The Role of Potassium and Calcium Ions in the Effect of Epinephrine on Cardiac Cyclic Adenosine 3′,5′-Monophosphate, Phosphorylase Kinase, and Phosphorylase

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(Received May 9, 1968)

SUMMARY

The effect of epinephrine on the biochemical sequence of events leading to the activation of glycogen phosphorylase was studied in the isolated, perfused rat heart. In arrested preparations, perfused with a medium either devoid of Ca^{++} or containing a depolarizing concentration of K^+, the epinephrine-induced activation of phosphorylase was markedly diminished. In the absence of Ca^{++}, however, the epinephrine-induced rise in cyclic adenosine 3′,5′-monophosphate (cyclic AMP) concentration and the activation of phosphorylase kinase were not greatly altered. This suggested that the effect of omission of Ca^{++} occurred at the phosphorylase *b* to *a* conversion step.

The effect of epinephrine on phosphorylase activation was blocked simultaneously with the cessation of contraction following perfusion with a Ca^{++}-deficient medium. The store of Ca^{++} required for the maintenance of contractile activity appeared to be similar to that necessary for the conversion of phosphorylase *b* to *a*.

Elevation of Ca^{++} in the perfusion medium resulted in activation of phosphorylase but not of phosphorylase kinase; nor was there an increase in cyclic AMP concentration. This observation also suggested that Ca^{++} is required for the catalytic activity of phosphorylase kinase.

In the heart depolarized with K^+, only a small and transient increase in cyclic AMP concentration occurred in response to epinephrine. Activation of phosphorylase kinase was considerably less than that observed in the beating heart. This suggested that an excess of K^+ interfered with the action of epinephrine at a site at or before adenyl cyclase.

Thus the activation of phosphorylase in cardiac muscle can be the consequence of one of the following events: (a) the activation of phosphorylase kinase as a result of a rise in cyclic AMP concentration; (b) an increase in the catalytic activity of phosphorylase kinase as a result of an increase in the concentration of Ca^{++} available to the enzyme; or (c) a combination of these two mechanisms.

This study was supported by Research Grant HE 04626 from the National Heart Institute, by Career Development Award GM 6257 (S.E.M.), and by the Interdepartmental Cardiovascular Training Program of Emory University School of Medicine, sponsored by the National Heart Institute (Training Grant 5T1 HE 5380). This is Publication 884 of the Division of Basic Health Sciences.

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INTRODUCTION

In 1956 Krebs and Fischer (1) demonstrated that the interconversion of the two forms of muscle glycogen phosphorylase *in vitro* involved the phosphorylation and dephosphorylation of the enzyme. It was subsequently shown that the terminal phosphate of ATP was transferred to phosphorylase *b* according to the following reaction (2): 2 phosphorylase *b* + 4 ATP → phosphorylase *a* + 4ADP. Krebs *et al.*

(3) have shown that phosphorylase kinase, the enzyme that catalyzes the above reaction, also exists in two forms. Conversion of the inactive form of phosphorylase kinase to the active form was accomplished by incubation of the enzyme with ATP and Mg^{++}. The similarity of the rate of activation and rate of phosphorylation of the enzyme suggested that this activation mechanism involved the transfer of the terminal phosphate of ATP to the inactive form of the enzyme (4).

The conversion of the inactive form of phosphorylase kinase to its active form has now been clearly demonstrated in both skeletal (5) and heart muscle (6) *in situ*. It has been proposed that a factor which regulates this activation of phosphorylase kinase is the tissue concentration of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (7). This nucleotide has been shown to accelerate the activation and phosphorylation of the kinase *in vitro* (4). The tissue concentration of cyclic AMP is determined by the catalytic activity of adenyl cyclase and cyclic nucleotide phosphodiesterase, the enzymes responsible for the production and destruction of the nucleotide, respectively. It has been proposed that catecholamines activate phosphorylase through their stimulant action on adenyl cyclase (8, 9), while other agents, such as the methylxanthines, exert their effect through an inhibitory action on phosphodiesterase activity (10).

