Mechanism of Activation of a Cyclic Adenosine 3':5'-Monophosphate Phosphodiesterase from Bovine Heart by Calcium Ions

IDENTIFICATION OF THE PROTEIN ACTIVATOR AS A Ca²⁺ BINDING PROTEIN

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SUMMARY

Bovine heart cyclic adenosine 3':5'-monophosphate (cAMP) phosphodiesterase requires a heat-stable protein activator for full enzymic activity. Enzyme preparations, largely freed of the protein activator, possess low enzyme activity which is independent of Ca²⁺. Addition of excess protein activator stimulates the enzyme activity 6- to 10-fold. This activation by the protein activator is shown to be completely dependent on the presence of low concentrations of Ca²⁺. The concentration of Ca²⁺ required to give 50% of the maximal activation is 2.3 μM. An equilibrium binding study has shown that ⁴⁰Ca binds to the protein activator. A Scatchard plot exhibits two linear regions suggesting the presence of two sets of Ca²⁺ binding sites on the protein with different affinities: one high affinity site and two low affinity sites per protein activator. The dissociation constants for Ca²⁺ bound at the high and low affinity sites are 3 and 12 μM, respectively. The results suggest that the complex of Ca²⁺ and the protein activator is the true activator for cAMP phosphodiesterase.

The understanding of regulatory properties of cAMP phosphodiesterase is fundamental to the delineation of how cAMP effects on various physiological and biochemical functions are controlled. Degradation of cAMP to 5'-AMP by this enzyme is the only well-established mechanism for the disposal of this cyclic nucleotide in mammalian cells. Sutherland and Rall (1) were the first to demonstrate this enzyme. Subsequently, Butcher and Sutherland (2) and Drummond and Perrot-Yee (3) partially purified this enzyme from bovine heart and rabbit brain, respectively. Recent studies have indicated that in any of the mammalian tissues examined, cAMP phosphodiesterase is present in multiple forms having different molecular weights and catalytic properties (4, 5). Of particular interest is the observation of Kakiuchi and Yamazaki (6) that a rat brain cAMP phosphodiesterase is inhibited by low concentrations of EGTA, thus indicating that it is a Ca²⁺-dependent enzyme. More recently, Miki and Yoshida (7) have shown that in the rat this Ca²⁺-dependent enzyme is restricted to the cerebrum. The present study shows that a Ca²⁺-dependent cAMP phosphodiesterase also exists in bovine heart.

Cheung (8), Goren and Rosen (9), and Kakiuchi et al. (10) have independently shown that a specific protein activator of cAMP phosphodiesterase is present in several mammalian tissues. Recently, a procedure has been developed to purify this protein activator from bovine heart to apparent homogeneity (11). In addition, an enzyme preparation largely freed of the protein activator has been obtained from bovine heart (11). The present study shows that the activation of this cAMP phosphodiesterase is completely dependent on the addition of the protein activator and Ca²⁺.

MATERIALS AND METHODS

Materials: Beef hearts were obtained fresh from a local slaughterhouse, Burns Food Ltd. of Winnipeg. The fresh hearts were cut into small pieces (approximately 1 cubic inch) and stored frozen at −20°C for 1 to 4 weeks prior to use. DEAE-cellulose (medium capacity) and Chelex 100 (minus 400 mesh) were obtained from Bio-Rad. Ammonium sulfate, ammonium molybdate, strontium chloride, and cupric sulfate were all A.C.S. certified grade obtained from Fisher. Naphthalene, 2,5-di-phenyloxazole (POPOP) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) were all "scintanalyzed" grade purchased from Fisher. Radiotracer ⁴⁰Ca was obtained from Amersham-Searle as the chloride with a specific activity of 0.037 mCi per μg of calcium. Cobalt chloride, barium chloride, nickelous sulfate, and ferrous sulfate were all A.C.S. certified grade obtained from Fisher. Magnesium acetate, manganese sulfate, and zinc chloride were Analar certified grade purchased from British Drug House. Sephadex G-100, G-50, and G-25 were the products of Pharmacia. Cyclic AMP, 5'-nucleotidase (Sigma Grade II) purified from...
the venom of <i>Crotalus adamanteus</i>, Tris base (reagent grade), and ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid were obtained from Sigma. Ultrafiltration membranes PM-10 and UM-2 were the products of Amicon Corp.

