CALMODULIN STIMULATES ADENYLATE CYCLASE ACTIVITY AND INCREASES DOPAMINE ACTIVATION IN BOVINE RETINA¹

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Abstract

Adenylate cyclase activity in bovine retina is highly responsive to Ca^{2+} and the endogenous Ca^{2+} -binding protein, calmodulin (CaM). CaM stimulated adenylate cyclase activity in washed particulate fractions of bovine retina by 6.6-fold in a Ca^{2+} -dependent manner. Activation of adenylate cyclase activity by CaM was maximal at 0.12 μM free Ca^{2+} . The apparent K_a for calmodulin stimulation of adenylate cyclase was 67 nM and the apparent $V_{\max}$ was 116 pmol/min/mg of protein above basal activity. Adenylate cyclase activity in bovine retina was stimulated approximately 50% by guanosine 5'-triphosphate (GTP), but the nonhydrolyzable GTP analogue, guanosine-5'-(β,γ -imido)triphosphate (Gpp(NH)p), was able to activate the enzyme nearly 5-fold. CaM and Gpp(NH)p appeared to be partially competitive activators of adenylate cyclase in the retina particulate fraction. Dopamine stimulated adenylate cyclase activity in the presence of GTP with an apparent K_a of 1.0 μM and an apparent $V_{\max}$ of 66 pmol/min/mg of protein. Ca^{2+} and CaM increased the apparent $V_{\max}$ of the dopamine-stimulated adenylate cyclase activity more than 2-fold to a level of 146 pmol/min/mg of protein but did not alter the apparent K_a . This suggests that CaM is an endogenous modulator of dopamine-stimulated adenylate cyclase activity in the retina. CaM-stimulated adenylate cyclase activity may be a common component to retina since we found this activity in retinas from rabbit, rat, and goldfish as well as cow.

It is well established that Ca^{2+} and cAMP are interrelated second messengers for many hormones and neurotransmitters (Rasmussen and Goodman, 1977). In a variety of tissues, Ca^{2+} increases cAMP synthesis and/or degradation by binding to the endogenous Ca^{2+} -binding protein, calmodulin (CaM), and increasing adenylate cyclase or phosphodiesterase activities (Cheung, 1980). Ca^{2+} - and CaM-stimulated adenylate cyclase activity is located primarily in brain (Cheung, 1980) but has also been reported in secretory tissues such as the pituitary (Brostrom et al., 1982) and pancreatic islets (Valverde et al., 1979). Two components of adenylate cyclase activity have been identified in areas of the brain such as striatum (Treisman et al., 1983) and cerebral cortex (Brostrom et al., 1977). One component can be activated by submicromolar concentrations of free Ca^{2+} and CaM, whereas the other component is insensitive to CaM and inhibited by concentrations of free Ca^{2+} greater than 1 μM . There is significant evidence suggesting a role for CaM in modulating dopaminergic activity in rat striatum (Gnegy, 1982). In this tissue, CaM not only can stimulate basal adenylate cyclase activity but also can increase the sen-

sitivity of adenylate cyclase for dopamine (DA) by 3- to 4-fold (Gnegy and Treisman, 1981). Similarly, other studies have demonstrated a relationship between CaM content and DA-stimulated adenylate cyclase activity in striatum and dopaminergic hyper- and hyporesponsive states (Hanbauer et al., 1980; Gnegy, 1982).

In an effort to explore further a relationship between CaM- and DA-stimulated adenylate cyclase we have investigated these activities in bovine retina. Retina is a neural tissue with relatively few cell types. Neural retina contains a high concentration of DA that is located primarily in certain groups of amacrine cells or interplexiform cells, depending on the species (Lolley, 1980). DA-stimulated adenylate cyclase activity has been measured in homogenate (Watling and Dowling, 1981) or membrane fractions (Clement-Cormier and Redburn, 1978; Redburn et al., 1980) as well as intact retina (Brown and Makman, 1972) of several species. The postsynaptic DA receptors in the retina are predominantly coupled to a stimulation of adenylate cyclase activity (D-1 receptors) (Schorderet, 1977; Watling et al., 1979). Recently, however, the presence of D-2 receptor-binding sites has been identified in mammalian retina (Makman et al., 1980; Watling and Iversen, 1981). The presence of a Ca^{2+} -dependent adenylate cyclase activity has been demonstrated indirectly in mammalian retina (DeVries et al., 1982; Ferrendelli et al., 1982). In these studies it was shown that the chelating agent EGTA can inhibit basal adenylate cyclase activity in retina, suggesting the presence of a Ca^{2+} -activated adenylate cyclase activity. We have examined the stimulation

