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Calmodulin May Play a Pivotal Role in Neurotransmitter-Mediated Inhibition and Stimulation of Rat Cerebellar Adenylate Cyclase

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SUMMARY

Adenylate cyclase activity was stimulated 2.5-fold by exogenous Ca^{2+}/calmodulin (CaM) (1 μM) in rat cerebellar plasma membranes which had been depleted of endogenous Ca^{2+}/CaM. In EGTA-washed membranes, phenylisopropyladenosine (an adenosine receptor agonist) was unable to inhibit adenylate cyclase activity unless exogenous Ca^{2+}/CaM was included in the assay. Conversely, isoproterenol (a β-adrenergic receptor agonist) was able to stimulate adenylate cyclase activity only in the absence of Ca^{2+}/CaM. The regulation of adenylate cyclase activity by guanyl-5′-yl-imidodiphosphate [Gpp(NH)p, a nonhydrolyzable guanine nucleotide analog, used to monitor interactions between guanine nucleotide-binding proteins and the catalytic unit of adenylate cyclase] was similar to that of phenylisopropyladenosine and isoproterenol; i.e., Gpp(NH)p produced inhibition exclusively in the presence of Ca^{2+}/CaM, whereas only stimulation was observed in the absence of Ca^{2+}/CaM. These results suggest that changes in intracellular Ca^{2+} concentrations may determine whether adenylate cyclase can be stimulated or inhibited by neurotransmitters.

Neurotransmitters may either stimulate or inhibit adenylate cyclase activity through distinct receptor subclasses (e.g., the β and α_2 receptors for norepinephrine) (1, 2). Receptor-mediated regulation of adenylate cyclase activity is mediated by GTP-binding proteins (N proteins). Distinct N proteins, N_i and N_s, couple inhibitory and stimulatory receptor occupation, respectively, to alterations in adenylate cyclase activity (3). However, adenylate cyclase activity may also be regulated by mechanisms not directly related to receptor occupation. CaM, in the presence of low μM concentrations of free Ca^{2+}, increases the adenylate cyclase activity of all brain regions (4, 5) as well as that from several peripheral tissues, such as pancreatic islets (6), adrenal medulla (7), smooth muscle (8), enterocytes (9), myocardium (10), thyroid (11), and platelets (12). Ca^{2+}/CaM appears to stimulate the catalytic unit of adenylate cyclase directly (4, 5, 13, 14). It has been proposed that a distinct, CaM-sensitive isozyme of adenylate cyclase is present in those tissues in which CaM-dependent stimulation of adenylate cyclase has been observed (4, 13, 15, 16). Some widely used therapeutic agents, including vinca alkaloids (17), local anesthetics (18), benzodiazepines, tricyclic antidepressants, and, most notably, phenothiazine antipsychotics (19), antagonize CaM-stimulated processes. However, the significance of these effects on the regulation of adenylate cyclase by CaM has been difficult to assess, due to the relative low potency and membrane-perturbing effects of these compounds (20). In addition, the high endogenous concentrations of CaM in many brain regions (21) renders detection of the effects of CaM on a particular process difficult to discern.

Depletion of endogenous Ca^{2+} and CaM by washing tissue with cation chelating agents such as EGTA, followed by readdition of exogenous Ca^{2+} and CaM, or titration of Ca^{2+} concentrations against buffers containing EGTA, has allowed some evaluation of the role of CaM in neurotransmitter-regulated adenylate cyclase. Gnegy and co-workers (22, 23) have demonstrated that full expression of the stimulatory effects of dopamine on rat striatal adenylate cyclase requires the presence of CaM. Girardot *et al.* (24) and Perez-Reyes and Cooper (25, 25a) have shown that guanine nucleotide-mediated inhibition of rat hippocampal and cortical adenylate cyclase activity is dependent upon the presence of Ca^{2+}/CaM, while Yeager *et al.* (26) have demonstrated that adenosine, P-site-mediated inhibition of adenylate cyclase activity in bovine cerebral cortex is

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ABBREVIATIONS: Ni, inhibitory guanine nucleotide-regulatory protein; N_s, stimulatory guanine nucleotide-regulatory protein; CaM, calmodulin; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N′*-tetraacetic acid; BSA, bovine serum albumin (Cohn fraction V); PIA, (−)-N6-(phenylisopropyl)adenosine; ISO, DL-isoproterenol HCl; ANOVA, analysis of variance; Gpp(NH)p, guanyl-5′-yl-imidodiphosphate; IP_3, inositol trisphosphate.

dependent upon the presence of CaM. In the cerebellum, Malnoe *et al.* (27) have suggested that a synergistic interaction exists between ISO- and CaM-mediated stimulation of enzyme activity, while the inhibition produced by Gpp(NH)p appears to be dependent upon the presence of CaM (28).

