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CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITIES OF PIG CORONARY ARTERIES*

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Summary

Most (85% or more) of the cyclic nucleotide phosphodiesterase (3′:5′-cyclic-AMP 5′-nucleotidohydrolase, EC 3.1.4.17) activity of pig coronary arteries was found in the 40 000 × *g* supernatant fraction of homogenates of the intima plus media layer. Chromatography of the soluble fraction of this layer on DEAE-cellulose resolved two phosphodiesterase activities and a heat stable, non-dializable activator. Peak I activity had apparent K_m values of 2—4 μM for cyclic GMP and 40—100 μM for cyclic AMP. Peak II activity was relatively specific for cyclic AMP and exhibited apparent negatively cooperative behavior. Peak I but not peak II activity could be stimulated 3—8-fold by the addition of the boiled activator fraction or a boiled crude supernatant fraction. Cyclic AMP hydrolysis by peak I or peak II was more rapid in the presence of Mn^{2+} than Mg^{2+}, but the latter promoted hydrolysis of cyclic GMP by peak I more effectively than did Mn^{2+} in the presence of activator.

In the absence of added metals, ethylene bis(oxyethylenenitrilo)tetraacetic acid (EGTA) and EDTA both inhibited hydrolysis of cyclic AMP and cyclic GMP by phosphodiesterase activities in the supernatant fraction and in peak I, but EDTA produced more complete inhibition at lower concentrations than did EGTA.

Imidazole (1 μM to 10 mM) had virtually no effect on the hydrolysis of cyclic AMP or cyclic GMP catalyzed by either of the two separated peaks or by total phosphodiesterase activities in crude supernatant or particulate fractions.

Introduction

The importance of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) as

Abbreviation: EGTA, ethylene bis(oxyethylenenitrilo)tetraacetic acid.

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an intracellular mediator of hormone action in a variety of tissues is well established [1], and an increasing amount of evidence suggests a regulatory role for cyclic AMP in vascular and other smooth muscle [1—4]. Although the physiological role of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) in smooth muscle and other tissues is not clear, cyclic GMP levels are increased by cholinergic and other agents that produce contraction of smooth muscle [5—9]. It has been suggested that cyclic AMP and cyclic GMP may play functionally antagonistic roles in many situations, including the control of vascular smooth muscle tone [9,10].

Enzymes of cyclic AMP and cyclic GMP metabolism (adenylate cyclase, guanylate cyclase and cyclic nucleotide phosphodiesterases) are of interest as possible sites of control of cyclic nucleotide levels and thus of regulatory functions mediated by these nucleotides. The levels of cyclic nucleotides depend upon rates of both formation and degradation, and cyclic nucleotide phosphodiesterases [11] represent the only well-established pathway for degradation. Methylxanthines, papaverine and other inhibitors of cyclic nucleotide phosphodiesterase activity can produce relaxation of vascular and other smooth muscle, and there is a superficial positive correlation between potency to inhibit phosphodiesterases and potency to relax smooth muscle [12—16].

Cyclic nucleotide phosphodiesterases of vascular smooth muscle have not been well characterized. Elucidation of the properties of these enzymes should aid in understanding the roles of cyclic AMP and cyclic GMP in vascular smooth muscle. The aim of the present study was to determine some of the fundamental characteristics of cyclic nucleotide phosphodiesterases from the media-intima layer of pig coronary arteries, tissues in which cyclic AMP and cyclic GMP levels can be increased by a phosphodiesterase inhibitor and by other vasoactive agents [17].

Experimental procedures

Materials

Hearts from pigs of both sexes and varying ages were obtained from a local slaughterhouse within 20 min after slaughter of the animals and kept packed in ice until dissection of the vessels which was completed within 2 h. DEAE-cellulose (0.95 mequiv./mg, coarse grade) was obtained from Sigma. Cyclic AMP and cyclic GMP (Schwartz) were prepared as stock solutions and used without further purification. Tritiated cyclic nucleotides obtained from New England Nuclear Corp. were purified on Dowex-50 cation exchange resin columns [18].

