tissues display an adenylate cyclase activity that is sensitive to an endogenous Ca^{2+}-binding protein, calmodulin (CaM). These include brain, retina, pancreatic islets, adrenal medulla, kidney, and heart (Manalan and Klee, 1984; MacNeil et al., 1985). The response of adenylate cyclase to CaM in nervous tissue, however, is significantly greater than that in other tissues. Much of the evidence to date suggests that CaM stimulates adenylate cyclase activity by binding directly to the catalytic moiety of the enzyme (Salter et al., 1981; Yeager et al., 1985). Although guanyl nucleotides are not required for stimulation of adenylate cyclase by CaM (Heideman et al., 1982; Seamon and Daly, 1982), CaM can affect the activation of guanyl nucleotide-mediated activities of adenylate cyclase (Brostrom et al., 1978; Treisman et al., 1983; Malnoe and Cox, 1985). We have shown that CaM can increase the activation of adenylate cyclase by dopamine (DA) in rat striatum and bovine retina (Gnegy and Treisman, 1981; Gnegy et al., 1984). In addition, stimulation of adenylate cyclase by CaM is increased after treatment of bovine striatal homogenates with cholera toxin (Mickevicius et al., 1986). Regulation of a β-adrenergic-stimulated adenylate cyclase by CaM from bovine cerebellum has also been demonstrated (Malnoe et al., 1983). These data strongly suggest that CaM and the guanyl nucleotide binding protein that mediates stimulation of adenylate cyclase (N_s) can interact to regulate the catalytic activity of adenylate cyclase.

We examined more closely the concentration requirements for CaM in affecting the activation of basal and GTP- and DA-stimulated adenylate cyclase activities in bovine striatum. We report here that the concentration of CaM required to affect maximally both the GTP- and DA-stimulated adenylate cyclase

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Abbreviations used: CaM, calmodulin; DA, dopamine; N_i and N_s, guanine nucleotide-binding proteins mediating inhibition and stimulation of adenylate cyclase.

activities is significantly less than the concentration of CaM needed to support maximal stimulation of basal adenylate cyclase activity. A similar effect of CaM on guanyl nucleotide-stimulated adenylate cyclase in rat cerebral cortex has been reported by Brostrom et al. (1978). In this study, we demonstrate a greater sensitivity for CaM in affecting a hormone-stimulated adenylate cyclase activity as compared to basal activation. In addition, the potentiation of GTP-stimulated adenylate cyclase activity by CaM was biphasic with respect to $MgCl_2$ concentration. The differential effect of CaM on adenylate cyclase activities in bovine brain suggests that CaM regulates this system by multiple mechanisms.

MATERIALS AND METHODS

Materials

[α-^{32}P]ATP (specific activity, 38 Ci/mmol) and [^{3}H]-cyclic AMP (41.7 Ci/mmol) were purchased from Amersham Searle (Arlington Heights, IL, U.S.A.). Cyclic AMP, EGTA, phosphoenol pyruvate, ATP, 3-hydroxytyramine HCl (DA), and HEPES were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). GTP and pyruvate kinase were from Boehringer-Mannheim (Indianapolis, IN, U.S.A.).

Preparation of bovine striatal particulate fractions and adenylate cyclase assay

Bovine striatum was dissected at a local slaughterhouse and immediately placed in liquid N_2. Tissue was stored at −70°C. A particulate preparation was prepared by thawing tissue and homogenizing it in 9 volumes of 20 m*M* HEPES:Na, pH 7.5, containing 5 m*M* $MgCl_2$ and 1.2 m*M* EGTA (buffer A). The homogenate fraction was centrifuged at 27,000 *g* for 20 min. The pellet was washed two more times in buffer A and resuspended in this buffer to a concentration of approximately 4 mg/ml protein. This preparation was stable to freezing at −70°C for at least 3 months. In designated experiments, the frozen preparation was thawed, homogenized in 100 volumes of buffer A, centrifuged as described above, and resuspended in buffer A to a concentration of 4 mg/ml or less.