Previous work from this laboratory (24, 25) and others (28) has demonstrated that Gpp(NH)p-mediated inhibition is strictly dependent upon Ca^{2+}/CaM in rat hippocampus and cortex, and bovine cerebellum, respectively. However, recently it has become clear that guanine nucleotide-binding proteins probably regulate not only adenylate cyclase (i.e., via N_i and N_s) but also phospholipase C (29, 30) and ion channels (31). Thus, nonhydrolyzable guanine nucleotide analogs, such as Gpp(NH)p, may access several different types of regulatory proteins within a plasma membrane preparation and their effects may not necessarily mimic those of the specific, GTP-utilizing regulatory proteins associated with stimulatory and inhibitory receptors that regulate adenylate cyclase. Therefore, the present study utilized PIA, an adenosine, A-1 receptor agonist, and ISO, a β-adrenergic agonist, to determine whether CaM could regulate receptor-mediated inhibition and stimulation, respectively, of adenylate cyclase in rat cerebellar plasma membranes.

Materials and Methods

Preparation of rat cerebellar membranes. The protocol for preparation of rat cerebellar plasma membranes was similar to that previously described for striatal plasma membranes (32). Freshly dissected cerebella from unanesthetized male Sprague-Dawley rats (170–250 g, sacrificedby decapitation) were placed in a buffer containing 50 mM Tris (pH 7.4), 1 mM dithiothreitol, 0.1 mM EDTA, 1 mg/ml leupeptin, 1 mg/ml pepstatin, 6000 units/ml aprotinin, and 1 mg/ml trypsin inhibitor, to which 10% sucrose (w/w) was added. The tissue was homogenized by hand and the suspension centrifuged at 121 × *g* for 10 min; the pellet was discarded. Subsequently, the supernatant was centrifuged at 12,000 × *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in preparation buffer (without sucrose), plus 0.5 M NaCl. This suspension was placed on ice for 10 min before centrifugation at 12,000 × *g* for 10 min. The pellet was resuspended in preparation buffer without sucrose and centrifuged at 12,000 × *g* for 10 min. This pellet was resuspended in a small volume of preparation buffer containing sucrose (10%). Aliquots (2–2.5 ml) were layered on continuous 30–38% sucrose gradients. The gradients were centrifuged at 82,500 × *g* for 1 hr 4°. The main turbid band (at ~35% sucrose) in each tube was collected and these fractions were pooled. This suspension was diluted approximately 4-fold with preparation buffer without sucrose and centrifuged at 12,000 × *g* for 10 min. The pellet was resuspended in the same buffer at 0.5–1 mg of protein per ml. Aliquots were frozen and stored in liquid nitrogen.

Adenylate cyclase assay. In order to remove endogenous Ca^{2+} and calmodulin, extensive washes of the membranes were employed prior to the adenylate cyclase assay. Frozen membranes were thawed and diluted 3-fold with buffer consisting of 50 mM Tris (pH 7.4), 1 mM EGTA, and 1 mg/ml BSA. This suspension was centrifuged at 12,000 × *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in 3 ml of the above buffer. This process was repeated twice (for a total of three washes). Following the third centrifugation, the pellet was suspended in 40 mM Tris (pH 7.4), 0.8 mM EGTA, and 0.8 mg/ml BSA to give a final EGTA concentration of 200 μM in the adenylate cyclase assay.

Adenylate cyclase activity was measured by the method of Salomon *et al.* (33). The assay mixture contained: α-[^{32}P]ATP (1 μCi), 0.1 mM cyclic AMP, 80 mM Tris (pH 7.4), 0.2 mM ATP, 20 mM NaCl, 10 μM GTP, 1 unit/ml adenosine deaminase, 4 mM creatine phosphate, 25 units/ml creatine phosphokinase, and 0.2 mg/ml BSA. The final assay volume was 100 μl. In most cases, 10 μg of membrane protein, as determined by the method of Lowry *et al.* (34), were assayed. Reactions were conducted for 10 min at 24° and were stopped by the addition of 0.1 ml of 2% sodium lauryl sulfate containing 1.3 mM cyclic AMP and 49 mM ATP. Recovery was monitored with 15,000 cpm of ^{3}H-cyclic AMP.