Assay procedures

The assay of phosphodiesterase activity was in principle that described by Butcher and Sutherland [19] with the tritiated substrate modification of Beavo et al. [20]. The phosphodiesterase preparation was incubated routinely at 30° C for 30 min with indicated concentrations of tritiated cyclic nucleotide in a medium containing 10 μmol of Tris · HCl (pH 7.5) and, unless specified otherwise, 0.5 μmol of $MgCl_2$ in a final volume of 250 μl. Dilutions of phosphodiesterase preparations to give 5—20% conversion of substrate to product were

made in 40 mM Tris · HCl (pH 7.5) containing 1 mg/ml of bovine serum albumin (Fraction V, Sigma). Other divalent cations, EDTA (ethylenediaminetetraacetic acid, disodium salt), EGTA (ethylene bis(oxyethylenenitrilo)tetraacetic acid) and a heat stable non-dialyzable activator of phosphodiesterase (see below) were included in the incubation mixture as indicated. Product accumulation was determined to be linear with time and with enzyme dilution under all conditions studied. All determinations were made in duplicate.

The reaction was started by addition of the phosphodiesterase preparation and stopped by the addition of 25 μl of an aqueous solution of EDTA (50 mM) and theophylline (30 mM). Excess *Crotalus atrox* venom (0.1 mg) was then added in a volume of 10 μl, and the solution was incubated for another 10 min at 30° C. Control (blank) values were obtained by replacing the phosphodiesterase preparation with its carrier solution. Neither the EDTA/theophylline mixture nor any of the cations, in the amounts used, interfered with the effectiveness of the venom nucleotidase step. After incubations were completed, the reaction mixture was diluted to 1 ml with an aqueous solution of adenosine (0.1 mM) and guanosine (0.1 mM). The entire mixture was then applied to a column (2 × 0.7 cm) of QAE Sephadex A-25 (formate form) which had been washed with 3 ml of 20 mM NH_4CHO_2 (pH 7.4) [21]. The assay tube was rinsed with 1 ml of 20 mM NH_4CHO_2 (pH 7.4) which was then applied to the column. The column was then washed with 3 ml of 20 mM NH_4CHO_2 (pH 7.4). The total eluate was collected and the tritiated, dephosphorylated products were counted in 15 ml of tT-21 scintillation fluid [22] in a liquid scintillation spectrometer. No nucleotides were eluted by the above procedure and more than 95% of the original radioactivity was eluted as dephosphorylated product when the cyclic nucleotides were completely hydrolyzed by excess phosphodiesterase and venom.

Homogenate preparation

The right coronary, anterior descending and circumflex arteries were dissected from fresh cold pig hearts and placed in a cold solution containing 40 mM Tris · HCl (pH 7.5), sucrose (0.25 M) and EDTA (0.1 mM). The arteries were everted, and the intima-media layers were removed and placed in the above buffer. The tissue was blotted on filter paper, weighed, minced with scissors and homogenized in 9 ml per g of the buffer at 4° C with an Ultra-Turrax homogenizer (Jahnke and Kunkel, Staufen, Germany). Supernatant and particulate fractions were obtained by centrifugation of this homogenate for 30 min at 40 000 × *g* at 0° C. Particulate fractions were washed twice in the homogenization medium, being resuspended by short treatments with the Ultra-Turrax homogenizer. Protein was determined as described by Lowry et al. [23].

Separation of phosphodiesterases

The supernatant fraction used for column chromatography was prepared as above except that the tissue was homogenized in 4 ml/g of a solution containing 20 mM Tris · HCl (pH 7.5), 2 mM $MgCl_2$ and 1 mM dithiothreitol. This and subsequent manipulations were carried out at 4° C.

DEAE-cellulose was washed according to the method of Cheung [24] and

then equilibrated with the Tris · HCl homogenization buffer. The homogenate supernatant fraction (8 ml) was applied to a 10 × 0.9 cm column of DEAE-cellulose, and the column was then washed with 80—100 ml of the homogenization medium. This wash fluid contained 30—50% of the protein in the original supernatant fluid and no measurable phosphodiesterase activity. The column was developed with the same buffer containing an exponential gradient of $(NH_4)_2SO_4$ [24]. The mixing tank contained 400 ml of buffer and the addition tank contained the buffer made 0.5 M with $(NH_4)_2SO_4$. The flow rate was 0.6 ml/min and 60 fractions of 8 ml each were collected. Each collection tube contained 8 mg of bovine serum albumin dissolved in 0.2 ml of water. Appropriate fractions from separated peaks I and II of phosphodiesterase activity (Fig. 1) were pooled, concentrated to approximately 14% of the original volume in an Amicon ultrafiltration cell with an UM-10 membrane and dialized for 24 h against 100 vol. (3 changes) of the above buffer.