Adenylate cyclase activity of the washed particulate fraction was determined in an assay (200 μl volume) containing 20 m*M* HEPES:Na, pH 7.5, 5 m*M* $MgCl_2$, 2 m*M* cyclic AMP, 4 m*M* phosphoenol pyruvate, 20 μg pyruvate kinase, 0.12 m*M* isobutylmethylxanthine, 100 μg or less of particulate membrane protein, 0.15 m*M* EGTA, and 0.5 m*M* [^{32}P]ATP (1 μCi per assay), with or without various effectors, such as GTP, DA, Ca^{2+}, and CaM. Assays were incubated for 6 or 8 min at 37°C, and the reaction was stopped by heating for 1 min at 95°C. Linearity was maintained in the presence of all activators for 8–10 min. A solution containing 20 m*M* ATP and 0.7 m*M* [^{3}H]cyclic AMP (200 μl volume) was then added to the tubes. The particulate material was centrifuged and the ^{32}P-labelled cyclic AMP in the supernatant fraction was determined by the method of Krishna et al. (1968). Recovery of cyclic AMP was measured using the [^{3}H]cyclic AMP and was usually 80–90%. Assays measuring stimulation of adenylate cyclase by DA contained 1 μ*M* GTP. DA-stimulated adenylate cyclase activity is defined as the picomoles of cyclic AMP formed per minute per milligram of protein above that activity produced in the presence of GTP alone or GTP and CaM. Protein was determined by the method of Lowry et al. (1951). Free or effective concentrations of Ca^{2+} were calculated using a dissociation constant for Ca-EGTA of 4.08×10^{-8} *M* according to the method of Nanninga and Kempen (1971). In assays in which Ca^{2+} was present, 125 μ*M* $CaCl_2$ was added resulting in a free or effective concentration of Ca^{2+} of 0.12 μ*M*. Kinetic constants were determined by the method of Wilkinson (1961).

Preparation and measurement of CaM

CaM was purified from bovine testis by the method of Dedman et al. (1977) and demonstrated a single band on disc gel electrophoresis containing 12% polyacrylamide. The protein concentration was determined by ultraviolet absorption. The M_r determined by slab gel electrophoresis using standards of known molecular weight was 16,700. This value was used to calculate the concentrations of CaM reported in this study.

CaM was measured in the particulate fractions by using a radioimmunoassay (New England Nuclear, Boston, MA, U.S.A.). Homogenate or particulate fractions were homogenized in 1% Lubrol PX and diluted in the assay buffer provided. The samples were not heated, and the assay was performed using unheated CaM standards.

Measurement of nucleotide content

Protein was precipitated in the fresh or frozen particulate fractions by addition of an equal volume of 0.8 *M* $HClO_4$, followed by centrifugation at 4°C. Supernatants were neutralized by addition of KOH and $KHCO_3$. Nucleotides were quantitated by HPLC on a strong anion-exchange column (Shewach et al., 1985).

RESULTS

Activation of adenylate cyclase by GTP in the presence and absence of CaM

The dose-dependent activation of bovine striatal adenylate cyclase by GTP was examined in the presence of various concentrations of CaM. As shown in Fig. 1, GTP stimulated adenylate cyclase activity in the presence of all concentrations of CaM tested. In the absence of added CaM, GTP stimulated adenylate cyclase activity by 80 pmol/min/mg protein over basal activity at a maximum concentration of 10 μ*M*. In the presence of increasing concentrations of CaM, progressively lower concentrations of GTP were required to reach maximal activation. In the presence of concentrations of CaM at or greater than 90 nM, only 1 μ*M* GTP was required to stimulate maximally the adenylate cyclase activity. Table 1 summarizes the kinetic constants (EC_{50} and V_{max} values) for the data presented in Fig. 1. In the absence of CaM, the EC_{50} for stimulation of adenylate cyclase activity by GTP was 303 ± 56 n*M* (n = 5). The EC_{50} values steadily and significantly decreased with increasing concentrations of CaM. In the presence of 90 n*M* CaM and above, the EC_{50} for GTP stimulation was approximately 60 n*M*. At all concentrations of CaM, the apparent V_{max} for GTP stimulation was slightly decreased. At high GTP concentrations, the inhibi-