Determination of free divalent cation concentration. Free concentrations of Ca^{2+} were calculated as previously described (24, 32). This involved an iterative computing procedure for the solution of the equations describing the complexes formed in a mixture comprising all of the ingredients involved in the assay of adenylate cyclase that can affect free divalent cation concentration, i.e., ATP, GTP, EGTA, H^+, Mg^{2+}, Ca^{2+}, and Na^+. The log of the association constants used were: H^+ to $EGTA^{4-}$, 9.46; H^+ to $HEGTA^{3-}$, 18.31; H^+ to H_2EGTA^{2-}, 20.99; H^+ to H_3EGTA^-, 22.99; Ca^{2+} to $EGTA^{4-}$, 10.97; Ca^{2+} to $HEGTA^{3-}$, 14.78; Mg^{2+} to $EGTA^{4-}$, 5.28; Mg^{2+} to $HEGTA^{3-}$, 12.90; H^+ to ATP^{4-}, 6.51; H^+ to $HATP^{3-}$, 10.57; Ca^{2+} to ATP^{4-}, 3.77; Ca^{2+} to $HATP^{3-}$, 8.46; Mg^{2+} to ATP^{4-}, 4.06; Mg^{2+} to $HATP^{3-}$, 8.61; Na^+ to ATP^{4-}, 1.10 (35).

Statistics. The data are presented at the mean ± standard error of at least six determinations from two membrane preparations. Data were analyzed by two-way ANOVA using the presence or concentration of Ca^{2+}/CaM or drug as within group variables. When significant interactions were found, Duncan's or Dunnett's multiple comparison test (36) was employed where appropriate.

Materials. CaM (from bovine brain, activity reported to be greater than 40,000 units/mg of protein), GTP, BSA (Cohn fraction V), adenosine deaminase (1458 units/ml, from calf intestinal mucosa, in glycerol), leupeptin, trypsin inhibitor, and ISO were from Sigma. Aprotinin was from Calbiochem, and pepstatin, Gpp(NH)p and PIA were from Boehringer-Mannheim. All other drugs and reagents were obtained from standard sources.

Results

The adenylate cyclase activity of cerebellar plasma membranes, washed with a buffer containing EGTA (as described in Materials and Methods), was insensitive to concentrations of CaM up to 10 μM in the absence of added Ca^{2+} (Fig. 1a). Similarly, in the absence of added CaM, increasing free Ca^{2+} concentrations evoked no change in activity until a concentration of 20 μM, whereafter inhibition of adenylate cyclase activity was observed (Fig. 1b). However, in the presence of 2 μM free Ca^{2+}, CaM stimulated adenylate cyclase activity in a concentration-dependent manner by up to 250% of basal activity (Fig. 1a). Similarly, in the presence of 1 μM CaM, concentrations of free Ca^{2+} from 0.4 to 10 μM stimulated adenylate cyclase activity by 2.5-fold (Fig. 1b). Thus, washing membranes with EGTA appears to remove endogenous Ca^{2+} and CaM and permits control of Ca^{2+} and CaM concentrations in subsequent experiments.

To determine whether the presence of Ca^{2+}/CaM influences neurotransmitter-mediated inhibition of adenylate cyclase activity, PIA dose response experiments were performed in the absence or presence of Ca^{2+}/CaM in EGTA-washed membranes (Fig. 2). In the absence of Ca^{2+}/CaM, no inhibition of adenylate cyclase activity was observed. In the presence of Ca^{2+}/CaM, PIA significantly inhibited adenylate cyclase activity at concentrations from 0.01 to 10 μM (maximum inhibition was 20% of basal activity). Thus, the inhibitory effects of PIA could only be observed in the presence of Ca^{2+}/CaM-mediated stimulation.

To confirm this observation, a Ca^{2+} dose response experiment (in the presence of 1 μM CaM) was performed in the presence or absence of 1 μM PIA (Fig. 3). At concentrations of free Ca^{2+} below 0.4 μM, PIA was unable to inhibit adenylate cyclase