**Preparation of Activator-deficient cAMP Phosphodiesterase—**

Frozen beef heart (500 g) was thawed, put through a meat mincer, and homogenized for 10 s at low speed in a Waring Blender with 2.5 volumes of 0.1 M Tris-HCl-2 mM EDTA, pH 7.5. The homogenate was centrifuged at 10,000 × g at 4°C for 20 min. The supernatant solution was titrated to pH 8.6 with 1 N NaOH, and powdered ammonium sulfate was added to give 50% saturation. This was immediately centrifuged at 10,000 × g at 4°C for 20 min.

The supernatant fluid was used for the preparation of the protein activator. The pellet was dissolved in a minimal volume of a solution containing 0.02 M Tris-HCl, 0.08 M NaCl, 1 mM Mg<sup>2+</sup>, and 1 mM imidazole, pH 7.5, and the solution was dialyzed overnight against the same buffer. The dialyzed enzyme was frozen and thawed and then centrifuged at 100,000 × g for 1 hour at 4°C. The supernatant solution was applied to a DEAE-cellulose column (4.5 × 30 cm) which had been equilibrated with a solution containing 0.02 M Tris-HCl, 0.08 M NaCl, 1 mM Mg<sup>2+</sup>, and 1 mM imidazole, pH 7.5. The column was washed with the same buffer until no more protein was eluted. A negligible amount of enzyme activity was eluted by this buffer. The column was then developed with the same buffer containing 0.22 mM NaCl. The eluted protein fractions which contained the activator-deficient cAMP phosphodiesterase were pooled and dialyzed against 0.02 M Tris-HCl containing 1 mM Mg<sup>2+</sup> and 1 mM imidazole, pH 7.5. The dialyzed sample was stored frozen at −20°C in small aliquots until use. The maximal activation of the enzyme by the protein activator varied from 6- to 10-fold depending on the preparation.

**Preparation of Protein Activator of cAMP Phosphodiesterase—**

The protein activator was prepared from the 50% ammonium sulfate supernatant by a procedure described previously (11). Since it was found that the purified samples sometimes contained a small amount of low molecular weight contaminant which could be removed by gel filtration on a Sephadex G-50 column, the protein activator preparations used for the Ca<sup>2+</sup> binding study were always subjected to filtration on a Sephadex G-50 column.

**Assay of cAMP Phosphodiesterase—**
The enzyme activity was measured by the method of Butcher and Sutherland (2) with a slight modification (12). This procedure involved the conversion of 5'-AMP, the product of the cAMP phosphodiesterase reaction, to adenosine and inorganic phosphate by 5'-nucleotidase. The 5'-nucleotidase reaction was carried out either concurrently with the phosphodiesterase reaction (a one-stage assay) or after the termination of the phosphodiesterase reaction by boiling (a two-stage assay). The reaction mixture, in a volume of 0.9 ml, contained, in addition to cAMP phosphodiesterase and the protein activator, 25 mM Tris, 25 mM imidazole, 3 mM magnesium acetate, 1.2 mM cAMP, and 0.2 unit of 5'-nucleotidase. In experiments (Figs. 3-5) in which it was necessary to control precisely the concentration of calcium, the cAMP phosphodiesterase activity was assayed by the two-stage method. 5'-Nucleotidase retained an appreciable amount of calcium even after deactivation by gel filtration with Sephadex G-25.

One unit of cAMP phosphodiesterase activity is equivalent to the amount of enzyme which, when maximally activated by both the protein activator and Ca<sup>2+</sup>, hydrolyzed 1 μ mole of cAMP per min at 30°C under standard conditions.