After concentration and dialysis, pooled fractions from peak I or peak II were separately re-chromatographed to remove contaminating peaks II and I, respectively. The appropriate fractions, which were of the same elution volume as in the first fractionation, were again pooled, concentrated, dialyzed, and then stored at —70° C in small aliquots.

Preparation of activator

A heat-stable, non-dialyzable activator of phosphodiesterase, presumably the same as or similar to protein activators first described by Cheung [25,26] and subsequently by other investigators [27—30], was prepared by boiling the supernatant fraction for 5 min and then dialyzing it for 24 h against 100 vol. (2 changes) of a solution containing 20 mM Tris · HCl buffer (pH 7.5). When 10 μl (10—15 μg protein) of the resulting preparation were diluted with 40 μl of 40 mM Tris · HCl buffer (pH 7.5) containing 2 mg/ml bovine serum albumin and added to the assay mixture, maximal stimulation of activator-deficient enzyme (peak I) resulted. Unless specified otherwise, 50 μl of this preparation were used in the assay.

Protein activator, bovine serum albumin and phosphodiesterase preparations were treated for the cation studies by addition of EGTA, 2.2 mM final concentration, followed by dialysis for 36 h against 100 vol. (3 changes) of a solution containing 20 mM Tris ·HCl (pH 7.5), 50 μM EGTA and 1 mM dithiothreitol, and then for another 36 h against 100 vol. (3 changes) of the same solution without EGTA.

Results

Distribution of phosphodiesterase activity in pig coronary arteries

The bulk of the phosphodiesterase activity of pig coronary arteries (as detected at 1 μM substrate concentration) was contained in the 40 000 × *g* supernatant fraction of the inner layer (Table I), which was predominantly intima-media. The washed, 40 000 × *g* particulate fraction from the inner layer contained about 14% of the cyclic AMP hydrolytic activity and about 4% of the cyclic GMP hydrolytic activity of the whole homogenate.

The small amount of phosphodiesterase activity detected in the outer

TABLE I

DISTRIBUTION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITY IN PIG CORONARY ARTERIES

Cyclic nucleotide phosphodiesterase activity was measured as described in Experimental procedures. Values are ± S.E. of the number of preparations indicated in parentheses. The activity of each preparation was determined with at least two concentrations of protein. Substrate concentration was 1 μM.

	Activity (pmoles · (mg tissue · 30 min)$^{-1}$)	
	Cyclic AMP	Cyclic GMP
Outer layer		
Homogenate	22 ± 3 (3)	48 ± 8 (3)
Inner layer		
Homogenate	127 ± 18 (5)	456 ± 76 (5)
40000 × *g* Supernatant	110 ± 14 (6)	434 ± 69 (6)
40000 × *g* Particles	21 ± 5 (6)	19 ± 4 (6)

layer (predominantly adventitia) could have been due to small amounts of the media or of fat that were not removed during dissection. At most, the outer layer contained 17% of the total phosphodiesterase activity of the vessels. This activity was not studied further.

Separation of activities by DEAE-cellulose chromatography

Two peaks of phosphodiesterase activity were resolved by DEAE-cellulose chromatography of the 40 000 × *g* supernatant fraction (Fig. 1). In addition, a heat-stable, non-dialyzable activator was recovered in tubes 26—38 (data not shown). Peak I phosphodiesterase hydrolyzed both cyclic GMP and cyclic AMP, but it displayed a much greater activity with cyclic GMP as substrate at

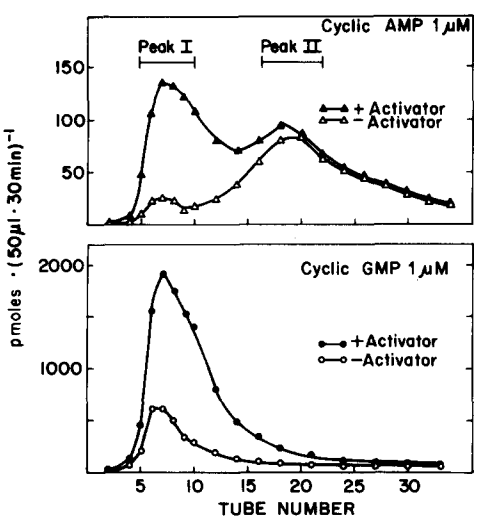

Fig. 1. Separation of cyclic nucleotide phosphodiesterase activities by DEAE-cellulose chromatography. Experimental details are given in Experimental procedures. Cyclic GMP (○,●) and cyclic AMP (△,▲) phosphodiesterase activities were assayed in the presence (●,▲) or absence (○,△) of activator as described in the text. Results are means of two determinations, and are representative of 12 preparations.

micromolar concentrations. Very low cyclic GMP phosphodiesterase activity in peak II made it impractical to study this nucleotide as a substrate, but peak II displayed good activity against cyclic AMP.