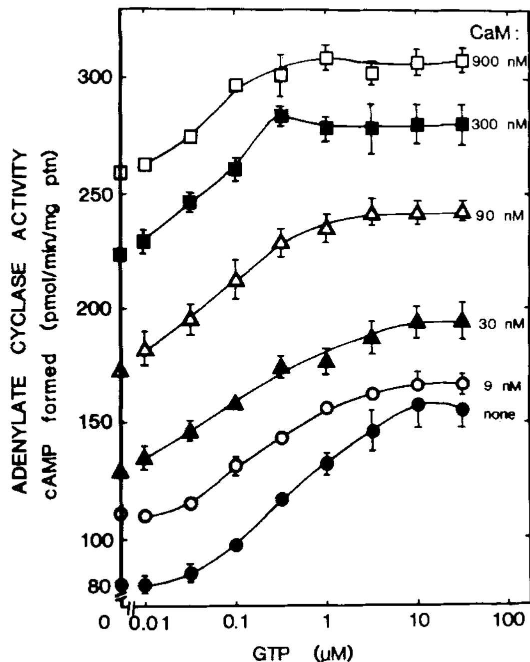

FIG. 1. Effect of CaM on the activation of adenylate cyclase by GTP in bovine striatal membranes. The concentration-dependent activation of adenylate cyclase by GTP was measured in an EGTA-washed striatal particulate preparation in the presence of the given concentrations of CaM. The effective concentration of Ca^{2+} in these assays was 0.12 μM. Results are presented as the means ± SEM of five separate experiments.

tory GTP-binding protein, N_i, may be more active in the presence of CaM (Girardot et al., 1983), which may result in an apparent inhibition of the N_s-mediated stimulatory activity. Also shown in Table 1 is the stimulation of basal adenylate cyclase by CaM. CaM stimulated basal activity approximately 3.5-fold, with a maximum near 3 μM. The EC_{50} for stimulation of basal activity by CaM is 110 ± 23 nM (n = 4). The apparent V_{max} is 194 ± 8 pmol/min/mg protein.

The effect of CaM in increasing the sensitivity of adenylate cyclase to GTP, as well as the activation of basal activity, was dependent upon both Ca^{2+} and CaM. No increase in GTP sensitivity was seen in the presence of Ca^{2+} alone. Ca^{2+} increased basal adenylate cyclase activity by only 15% in the absence of exogenous CaM. This may be due to the presence of endogenous CaM remaining in the particulate fraction after washing, as discussed below.

Activation of adenylate cyclase by DA in the presence of CaM

Figure 2 demonstrates the dose-dependent activation of bovine striatal adenylate cyclase by DA in the presence and absence of 150 nM CaM. One micromolar GTP was used to support DA stimulation. The inset to Fig. 2 demonstrates the DA-stimulated adenylate cyclase activity. As shown in Fig. 2, activation of adenylate cyclase by DA was significantly greater in the presence than in the absence of CaM. The apparent V_{max} for DA-stimulated adenylate cyclase activity in these experiments was 91 ± 4 pmol/min/mg protein in the absence of CaM and 139 ± 8 pmol/min/mg protein when measured in the presence of Ca^{2+} and CaM ($p \leq 0.001$, n = 4). There was also a small change in the EC_{50} for DA in the presence of CaM. The EC_{50} was reduced from 2.8 ± 0.4 μM in the absence of CaM to 1.3 ± 0.5 μM in the presence of CaM. Due to interassay variability in the EC_{50} values, this decrease is significant only when using paired t analysis ($p \leq 0.005$). In each experiment, however, on three different days, the EC_{50} for DA was decreased by 50% in the presence of CaM.

The concentration dependence of CaM for the potentiation of DA-stimulated adenylate cyclase activity was determined. The effect of various concentrations of CaM on the activation of adenylate cyclase by 50 μM DA is shown in Table 2. CaM increased the DA-stimulated activity by 60% from 63 to 105 pmol/min/mg protein. A maximal effect was achieved at 90 nM CaM. These values are slightly lower than those

TABLE 1. *Calmodulin stimulates basal adenylate cyclase and lowers the EC_{50} for GTP*

		Adenylate cyclase activity		Kinetic parameters for GTP-stimulated adenylate cyclase	
[CaM] (nM)	n	Total[a]	CaM-Stimulated[a]	EC_{50} (nM)	V_{max}[a]
0	5	78 ± 3.6	—	303 ± 56	76 ± 8.6
9	5	111 ± 4	33	228 ± 42	57 ± 1
30	5	128 ± 6	50	94 ± 13[b]	60 ± 6.7
90	5	172 ± 6	94	66 ± 13[c]	69 ± 4
300	5	223 ± 5	145	67 ± 10	63 ± 8.5
900	4	258 ± 16	180	60 ± 10[d]	52 ± 3.7
3,000	4	268 ± 25	190	ND	ND

[a] Values given are means ± SEM and are expressed as picomoles per minute per milligram of protein.
[b] $p < 0.01$.
[c] $p < 0.005$.
[d] $p < 0.02$.