**Assay of Protein Activator of cAMP Phosphodiesterase—**
The protein activator was assayed by a previously described procedure (11) which involved the measurement of the extent of stimulation of a fixed amount of activator-deficient beef heart cAMP phosphodiesterase and then compared with a standard curve. Since it was found during this study that Ca<sup>2+</sup> could effect the activation of cAMP phosphodiesterase by the protein activator, 0.1 mM Ca<sup>2+</sup> was routinely included in the reaction mixture for activator assays. One unit of protein activator activity is defined as the amount which is required to give 50% stimulation of 0.012 unit of cAMP phosphodiesterase.

**Removal of Ca<sup>2+</sup> from Reagents—**Chelex 100, a resin specific for chelating divalent cations, was used for removing Ca<sup>2+</sup> from all of the stock solutions. The resin was washed once with 1 N HCl and then with 1 N NaOH prior to the packing of the column. The packed columns were then washed with double distilled water. Double distilled water, Tris-HCl (0.3 M), and imidazole (0.3 M) were separately treated for the removal of Ca<sup>2+</sup> by passage through Chelex 100 columns (20 × 3 cm). cAMP solutions (10.8 mM) were passed through a Chelex 100 column (6 × 1.5 cm) to remove Ca<sup>2+</sup>. Plastic columns and connections were used in the column chromatography. The purified reagents were always stored in plastic containers and all reactions were carried out in plastic vessels. A Perkin-Elmer atomic absorption spectrophotometer, model 303, was used to monitor the concentration of calcium in these stock reagents. The limit of detection of calcium by this instrument is 4 ppm. After Chelex 100 treatment, the calcium content of stock reagents was below this limit of detection.

Calcium was removed from the protein activator and cAMP phosphodiesterase by treatment with 0.5 mM EGTA for 20 min at 4°C; these materials were then desalted by gel filtration through Sephadex G-25 columns (30 × 1.5 cm). Chelex 100-treated water and buffer were used at all steps. Atomic absorption spectrophotometry showed that the Ca<sup>2+</sup> in both the enzyme and the protein activator as less than 4 ppm.

Magnesium acetate and cobalt chloride were essentially free of calcium contamination. Strontium chloride was contaminated with Ca<sup>2+</sup> to the extent of 0.002%. Since the highest concentration of Sr<sup>2+</sup> used was 0.22 mM, the resultant contribution of Ca<sup>2+</sup> was less than 10<sup>−4</sup> M.

**Binding of Ca<sup>2+</sup> by Purified Protein Activator of cAMP Phosphodiesterase—**
The gel filtration method of Hummel and Dreyer (13), as modified by Fairclough and Fruton (14), was used to determine the binding of Ca<sup>2+</sup> by the purified protein activator of cAMP phosphodiesterase. A column (26 × 0.9 cm) of Sephadex G-25 was equilibrated at 24°C with buffer containing 25 mM Tris-HCl, 25 mM imidazole, and 3 mM magnesium acetate with a known concentration of Ca<sup>2+</sup> plus 4Ca<sup>2+</sup>. The column used was a plastic Pharmacia preparative K9/30 column. Chelex 100-treated reagents were used throughout. Desalted protein activator, 80.4 μg in 0.4 ml, was used for each experiment. The gel filtration was carried out at 24°C at a flow rate of 3 ml per hour and 0.6 ml fractions were collected. Aliquots of each fraction were analyzed for radioactivity in duplicate in a Beckman LS-250 liquid scintillation spectrometer. The scintillator mixture was composed of 125 g of naphthalene, 7.5 g of 2,5-diphenyloxazole, and 0.375 g of 1,4-bis[2-(5-phenyloxazoyl)]-benzene per liter of dioxane.
RESULTS

**EGTA Inactivation of Bovine Heart cAMP Phosphodiesterase**—Kakuiuchi and Yamazaki (6) have found that low concentrations of EGTA inhibit rat brain cAMP phosphodiesterase. Fig. 1 shows that this chelating agent in the presence of excess Mg^{2+} also inactivates cAMP phosphodiesterase of crude extracts of bovine heart to a maximum of 67\% inhibition. If 30 μM Ca^{2+} is added to the enzyme assay mixture, much higher concentrations of EGTA are needed to inhibit the enzyme. This result suggests that a Ca^{2+}-dependent cAMP phosphodiesterase exists in bovine heart. In the absence of the chelating agent, however, 30 μM Ca^{2+} activates the enzyme by less than 10\% (Fig. 1).