Peak I phosphodiesterase activity was increased 3—8-fold (depending upon the enzyme preparation) by the addition of saturating amounts of boiled, dialyzed supernatant fluid or of the boiled activator-containing fractions from the column. After being re-chromatographed to remove overlapping peak I, peak II activity was not changed by the addition of activator preparations. Total phosphodiesterase activity with either nucleotide recovered in fully activated peak I and peak II amounted to 70—80% of that applied to the column.

Kinetic behavior

Cyclic GMP phosphodiesterase activity in the crude supernatant fraction displayed straightforward kinetic behavior with an apparent K_m of 2—4 μM (Fig. 2, lower panel). Cyclic AMP phosphodiesterase activity in the supernatant fraction (Fig. 2, upper panel) displayed anomalous kinetic behavior. This anomalous kinetic behavior could be the result of two enzymes with different K_m values or of one enzyme displaying negatively cooperative behavior [32]. Both cyclic GMP and cyclic AMP phosphodiesterase activities in the particulate fraction displayed similar anomalous kinetic behavior (data not shown).

When phosphodiesterase activities were separated on DEAE-cellulose columns, peak I gave essentially straight kinetic plots with either cyclic AMP or

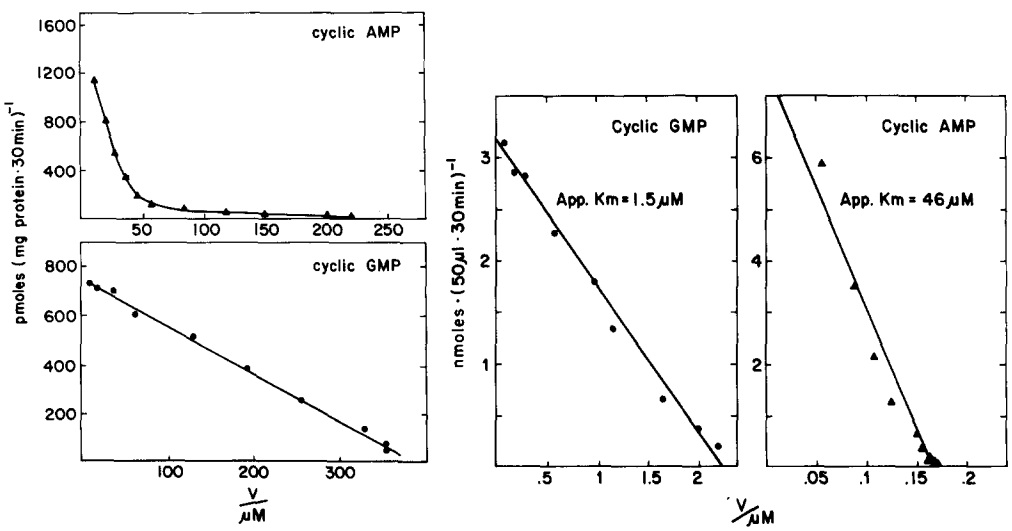

Fig. 2. Hofstee plots [31] of cyclic AMP (upper) and cyclic GMP (lower) hydrolysis by a crude supernatant fraction of the intima-media layer of pig coronary arteries. Substrate concentrations ranged from 0.1 to 100 μM. Results are means of duplicate determinations and are representative of three experiments with different preparations.

Fig. 3. Hofstee plots [31] of cyclic AMP (right panel) and cyclic GMP (left panel) hydrolysis by peak I eluted from a DEAE-cellulose column. Substrate concentrations ranged from 0.1 to 100 μM, and a saturating amount of the activator preparation (15 μg protein) was present during the incubation. Results are means of duplicate determinations and are representative of experiments with four different preparations.