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of adenylate cyclase activity in bovine retina by Ca^{2+} and CaM, as well as by guanyl nucleotides and DA. Guanyl nucleotides bind to a guanyl nucleotide-binding protein (N) in membranes which then acts as the transducer for receptor-adenylate cyclase-coupled systems (Rodbell, 1980). Guanyl nucleotides are required for DA stimulation of adenylate cyclase activity (Gnegy and Treisman, 1981). Interactions between guanyl nucleotides and CaM have been reported in both striatum and cerebral cortex (Brostrom et al., 1978; Treisman et al., 1983), although evidence suggests that CaM acts at the catalytic subunit (C) of adenylate cyclase (Salter et al., 1981). An effect of CaM on DA-stimulated adenylate cyclase could occur through an interaction of CaM and guanyl nucleotides; therefore, we studied the activation of adenylate cyclase in the presence of both the guanyl nucleotides and CaM.

We found that adenylate cyclase activity in bovine retina is highly responsive to CaM; CaM can stimulate adenylate cyclase activity at least 6- to 7-fold in a Ca^{2+} -dependent manner. Many characteristics of the CaM-sensitive adenylate cyclase activity in bovine retina are very similar to those in bovine and rat striatum and cerebral cortex. Our data further demonstrate that CaM was able to increase the maximal velocity of DA-stimulated adenylate cyclase activity more than 2-fold, suggesting that CaM is an endogenous modulator of DA-stimulated adenylate cyclase activity in retina.

Materials and Methods

Particulate preparation. Bovine eyes were obtained from the local slaughterhouse and retinas were removed within 15 min of the animals' deaths. Retinas were dissected free from the sclera at 4°C, as described by Watling and Iversen (1981), taking care to tease the retina from the pigment epithelium. Retinal 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, retinas were homogenized in 9 vol of 10 mM Tris maleate buffer, pH 7.5, containing 1 mM MgSO_4 and 1.2 mM EGTA. The homogenate fraction was centrifuged at $27,000 \times g$ for 20 min, resuspended in the same buffer, and centrifuged a second time at $27,000 \times g$. 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 (200- μl volume) containing: 80 mM Tris maleate buffer, pH 7.5, 5 mM MgSO_4 , 2 mM cAMP, 4 mM phosphoenolpyruvate, 20 μg of pyruvate kinase, 0.12 mM isobutylmethylxanthine, 100 to 150 μg of particulate membrane protein, 0.15 mM EGTA, and 1 mM [α - ^{32}P]ATP (1 μCi /assay), with or without additions such as guanosine-5'-(β , γ -imido)triphosphate (Gpp(NH)p), guanosine 5'-triphosphate (GTP), CaCl_2 , and CaM. Assays were incubated for 10 min and the reaction was stopped by heating for 1 min at 95°C. A solution (200 μl) containing 20 mM ATP and 0.7 mM [^3H]cAMP was then added to the tubes. The particulate material was centrifuged and the ^{32}P -labeled cAMP in the supernatant fraction was determined by the method of Krishna et al. (1968). Recovery of the cAMP was measured using the [^3H]cAMP and was usually 80 to 90%. 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). 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, TX). Kinetic constants were determined by the method of Wilkinson (1961).