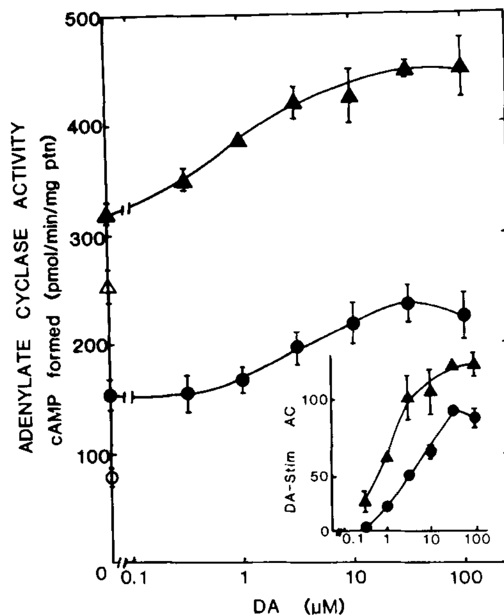

FIG. 2. Effect of CaM on the activation of adenylate cyclase by DA in a bovine striatal particulate fraction. The concentration-dependent activation of adenylate cyclase by micromolar levels of DA was measured in EGTA-washed membranes in the absence (●) and presence (▲) of 150 nM CaM and 0.12 μM Ca^{2+}. The concentration of GTP used to support DA stimulation was 1 μM. GTP-stimulated activity is represented by the solid symbols on the ordinate. Adenylate cyclase activity in the absence (basal, ○) and presence of CaM (△) is also shown on the ordinate. The inset shows the DA-stimulated adenylate cyclase activity calculated from the main figure determined in the presence of GTP (●) or GTP, CaM, and Ca^{2+} (▲). The concentration of DA in micromolar units is given on the abscissa. Results are presented as the means ± SEM for three (CaM and GTP) and four (GTP alone) separate experiments.

reported above, because they were from a different bovine preparation that had been frozen and washed two extra times. The basal adenylate cyclase activity was similarly reduced 30% in these experiments.

TABLE 2. *Dose-dependent increase in DA-stimulated adenylate cyclase activity by CaM*

CaM (nM)	DA-stimulated activity[a] (pmol/min/mg protein)
0	63 ± 1.7
9	71 ± 5
30	79 ± 8
45	94 ± 12[b]
90	101 ± 5[c]
900	105 ± 11[d]

Values given are means ± SEM of three separate experiments.
[a] DA-stimulated activity was determined in the presence of 50 μM DA and 1 μM GTP.
[b] $p < 0.05$.
[c] $p < 0.001$.
[d] $p < 0.01$.

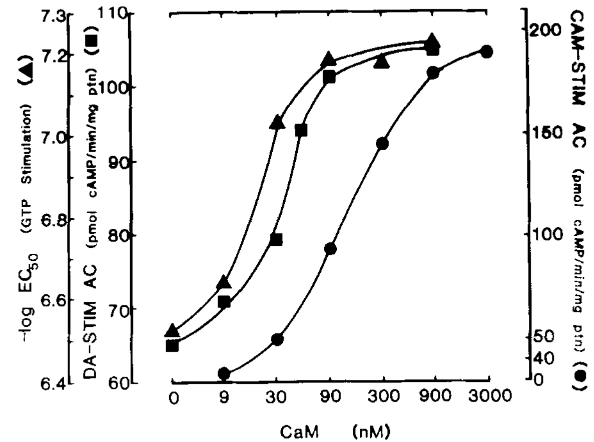

FIG. 3. Effect of CaM on the EC_{50} for GTP stimulation, the DA-stimulated activity, and the activation of basal adenylate cyclase activity. The $-\log EC_{50}$ for GTP-stimulated adenylate cyclase, the DA-stimulated activity, and the CaM-stimulated activity were plotted for each CaM concentration tested. Data are taken from Tables 1 and 2.