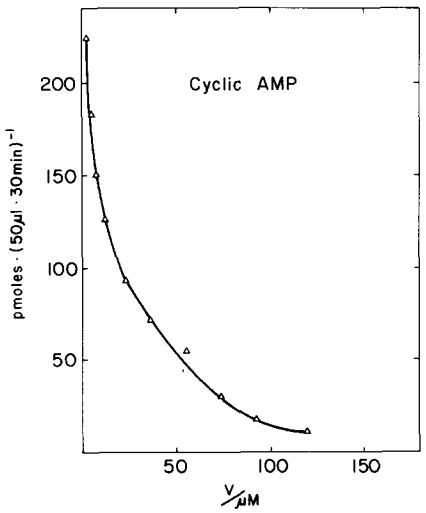

Fig. 4. Hofstee plot of cyclic AMP hydrolysis by peak II eluted from a DEAE-cellulose column (see Fig. 1). Substrate concentrations ranged from 0.1 to 100 µM. Results are means of duplicate determinations and are representative of experiments with four different preparations.

cyclic GMP as substrate (Fig. 3); slight curvature of cyclic AMP kinetic plots was seen with some preparations. Apparent K_m values were 40—100 µM for cyclic AMP and 2—4 µM for cyclic GMP; these were not measurably altered by the addition of the activator. Activation thus was due to changes in V under these conditions. Peak II displayed anomalous kinetic behavior toward cyclic AMP (Fig. 4) qualitatively similar to that seen in the crude fractions (Fig. 2). This behavior was unaffected by the presence or absence of the activator.

Effect of divalent cations

Both cyclic AMP and cyclic GMP phosphodiesterase activities in the dialyzed supernatant and washed particulate fractions (Fig. 5) were detectable without any added metal, and they were increased by Mg^{2+} and inhibited by added Ca^{2+} as has been reported for phosphodiesterase activities from other tissues [33,34]. However, the effects of Mn^{2+} on supernatant and particulate enzyme activities were quite different with the two substrates. Cyclic AMP phosphodiesterase activity in the presence of Mn^{2+} was much greater than that in the presence of Mg^{2+} at low concentrations, but little or no increase in cyclic GMP phosphodiesterase activity was observed with Mn^{2+} at any concentration. The data shown in Fig. 5 were obtained at 1 µM substrate; experiments carried out at 100 µM substrate yielded indistinguishable results in terms of relative effects of the cations.

Isolated peak I phosphodiesterase activity (in the presence or absence of added activator, at 1 µM substrate concentration) hydrolyzed both cyclic AMP and cyclic GMP in the absence of added metal (Fig. 6). In the absence of activator (right panels), Mn^{2+} increased the activity against either substrate much more effectively than did Mg^{2+}. Ca^{2+} either had no effect or was inhibitory at all concentrations in the presence (data not shown) or absence of Mg^{2+} or Mn^{2+}.

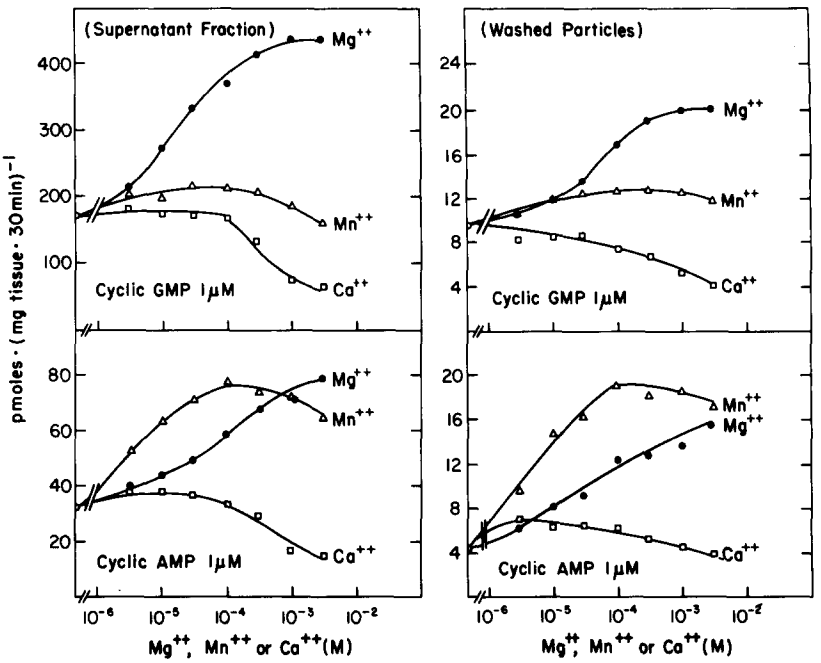

Fig. 5. Effect of Mg^{2+}, Mn^{2+} and Ca^{2+} on phosphodiesterase activity from 40 000 X *g* washed particulate and dialyzed supernatant fractions of the intima-media layer of pig coronary arteries. The enzyme preparation was dialyzed as described in Experimental procedures. Results are means of eight determinations.