Hypotheses concerning other mechanisms of the regulation of phosphorylase kinase activity have been proposed. These have been derived from the studies of the effects of Ca^{++} on kinase activity *in vitro*. Fischer and Krebs (11) showed that the addition of Ca^{++} to skeletal muscle extracts resulted in the conversion of phosphorylase *b* to *a*. A later report by Meyer *et al.* (12) demonstrated that Ca^{++} and a protein factor, kinase-activating factor, caused the conversion *in vitro* of inactive phosphorylase kinase to its active form. The physiological significance of this activation pathway has been questioned, however, since it appeared to be irreversible (12). Recently kinase-activating factor was identified as a nonspecific proteolytic enzyme, and the activation of phosphorylase kinase produced by proteolysis *in vitro* was shown to be unlike the activation observed in intact tissue (13).

A second effect of Ca^{++} was also reported by Meyer *et al.* (12). These authors showed that phosphorylase kinase was inhibited by EDTA and that this inhibition was specifically reversed by Ca^{++}. Although it has not yet been possible to demonstrate Ca^{++} stimulation of the enzyme clearly in the absence of chelators, it has been proposed that phosphorylase kinase requires Ca^{++} for activity (13, 14).

Experiments on intact tissues have also suggested that Ca^{++} may be involved in the cardiac phosphorylase activation pathway. Ellis and Vincent (15) reported that the epinephrine-induced phosphorylase activation in isolated guinea pig hearts was abolished when the hearts were perfused with a medium devoid of Ca^{++}. Friesen *et al.* (16) have shown that rapid elevation of the Ca^{++} concentration in the perfusion medium of the isolated rat heart produced an increase in phosphorylase *a* activity.

In order to discuss clearly the various control mechanisms in the phosphorylase-activating pathway, we are adopting the terminology suggested by Krebs *et al.* (13). Activation refers only to an effect in which a new molecular form of the enzyme is produced. The conversion of the inactive form of phosphorylase kinase to the active form is an example of an activation process. *Stimulation* refers only to an increase in the activity of a single molecular form of the enzyme.

The purpose of this investigation was to characterize further the role of Ca^{++} in the phosphorylase activation reactions.

METHODS

Perfused hearts. Male Sprague-Dawley rats (200–300 g) were anesthetized with ether. The hearts were removed rapidly and cooled in perfusion medium at 0°. After cessation of ventricular contraction, a glass cannula was inserted in the aorta and the other end of the cannula was connected to the perfusion apparatus. The

perfusion medium was pumped at a constant rate of 7 ml/min by a Holter pump. The temperature of the medium at the aortic cannula was 37°. The normal medium was a modified Chenoweth-Koelle (17) medium of the following composition: NaCl, 119 mEq/liter; KCl, 5.6 mEq/liter; $CaCl_2$, 3.2 mEq/liter; $MgCl_2$, 2.0 mEq/liter; EDTA, 0.05 mM; dextrose, 10 mM; and $NaHCO_3$, 25 mM, equilibrated with 95% O_2–5% CO_2. This medium was altered to provide special perfusion conditions as follows: when Ca^{++} was omitted, the concentration of NaCl was 122 mEq/liter. The medium used to produce K^+ depolarization was identical with the normal medium except that KCl was present at 56 mEq/liter and NaCl at 73 mEq/liter. The high Ca^{++} medium contained 9.6 mEq of $CaCl_2$ per liter.

Contractile activity of the perfused rat heart was measured with a force transducer (Grass FT-03) connected to the apex of the heart via a string run through a single pulley.