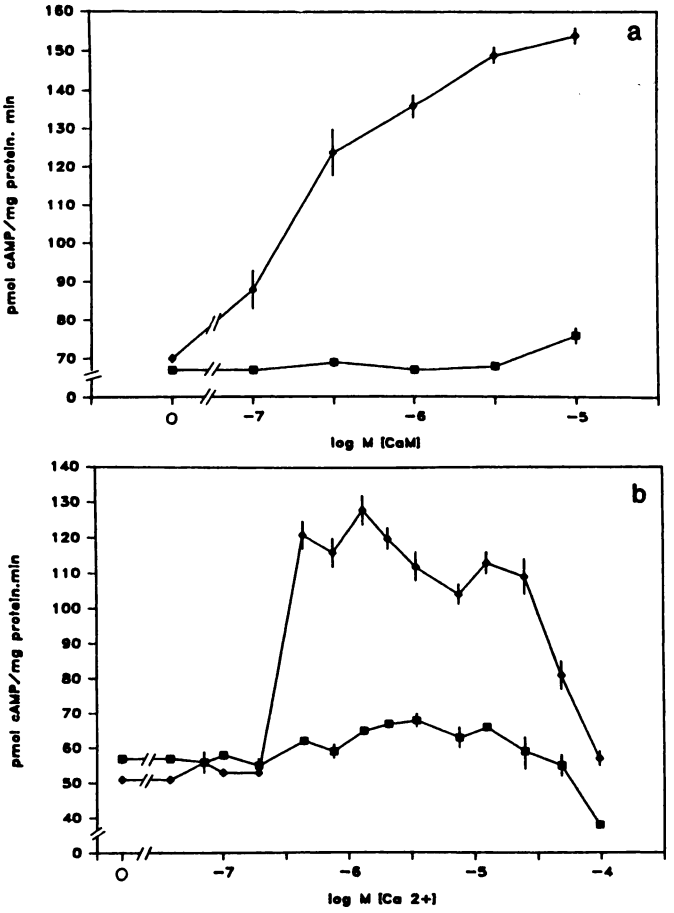

Fig. 1. a. CaM dose response curves in EGTA-washed membranes (see Materials and Methods) in the absence (□) or presence (◇) of a calculated free Ca^{2+} concentration of 2 μM. b. Ca^{2+} dose response curves in EGTA-washed membranes in the absence (□) or presence (◇) of 1 μM CaM.

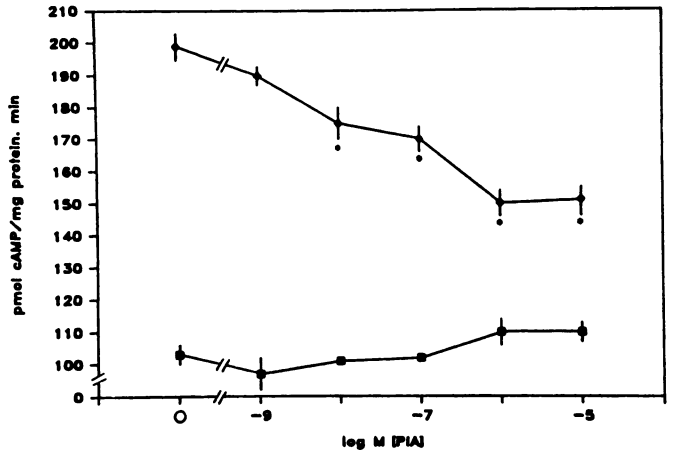

Fig. 2. PIA dose response curves in EGTA-washed membranes in the absence (□) or presence (◇) of Ca^{2+}/CaM (1.3 and 1 μM, respectively). *, significant difference from the absence of PIA (ANOVA followed by Dunnett's multiple comparison test, $p < 0.05$).

activity. At free Ca^{2+} concentrations from 0.4 to 100 μM, PIA significantly inhibited adenylate cyclase activity. The stimulation of adenylate cyclase activity by these concentrations of Ca^{2+} coincided with the appearance of PIA-mediated inhibition.

Since the inhibition produced by PIA was observed only when adenylate cyclase activity was stimulated by CaM, the possibility existed that any agent capable of stimulating enzyme

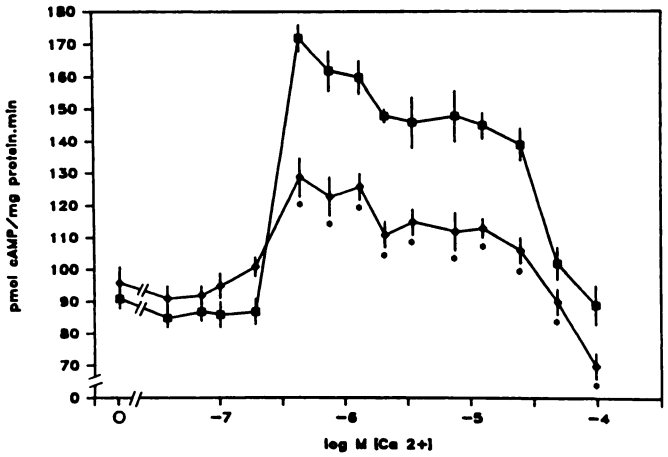

Fig. 3. Ca^{2+} dose response curves (in the presence of 1 μM CaM) in EGTA-washed membranes in the absence (□) or presence (◇) of 1 μM PIA. *, a significant difference from the absence of PIA (ANOVA followed by Duncan's test, $p < 0.05$).

