Dissociations between Changes in Myocardial Cyclic Adenosine Monophosphate and Contractility

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ABSTRACT

The relationship between changes in the myocardial concentration of adenosine 3'-5'-cyclic monophosphate (cyclic AMP) and cardiac contractility was studied in guinea pig and rat myocardium. When isolated perfused guinea pig hearts were perfused with $10^{-5}$M papaverine, a potent inhibitor of cyclic AMP phosphodiesterase activity, myocardial cyclic AMP concentration increased significantly from $1.7 \pm 0.2$ (SE) pmoles/mg protein ($N = 12$) to $3.3 \pm 0.2$ pmoles/mg protein ($N = 12$), and the percent of phosphorylase $a$ activity increased from $21 \pm 3\%$ ($N = 6$) to $44 \pm 8\%$ ($N = 6$) ($P < 0.01$). However, perfusion with papaverine had no effect on contractility in the absence or the presence of exogenous epinephrine. In perfused rat hearts, $10^{-5}$M glucagon increased myocardial cyclic AMP concentration from $1.5 \pm 0.1$ pmoles/mg protein ($N = 12$) to $2.6 \pm 0.1$ pmoles/mg protein ($N = 12$) ($P < 0.001$). In contrast, cyclic AMP levels did not increase detectably in guinea pig hearts perfused with glucagon. Glucagon increased adenylate cyclase activity more than twofold in rat myocardial broken cell preparations but failed to stimulate the enzyme in preparations from guinea pigs. Despite these differences, the positive inotropic effects of glucagon on rat and guinea pig hearts were very similar over a wide dose range. Thus, with both papaverine and glucagon, changes in cardiac contractility were dissociated from stimulation of adenylate cyclase activity, increases in myocardial cyclic AMP levels, and conversion of phosphorylase $b$ to phosphorylase $a$ in perfused hearts.

KEY WORDS isolated perfused hearts adenylate cyclase phosphodiesterase catecholamines

Substantial evidence suggests that changes in myocardial adenosine 3'-5'-cyclic monophosphate (cyclic AMP) are associated with alterations in cardiac contractility. However, it has been difficult to determine whether a cause and effect relationship exists between the two. The lack of a positive inotropic effect of exogenous cyclic AMP on isolated cardiac muscle in some studies has been attributed to poor penetration of the nucleotide into myocardial cells (1). However, exposure of perfused hearts to exogenous cyclic AMP at low temperature (2) or in the presence of dimethylsulfoxide (3), procedures that promote the entry of cyclic AMP into cells, results in conversion of phosphorylase $b$ to phosphorylase $a$ but does not appear to increase cardiac contractility.

The present study was stimulated by two preliminary observations. First, the adenylate cyclase activity of the particulate fraction from guinea pig myocardium does not appear to be stimulated by glucagon, although the peptide exerts the anticipated positive inotropic effects on the isolated heart. Second, papaverine, a potent phosphodiesterase inhibitor, does not exert a positive inotropic effect on isolated guinea pig hearts. In the present study, additional experiments were performed to examine these apparent dissociations between changes in contractility and alterations in myocardial cyclic AMP concentration and to explore some of their implications.

Methods

ISOLATED PERFUSED HEART PREPARATIONS

Male Hartley guinea pigs weighing 350-500 g and male albino Sprague-Dawley rats weighing 250-350 g were used. The animals were killed by stunning, and perfusion of the excised hearts was started within 30 seconds. Isovolumic left heart preparations were perfused with Krebs-bicarbonate buffer at a flow rate of 4.5 ml/min 100 g$^{-1}$ body weight; a roller pump (Gilford model MP-4) was used as previously described (4). Perfusion pressure under these conditions averaged $79 \pm 2$ mm Hg ($N = 21$) and declined slightly in the presence of $10^{-5}$M papaverine.
in the perfusate to 71 ± 4 mm Hg (P < 0.05). In some experiments, perfusions were performed at a constant pressure of 80 mm Hg. The perfusion medium had the following millimolar composition: NaCl 118, NaHCO3 25, KCl 4.7, CaCl2 2.5, MgSO4·7H2O 1.2, KH2PO4 1.2, and Na2EDTA 0.1. The medium was equilibrated with 95% O2-5% CO2 to a pH of 7.38 at 37°C. The manometric system used to measure left ventricular pressure and its first derivative, dP/dt, consisted of an intraventricular balloon, a short cannula, and a Statham P23Db transducer. All hearts were paced at a rate of 300 beats/min with a bipolar electrode attached to the right atrium; the stimulus, which was 1.5 msec in duration and 10% above threshold, was generated by a Grass model S4K stimulator.