CaM preparation. CaM was purified from bovine brain by the method of Dedman et al. (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 $(\text{NH}_4)\text{HCO}_3$, and lyophilized. The CaM was redissolved in 10 mM Tris maleate buffer, pH 7.5. The protein concentration was determined by UV absorption (Dedman et al., 1977) and the method of Lowry et al. (1951). The molecular weight determined by sodium dodecyl sulfate slab gel electrophoresis using standards of known molecular weight was 16,700. This mass was used to calculate the concentrations of CaM reported in this study.

Determination of CaM content. CaM was assayed by its ability to stimulate CaM-deficient phosphodiesterase activity in the phosphodiesterase assay described previously (Gnegy et al., 1977). The CaM content (in nanograms) was determined from a standard curve using highly purified CaM.

Materials. Bovine eyes were obtained fresh from a local slaughterhouse. [α - ^{32}P]ATP (specific activity, 38 Ci/mmol) was purchased from Amersham Searle Corp. (Arlington Heights, IL). cAMP, phosphoenolpyruvate, ATP, and 3-hydroxytyramine hydrochloride (dopamine, DA) were obtained from Sigma Chemical Co. (St. Louis, MO); pyruvate kinase was from Boehringer Mannheim (Indianapolis, IN). GTP and Gpp(NH)p (high pressure liquid chromatography purified) were purchased from International Chemical and Nuclear Corp. (Irvine, CA).

Results

CaM content in retinal particulate and cytosolic fractions. The CaM content as measured by phosphodiesterase activation in the cytosolic and particulate fractions prepared from bovine retina is shown in Table I. Bovine retina contained CaM in both fractions in concentrations comparable to that found in brain (Gnegy et al., 1977; Gnegy and Treisman, 1981). The data demonstrate that CaM was nearly twice as concentrated in the $27,000 \times g$ cytosolic fraction (11.4 ng of CaM/ μg of protein) as in the particulate fraction (5.9 ng of CaM/ μg of protein). Upon consideration of the total CaM content in the fractions it can be seen that CaM was equally distributed between the cytosolic and particulate fractions, which is virtually the same distribution that is found in brain (Gnegy et al., 1977).

Stimulation of retinal adenylate cyclase activity by Ca^{2+} and CaM. Particulate fractions washed with EGTA from whole bovine retina were used in all subsequent experiments. Depletion of endogenous Ca^{2+} and CaM from membranes is required in order to observe the effects of directly restoring CaM. We found that 60 to 70% of the endogenous CaM was removed by the washing procedure. The ability of CaM to stimulate adenylate cyclase in the retinal particulate fractions depleted of Ca^{2+} and CaM was determined. The activation of bovine retinal adenylate cyclase by CaM as a function of free Ca^{2+} is shown in Figure 1. Basal adenylate cyclase activity was 26 ± 3 (SEM) pmol/min/mg of protein. The adenylate cyclase activity was highly responsive to CaM and dependent upon Ca^{2+} . Ca^{2+} itself could stimulate adenylate cyclase activity in the washed particulate fraction 2.0-fold to a level of 50 pmol/min/mg of protein. However, in the presence of 280 nM CaM, a 6.6-fold stimulation was attained, reaching a maximum of 145 pmol of cAMP formed/min/mg of protein. The concentration of free Ca^{2+} at which CaM could maximally stimulate adenylate cyclase activity was 0.12 μM . The stimulation of the enzyme by CaM did not increase beyond this Ca^{2+} concentration and was inhibited by 30% with increasing concentrations of free Ca^{2+} . The basal

TABLE I

CaM content in fractions from bovine retina

Retina was homogenized in 9 vol of 10 mM Tris maleate buffer, pH 7.5, containing 1 mM MgSO_4 . Particulate fractions were prepared from homogenate fractions as described under "Materials and Methods" using the above buffer. CaM content was determined as described under "Materials and Methods." Results are given as values $\pm$ SEM for three separate experiments.