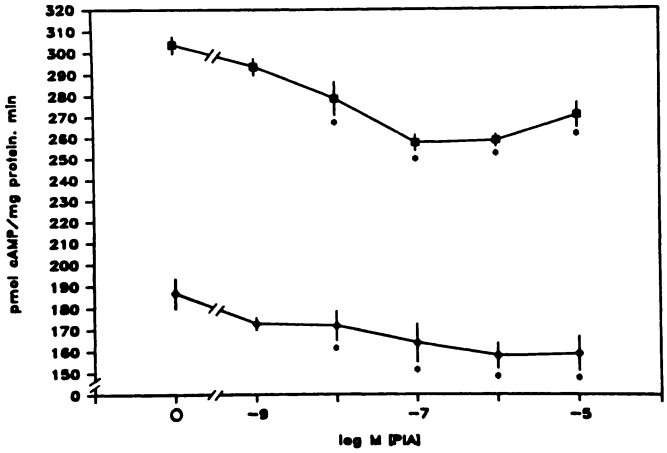

Fig. 4. PIA dose response curves in EGTA-washed membranes in the absence of Ca^{2+}/CaM and in the presence of 1 mM Mn^{2+} (□) or 3 μM (◇) forskolin. *, significant difference from the absence of PIA (ANOVA followed by Dunnett's multiple comparison test, $p < 0.05$).

activity would permit observation of PIA-mediated inhibition. To test this possibility, PIA dose response experiments were performed on EGTA-washed membranes in the absence of Ca^{2+}/CaM and in the presence of either 3 μM forskolin or 1 mM manganese (Mn^{2+}) (Fig. 4). PIA produced inhibition of either forskolin- or Mn^{2+}-stimulated adenylate cyclase activity to an extent similar to that of the inhibition observed in the presence of Ca^{2+}/CaM (cf. Figs. 4 and 2). Thus, stimulation of adenylate cyclase activity by either Ca^{2+}/CaM, Mn^{2+}, or forskolin permits neurotransmitter-mediated inhibition of enzyme activity to be observed.

To determine whether CaM also regulates neurotransmitter-mediated stimulation of adenylate cyclase activity, ISO dose response experiments were performed in the presence or absence of Ca^{2+}/CaM (Fig. 5). ISO evoked a modest but significant (ANOVA followed by Dunnett's multiple comparison test, $p < 0.05$) stimulation of adenylate cyclase activity (maximum stimulation was 20% of basal activity) in the absence of Ca^{2+}/CaM, but was unable to increase enzyme activity in the presence of Ca^{2+}/CaM (ANOVA, $p > 0.15$). These data suggest that neurotransmitter-mediated stimulation of adenylate cyclase may only occur in the absence of Ca^{2+}/CaM. To determine whether

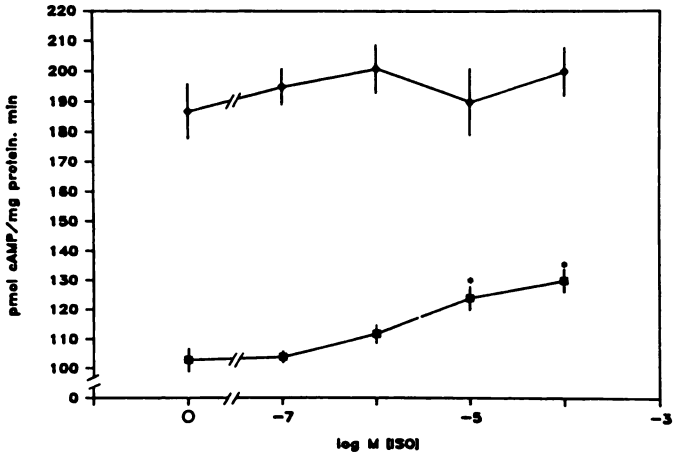

Fig. 5. ISO dose response curves in EGTA-washed membranes in the absence (□) or presence (◇) of Ca^{2+}/CaM (1.3 and 1 μM, respectively). *, significant difference from the absence of ISO (ANOVA followed by Dunnett's multiple comparison test, $p < 0.05$).