Pharmacological experiments were performed after a 60-minute equilibration period. During control periods, the diluent without the test drug was infused at a rate equal to that used in the subsequent drug infusion. Papaverine was dissolved in Na2EDTA (0.1 mM, pH 7.4) and glucagon in glycol-glycine buffer (150 mM, pH 9.4) (5). These agents were infused at a constant flow rate (<0.1 ml/min) with a syringe pump (Harvard model 975) 4 cm upstream from the aortic root. Concentrations given in Results refer to final concentrations reaching the heart.

During control periods or at selected times during infusions of glucagon or papaverine, hearts for biochemical analyses were quickly frozen with Wollenberger clamps precooled in liquid nitrogen. Krebs buffer containing more than 5 x 10^{-6} M papaverine or more than 2 x 10^{-6} M glucagon did not form stable solutions, as reflected by the stability of the optical densities of the solutions at 247 nm (6) and 280 nm (7), respectively. Such concentrated solutions were not utilized in the present series of experiments, since they markedly impaired the function of the perfused hearts.

**BIOCHEMICAL PROCEDURES**

**Tissue Preparation and Extraction.**—For assays of adenylate cyclase and cyclic AMP phosphodiesterase activities, broken cell preparations were prepared from fresh hearts as previously described (8). In some experiments the homogenization step for the preparation of broken cells was modified; a single, 10-second burst with a Polytron shearing homogenizer (model PT20) was utilized. For assays of phosphorylase and myocardial cyclic AMP, fast-frozen myocardial samples from perfused hearts were stored at -65°C. Samples for determination of phosphorylase activity were homogenized and treated as recently reported (9). Homogenization and extraction of samples for cyclic AMP assays were performed as described by Wastila et al. (10).

**Chemical Assays.**—Cyclic AMP in myocardial extracts was assayed by the method of Wastila et al. (10), which is based on the activation of protein kinase from skeletal muscle.

Adenylate cyclase activity was assayed at 30°C in a final volume of 1.2 ml of incubation mixture containing 2 mM adenosine triphosphate (ATP), 4 mM MgCl2, 40 mM Tris (pH 7.4), 0.01 mM Ro20-1724 (4-[3-butyloxy, 4-methoxy-benzyl]-2-imidazolidinone), 1 ng/ml of bovine serum albumin, 1 mM dithiothreitol, and selected activators (glucagon, epinephrine, or NaF). Ro20-1724 is a phosphodiesterase inhibitor (11). The reaction was initiated by the addition of 0.2 ml of the broken cell preparation (~2-3 mg of protein) and terminated by the addition of 0.5 ml of the reaction mixture to 0.1 ml of 50% trichloroacetic acid. In some experiments, 10 mM theophylline-7-acetate or 0.01 mM papaverine-HCl was used instead of Ro20-1724 to inhibit phosphodiesterase activity. In some assays, an ATP regenerating system consisting of 3 mM phosphonoenolpyruvate and 30 IU/ml of pyruvate kinase was included. Some assays were performed in the presence of 0.1 mM guanosine triphosphate (GTP), a known cofactor of adenylate cyclase systems (12). Adenylate cyclase activity was assessed by measuring the rate of accumulation of cyclic AMP, assayed by the protein kinase activation method (10).

Phosphodiesterase activity was assayed by two methods. In the first, samples were incubated at 30°C in a medium containing 0.4 mM cyclic AMP (0.02 μC-γ-cyclic AMP/ml assay mixture), 4 mM MgCl2, and 40 mM Tris-HCl (pH 7.4). The reaction was started by adding 0.1 ml of the broken cell preparation (1.0-1.5 mg of protein) to a final volume of 1.0 ml and terminated by adding 0.2 ml of 7.2% ZnSO4·7 H2O (w/v) to the assay mixture followed by 0.2 ml of 6% BaOH (w/v) (15). Samples were centrifuged at 4000 g for 15 minutes, and the supernatant fraction was subjected to a second barium-zinc precipitation. Radioactivity was measured by liquid scintillation spectrometry.