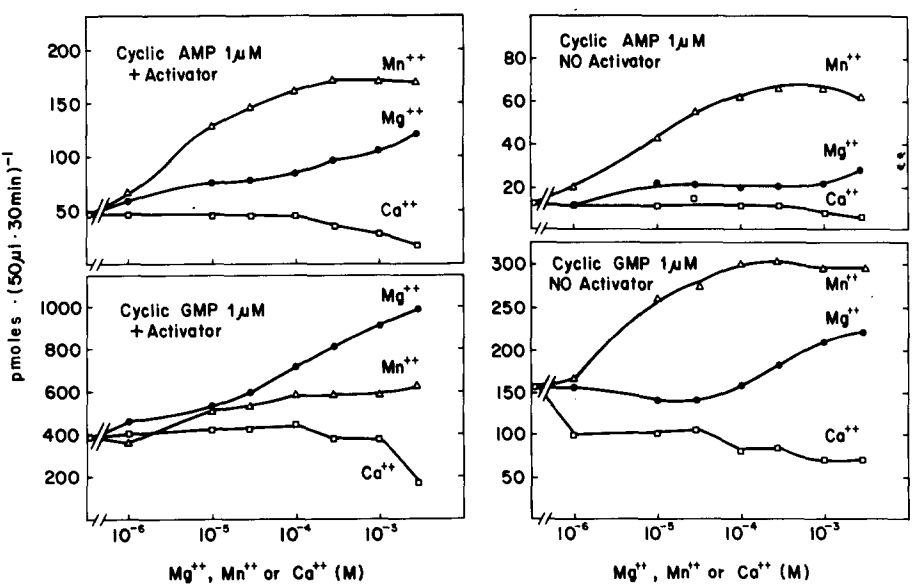

Fig. 6. Effects of Mg^{2+}, Mn^{2+} and Ca^{2+} on peak I activity in the absence (right panels) or presence (left panels) of activator. The enzyme preparation was dialyzed as described in Experimental procedures. Data are means of duplicate determinations and are representative of experiments with three different preparations.

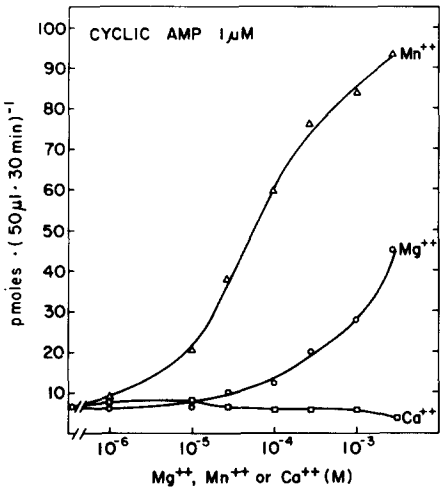

Fig. 7. Effects of Mg^{2+}, Mn^{2+} and Ca^{2+} on peak II phosphodiesterase activity. The enzyme preparation was dialyzed as described in Experimental procedures. Data are means of duplicate determinations and are representative of experiments with three different preparations.

In the presence of activator, peak I phosphodiesterase activity against cyclic GMP was most effectively increased by Mg^{2+}, while activity against cyclic AMP was increased most effectively by Mn^{2+} (Fig. 6, left panels). Ca^{2+} again was without effect or was inhibitory in the presence (data not shown) or absence of Mg^{2+} or Mn^{2+}.

Hydrolysis of cyclic AMP by peak II activity was increased more effectively by Mn^{2+} than by Mg^{2+} (Fig. 7). Again, Ca^{2+} was without effect or inhibitory either alone or in combination (data not shown) with Mg^{2+} or Mn^{2+}. The activator was without effect upon peak II activity in the presence of Mn^{2+} or Mg^{2+} or of combinations of 2 mM Mg^{2+} and 2 mM Mn^{2+} or Ca^{2+}.