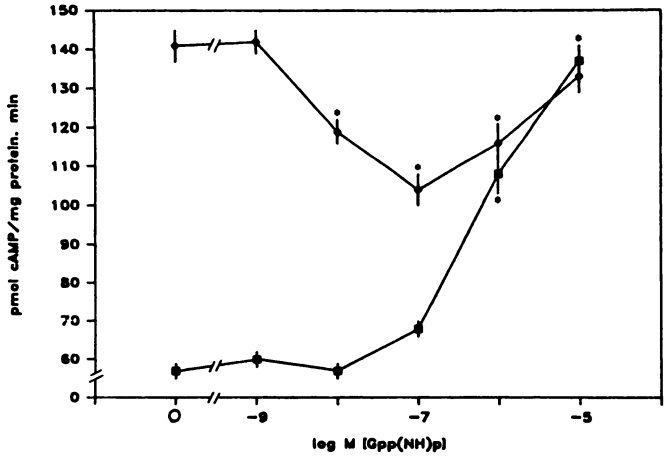

Fig. 7. Gpp(NH)p dose response curves in EGTA-washed membranes in the absence (□) or presence (◇) of Ca^{2+}/CaM (1.3 and 1 μM, respectively). *, significant difference from the absence of Gpp(NH)p (ANOVA followed by Dunnett's multiple comparison test, $p < 0.05$).

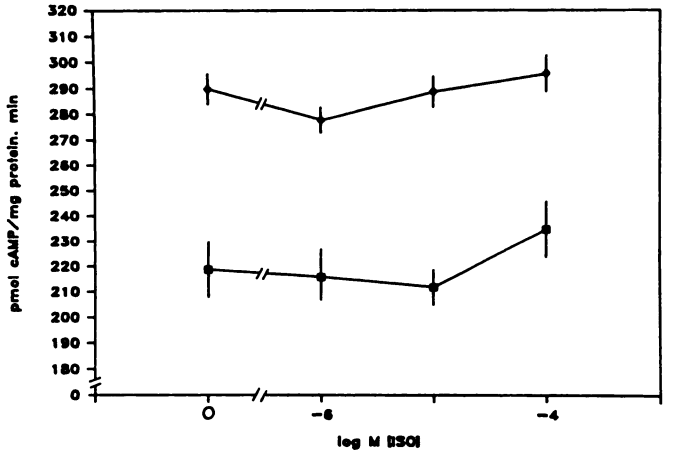

Fig. 6. ISO dose response curves in EGTA-washed membranes in the absence of Ca^{2+}/CaM and in the presence of 1 mM Mn^{2+} (◇) or 3 μM forskolin (□). No concentrations of ISO produced significant stimulation under these conditions (ANOVA, $p > 0.15$).

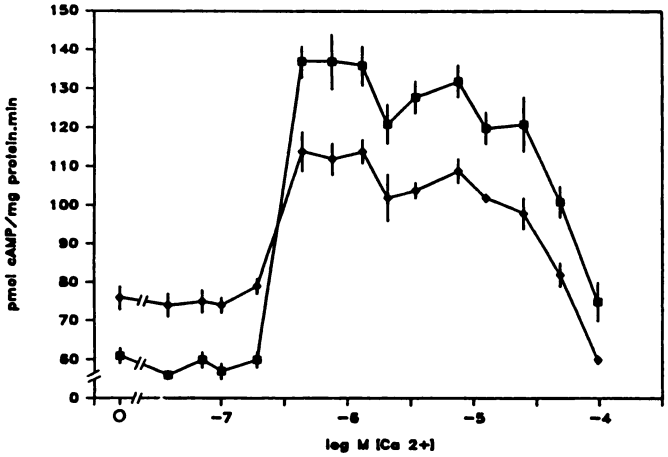

Fig. 8. Ca^{2+} dose response curves in EGTA-washed membranes in the absence (□) or presence (◇) of 0.3 μM Gpp(NH)p. At all Ca^{2+} concentrations and in the absence of Ca^{2+}, values in the presence of Gpp(NH)p are significantly different from those in the absence of Gpp(NH)p (ANOVA followed by Duncan's test, $p < 0.05$).

activation of adenylate cyclase activity by agents other than Ca^{2+}/CaM also prevents ISO-mediated stimulation, ISO dose response experiments were performed in the absence of Ca^{2+}/CaM and in the presence of either 3 μM forskolin or 1 mM Mn^{2+} (Fig. 6). Again, ISO was unable to stimulate adenylate cyclase activity, the presence of either of these activators. Thus, activation of adenylate cyclase by Ca^{2+}/CaM, Mn^{2+}, or forskolin precludes further neurotransmitter-mediated stimulation of enzyme activity.