In other experiments, phosphodiesterase activity was assayed by another procedure to validate results obtained with the barium-zinc method. In this second procedure, cyclic AMP was converted to adenosine with 5'-nucleotidase. Samples were assayed at 30°C in a final volume of 1.0 ml containing 40 μmoles of Tris, (pH 7.4), 8 μmoles of MgCl2, 6 pmoles of 3H-cyclic AMP (25 c/m mole), and selected concentrations of unlabeled cyclic AMP. The reaction was started by adding 0.1 ml of the broken cell preparation and terminated by adding 80 μmoles of HCl, 1.4 μmoles of cyclic AMP, and 1.6 μmoles of adenosine 5'-monophosphate (5'-AMP) in 0.25 ml. After heating for 4 minutes at 75°C, 80 μmoles of NaOH and 80 μmoles of Tris (pH 8.0) in 0.15 ml were added followed by 0.25 ml of 100 mM Tris (pH 8.0) containing 0.60 mg of Crotalus adamanteus venom (Sigma). The incubation with 5'-nucleotidase proceeded for 30 minutes at 37°C; at the end of the incubation, samples were diluted and centrifuged, and 3H-adenosine was isolated for radioassay as described by Murad et al. (14).

Glycogen phosphorylase was assayed by measuring glucose-1-phosphate production in the presence and the absence of 5'-AMP (15).

Protein was measured by the method of Lowry et al. (16) with bovine serum albumin as the standard.

**MATERIALS**

Nucleotides, glucose-1-phosphate, phosphoenolpyruvate, β-glycerophosphate, bovine serum albumin (Cohn fraction V), glycogen, and snake venom were purchased from Sigma Chemical Company. Enzymes used in the phosphorylase assay and pyruvate kinase were obtained from Boehringer Mannheim. Casein was obtained from Matheson, Coleman and Bell. QAE-Sephadex A-25 was purchased from Pharmacia. Glucagon and papaverine...
Effects of epinephrine on perfused guinea pig hearts in the absence and the presence of papaverine. Isovolumic left heart preparations were perfused as described in Methods. Epinephrine was infused during the last 15 seconds (horizontal bars) of 4-minute infusions of diluent alone (left) or of diluent with $10^{-7}$M papaverine (right). A: $10^{-7}$M epinephrine. B: $10^{-6}$M epinephrine. Effects of epinephrine on mechanical performance were unchanged by papaverine. LVP = left ventricular pressure and $dP/dt$ = first derivative of left ventricular pressure.

were purchased from Eli Lilly. Solutions of l-epinephrine bitartrate (Nutritional Biochem) were formulated immediately before use. Radioactively labeled nucleotides were obtained from New England Nuclear Corporation. Ro20-1724 was a gift.1

Results

EFFECTS OF PAPAVERINE ON PERFUSED GUINEA PIG HEARTS

Mechanical Performance.—Papaverine in concentrations ranging from $10^{-9}$ to $10^{-4}$M administered for 15-second to 4-minute intervals had no effect on left ventricular pressure and its first derivative, $dP/dt$, in any of the 21 guinea pig preparations tested. Furthermore, increases in peak left ventricular pressure in response to infusions of $10^{-8}$-$10^{-5}$M epinephrine for 15 or 30 seconds were not altered by preequilibrating the hearts for 4 minutes with perfusate containing $10^{-3}$M papaverine (Figs. 1 and 2). Similarly, in ten additional experiments, papaverine administered at a lower concentration ($10^{-7}$M) for 1 minute did not alter contractility and did not enhance the mechanical response to a subsequent infusion of $10^{-7}$M epinephrine for 15 or 30 seconds. Papaverine could be a myocardial depressant, thereby masking positive inotropic effects associated with increased cyclic AMP concentration. However, the results with epinephrine administered in the presence of papaverine militate against this possibility.