Fraction	CaM Content	
	Concentration	Total CaM
	$\mu\text{g}/\text{mg}$ of membrane protein	mg/gm of tissue wet weight
Homogenate	5.5 ± 0.6	427 ± 32
Particulate	5.9 ± 1.0	210 ± 22
Soluble	11.4 ± 1.5	243 ± 30

Figure 1. Effects of calcium and CaM on basal adenylate cyclase activity in bovine retinal particulate fractions. Adenylate cyclase assays were performed in an EGTA-washed retinal particulate fraction as described under "Materials and Methods" with (▲) and without (●) the addition of 280 nM highly purified CaM. The concentration of free Ca^{2+} was determined as described under "Materials and Methods." Each point is the average of duplicate determinations from two different experiments that did not vary by more than 5%.

adenylate cyclase activity, however, was unaffected by increasing free Ca^{2+} to a level of 200 μM , a concentration that greatly inhibits adenylate cyclase activity in striatum (Gnegy and Treisman, 1981).

The concentration dependence for CaM in activation of bovine retina adenylate cyclase is illustrated in Figure 2. Activation of the cyclase is maximal at 560 nM CaM, at a level of 150 pmol/min/mg of protein. The apparent K_a for CaM activation was 64 ± 7 nM ($N = 3$). The apparent V_{max} for the stimulation of adenylate cyclase by CaM above basal activity was 116 ± 8 pmol/min/mg of protein ($N = 3$).

The occurrence of CaM-stimulated adenylate cyclase activity was not unique to bovine retina. We found that a maximal concentration of 1.4 μM CaM, in the presence of 0.12 μM free Ca^{2+} , could stimulate adenylate cyclase activity in several species to the following degrees: rat, 5.2-fold; rabbit, 3-fold; and goldfish, 6-fold.

Effect of guanyl nucleotides on adenylate cyclase activity in the bovine retina particulate preparation. GTP stimulates basal adenylate cyclase activity in many tissues and is required for hormone and neurotransmitter activation of the enzyme through its interaction with the GTP-binding protein, N (Rodbell, 1980). We examined the ability of GTP and its nonhydrolyzable analogue, Gpp(NH)p, to activate adenylate cyclase activity in particulate preparations of bovine retina. As the data in Table II demonstrate, GTP was not a potent activator of retinal adenylate cyclase activity. GTP stimulated basal adenylate cyclase activity maximally by only 53%. Stimulation was no greater at 100 μM GTP than at 10 μM GTP. CaM and Ca^{2+} did not significantly alter the response to GTP; 10 μM GTP was required to measure significant activation by GTP in the presence of Ca^{2+} and CaM. The activation by the two agents did not appear to be interactive, suggesting that they stimulate adenylate cyclase activity independently.

In contrast to the weak stimulation afforded by GTP, the nonhydrolyzable analogue of GTP, Gpp(NH)p, stimulated adenylate cyclase activity maximally by 4.8-fold (Fig. 3). The Gpp(NH)p activation curve was biphasic; maximal activation occurred at 100 μM Gpp(NH)p but decreased at 1 mM. Kinetic analysis of the activation portion of the curve revealed an

apparent K_a for Gpp(NH)p of 3.5 ± 0.9 μM and an apparent V_{max} of 117 ± 5 pmol/min/mg of protein for cAMP formed above basal activity ($N = 4$). Adenylate cyclase activity in the presence of CaM and Gpp(NH)p was not purely additive at every concentration of Gpp(NH)p (Fig. 3). Activation by 280 nM CaM was greatest at concentrations of Gpp(NH)p from 0 to 1 μM ; at these concentrations CaM stimulated adenylate cyclase activity by approximately 114 to 96 pmol/min/mg of protein above basal levels. At 100 μM Gpp(NH)p, however, the stimulation produced by CaM above that of Gpp(NH)p was 70 pmol/min/mg of protein. Similarly, CaM appeared to decrease the extent of stimulation of adenylate cyclase activity by Gpp(NH)p. In the presence of 280 nM CaM, there was no significant activation by Gpp(NH)p below 100 μM Gpp(NH)p in contrast to the significant stimulation by 1 to 10 μM Gpp(NH)p apparent in the absence of CaM. The amount of activation by 100 μM Gpp(NH)p was reduced in the presence of CaM from 114 to 78 pmol/min/mg of protein for cAMP above basal levels. The results suggest that, although both CaM and Gpp(NH)p can activate retinal adenylate cyclase activity, they appear to have a partially competitive rather than a strictly additive interaction.