Nonhydrolyzable guanine nucleotide analogs [e.g., Gpp(NH)p] are often used as monitors of the interaction between N proteins and the catalytic unit of adenylate cyclase. Therefore, it was of interest to determine whether the stimulatory and inhibitory actions of Gpp(NH)p on adenylate cyclase activity were regulated by CaM in a fashion similar to that of ISO and PIA. Gpp(NH)p dose response experiments were performed on EGTA-washed membranes in the presence and absence of Ca^{2+}/CaM (Fig. 7). In the absence of Ca^{2+}/CaM, Gpp(NH)p stimulated adenylate cyclase activity at concentrations above 0.1 μM. Inhibition of activity was not observed at any concentration tested. Conversely, in the presence of Ca^{2+}/CaM, inhibition was observed between 0.01 and 1 μM Gpp(NH)p, with a return to basal activity at 10 μM. Stimulation was not observed at any concentration tested. Thus, the regulation of Gpp(NH)p-mediated inhibition and stimulation of adenylate cyclase activity by CaM appeared to be identical to CaM regulation of neurotransmitter-mediated stimulation and inhibition. To confirm this observation, a detailed Ca^{2+} dose response experiment was performed in the presence or absence of 0.3 μM Gpp(NH)p (Fig. 8). At Ca^{2+} concentrations below 0.4 μM, Gpp(NH)p significantly stimulated adenylate cyclase activity, whereas at concentrations above 0.4 μM Ca^{2+}, Gpp(NH)p significantly inhibited activity. Thus, in the absence of Ca^{2+}/CaM-mediated stimulation, Gpp(NH)p only stimulated activity, whereas in the presence of Ca^{2+}/CaM-mediated stimulation, the same concentration of Gpp(NH)p was only able to inhibit adenylate cyclase activity.

Discussion

Elucidation of the regulatory significance of the stimulation of adenylate cyclase by Ca^{2+}/CaM has been impeded by the lack of anti-CaM agents which are effective in whole cells or

plasma membrane preparations. Repeated washing of membranes with a buffer containing EGTA effectively eliminates any contribution to basal adenylate cyclase activity made by endogenous Ca^{2+} and CaM. Addition of exogenous Ca^{2+} and CaM to these membranes, as adapted in the present study, allows the control of Ca^{2+}/CaM concentrations and the degree of stimulation of adenylate cyclase activity that is produced by Ca^{2+}/CaM. This protocol effectively "reconstitutes" Ca^{2+}/CaM-dependent adenylate cyclase activity.