Comparison of the concentration dependence of CaM on basal, GTP-, and DA-stimulated adenylate cyclase activities

The above experiments demonstrate that there is a differential concentration requirement for CaM to increase basal adenylate cyclase activity as compared to its ability to increase both the sensitivity for GTP and the maximal response of adenylate cyclase to DA. The concentration dependence for CaM in altering these three activities is shown in Fig. 3. The concentrations of CaM that effect the increase in GTP sensitivity and response to DA are three to 10 times less than those required to activate basal adenylate cyclase activity. The EC_{50} for CaM in activation of adenylate cyclase activity was 110 nM. The EC_{50} for CaM in increasing the sensitivity to adenylate cyclase to GTP was only 11 nM, and that for increasing the response to DA was 35 nM. Thus, the processes involved in altering N_s-mediated adenylate cyclase activities are much more sensitive to CaM than those involved in increasing basal adenylate cyclase activity.

Effect of $MgCl_2$ on the GTP and CaM potentiated adenylate cyclase activity

The effect of $MgCl_2$ concentration on the CaM plus GTP-potentiated adenylate cyclase activity was examined and is shown in Fig. 4. Depicted in Fig. 4 are the GTP-, CaM-, and GTP plus CaM-stimulated activities. Concentrations of GTP (0.3 μM) and CaM (300 nM) were chosen at which the potentiating interaction was observed. GTP-stimulated adenylate cyclase activity was slightly enhanced by the higher $MgCl_2$ concentrations in the assay, whereas the CaM-stimulated activity was greatest at the lower concentrations of $MgCl_2$. This decrease in CaM-stim-

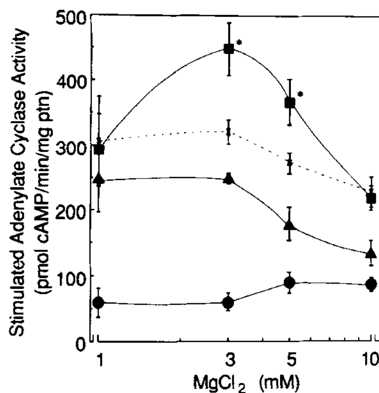

FIG. 4. Effect of $MgCl_2$ on the GTP- plus CaM-stimulated activity. The GTP- (●), CaM- (▲), and GTP plus CaM- (■) stimulated adenylate cyclase activities were measured in an EGTA-washed striatal particulate preparation in the presence of various concentrations of $MgCl_2$. Concentrations of GTP and CaM were 0.3 μM and 300 n*M*, respectively. Activity is presented as the stimulated activity above the basal activities for the respective $MgCl_2$ concentrations. Basal activities were as follows: 1 m*M* $MgCl_2$: 144 ± 42; 3 m*M* $MgCl_2$: 221 ± 38; 5 m*M* $MgCl_2$: 218 ± 37; and 10 m*M* $MgCl_2$: 269 ± 54 pmol/min/mg protein. The dashed line depicts the theoretical additive values for GTP- and CaM-stimulated activities. Results are presented as the means ± SEM of four separate experiments. The asterisks indicate a significant difference of the measured GTP plus CaM-stimulated activity above the theoretical added values ($p < 0.05$).

ulated adenylate cyclase appears to be due to a steady increase in basal activity with no significant change in the total activity measured with Ca^{2+} and CaM (see Fig. 4 legend). In the presence of GTP and CaM, the measured activity was greater than additive only in the presence of 3 and 5 m*M* $MgCl_2$. At 1 and 10 m*M* $MgCl_2$, the GTP plus CaM-stimulated activities were additive. Thus, CaM plus GTP-potentiated adenylate cyclase activity was dependent on an optimal concentration of $MgCl_2$ in the assay (3–5 m*M*).

GTP and CaM content of particulate fractions from bovine striatum

The ability to detect the effect of CaM on increasing the sensitivity of adenylate cyclase for GTP, and especially for increasing the response to DA, was critically dependent on the concentration of protein in the assay. Activation of basal adenylate cyclase by CaM was linear to at least 1.5 mg/ml protein in the adenylate cyclase assay, but the potentiating response between GTP and CaM was most apparent at protein concentrations equal to or less than 0.5 mg/ml. There could be a factor interfering with the response that is diluted below a critical level at low protein concentrations. Another explanation is that endogenous levels of GTP or CaM could be high enough to mask the potentiating response. In fresh rat brain, for instance, only 50% of the endogenous CaM can be removed by washing the membranes several times with millimolar concentrations of EGTA (Gnegy and Treisman, 1981). The concentration of the endogenous ligands in the assay could be important as to the degree of the interactive response.