Effects of EDTA and EGTA

As shown on previous figures, phosphodiesterase activity was easily detectable in the absence of added divalent cation in both dialyzed crude supernatant fractions and in dialyzed peak I. Cyclic AMP and cyclic GMP phosphodiesterase activities from both sources were decreased by both EGTA and EDTA. With activities in supernatant fractions (Fig. 8, left panels), both EDTA and EGTA, at 0.1 mM decreased the rate of hydrolysis of either cyclic nucleotide, but EDTA produced more complete inhibition than did EGTA at this concentration. At 0.1 mM, EGTA decreased phosphodiesterase activity by 55—60%, but 90% inhibition was not achieved until the EGTA concentration was increased to 10 mM. EDTA, on the other hand, decreased activity by more than 90% at 0.1 mM.

Both chelators at 10 μM concentrations inhibited the activity of peak I in the presence of activator to approximately the level of activity seen in the absence of activator (Fig. 8, right panels). However, EDTA was a more effective inhibitor than EGTA at 0.1 mM and above in the presence or absence of activator. The inhibition patterns may suggest an involvement of more than one metal [35] in the maintenance of peak I activity in the presence of activator,

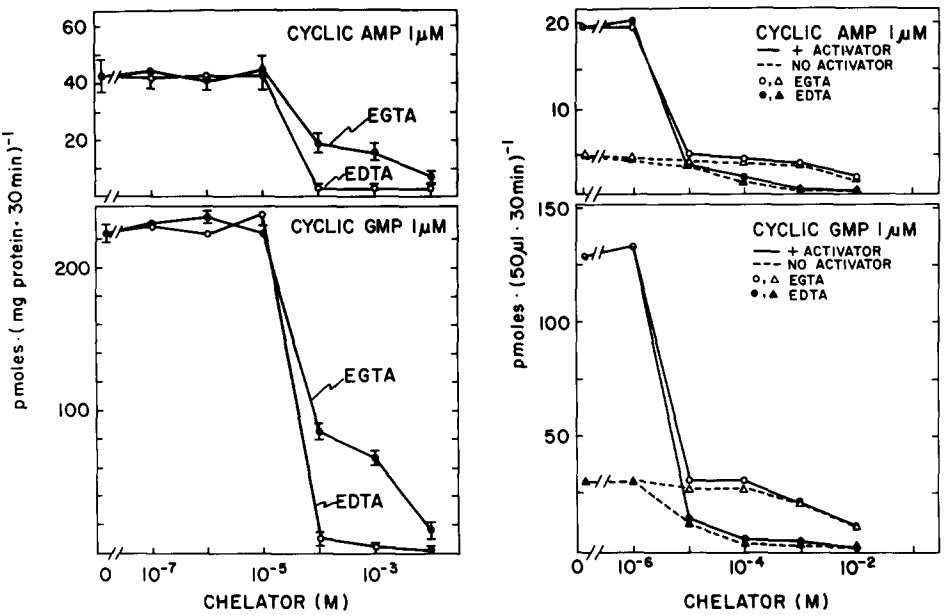

Fig. 8. Effect of EDTA and EGTA upon supernatant (left panels) and peak I (right panels) phosphodiesterase activity. Values for left panels are ± S.E. of six determinations with three different preparations. Values for right panels are means of duplicate determinations and are representative of experiments with three different preparations.

although direct inhibition by the chelating agents cannot be ruled out. Peak II activity was reduced so effectively by dialysis that study of its inhibition by chelators was impractical.

It is likely that the systems used for the divalent cation studies, although dialyzed against EDTA, contained one or more metals necessary for the action

TABLE II

EFFECT OF Ca^{2+} ON THE ACTIVATION OF PEAK I PHOSPHODIESTERASE IN THE PRESENCE OF EGTA

Activities are expressed as means of duplicate determinations and are representative of experiments with three different preparations.

Cation added (mM)*		Peak I phosphodiesterase activity (pmoles · (50 μl · 30 min)$^{-1}$)			
Mg^{2+}	Ca^{2+}	Cyclic AMP		Cyclic GMP	
		No activator	Saturating activator	No activator	Saturating activator
0	0	2	2	10	11
2	0	8.4	9.0	110	115
0	0.05	—	—	12	13
0	0.2	2	4.2	15	45
2	0.05	9	9.5	115	121
2	0.2	10	85	150	850

* The assay mixture contained 0.1 mM EGTA.

of the activator. When 0.1 mM EGTA was in the reaction mixture, Mg^{2+} did not support the activator's effect (Table II). When Ca^{2+} was added in excess of EGTA in the absence of added Mg^{2+}, there was minimal activator effect. This was in contrast to the marked effect that was seen when both Mg^{2+} and Ca^{2+} were present in excess of EGTA.