Experimental design. Three experimental preparations were used: the *normal perfused hearts* were equilibrated with normal perfusion medium for 30 min; the *no-calcium perfused hearts* were initially equilibrated with normal perfusion medium for 30 min and then switched to a calcium-free medium for an additional 30 min before drugs were given; and the *K^+-depolarized hearts* were equilibrated with normal medium for 30 min followed by a 5-min perfusion with high K^+ medium, after which drugs were given.

Drugs were injected in a volume of 0.2 ml just above the aortic cannula, the vehicle being the medium perfusing the heart at the time of injection. Hearts were frozen with modified Wollenberger clamps as previously reported (6).

In experiments in which the effect of the perfusion medium on nutritional blood flow was tested, ^{42}K, approximately 1 μC, was injected in 0.2 ml of the appropriate perfusion medium. The effluent from the heart was collected for 18 sec, at which time 200–500 mg of ventricle were cut off, rinsed four times in ice-cold medium, blotted, and weighed. The sample was then homogenized with 10 ml of 0.6 N perchloric acid at 0°. After centrifugation, the entire supernatant solution was counted as described below.

Biochemical measurements. Phosphorylase activity was measured in the direction of glucose 1-phosphate production (18). The phosphorylase kinase activity was determined in terms of the rate of conversion of crystalline rabbit muscle phosphorylase *b* to the *a* form (6). Cyclic AMP was determined by a modification (6) of the method of Hammermeister *et al.* (19), in which the effect of the nucleotide on purified muscle phosphorylase kinase activity is measured.

Data on phosphorylase activity are reported as the apparent fraction of the enzyme in the *a* form. Phosphorylase kinase data are presented as the ratio of activity at pH 6.8 to that at 8.2. This has been shown to be a valid index of the amount of the enzyme present in the active form (3).

Materials. Enzymes used in the phosphorylase assay were obtained from Boehringer Mannheim. Inulin-methoxy-^3H was obtained from New England Nuclear Corporation, and $^{42}K_2CO_3$ from Mallinckrodt Nuclear.

Counting methods. In experiments in which the washout of inulin-methoxy-^3H was studied, 1 ml of effluent from the perfused hearts containing the inulin was added to 15 ml of a solution containing 5 ml of Triton X-100 (Rohm and Haas) and 10 ml of toluene with 4 g of 2,5-diphenyloxazole and 0.1 g of bis(*O*-methylstyryl)-benzene per liter (20). The emitted fluorescence was measured in a Packard model 4321 liquid scintillation counter. The ^{42}K was measured in the same instrument by the Cerenkov radiation emitted from the Compton effect electrons induced by the gamma radiation from the isotope.

Drugs. Epinephrine bitartrate (Suprarenin) was diluted in the perfusion medium, containing 0.1% sodium metabisulfite.

Statistical methods. Statistical analysis was performed by means of Student's *t*-test.

RESULTS

Effects of epinephrine in the normal perfusion medium. Five seconds after the addition of 2 μg of epinephrine to the perfusion line, the cyclic AMP concentration in the rat hearts rose almost 2-fold, as did the activity of phosphorylase kinase (Fig. 1a and b). Phosphorylase *a* also increased significantly, from 5 to 12% (Fig. 1d). No attempt was made to determine the sequence of these changes by sampling at an earlier time after epinephrine. The subsequent pattern of change in cyclic AMP concentration appeared to be biphasic, while the activity of phosphorylase kinase and of phosphorylase continued to rise. Contractile amplitude reached a peak 15 sec after drug administration.