![Graph](https://example.com/graph1.png)

**Fig. 1.** Inhibition by EGTA of cAMP phosphodiesterase in crude extract of beef heart. Dialyzed 10,000 × g supernatant solution of a homogenate of beef heart was used for this experiment. cAMP phosphodiesterase activity was measured by the one-stage assay. No Ca^{2+} added, ○; Ca^{2+} (30 μM) added, ●.

Presumably, this is because a sufficient amount of endogenous Ca^{2+} is present in the assay mixture.

Enzyme preparations, largely freed of the protein activator by chromatography on DEAE-cellulose columns, always possess low but significant enzyme activity. Fig. 2 shows that this low basal enzyme activity is not inhibited by EGTA in the presence of excess Mg^{2+} and is therefore independent of Ca^{2+}. When excess protein activator is added to these activator-deficient enzyme preparations, the enzyme activity is stimulated 6- to 10-fold. This activation by the protein activator is completely abolished by low concentrations of EGTA in the presence of excess Mg^{2+}. This indicates that the activation of bovine heart cAMP phosphodiesterase requires the simultaneous presence of protein activator and low concentrations of Ca^{2+}.

**Ca^{2+} Activation of cAMP Phosphodiesterase**—Although the preceding results suggest that there is a Ca^{2+}-dependent cAMP phosphodiesterase in bovine heart, the possibility that EGTA has a direct effect on cAMP phosphodiesterase or that a metal ion other than Ca^{2+} is responsible for the enzyme activation cannot be excluded. To establish unequivocally that Ca^{2+} activates the enzyme, removal of contaminating Ca^{2+} in the reaction mixture and direct demonstration of the Ca^{2+} activation are essential. Fig. 3 shows that when reagents and protein samples relatively free of the contaminating Ca^{2+} are used, activation of bovine heart cAMP phosphodiesterase by Ca^{2+} can be shown. The dependence of the enzyme activity upon Ca^{2+} concentration has been examined at different levels of the protein activator. In the absence of the protein activator, increasing the Ca^{2+} concentration to 0.2 mM results in little enzyme activation. At a saturating level of the protein activator, Ca^{2+} can bring about a 10-fold increase in the enzyme activity. At a lower level of the protein activator, the maximal Ca^{2+} activation of bovine heart cAMP phosphodiesterase is also lowered. In addition to the extent of activation, the Ca^{2+} concentration...
required to achieve 50% of the maximal activation, $A_{50\%}$, also depends on the amount of the protein activator in the enzyme assay. The $A_{50\%}$ values at 1.4 and 13 units of the protein activator are 3.6 and 2.3 $\mu$M, respectively.

The results presented above demonstrate that activation of cAMP phosphodiesterase by Ca$^{2+}$ is dependent upon the presence of the protein activator, and the data of Fig. 4 show that the activation of the enzyme by the protein activator is dependent upon Ca$^{2+}$. In the absence of Ca$^{2+}$, cAMP phosphodiesterase activity is not stimulated by the protein activator. At 100 $\mu$M Ca$^{2+}$, however, cAMP phosphodiesterase is activated by increasing concentrations of the protein activator to a maximal activation of 600%. Both the extent of the enzyme activation and the concentration of the protein activator required for 50% maximal activation are functions of Ca$^{2+}$ concentration. At a lower concentration of Ca$^{2+}$, the enzyme is activated by the protein activator to a smaller extent, and more protein activator is needed to achieve 50% maximal activation of the enzyme. Thus, results in Figs. 3 and 4 indicate that activation of cAMP phosphodiesterase is achieved only when both Ca$^{2+}$ and the protein activator are present. Furthermore, the two activators may enhance each other's efficiency in the enzyme activation.