Effects of imidazole

Imidazole has been reported to increase cyclic nucleotide phosphodiesterase activity from beef heart at high cyclic AMP concentrations [19], but it apparently has little effect on a preparation from rabbit skeletal muscle at low substrate concentrations [34], and it has been reported to inhibit the hydrolysis of cyclic GMP and stimulate the hydrolysis of cyclic AMP by phosphodiesterase(s) from rat brain [36]. Cyclic GMP and cyclic AMP phosphodiesterase activities in supernatant fractions from pig coronary arteries were unaffected at 1 μM substrate by imidazole concentrations from 10 μM to 10 mM, and they were only slightly (about 30%) increased by 10 mM imidazole at 100 μM substrate. The effects of imidazole on particulate cyclic AMP and cyclic GMP phosphodiesterase activities at 1 and 100 μM substrate and on peak I and peak II activities at 1 μM substrate were indistinguishable from those on the activities in the supernatant fractions.

Discussion

The separation of two cyclic nucleotide phosphodiesterase activities from the supernatant fraction of pig coronary arteries reveals that a protein activator selectively affects a form of the enzyme (peak I) that hydrolyzes both cyclic AMP and cyclic GMP but that has a higher apparent affinity for the latter nucleotide. Others have found that phosphodiesterase activity which is activated by a protein activator can be separated from activity which is not affected by the activator [27,37]. The non-activatable form from coronary arteries (peak II) is relatively specific for cyclic AMP, having little if any activity against cyclic GMP. Thus if the activator is important in regulating levels of the two cyclic nucleotides in pig coronary arteries, it may be more important for cyclic GMP than cyclic AMP. A recent preliminary report also indicates similar selectivity by a phosphodiesterase activator from rat uterus [38].

The two forms of phosphodiesterase from pig coronary arteries (peaks I and II) correspond in at least their kinetic properties and substrate specificities to those found in a number of tissues [39]. Selective physiological regulation of these two forms is likely. In those cases in which the kinetic behavior of protein activator-sensitive phosphodiesterase has been described, the activator-sensitive phosphodiesterase has seemed to correspond in its properties to peak I [29,37,40]. A membrane-associated phosphodiesterase activity of fat cells, which resembles peak II in its kinetic properties has been shown to be selectively increased by insulin [41,42]. Furthermore, cyclic nucleotide phosphodiesterase activities superficially resembling peaks I and II have been reported to be under separate genetic control in chicken embryo fibroblasts [43]. The peak II-like activity was selectively increased (apparently induced) by treatment of the cells with a phosphodiesterase inhibitor or with analogs of cyclic

AMP. However, it should be emphasized that at 1 μM cyclic AMP, total phosphodiesterase activity of fully activated peak I from the coronary artery is approximately equal to the total activity of peak II, therefore regulation of either activity could affect cyclic AMP levels in this tissue.

The interaction of peak I with activator seems to require a metal, and others have demonstrated that Ca^{2+} is required for interaction of protein activators and phosphodiesterases isolated from other sources [26,27,29,30]. The data presented here only demonstrate that the inhibitory effects of EGTA can be overcome by Ca^{2+}. A dilemma is encountered whenever one is forced to try to demonstrate a metal effect by titrating a chelator with metal. Ca^{2+} could be acting to release another required metal from its complex with EGTA or it could simply be complexing the EGTA to relieve inhibition by free EGTA [44]. We have been unable, thus far, to demonstrate a specific metal requirement for the activator-enzyme interaction in the absence of EGTA. Attempts to remove metals from all components of the assay system have failed to produce a system that was dependent upon more than added Mg^{2+}.

Mn^{2+} supports peak I activity more effectively than does Mg^{2+} in all situations except for the hydrolysis of cyclic GMP in the presence of activator. Although the presence of two enzyme forms within peak I is possible this data also could indicate that the interaction of the activator with peak I confers different metal-dependent substrate binding properties than were present before activation. The differential effects of Mg^{2+} and Mn^{2+} are superficially similar to those recently reported by Lin et al. [26] who worked with an activator-sensitive phosphodiesterase from brain.

The possibility of selective control mechanisms for cyclic AMP and cyclic GMP phosphodiesterase activities gives the potential for selective control of the levels of these nucleotides in cells and thus for control of cyclic AMP or cyclic GMP sensitive processes, which may include contraction of vascular smooth muscle.

Acknowledgements

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