DA-stimulated adenylate cyclase activity in bovine retina. The effect of Ca^{2+} and CaM on DA-stimulated adenylate cyclase

Figure 2. Activation of bovine retina adenylate cyclase by CaM. Adenylate cyclase activity was assayed in an EGTA-washed particulate fraction as described under "Materials and Methods." All assays with CaM contained 0.12 μM free Ca^{2+} (100 μM added CaCl_2). Each point is the average of four separate experiments $\pm$ SEM.

TABLE II
Stimulation of bovine retina adenylate cyclase activity by GTP and CaM

GTP μM	Adenylate Cyclase Activity	
	-CaM	+CaM ^a
	pmol/min/mg of protein ^b	
0	34 ± 5	108 ± 5
0.1	40 ± 4	100 ± 4
1.0	42 ± 3	111 ± 7
10.0	52 ± 6^c	127 ± 3^c

^a The assay contained 280 nM CaM and 0.12 μM free Ca^{2+} (100 μM added CaCl_2).

^b Results are given as picomoles per minute per milligram of protein $\pm$ SEM for four separate experiments.

^c $p < 0.05$ as compared to value for no GTP within the respective group.

Figure 3. Activation of bovine retina adenylate cyclase by Gpp(NH)p in the absence and presence of Ca^{2+} and CaM. A bovine retina particulate fraction was prepared and adenylate cyclase was assayed as described under "Materials and Methods." The concentration dependence to Gpp(NH)p was measured in the absence (●) and presence (▲) of $0.12 \mu\text{M}$ Ca^{2+} ($100 \mu\text{M}$ CaCl_2 added in the assay) and 280 nM CaM. The results are the average of four separate experiments $\pm$ SEM.

activity in the bovine retina particulate fraction was examined. In the EGTA-washed retinal particulate preparations, GTP was required for stimulation of adenylate cyclase activity by DA. The stimulation of adenylate cyclase activity by various concentrations of DA in the retinal particulate fractions in the absence and presence of CaM is shown in Figure 4. Activation of adenylate cyclase in the presence of $1 \mu\text{M}$ GTP was maximal at $100 \mu\text{M}$ DA. The activation of adenylate cyclase in the presence of GTP, DA, and 280 nM CaM was greater than additive (Fig. 4A). Thus, DA was able to stimulate the production of more cAMP in the presence, as opposed to the absence, of Ca^{2+} and CaM. This is shown more clearly by the data in Figure 4B, which depict the DA-stimulated adenylate cyclase activity. DA-stimulated adenylate cyclase activity was defined as the picomoles of cAMP per minute per milligram of protein formed above the activity in the presence of GTP alone. Kinetic analysis revealed that CaM significantly increased the maximal velocity of the DA-stimulated adenylate cyclase activity. In the absence of Ca^{2+} and CaM, the apparent K_a for DA was $1.0 \pm 0.3 \mu\text{M}$ and the apparent V_{max} was $66 \pm 4 \text{ pmol/min/mg}$ of protein ($N = 4$). The apparent K_a for DA was not significantly affected by addition of Ca^{2+} and CaM and was $1.3 \pm 0.4 \mu\text{M}$ ($N = 4$). However, the apparent V_{max} of the DA-stimulated adenylate cyclase activity was significantly increased more than 2-fold by CaM to $146 \pm 15 \text{ pmol/min/mg}$ of protein ($p < 0.01$, as compared to the value obtained in the absence of CaM).