The solubility of papaverine-HCl in Krebs buffer is limited, even in the presence of 0.1 mM EDTA. At concentrations of papaverine exceeding $5 \times 10^{-4}$M or when single (bolus) injections were administered, a white precipitate was visible in the effluent, and a marked depression of contractility occurred. However, stable solutions of papaverine in Krebs buffer did not produce negative inotropic effects in any of the isovolumic hearts studied.

Cyclic AMP.—Infusions of $10^{-8}$M papaverine for 4 minutes resulted in significant increases in the myocardial concentration of cyclic AMP (Fig. 3). Infusions of $10^{-7}$M epinephrine for 30 seconds produced only slightly greater increases in cyclic AMP.

1 Generously supplied by Dr. S. E. Mayer, University of California, San Diego.
AMP concentration (Fig. 3). The greatest increase in myocardial cyclic AMP concentration was observed when \(10^{-7}\text{M}\) epinephrine was administered during the last 30 seconds of the 4-minute infusion of \(10^{-5}\text{M}\) papaverine (Fig. 3). Thus, papaverine, a known phosphodiesterase inhibitor, led to an additive effect on catecholamine-induced accumulation of cyclic AMP in the myocardium.

**Conversion of Phosphorylase b to Phosphorylase a.**—Four-minute infusions of \(10^{-5}\text{M}\) papaverine increased the percent of phosphorylase activity in the a form significantly (Fig. 4). Infusions of \(10^{-7}\text{M}\) epinephrine for 30 seconds increased phosphorylase a activity comparably (Fig. 4). When infusions of epinephrine were superimposed on infusions of papaverine, marked increases in phosphorylase a occurred (Fig. 4). These increases paralleled the changes in cyclic AMP levels in the same preparation (Fig. 3).

**EFFECTS OF PAPAVERINE ON CYCLIC AMP PHOSPHODIESTERASE ACTIVITY OF BROKEN CELL PREPARATIONS FROM GUINEA PIG MYOCARDIUM**

Concentrations of papaverine required to inhibit phosphodiesterase activity by 50% (\(I_{50}\)) in the presence of 400 \(\mu\text{M}\) cyclic AMP as substrate averaged 12.1 \(\mu\text{M}\) (\(N = 7\)) when phosphodiesterase activity was assayed by the barium-zinc precipitation method and 11.4 \(\mu\text{M}\) (\(N = 6\)) when it was assayed by measurement of adenosine as an end product. Thus, results with the two assay procedures corresponded closely. With the adenosine assay, \(I_{50}\) was 1.9 \(\mu\text{M}\) (\(N = 6\)) in the presence of 0.4 \(\mu\text{M}\) cyclic AMP. Corresponding \(I_{50}\) values for theophylline were 690 \(\mu\text{M}\) (\(N = 5\)) and 98 \(\mu\text{M}\) (\(N = 5\)) at the high and low substrate concentrations, respectively. Thus, papaverine was a substantially more potent inhibitor than was theophylline.

**EFFECTS OF GLUCAGON ON PERFUSED GUINEA PIG AND RAT HEARTS**

**Mechanical Performance.**—Infusions of \(10^{-9}-10^{-6}\text{M}\) glucagon for 30 seconds had marked and very similar positive inotropic effects in guinea pig and rat hearts (Figs. 5 and 6). Increases in left ventricular dP/dt paralleled increases in left ventricular pressure in both species. Base-line dP/dt in the guinea pig and rat averaged 1800 ± 80 mm Hg/sec (\(N = 18\)) and 1680 ± 70 mm Hg/sec (\(N = 13\)), respectively. Corresponding values at the highest concentration of glucagon (\(10^{-6}\text{M}\)) were 3800 ± 113 mm Hg/sec and 3350 ± 103 mm Hg/sec. The somewhat lower absolute values for dP/dt reflect the limited frequency response of hydraulic manometric systems.

**Cyclic AMP.**—Infusions of \(10^{-9}-10^{-5}\text{M}\) glucagon were maintained for 30 seconds, after which the hearts were quickly frozen with Wollenberger clamps. These infusions produced graded increases in myocardial cyclic AMP levels in rat hearts (Fig.
However, the same conditions failed consistently to increase the concentration of cyclic AMP in guinea pig hearts. The cyclic AMP concentration was not increased in guinea pig hearts frozen at earlier or later intervals after the onset of infusion of glucagon (5 seconds [N = 4], 20 seconds [N = 5], 60 seconds [N = 3], or 120 seconds [N = 4]). Thus, in contrast to the case in rat hearts, glucagon in concentrations that increased contractility markedly did not produce detectable changes in cyclic AMP in guinea pig hearts.