The present studies were undertaken to determine whether Ca^{2}/CaM regulation of adenylate cyclase had implications for the physiological regulation of the enzyme's activity. Evidence that Ca^{2+}/CaM regulates adenylate cyclase *in vivo* can be found in studies utilizing intact PC12 and GH_3 cells (37, 38) or primary cultures of pituitary cells (39). Additionally, Ca^{2+}/CaM-dependent stimulation of adenylate cyclase may play a role in the enhanced stimulation of adenylate cyclase produced by neurotransmitters in the presence of Ca^{2+}-mobilizing hormones (e.g., α_1-adrenergic potentiation of β-adrenergic stimulation of adenylate cyclase activity in brain slices) (40–42). Previous work from this laboratory (24, 25) and others (28) has demonstrated that Gpp(NH)p-mediated inhibition of adenylate cyclase is strictly dependent upon Ca^{2+}/CaM in rat hippocampus and cortex, and bovine cerebellum, respectively. However, recently it has become clear that guanine nucleotide-binding proteins probably regulate not only adenylate cyclase (i.e., via N_i and N_s) but also phospholipase C (29, 30) and ion channels (31). Thus, nonhydrolyzable guanine nucleotide analogs, such as Gpp(NH)p, may access several different types of regulatory proteins within a plasma membrane preparation. Therefore, it was important to demonstrate that receptor-mediated inhibition and stimulation of adenylate cyclase activity could also be regulated by Ca^{2+}/CaM. In the present study, inhibition of the adenylate cyclase activity of cerebellar membranes by both PIA and Gpp(NH)p was observed in the presence of CaM and low μM concentrations of Ca^{2+}. The maximum inhibition produced by PIA was 25–30% of CaM-stimulated activity. Although this inhibition is relatively modest, it is in agreement with most published studies of neurotransmitter-mediated inhibition of neuronal adenylate cyclase from this and other laboratories (e.g., Refs. 32 and 43–46) and hopefully does relate to the physiological role played by such receptor systems. Although PIA also produced inhibition in the presence of Mn^{2+} and forskolin, this discussion will focus on the implications of the regulation by Ca^{2+}/CaM, since the former two activators are pharmacological agents which are mainly of experimental utility, whereas calmodulin is present in whole cells and its role is of physiological relevance. The data suggest that under conditions in which Ca^{2+}/CaM does not stimulate adenylate cyclase, inhibitory neurotransmitters have little effect upon adenylate cyclase activity. For example, the free Ca^{2+} concentration within a quiescent neuron has been reported to be approximately 100 nM (47), and Ca^{2+}/CaM-dependent stimulation of adenylate cyclase activity would not be expected to occur at this concentration of free Ca^{2+} (see Fig. 1b). However, subsequent to occupation of a receptor linked to IP_3 production, with a concomitant increase in Ca^{2+} concentration (to approximately 0.8–1 μM) (48), Ca^{2+}/CaM-dependent stimulation of adenylate cyclase activity (up to 2.5-fold in cerebellar tissue) could occur. This stimulation by Ca^{2+} would permit the occupation of an inhibitory receptor to be effectively coupled to the inhibition of adenylate cyclase activity. Alternatively, depolarization of a neuron and a subsequent increase in intracellular free Ca^{2+} concentration could also presumably result in Ca^{2+}/CaM-dependent stimulation of adenylate cyclase activity, permitting neurotransmitter-mediated inhibition of adenylate cyclase to proceed.

With respect to stimulation of adenylate cyclase, again, only a relatively small increment of stimulation was produced by ISO (approximately 20% above basal). Plasma membrane preparations derived from a mammalian neuronal tissue are notoriously resistant to neurotransmitter-mediated stimulation of adenylate cyclase activity, with maximal stimulation of adenylate cyclase activity being approximately 50% above basal (e.g., Ref. 23). This degree of stimulation is generally obtained at higher temperatures, higher substrate (ATP) concentrations, and higher protein concentrations than those used in the present study. Thus, the stimulation produced by ISO in the present study is within the range of expected values. ISO- and Gpp(NH)p-mediated stimulation of adenylate cyclase activity occurred only in the absence of Ca^{2+}/CaM-dependent stimulation of enzyme activity. Thus, neurotransmitter-mediated stimulation of adenylate cyclase activity might only occur in a resting neuron, whose intracellular Ca^{2+} concentration is relatively low. However, in a depolarized cell, or following occupation of an IP_3-linked receptor, neurotransmitter-mediated stimulation of adenylate cyclase activity would be precluded due to stimulation of enzyme activity by Ca^{2+}/CaM.

The present demonstration that Ca^{2+}/CaM precludes ISO-mediated stimulation of rat cerebellar adenylate cyclase activity is not totally in agreement with a previous report of synergism between CaM- and ISO-mediated stimulation of bovine cerebellar enzyme activity (27). However, in the latter study, the degree of stimulation produced by 10 μM ISO (in the presence of 10 μM GTP) was actually higher in the absence than in the presence of Ca^{2+}/CaM. It is well established that dopamine-mediated stimulation of rat or bovine striatal adenylate cyclase activity is potentiated by Ca^{2+}/CaM (22, 23). However, the striatum differs from other brain regions with respect to the role of CaM in the inhibition of adenylate cyclase activity (32) and the same may be true of stimulation.

In summary, the permissive and nonpermissive effects of Ca^{2+}/CaM-dependent stimulation of adenylate cyclase upon neurotransmitter-mediated inhibition and stimulation, respectively, provide an attractive mechanism whereby neurotransmitter-mediated inhibition of adenylate cyclase might only occur in the presence of increased free Ca^{2+} ions (via production of IP_3 or depolarization), while neurotransmitter-mediated stimulation of enzyme activity might only occur in a quiescent neuron at low concentration of intracellular free calcium. In an *in vivo* situation, this could provide a precise feedback mechanism whereby neurotransmitter-mediated stimulation of adenylate cyclase activity would occur only in a resting cell, while inhibition would occur only in an activated neuron.

Acknowledgments

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