**Activation of cAMP Phosphodiesterase by Other Metal Ions**

Since all enzyme assays in the present study were carried out in the presence of 3 mM Mg$^{2+}$, the demonstration of the Ca$^{2+}$ activation indicates that Mg$^{2+}$ does not substitute for Ca$^{2+}$ in the activation of cAMP phosphodiesterase. However, Mg$^{2+}$ is essential for the catalytic activity of cAMP phosphodiesterase since the enzyme is inactive in the presence of Ca$^{2+}$ alone. Thus, the enzyme depends on both Mg$^{2+}$ and Ca$^{2+}$ for its full activity. To further study the specificity of Ca$^{2+}$ activation of cAMP phosphodiesterase, the enzyme activity in the presence of 3 mM Mg$^{2+}$ and one of several divalent metal ions has been examined. Table I shows that Sr$^{2+}$, Co$^{2+}$, and Mn$^{2+}$ are the only metal ions which exhibit significant enzyme activation at a concentration of 30 $\mu$M. The magnitudes of enzyme activation by these metals are, however, much less than that by Ca$^{2+}$.

In order to determine whether the low levels of activation achieved by Sr$^{2+}$ and Ca$^{2+}$ are due to low affinities of these metal ions for the binding site or low maximal enzyme activations, cAMP phosphodiesterase activity in the presence of excess protein activator has been examined as a function of the metal ion concentration. Fig. 5 shows that the enzyme is maximally activated by Sr$^{2+}$ and Co$^{2+}$ to 900 and 300%, respectively. Under the same condition, maximal Ca$^{2+}$ activation of the enzyme is 1000%. Concentrations of Sr$^{2+}$ and Co$^{2+}$ required to provide 50% maximal activation are 36.3 and 19.2 $\mu$M, respectively; about 10 to 20 times higher than that of Ca$^{2+}$. Thus, Ca$^{2+}$ appears to be the most effective metal activator for cAMP phosphodiesterase.

**Interaction of Ca$^{2+}$ and Protein Activator**

One possible ex-

TABLE I  

| Cation added | Enzyme activity | Stimulation |
|--------------|----------------|-------------|
| None         | 0.00306        | 617         |
| Ca$^{2+}$    | 0.0147         | 180         |
| Sr$^{2+}$    | 0.00375        | 87          |
| Mn$^{2+}$    | 0.00333        | 18          |
| Co$^{2+}$    | 0.0036         | 76          |
| Zn$^{2+}$    | 0.00287        | 40          |
| Ni$^{2+}$    | 0.00243        | 18          |
| Cu$^{2+}$    | 0.00242        | 18          |
| Ba$^{2+}$    | 0.00227        | 11          |
| Fe$^{2+}$    | 0.00217        | 5           |

**Fig. 5.** Activation of cAMP phosphodiesterase by divalent cations in the presence of excess protein activator. The enzyme activity was measured in the presence of 13 units of Ca$^{2+}$-free protein activator under conditions as described in the legend for Fig. 3. Activation by Co$^{2+}$, O; activation by Sr$^{2+}$, A; activation by Ca$^{2+}$, •.
planning for the mutual dependence of Ca\(^{2+}\) and the protein activator in the activation of cAMP phosphodiesterase is that the two activators have to combine to form a metal protein complex to activate the enzyme. The possible formation of the Ca\(^{2+}\)-protein activator complex has been investigated by the equilibrium binding technique on a Sephadex G-25 gel filtration column (13). Fig. 6 shows the elution profile for a typical binding experiment. The appearance of \(^{45}\)Ca peak and troughs in the profile is indicative of the binding of Ca\(^{2+}\) to the protein activator. The radioactivity peak coincides exactly with the activity peak of the protein activator. In most experiments, double troughs have been observed in the elution profile, but the origin and significance of the double troughs are not clear. For the calculation of the amount of bound Ca\(^{2+}\), only the data at peak regions have been used.