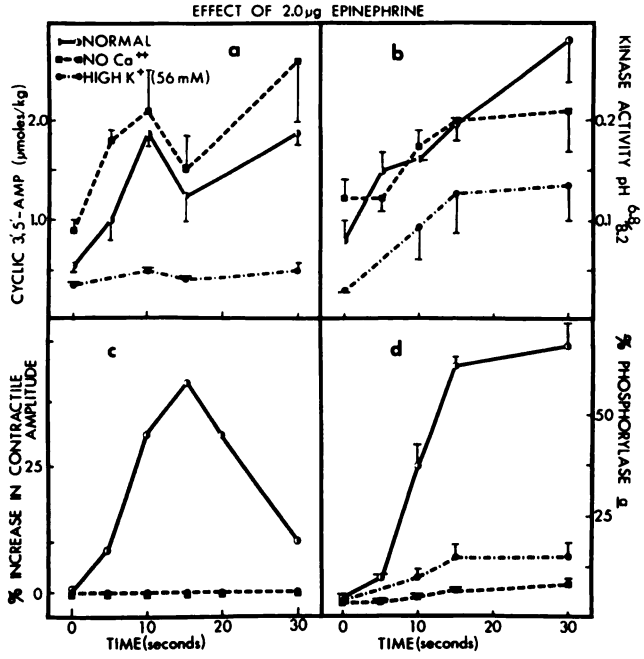

FIG. 1. *Effects of epinephrine on normal, no-calcium, and K+-depolarized hearts*

Epinephrine, 2 μg, was injected into the perfusate at zero time. Bars represent 1 standard error. In the contractile amplitude graph (c), the broken line represents data from both no-Ca^{++} and high-K^+ experiments.

We have used the pH 6.8:8.2 activity of phosphorylase kinase as an index of the amount of active enzyme present. Evidence *in vitro* suggests that the activity measured at pH 6.8 is essentially that of the active form of the enzyme, whereas at pH 8.2 the activities of both forms of the enzyme are measured. An increase in the pH 8.2 activity of the kinase was observed when the pH 6.8 activity was markedly elevated by epinephrine. This has been reported by other investigators (21) and is consistent with the pH-activity relationship of the two forms of the enzyme as reported by Krebs *et al.* (3).

Thus, in normal hearts an increased concentration in cyclic AMP was associated with sustained elevation of the catalytic activity of phosphorylase kinase and phosphorylase *a*. The correlation of the degree of activation of kinase (as expressed by the pH 6.8:8.2 activity) and the observed percentage of phosphorylase *a* is analyzed in Fig. 2. The sigmoid shape of the curve suggests that the catalytic activity of kinase is dependent upon an additional factor or factors besides its transformation to a new molecular form.

Effects of epinephrine in the absence of Ca^{++} from the perfusion medium. Perfusion

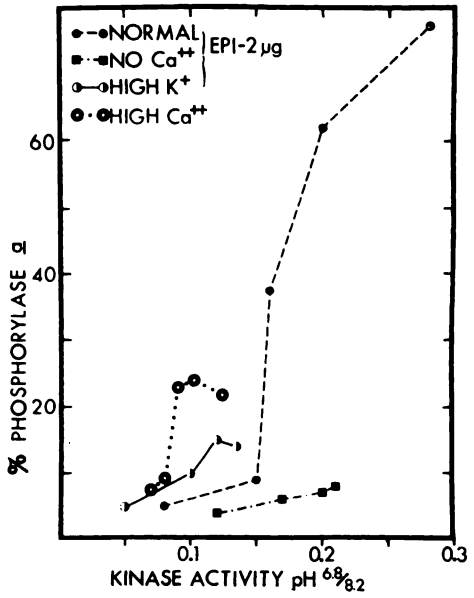

FIG. 2. *The effect of alteration in ionic composition and of the superimposed administration of epinephrine on the relation between phosphorylase kinase and phosphorylase a activities*

The data plotted here are the same as those used for Figs. 1 and 4.

of rat hearts with the no-calcium medium did not itself alter the fraction of phosphorylase in the *a* form nor the activity of phosphorylase kinase (Fig. 1d and b). However, the concentration of cyclic AMP was significantly higher (0.9 μmole/kg) than that of the normal hearts (0.5 μmole/kg) (Fig. 1a). Contractions were, of course, arrested and were not reinstituted after the administration of 2 μg of epinephrine (Fig. 1c). This drug, however, caused a rapid and marked increase in cyclic AMP concentration similar to that observed in control hearts (Fig. 1a). Other than a delay in the initial activation of kinase, the time course and magnitude of the activation of this enzyme were also unaltered (Fig. 1b). The catecholamine-induced rise in percentage of phosphorylase *a* was greatly diminished but not abolished. Total phosphorylase activity was not changed (13.1 units/g of heart).