The CaM and GTP contents of bovine striatal homogenates and EGTA-washed particulate fractions are shown in Table 3. The concentration of CaM in EGTA-washed particulate fractions in an assay containing 100 μg of protein is 16 n*M*, which is near the EC_{50} for CaM in increasing the sensitivity for GTP. Similarly, the concentration of GTP in an assay containing 100 μg of protein is 54 n*M*, which is approximately the EC_{50} for GTP in the presence of CaM. The fact that the membranes cannot be depleted of these ligands means that exact kinetic constants cannot be calculated, but it is apparent that lowering the concentrations to near the experimentally determined constants is important. Freeze-thawing the membranes and diluting them into large volumes of hypotonic medium did not reduce further the concentration of GTP or CaM in the membranes.

DISCUSSION

Evidence from recent studies suggests that CaM activates adenylate cyclase by acting at the catalytic subunit of adenylate cyclase (Coussen et al., 1985; Yeager et al., 1985; Smigel, 1986). Activation of basal adenylate cyclase activity by CaM does not require GTP (Heideman et al., 1982; Seamon and Daly, 1982); activation of a partially purified catalytic subunit by CaM was additive with N_s (Salter et al., 1981). Several studies, however, have found interactions and potentiating effects between CaM, neurotransmitters, and guanyl nucleotides, suggesting that the two activities are not totally independent (Brostrom et al., 1978; Ausiello and Hall, 1981; Gnegy and Treisman, 1981; Malnoe et al., 1983; Treisman et al., 1983; Panchenko and Tkachuk, 1984; Malnoe and Cox, 1985; Asano et al., 1986). Our laboratory has found that CaM enhances the stimulation by DA in rat and bovine striatum and bovine retina (Gnegy and Treisman, 1981; Gnegy et al., 1984; this article). Similarly, potentiating interactions have been found between CaM, guanyl nucleotides, and β-adrenergic receptor stimulation in bovine cerebellum (Malnoe et al., 1983; Malnoe and Cox, 1985) and rabbit heart (Pan-

TABLE 3. *Calmodulin and GTP contents of striatal homogenates and EGTA-washed particulate fractions*

	CaM		GTP	
	ng/μg protein	n*M* in assay[a]	nmol/mg protein	n*M* in assay[a]
Homogenate	2.05	—	ND	ND
EGTA-washed particulate	0.55	16	0.108	54

[a] Concentration in a 200-μl assay containing 100 μg of protein.

chenko and Tkachuk, 1984). Thus, it appears that CaM can both activate basal adenylate cyclase activity in an apparently GTP-independent manner and potentiate adenylate cyclase activities that involve the activating GTP-binding protein, N_s.

In this study, we investigated the ability of CaM to activate basal adenylate cyclase activity and potentiate N_s-mediated activities in a bovine striatal particulate fraction. We found that these activities were differentiated by their concentration requirements for CaM. CaM increased basal adenylate cyclase activity in bovine striatum by 3.5-fold. CaM also increased the sensitivity of adenylate cyclase for GTP in the activation of adenylate cyclase by fivefold, shifting the apparent activation constant for GTP from 300 n*M* (in the absence of CaM) to 60 n*M* (in the presence of CaM). In addition, CaM increased the maximal extent by which DA stimulated adenylate cyclase by 50–60%. Most notable were the concentration requirements for CaM in effecting these actions on adenylate cyclase. The EC_{50} for CaM in the stimulation of basal adenylate cyclase activity was approximately 110 n*M*. In contrast, this concentration of CaM was sufficient to affect maximally the GTP sensitivity, as well as activation of adenylate cyclase by DA. The EC_{50} for CaM in increasing the sensitivity for GTP was 11 n*M* and that for increasing maximal activation by DA was 35 n*M*. These data suggest that CaM has two distinct modes of action in the regulation of bovine striatal adenylate cyclase. A high-affinity interaction is involved with stimulation of adenylate cyclase by GTP and DA, whereas the activation of basal activity is a lower affinity interaction. The results of Brostrom et al. (1978) suggest that CaM and guanyl nucleotides regulate adenylate cyclase from rat cerebral cortex in a similar manner. They demonstrated that guanyl nucleotides potentiated activation of rat cortical adenylate cyclase activity by low concentrations of CaM. Our results in bovine striatum extend the mechanism to include regulation of hormone receptor-stimulated adenylate cyclase by CaM, a process which presumably requires the activation of N_s. A dual regulation by CaM was similarly suggested by the studies of MacNeil et al. (1984). In examining reconstitution of CaM-stimulated adenylate cyclase into CaM-depleted B16 melanoma membranes, it was found that concentrations of CaM that poorly stimulated basal adenylate cyclase activity could increase the prostaglandin E- plus forskolin-stimulated activity. It is not possible at this time to delineate whether the mechanism by which CaM affects the sensitivity to GTP is exactly the same as that by which it increases activation by DA. They could be separate events, because the EC_{50} for CaM in increasing DA activation was slightly higher than that measured for increasing the GTP sensitivity. Similarly, higher concentrations of GTP were required to support DA activation in the absence or presence of CaM (over 0.3 μM) than were involved in the potentiating interaction with CaM.