**Activation of Adenylyl Cyclase Activity by Glucagon in Broken Cell Preparations**

A representative example of a kinetic assay of adenylyl cyclase activity in the absence of glucagon and with $10^{-9}$M glucagon is illustrated in Figure 8. As can be seen, control activity in the guinea pig slightly exceeded that in the rat. With glucagon, only the activity in the rat appeared to be stimulated. Results of the adenylyl cyclase assays without and with glucagon are summarized in Figure 9. The rat exhibited a sigmoid log dose-response curve, but the guinea pig showed no stimulation of adenylyl cyclase at any concentration of glucagon. Since glucagon-sensitive adenylyl cyclase is a labile enzyme system, the unresponsiveness in the guinea pig preparations could reflect inactivation of the preparation in vitro. Nevertheless, the enzyme was activated by 10 mM NaF and $10^{-7}$M epinephrine, and the degree of

![Graph](image)

**FIGURE 6**

Effects of glucagon on isolated guinea pig hearts. Isovolumic left heart preparations were perfused as described in Methods. Values refer to peak responses to sustained infusions of glucagon. Control values (100%) of left ventricular pressure and $dP/dt$ in the guinea pig averaged $84 \pm 6$ (SE) mm Hg and $1800 \pm 80$ mm Hg/sec, respectively. Corresponding values in the rat were $78 \pm 4$ mm Hg and $1680 \pm 70$ mm Hg/sec. Vertical bars indicate $\pm$ 1 SE. The mechanical responses to glucagon in both species were similar.

![Graph](image)

**FIGURE 7**

Effects of glucagon on myocardial cyclic AMP concentration in perfused rat and guinea pig hearts. Hearts were rapidly frozen with Wollenberger clamps at the end of 30-second infusions of glucagon. Numbers in parentheses refer to the number of hearts studied with each concentration of glucagon. Vertical bars indicate $\pm$ 1 SF. The guinea pig heart did not exhibit an increase in cyclic AMP in response to glucagon, but the rat heart exhibited a dose-related increase in cyclic AMP.

**FIGURE 5**

Effects of glucagon on isolated guinea pig and rat hearts. Isovolumic left heart preparations were perfused as described in Methods. Arrows indicate the beginning and the end of infusions of glucagon. Values refer to peak responses to sustained infusions of glucagon. Control values (100%) of left ventricular pressure and $dP/dt$ in the guinea pig averaged $84 \pm 6$ (SE) mm Hg and $1800 \pm 80$ mm Hg/sec, respectively. Corresponding values in the rat were $78 \pm 4$ mm Hg and $1680 \pm 70$ mm Hg/sec. Vertical bars indicate $\pm$ 1 SE. The mechanical responses to glucagon in both species were similar.

**TABLE**

<table>
<thead>
<tr>
<th>Glucagon Concentration</th>
<th>Guinea Pig</th>
<th>Rat</th>
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<tbody>
<tr>
<td>$1 \times 10^{-9}$ M</td>
<td></td>
<td></td>
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<tr>
<td>$5 \times 10^{-9}$ M</td>
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<tr>
<td>$1 \times 10^{-7}$ M</td>
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LVP = left ventricular pressure and $dP/dt$ = first derivative of left ventricular pressure. The contractile response to glucagon was similar in the two species.
Assays of adenylate cyclase in broken cell preparations from guinea pig and rat myocardium without and with $10^{-6}$M glucagon. Assays were performed at 30°C in a final volume of 1.2 ml containing 2 mM ATP, 4 mM MgCl$_2$, 40 mM Tris (pH 7.4), 0.01 mM Ro20-1724, 1 mg/ml of serum albumin, 1 mM dithiothreitol, and 0.2 ml of the broken cell preparation. In contrast to the guinea pig preparation, the rat heart preparation exhibited increased cyclic AMP in response to glucagon.