The time course of the relationship between loss of contractility and the response of phosphorylase to epinephrine after the withdrawal of Ca^{++} was determined. Rat hearts were perfused for 30 min with normal medium containing inulin-methoxy-^3H (0.1 μC/ml) and then switched to a medium devoid of Ca^{++} and inulin. Epinephrine, 2 μg, was injected just before or at various times after this change of medium. Hearts were frozen 30 sec after the injection of the drug. The decline in the contractile activity of the hearts, the decrease of the epinephrine-induced phosphorylase activation, and the washout of the extracellular space marker, inulin-^3H, are shown in Fig. 3. The three curves have similar slopes after the first 10 sec on the no-calcium medium. The contractile activity and the effect of epinephrine on phosphorylase activity were almost abolished when the extracellular calcium ion concen-

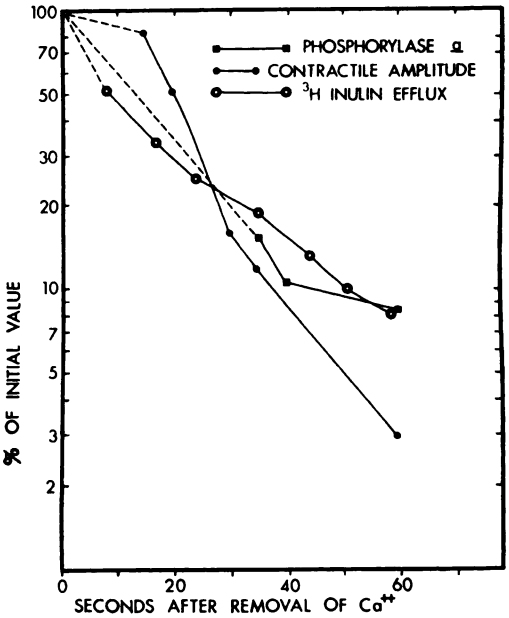

FIG. 3. *Effects of perfusion without calcium on the contractile activity and epinephrine-induced phosphorylase activation of the perfused heart*

Hearts were switched to a no-calcium perfusion medium at zero time. Points on the phosphorylase line refer to the time of sampling, which was 30 sec after the drug was injected. Inulin-^3H, 0.1 μC/ml, was present in the normal medium to determine the washout of the extracellular components after the switch of media. The broken line represents extrapolation of experimental data.

tration was decreased to about 0.25 mEq/liter.

The possibility that omission of Ca^{++} from the perfusion medium altered the fraction of medium, and therefore of epinephrine, which was delivered to coronary capillaries, was tested with the technique adapted from Sapirstein (22). No differences in uptake of ^{42}K were observed between normal (285 ± 5 cpm/mg of heart) and no-calcium hearts (276 ± 9) 20 sec after administration of the tracer. Therefore, the marked reduction in the response of phosphorylase activity to epinephrine in the absence of Ca^{++} cannot be explained on the basis of altered nutritive coronary circulation.

Thus, reduction of intracellular Ca^{++} in the heart resulted in a rapid loss of the ability of epinephrine to induce the transformation of phosphorylase *b* to *a*. The fact that the drug produced a similar degree of activation of phosphorylase kinase to that observed in normal hearts (Fig. 2) suggests that the omission of Ca^{++} diminished the catalytic activity of the active form of this enzyme.