In a preceding section, it has been shown that Sr\(^{2+}\) can replace Ca\(^{2+}\) in the activation of cAMP phosphodiesterase. This metal ion, therefore, is expected to compete for Ca\(^{2+}\) binding sites on the protein activator if Ca\(^{2+}\) binding is indeed involved in the enzymatic activation. As is shown in Fig. 6, binding of Ca\(^{2+}\) to the protein activator may be significantly reduced in the presence of 500 \(\mu\)M Sr\(^{2+}\). At a free Ca\(^{2+}\) concentration of 0.7 \(\mu\)M, the amount of Ca\(^{2+}\) bound per mole of protein is changed from 0.205 in the absence of Sr\(^{2+}\) to 0.092 mole in the presence of Sr\(^{2+}\). Although the result does not show that the two ions compete for the same binding site, it does agree with such an interpretation.

The stoichiometry of the interaction between Ca\(^{2+}\) and the protein activator and the dissociation constant for the complex have been determined from a Scatchard plot (Fig. 7). The Scatchard plot consists of two linear regions, thus having two different slopes. The result suggests that there are two types of Ca\(^{2+}\) binding sites on the protein activator having different affinities. From the slopes, the dissociation constants of Ca\(^{2+}\) for the high and low affinity sites are calculated to be 2.9 and 11.8 \(\mu\)M, respectively. Since kinetic studies indicate that Ca\(^{2+}\) concentration required for 50% enzyme activation at a saturating amount of the protein activator is 2.3 \(\mu\)M, it may be suggested that only the high affinity Ca\(^{2+}\) binding site is involved in the enzyme activation. The stoichiometry of the interaction between Ca\(^{2+}\) and the protein activator may be calculated from the intercepts on the horizontal axis of the Scatchard plot. Extrapolated lines for the high and low affinity sites intersect at 1.04 and 3.25 moles per mole of the protein activator, respectively. This indicates that there is one high affinity Ca\(^{2+}\) binding site and 2 to 3 low affinity Ca\(^{2+}\) binding sites per molecule of the activator.

**DISCUSSION**

Based on experiments carried out in EGTA-Ca\(^{2+}\) buffer, Kakiiuchi and his co-workers (6) have concluded that a Ca\(^{2+}\)-dependent cAMP phosphodiesterase is present in rat brain. In the present study, a similar enzyme is also found in bovine heart. Brostrom et al. (15), in their study of Ca\(^{2+}\) activation of phosphorylase kinase, have indicated the advisability of the use of Ca\(^{2+}\)-free reagents rather than EGTA-Ca\(^{2+}\) buffer to control the concentration of free Ca\(^{2+}\) in the study of Ca\(^{2+}\) effects on enzymes. This is because EGTA also chelates other metal ions, and the possibility of a direct effect of EGTA on the enzyme activity is difficult to rule out completely. In addition, Ca\(^{2+}\) concentrations in enzyme assays can be more accurately determined if Ca\(^{2+}\)-free reagents are used.

Kakiiuchi and his co-workers (16) observed that the protein activator caused an increase in the maximum Ca\(^{2+}\) activation of rat brain cAMP phosphodiesterase. Furthermore, in the presence of the protein activator, a significantly lower concentration of Ca\(^{2+}\) was needed to activate the rat brain cAMP phosphodiesterase (18). These observations are confirmed and extended in the present study with the bovine heart enzyme. The activation of the enzyme requires the simultaneous presence of both Ca\(^{2+}\) and the protein activator. This may be explained by postulating that the enzyme is activated by the complex of the protein activator and Ca\(^{2+}\). Such a postulate is supported by the observation that the purified activator binds Ca\(^{2+}\). It is significant that the observed dissociation constant for the Ca\(^{2+}\)
bound at the high affinity site of the protein activator is very similar to the kinetic constant of Ca\(^{2+}\) activation of the enzyme. Furthermore, Sr\(^{2+}\), which can substitute for Ca\(^{2+}\) in the enzyme activation, is shown to reduce the Ca\(^{2+}\) binding to the protein activator.