Discussion

The highest concentration of CaM- and Ca^{2+} -stimulated adenylate cyclase activity occurs in brain, although CaM has

Figure 4. A, Activation of retinal adenylate cyclase by DA in the absence and presence of Ca^{2+} and CaM. Adenylate cyclase activity was measured in a washed particulate fraction as described under "Materials and Methods." Each assay contained $1 \mu\text{M}$ GTP and various concentrations of DA in the absence (●) and presence (▲) of $0.12 \mu\text{M}$ free Ca^{2+} ($100 \mu\text{M}$ CaCl_2) and 280 nM CaM. For adenylate cyclase, activity in the presence of $1 \mu\text{M}$ GTP and GTP + Ca^{2+} and CaM was 42 ± 3 and $122 \pm 13 \text{ pmol/min/mg}$ of protein, respectively. Each point is the average of four separate experiments $\pm$ SEM. B, Effect of Ca^{2+} and CaM on DA-stimulated adenylate cyclase activity in rat particulate fractions. DA-stimulated adenylate cyclase activity was defined as the picomoles of cAMP per minute per milligram of protein formed above the activity in the presence of GTP alone (●) or GTP plus Ca^{2+} and CaM (▲). The DA-stimulated adenylate cyclase activity was calculated from the data presented in A. Each point is the average of four separate experiments $\pm$ SEM.

been reported to stimulate adenylate cyclase activity in several peripheral secretory tissues such as the pituitary and pancreatic islets (Brostrom et al., 1982; Valverde et al., 1979). In this report, we have demonstrated CaM-stimulated adenylate cyclase activity in another neural tissue, the retina. Our results demonstrate that adenylate cyclase activity in bovine retina is highly sensitive to CaM. In the presence of Ca^{2+} , CaM could stimulate the enzyme 6.6-fold. The enzyme was significantly stimulated by DA and Gpp(NH)p, whereas GTP was a weak activator. Furthermore, CaM affected the activation of adenylate cyclase by both Gpp(NH)p and DA, demonstrating an interaction with these agents. CaM-stimulated adenylate cyclase activity is likely to exist in the retina of many species since we were able to measure 3- to 7-fold stimulation by CaM in bovine, rat, rabbit, and goldfish retina.

The characteristics of the activation of bovine retina adenylate cyclase by CaM are similar to those we reported previously in rat striatum (Gnegy and Treisman, 1981), although there appears to be more CaM-sensitive adenylate cyclase activity in retina as compared to CaM-insensitive adenylate cyclase activity. The stimulation by CaM was totally dependent upon the presence of Ca^{2+} . Maximal activation by CaM was attained at $0.12 \mu\text{M}$ free Ca^{2+} , which is the maximally effective concentration of Ca^{2+} for CaM activation found in rat striatum (Gnegy and Treisman, 1981) and rat cerebral cortex (Brostrom et al., 1977). Neither the basal adenylate cyclase activity nor the activation by CaM was strongly inhibited by high free Ca^{2+} in the retina, unlike the CaM activation in brain. In bovine retina, free Ca^{2+} itself was able to activate adenylate cyclase 2-fold. This may reflect either an activation by endogenous CaM that could not be depleted from the membranes or a separate action of Ca^{2+} in stimulation of adenylate cyclase. The kinetic constants obtained for CaM-stimulated adenylate cyclase activity in bovine retina were very similar to those obtained in rat striatum. The apparent K_s for CaM in activation of adenylate cyclase in retina and striatum are 67 and 70 nM (Gnegy and Treisman, 1981), respectively. The apparent V_{max} s are also similar in retina and striatum, 116 and 110 pmol/min/mg of protein, respectively (Gnegy and Treisman, 1981), despite the fact that CaM stimulates adenylate cyclase 6.6-fold in the retina and 2-fold in the striatum. Therefore, CaM-stimulated adenylate cyclase in retina has many characteristics similar to those in brain.