Effect of elevated Ca^{++} concentration on cardiac phosphorylase activation. Rat hearts were perfused with normal medium for 20 min, after which they were rapidly switched to a medium which contained 3 times the normal Ca^{++} concentration (9.6 mEq/liter). This caused a significant increase in the percentage of phosphorylase *a* (Fig. 4), which returned to control values 2 min after the Ca^{++} perfusion had been initiated. No activation of phosphorylase kinase was observed. Cyclic AMP concentration significantly decreased during this same period from the control value of 0.69 μmole/kg to 0.42 μmole/kg.

Thus the mechanism of action of Ca^{++} is to stimulate the activity of phosphorylase kinase in catalyzing the conversion of phosphorylase *b* to *a*, rather than to cause activation of the kinase (Fig. 2).

Effects of epinephrine in the presence of high K^+ concentration. Depolarization of the perfused hearts by 56 mM K^+ produced a transient increase (20 sec) in the percentage of phosphorylase *a* (to 31%). Ellis and Vincent (15) had reported a similar but longer effect (90 min) of K^+ in the isolated, perfused guinea pig heart. We found that this effect was abolished by prior treatment of the animals for 3 days with reserpine, 2.5 mg/kg, or by adding pronethalol to the perfusion medium at a concentration of 2 μg/ml. The more sustained effect on guinea pig heart is probably a

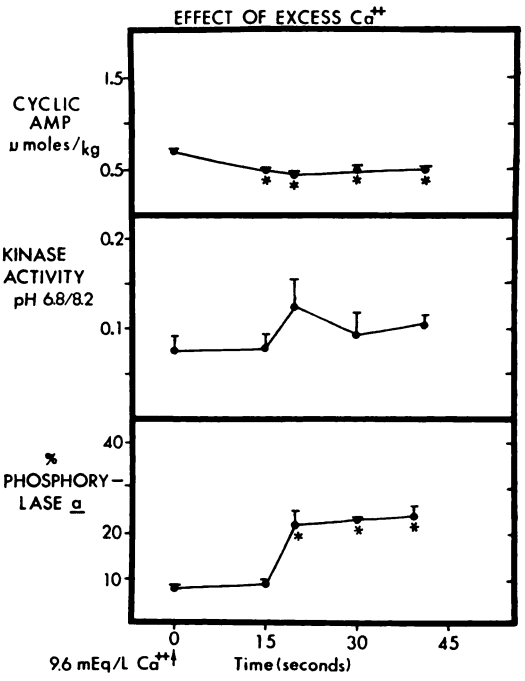

FIG. 4. *Effect of elevated calcium ion concentration on cardiac phosphorylase activation*

Bars represent 1 standard error. Asterisks indicate points different from the control at $p < 0.05$.

function of temperature (37° in our experiments, 27° in those of Ellis and Vincent) and the greater catecholamine content of guinea pig heart (23). Because of the phosphorylase-activating effect of depolarization, drugs were not administered to depolarized hearts until 5 min after the switch to the high-potassium medium was accomplished.

At this time neither the cyclic AMP concentration nor the percentage phosphorylase *a* was altered when compared to normal hearts, but the pH 6.8:8.2 activity ratio of phosphorylase kinase was significantly re-

duced (Fig. 1). Epinephrine, 2 μg, did not reinstitute contractions (Fig. 1c). A significant rise in cyclic AMP concentration was observed only after 10 sec (Fig. 1a). Activation of phosphorylase kinase occurred with a change comparable to that seen in the normal and no-calcium experiments. However, the maximum activity ratio attained (0.14) was only half of that induced by epinephrine in normal hearts. Phosphorylase activation was slight and significantly different from the zero time value only at the 15- and 30-sec intervals.

Since K^+ is known to increase the tone of smooth vascular muscle, it was conceivable that the decreased biochemical responses to epinephrine were due to shunting of the catecholamine through nonnutritive blood vessels. The uptake of ^{42}K was lower in hearts perfused with the high-K^+ medium (93.2 ± 6.7 cpm/mg) when compared to normal hearts (207 ± 17 cpm/mg). However, the dose of epinephrine used, 2 μg, was supramaximal, and a 55% reduction in delivery of the drug to heart capillaries should still produce near maximal increases in cyclic AMP concentration and kinase and phosphorylase activities.