The fact that the functions and the metabolism of cAMP and Ca\(^{2+}\) are closely related has been pointed out in a review by Rasmussen et al. (17). In the heart, both Ca\(^{2+}\) and cAMP have been implicated in the hormone-stimulated myocardial contraction. Several groups of investigators (18-20) have suggested that cAMP may control the free Ca\(^{2+}\) level in cardiac muscle by facilitating the inflow of Ca\(^{2+}\) into the cell. Kirschberger et al. (21) have shown that the uptake of Ca\(^{2+}\) by cardiac microsomes may be stimulated by cAMP in the presence of cAMP-dependent protein kinase. These observations may suggest that the isotropic effect of cAMP results from its effect on Ca\(^{2+}\) metabolism. The present observation that cAMP phosphodiesterase from bovine heart is activated by Ca\(^{2+}\) suggests that the concentration of cAMP could in turn be regulated by Ca\(^{2+}\) in cardiac muscle. That this Ca\(^{2+}\) activation of the enzyme is operative in intact hearts is supported by the observation of Nam et al. (29) that cAMP concentrations in rat hearts can be increased or decreased upon perfusion of the hearts with a Ca\(^{2+}\)-free or a Ca\(^{2+}\)-rich medium, respectively. Furthermore, the range of Ca\(^{2+}\) concentrations effective in the activation of cAMP phosphodiesterase, 1 to 10 \(\mu\)M, also suggests that this Ca\(^{2+}\) activation may have an important regulatory role in the cardiac contraction. It has been suggested (23) that the Ca\(^{2+}\) level during the myocardial contraction cycle fluctuates in the range of 0.1 to 10 \(\mu\)M. Although it is not clear as to how Ca\(^{2+}\) activation of cAMP phosphodiesterase contributes to the regulation of myocardial contraction, it could conceivably be an important mechanism for the removal of the excess cAMP used for the excitation of the muscle. In addition, the Ca\(^{2+}\) activation of cAMP phosphodiesterase could even be involved in the control of myocardial contraction in the absence of the hormonal stimulation.

Brooker (24) has recently demonstrated the fluctuation of cAMP concentration during the contraction cycle of electrically stimulated frog ventricle strips. It is interesting to note that the activation of cAMP phosphodiesterase by Ca\(^{2+}\) is analogous to the Ca\(^{2+}\) activation of myosin ATPase in that both depend on the binding of Ca\(^{2+}\) to specific protein (25). Furthermore, both these Ca\(^{2+}\) binding proteins are highly acidic with molecular weights of about 20,000 (11, 26).

Recently, we have shown that the interaction between bovine heart cAMP phosphodiesterase and the protein activator may be modulated by cAMP (11, 12). That the protein activator has an absolute requirement for Ca\(^{2+}\) for its action suggests that Ca\(^{2+}\) and cAMP may interact synergetically in the activation of cAMP phosphodiesterase. The significance of such interaction in the regulation of intracellular concentrations of cAMP and Ca\(^{2+}\) is, however, not clear.

Bovine heart cAMP phosphodiesterase has recently been extensively purified in two different laboratories (27, 28). In both cases, two major molecular forms of the enzymes have been observed. Hrapchak and Rasmussen (27) did not detect the existence of any specific intracellular protein activator. On the other hand, Goren and Rosen (28) reported that their cAMP preparations could be activated 100% by a nondialyzable activator. The present study shows that the protein activator can activate the activator-deficient cAMP phosphodiesterase 6- to 10-fold. The reason for this discrepancy is not clear and is currently under investigation.

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