Our data demonstrated that CaM and DA could synergistically activate adenylate cyclase activity in retina. In the retinal particulate fractions, CaM increased the apparent V_{max} for DA-stimulated adenylate cyclase activity more than 2-fold but did not affect the apparent K_a for DA. On the other hand, in rat striatum CaM could increase the affinity of adenylate cyclase for DA 3- to 4-fold but did not alter the apparent V_{max} for the reaction (Gnegy and Treisman, 1981). Therefore, the interaction of CaM with the components involved in DA-stimulated adenylate cyclase activity may be substantially different in retina from that in striatum. The molecular mechanism by which CaM affects basal or hormone-sensitive adenylate cyclase activity is unknown. Evidence suggests that CaM acts at the catalytic subunit in brain and does not require GTP for its activation (Salter et al., 1981; Heideman et al., 1982). Our results suggest that CaM and DA affect the same catalytic activity. CaM may increase an interaction between GTP-occupied N and C proteins in the retina. It is also possible that CaM could bind to a factor inhibitory to DA stimulation. A synergistic activation of adenylate cyclase by CaM and β -adrenergic agonists in bovine cerebellum has been reported (Malnoë et al., 1983), suggesting that CaM can similarly modulate the activity of other neurotransmitters in neural tissue.

Adenylate cyclase activity in bovine retina was also stimulated by guanyl nucleotides. GTP, the endogenous ligand for

the guanyl nucleotide-binding protein, could only minimally activate the adenylate cyclase as compared to the nonhydrolyzable analogue, Gpp(NH)p. This could be because endogenous guanosine 5'-diphosphate (GDP) is very tightly bound to N protein and is unable to be displaced by GTP. DA is most likely stimulating a nucleotide exchange reaction between GDP and GTP at protein N (Cassel and Selinger, 1978). CaM did not appreciably affect the activation by GTP but did have a limited interaction with Gpp(NH)p. CaM and Gpp(NH)p appeared to be partially competitive activators of adenylate cyclase, since one could decrease the activity of the other. CaM could act at a site on protein C that is affected by Gpp(NH)p-occupied protein N. Another explanation for the fact that CaM blocks the adenylate cyclase activation by low concentrations of Gpp(NH)p is that CaM and Ca^{2+} could be activating another adenylate cyclase system responsive to an inhibitory N protein (N_i) (Rodbell, 1980). Girardot et al. (1983) have shown that Ca^{2+} and CaM are required for inhibition of adenylate cyclase activity by guanyl nucleotides and hormones in rat hippocampal membranes. Thus, CaM may be able to affect both activation and inhibition of adenylate cyclase by hormones and guanyl nucleotides in neural tissue.

We do not know, of course, whether the CaM and DA-stimulated adenylate cyclase activities are in the same cell types in the retina. DA is located primarily in a certain type of amacrine cell in the inner nuclear layer of the retina or in interplexiform cells in species such as the teleost fish (Lolley, 1980). DeVries et al. (1982) have shown that adenylate cyclase is located primarily in the inner plexiform and photoreceptor cell layers in the rabbit and ground squirrel. The adenylate cyclase activity in the inner plexiform layer of the retina was inhibited by EGTA, suggesting that this layer contains a Ca^{2+} -activatable enzyme form. These investigators characterized the inner plexiform layer of rabbit and ground squirrel retina as containing an adenylate cyclase that is stimulated by Ca^{2+} , Gpp(NH)p, and DA. There were forms of adenylate cyclase in other layers which were not stimulated by Ca^{2+} ; indeed, EGTA stimulated these forms, suggesting that Ca^{2+} itself would be inhibitory.

In summary, we have identified a Ca^{2+} - and CaM-stimulated adenylate cyclase in bovine retina as well as retina from other species. This adenylate cyclase may be identical to a DA-stimulated adenylate cyclase since CaM can directly increase the V_{max} for DA. Our studies with CaM and Gpp(NH)p further suggest an interaction between CaM and guanyl nucleotides in the activation of adenylate cyclase. The evidence suggests that the CaM-stimulated adenylate cyclase can be stimulated to some extent by Gpp(NH)p, and these agents may be partial competitive activators of the cyclase. Although CaM has also been shown to affect DA-stimulated adenylate cyclase activity in rat striatum, the molecular mechanism of this action may be different in retina. Preliminary studies in our laboratory further strengthen a relationship between DA-, Gpp(NH)p-, and CaM-stimulated adenylate cyclase activities in bovine retina by showing that these activities are similarly regulated by lighting conditions.

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