Thus, an excess of K^+ in the medium was shown to lower the degree of activation of phosphorylase kinase, and, while epinephrine produced an increase in the pH 6.8:8.2 activity of the enzyme, this was not sufficient to catalyze extensive conversion of phosphorylase *b* to *a* (Fig. 2). The primary consequence of K^+ depolarization appears to be on the catecholamine-sensitive synthesis of cyclic AMP.

DISCUSSION

Danforth, Helmreich, and Cori (24) have suggested that the rate-limiting step in the interconversion of the two forms of glycogen phosphorylase is the phosphorylase *b* to *a* reaction. Our study indicates that the rate of this reaction in cardiac muscle is regulated by two mechanisms. One involves the activation of phosphorylase kinase, in which the inactive form of this enzyme is converted to an active form. The other involves the stimulation of the catalytic activity of the kinase by Ca^{++}.

The existence of a phosphorylase kinase activation mechanism had been demonstrated previously in perfused rabbit hearts (19) and in the rat heart *in situ* (6). Similar findings in the perfused rat heart are reported here. In both rat studies, the time course of the change in the concentration of cyclic AMP and the degree of activation of phosphorylase kinase is consistent with the hypothesis that the cyclic nucleotide is the mediator of this activation process.

The demonstration that an elevation of Ca^{++} concentration caused an increase in cardiac phosphorylase *a* activity without an activation of phosphorylase kinase or an increase in the cyclic AMP concentration suggests the existence of a second mechanism for the regulation of the phosphorylase *b* to *a* reaction. Experiments *in vitro* reported by Krebs *et al.* (13) and Ozawa and Ebashi (14) indicated that purified skeletal muscle phosphorylase kinase requires Ca^{++} for activity. Our results suggest that the Ca^{++} concentration in the beating heart is not sufficient for maximal stimulation of phosphorylase kinase activity and that an increased concentration of this ion enhances the catalytic activity of this enzyme. This interpretation is consistent with the sensitivity of kinase activity to Ca^{++} and estimation of free intracellular Ca^{++} concentration in muscle. The catalytic activity of purified muscle enzyme is enhanced to half its maximum value by about 10^{-7} M Ca^{++} (25). The free Ca^{++} concentration in cardiac muscle has been estimated to be less than 10^{-7} M by determinations *in vitro* of the threshold concentration of Ca^{++} which produced myofibrillar syneresis or ATPase activation (26).

The role of Ca^{++} in the catalytic activity of cardiac phosphorylase kinase is further illustrated by the observation that epinephrine substantially activated this enzyme in the no-calcium perfused hearts, presumably because of increased cyclic AMP. However, it produced little net conversion of phosphorylase *b* to *a*. This indicates that Ca^{++} is not required for the conversion of the inactive to active form of phosphorylase kinase. However, it should

be noted that lack of Ca^{++} delays epinephrine-induced activation of phosphorylase kinase about 5 sec relative to that in control experiments. This is consistent with the postulated role of Ca^{++} in the autocatalytic activation of kinase, but not in that catalyzed by a phosphorylase kinase kinase.[2]

The observation that the diminution of the epinephrine-induced phosphorylase activation coincided with the decline of contractile activity of hearts perfused with no-calcium medium suggests that the store of this ion necessary for contractile activity and that necessary for phosphorylase kinase activity are the same. An alternative hypothesis is that the store of Ca^{++} responsible for stimulation of kinase activity can be mobilized and made available to the enzyme only when the excitation-contraction coupling processes of cardiac muscle are intact.