![Figure 8](image)

**Figure 8**

Effects of epinephrine ($10^{-9}$M) and NaF ($10^{-2}$M) on adenylate cyclase activity of broken cell preparations from guinea pig and rat myocardium. Assay conditions were the same as those described in Figure 8. Vertical bars indicate ± 1 SE. Both rat and guinea pig heart preparations were responsive to stimulation by epinephrine or NaF.

![Figure 9](image)

**Figure 9**

Effect of glucagon on adenylate cyclase activity of broken cell preparations from guinea pig and rat myocardium. Assay conditions were the same as those described in Figure 8. Numbers in parentheses refer to the number of broken cell preparations studied at each concentration of glucagon. Vertical bars indicate ± 1 SE. Rat heart preparations, but not guinea pig heart preparations, were stimulated by glucagon.

![Figure 10](image)

**Figure 10**

C: Control  
E: Epinephrine ($10^{-9}$M)  
F: NaF ($10^{-2}$M)

Discussion

Results of the present study indicate that increases in myocardial cyclic AMP concentration produced by papaverine in the isolated guinea pig heart are not accompanied by increases in contractility. Furthermore, in this species, in contrast to the rat, glucagon appears to augment contractility.
without increasing myocardial cyclic AMP levels and to be ineffective in stimulating adenylate cyclase activity of broken cell preparations.

In the isovolumic left heart preparations used, coronary flow, heart rate, and muscle length, factors known to influence the mechanical performance of cardiac muscle, were controlled. Under these conditions, papaverine did not increase contractility of the guinea pig myocardium. However, since papaverine did not reduce the contractile response of these hearts to epinephrine, it apparently did not interfere with processes mediating the positive inotropic effect of catecholamines.

Previous reports on the effects of papaverine on cardiac muscle have been inconsistent. In early studies, bolus injections of papaverine were found to markedly depress isolated rabbit hearts (17, 18). However, the limited solubility of the agent was apparently not fully appreciated. Recently, papaverine has been reported to exert modest positive inotropic effects on isolated guinea pig hearts perfused at low pressure (60 cm H₂O) (19). Improved mechanical performance in these preparations may have reflected increased or redistributed coronary flow resulting from the vasodilating effects of papaverine. In the isolated guinea pig heart, papaverine has been reported to have no definite positive inotropic effect (20) and to produce no potentiation of catecholamine-induced positive inotropic responses (21), findings that appear to agree with the results of the present study.

Papaverine produced significant increases in cyclic AMP in perfused guinea pig hearts, as anticipated in view of its inhibitory effects on phosphodiesterase. Maximum increases in myocardial cyclic AMP produced by papaverine were of the same order of magnitude as those produced by 10⁻⁷M epinephrine, which markedly increased contractility. Increases in cyclic AMP produced by papaverine were associated with conversion of phosphorylase b to phosphorylase a, perhaps mediated by the activation of protein kinase by cyclic AMP. The present findings do not support the hypothesis that cyclic AMP per se mediates the inotropic effects of catecholamines. However, cyclic AMP may be compartmentalized in cells such that increases in average concentrations may occur without altering the cyclic AMP pool(s) involved in the regulation of contractility. Results reported by Brooker (22) based on experiments with frog ventricle exposed to N-isopropylmethoxamine, epinephrine, and norepinephrine suggest that a particular pool of intracellular cyclic AMP may be involved in mediating the positive inotropic effects of catecholamines and that this pool may not be related to pools of excess cyclic AMP produced during humoral stimulation of the myocardium. Other phosphodiesterase inhibitors have been reported to have no inotropic effect on the mammalian myocardium (23). On the other hand, theophylline may have positive inotropic effects at concentrations insufficient to inhibit phosphodiesterase (24). As may be the case for lipolysis in fat cells, effects of methylxanthines need not be due to inhibition of phosphodiesterase activity (25).

Results in the present study must be considered in connection with the possibility that papaverine exerted a direct myocardial depressant effect, masking the positive inotropic effects associated with increased intracellular cyclic AMP levels. However, the unimpaired mechanical response to the infusion of epinephrine in the presence of papaverine and the lack of detectable depression of baseline contractility by papaverine alone militate against this interpretation. Furthermore, a cancellation reflecting opposing effects producing no net change in mechanics over a 10⁻⁸-10⁻⁵M concentration range of papaverine appears unlikely. In contrast to the lack of effects on mechanics, even low concentrations of papaverine (<10⁻⁷M) produced an average increase of 35% in myocardial cyclic AMP concentration (N = 10) (P < 0.05).