Exploration of the $MgCl_2$ concentration requirements for the GTP and CaM potentiating interaction revealed that this response was biphasic with respect to $MgCl_2$. The potentiating interaction was observed at 3 and 5 m*M* $MgCl_2$, but did not occur at all at 1 or 10 m*M* $MgCl_2$. Regulation of hormone-sensitive adenylate cyclase activity by Mg^{2+} is known to be complex, appearing to have multiple sites of action. In addition to its role as the substrate (Mg^{2+}-ATP), for the adenylate cyclase catalytic reaction, Mg^{2+} appears to interact with hormone receptors (Heidenreich et al., 1982) and guanyl nucleotide-binding proteins (Gilman, 1984), as well as at the catalytic unit (C) of adenylate cyclase at a nonsubstrate site (Bender and Neer, 1983). Mg^{2+} has also been shown to bind to CaM (Wolff et al., 1977). Because the Mg^{2+} regulation occurs in the absence of a hormone, we can rule out effects of Mg^{2+} on the receptor in this GTP and CaM potentiating interaction. Mg^{2+} appears to be involved in dissociation of GTP-binding proteins into their α and $\beta\gamma$ subunits. Katada et al. (1987) showed that $\beta\gamma$ subunits of guanyl nucleotide-binding proteins potently inhibit CaM-sensitive adenylate cyclase activity, as well as bind to CaM-Sepharose in a Ca^{2+}-dependent manner. Therefore, it is attractive to suggest that Mg^{2+} and CaM could regulate the subunit association/dissociation state of N_s. It is also attractive to suggest that Mg^{2+} could modulate an interaction between N_s and CaM at the catalytic unit. These studies, as well as those of Brostrom et al. (1977), are consistent with the hypothesis that CaM and the α subunit of N_s allosterically modulate each other's binding at the catalytic unit to produce a synergistic activation of catalytic activity. With regard to this hypothesis, CaM appears to share the ability to dually regulate adenylate cyclase activity with forskolin, possibly by similar mechanisms. Numerous studies have shown that a component of forskolin's activation of adenylate cyclase is dependent upon N_s (Daly et al., 1982; Darfler et al., 1982; Green and Clark, 1982; Morris and Bilezikian, 1983), whereas it has an activating effect on catalytic activity that is independent of N_s (Seamon and Daly, 1981; Sano et al., 1983). Guanyl nucleotides have been shown to increase the sensitivity of forskolin in the activation of adenylate cyclase (Green and Clark, 1982), as well as the affinity of forskolin for its binding site(s) (Seamon et al., 1985). Forskolin has also been shown to increase the size of adenylate cyclase (Bouhelal et al., 1985), as well as decrease the rate of deactivation of a 5′-guanylylimidodiphosphate-activated adenylate cyclase (Yamashita et al., 1986). These data have been interpreted as an ability of forskolin to stabilize the N_s–C interaction and thereby prolong the activated enzyme state. CaM and forskolin, however, do not appear to act at the same site to elicit their effects on

the adenylate cyclase enzyme. In fact, CaM and forskolin demonstrate potentiating interactions with each other (Malnoe and Cox, 1985; Harrison and Gnegy, 1986). CaM, rather than forskolin, however, is an endogenous regulator of adenylate cyclase and thereby could serve the cell in a functional role in hormonal signalling mechanisms. Potentiating interactions between CaM and guanyl nucleotide-dependent adenylate cyclase activities clearly demonstrate potential direct interactions between calcium and cyclic AMP signal transduction systems.

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