K^+-induced depolarization appeared to interfere with the mechanism that promotes the activation of phosphorylase kinase, since only a small fraction of the kinase was present in the active form at zero time and during the response to epinephrine in the depolarized heart. The relatively small epinephrine-induced increase in cyclic AMP concentration in these hearts could account for the diminished activation of kinase. These observations suggest that excess K^+ interferes with the action of epinephrine on adenyl cyclase or an antecedent site. Studies (27) of the effect of K^+ depolarization on the isolated rat diaphragm indicate that excess K^+ had considerably different effects on skeletal muscle. Excess K^+ itself caused an increase in the cyclic AMP concentration, and epinephrine caused a further increase, comparable to the effect of the drug in the normal diaphragm.

Evidence that the amount of Ca^{++} available to phosphorylase kinase represents a mechanism for the physiological control of the activity of this enzyme suggests that the effects of drugs and hormones on the activation of phosphorylase involve more than increased production of cyclic AMP followed by activation of kinase. The effects of methylxanthines on cardiac phosphorylase activity and their ability to potentiate epinephrine-induced phosphorylase activation (28) have been attributed to their inhibition of cyclic nucleotide phosphodiesterase, although the actual concentration of this nucleotide was not measured. It is conceivable that both these effects are explained by an action of the methylxanthines on the release of intracellular Ca^{++}, an action reported in several studies (29, 30). The activation of phosphorylase in cardiac muscle by hypoxia (7) and in skeletal muscle by motor nerve stimulation (5) cannot be explained by the cyclic AMP-mediated mechanism. The role of calcium ion in these phenomena should be investigated.

It is possible that pharmacological agents may augment phosphorylase *b* to *a* conversion in cardiac tissue by a combination of the two mechanisms discussed above. Epinephrine may act by activating phosphorylase kinase through cyclic AMP and increase the activity of the kinase by mobilizing cardiac stores of Ca^{++}. Evidence supporting the latter action of the catecholamine has been reported (31).

Since Ca^{++} appears to be involved in the final pathway of phosphorylase activation, phosphorylase *b* to *a* conversion, any stimulus of cardiac phosphorylase activation, regardless of its mechanism of action, should be blocked by calcium removal. This hypothesis was supported by the observation[3] that glucagon no longer effected *b* to *a* conversion in the Ca^{++}-deficient, perfused rat heart.

That Ca^{++} may be involved in the regulation of the cardiac cyclic AMP concentration is suggested by two observations in this report. Removal of Ca^{++} caused a significant elevation in cyclic AMP (Fig. 1a). Excess Ca^{++} decreased the concentration of the cyclic nucleotide (Fig. 4). Ca^{++} may inhibit the activity of adenyl cyclase, possibly by competition with Mg^{++}, a cofactor of the enzyme (32). An alternative

[2] E. G. Krebs, personal communication.

[3] Unpublished observation.

hypothesis is that Ca^{++} stimulates the activity of cyclic nucleotide phosphodiesterase. There are, however, no reports of such a direct effect of the ion on this enzyme. The possibility that Ca^{++} increases phosphodiesterase activity indirectly by lowering the concentration of ATP, an inhibitor of the enzyme (33), was tested. Perfusion of hearts with the high-Ca^{++} medium did cause a significant reduction in heart ATP concentration 20 sec after the switch to this medium (from a control value of 3.82 μmoles/g to 3.23 μmoles/g). However, the decrease in cyclic AMP concentration was observed after only a 15-sec perfusion with the medium containing the high Ca^{++} concentration. Further studies of the effects of physiological ions on the enzymes responsible for the production and destruction of cyclic AMP are needed for a clearer understanding of the effect of Ca^{++} on this important nucleotide. Such studies should test the provocative hypothesis recently proposed by Rasmussen and Tenenhouse (34), that the transformation of ATP to cyclic AMP at the cell membrane causes Ca^{++} to become available. Thus Ca^{++} may integrate the variety of metabolic events that have been attributed to cyclic AMP as the mediator in the effects of hormones (7).

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