Glucagon exhibited marked and comparable positive inotropic effects in rat and guinea pig hearts but increased myocardial cyclic AMP only in the former. In guinea pig hearts, glucagon failed to increase cyclic AMP significantly even in concentrations approaching the limits of solubility in Krebs buffer. Although the adenylate cyclase activity of broken cells prepared from guinea pig myocardium was readily activated by NaF and epinephrine, glucagon did not appear to stimulate its activity. This phenomenon could be attributed to the lability of glucagon-sensitive adenylate cyclase from this species in vitro. However, increased lability in vitro would not explain the failure of glucagon to increase the concentration of cyclic AMP in perfused hearts. Although small changes in cyclic AMP concentrations not detectable with the assay used could have occurred, our results suggest that changes in contractility induced by glucagon in guinea pig hearts do not depend on increases in myocardial cyclic AMP concentration.

In the present study, increases in myocardial cyclic AMP induced by papaverine were associated with conversion of phosphorylase b to phosphorylase a. Dissociations between conversion of phos-
phorylase and cardiac contractility have been reported repeatedly (9, 26-28). Recently, such dissociations have been produced by exposing isolated hearts to exogenous cyclic AMP in the presence of hypothermia or dimethylsulfoxide (2, 3). In the open-chest rat, an increase in myocardial cyclic AMP and phosphorylase a activity during anoxia is not associated with a positive inotropic response even though such a response is obtained with epinephrine (9). Thus, intracellular accumulations of cyclic AMP effected by different mechanisms and capable of inducing expected intracellular metabolic events have failed to produce increases in cardiac contractility.

Cyclic AMP plays an important role in the regulation of several metabolic pathways yielding energy necessary for the performance of muscular work (29). The frequent associations between increases in cyclic AMP in myocardium and increases in contractility could therefore reflect enhanced metabolism. In the present study, accumulation of myocardial cyclic AMP occurred without increases in contractility, and increases in contractility were produced by glucagon without concomitant increases in cyclic AMP. Although increases in cyclic AMP and contractility may not be causally related, it is possible that interventions promoting energy-yielding reactions in muscle including activation of glycolysis by a cyclic AMP-dependent mechanism (29, 30) may enhance the contractile performance of the heart under certain circumstances. In experiments in which mechanical performance was limited by insufficient energy supply, increases in the concentration of glucose provided to cardiac muscle increased contractility by a nonosmotic mechanism (4, 31). Since increased mechanical work of the heart can occur only in association with energy-yielding chemical reactions, the latter are difficult to dissociate from metabolic reactions regulating the inotropic state of the myocardium.

Results of the present investigation indicate that selected experimental interventions increase myocardial cyclic AMP associated with increased phosphorylase a activity without augmenting cardiac contractility. In addition, they indicate that augmentation of contractility by glucagon can be dissociated from accumulation of cyclic AMP in perfused guinea pig hearts and broken cell preparations from the same species. Thus, these findings suggest that increased contractility resulting from hormonal stimulation is not directly related to changes in average concentrations of myocardial cyclic AMP.

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References

1. Robison GA, Butcher RW, Øye I, Morgan HE, Sutherland EW: Effect of epinephrine on adenosine-3',5'-phosphate levels in the isolated perfused rat heart. Mol Pharmacol 1:168-177, 1965
2. Langlet A, Øye I: Role of cyclic 3',5'-AMP in the cardiac response to adrenaline. Eur J Pharmacol 12:137-144, 1970
17. Melville KL, Lu FC: Effects of epinephrine, aminophylline, nitroglycerine and papaverine on coronary inflow and on the heart contraction, as recorded concurrently. J Pharmacol Exp Ther 99:286-303, 1950
Hemmstoffen. Naunyn Schmiedebergs Arch Pharmacol 267:99-113, 1970
27. Mayer SE, Cotten MDeV, Moran NC: Dissociation of the augmentation of cardiac contractile force from activation of myocardial phosphorylase by catecholamines. J Pharmacol Exp Ther 139:275-282, 1963
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