The Effect of Epinephrine on Adenosine 3′,5′-Phosphate Levels in the Isolated Perfused Rat Heart

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SUMMARY

The effect of epinephrine on cyclic AMP levels, phosphorylase activity, glycogen synthetase activity, and myocardial contractility was studied using the isolated perfused working rat heart.

A single dose of epinephrine increased the level of cyclic AMP from 0.42 to 1.42 mμmoles/g within 3 sec of its administration, after which it rapidly declined toward the control level. The force of contraction meanwhile rose to 137% of its control value, but did not reach this peak until 20 sec after the injection. The phosphorylase a activity increased from an apparent value of 18.7% of total phosphorylase activity to 66.0%, but required 45 sec to obtain this maximum. No change in the glycogen synthetase activity could be detected.

Nethalide blocked the epinephrine-induced rise in the cyclic AMP concentration as well as the inotropic effect. *N*-Isopropylmethoxamine did not block either of these effects. It was suggested that epinephrine acts first on the membrane adenyl cyclase system to increase the rate of formation of cyclic AMP, which then in turn acts as a second messenger to initiate the inotropic response.

It was not possible to elicit a positive inotropic effect with cyclic AMP or several of its derivatives. Evidence was presented to show that cyclic AMP, when added to the perfusate, does not gain access to the intracellular fluid in concentrations comparable to those seen after the administration of epinephrine.

INTRODUCTION

The positive inotropic effect of epinephrine has been extensively investigated within recent years, but the biochemical basis of this action remains obscure (1–3). Recently evidence has accumulated to suggest that adenosine 3′,5′-phosphate (cyclic AMP) (4), which is known to be involved as a second messenger in a variety of hormonal responses (5), may also be involved in the inotropic response to epinephrine. Murad *et al.* (6) found that the relative potencies of a series of catecholamines in stimulating adenyl cyclase activity in broken cell preparations were similar to their potencies as inotropic agents *in vivo*. These investigators also showed that the cyclase stimulation, like the inotropic effect, was blocked by dichloroisoproterenol (DCI). Rall and West (7), working with electrically driven segments of rabbit atria, observed a potentiation by theophylline of the inotropic effect of norepinephrine. Theophylline is known to be an *in vitro* inhibitor of the phosphodiesterase that converts cyclic AMP to 5′-AMP (8). Preliminary experiments in this laboratory (9)

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indicated that the levels of cyclic AMP in the isolated perfused rat heart were elevated by the addition of epinephrine to the perfusate, and this has also been shown by Hammermeister *et al.* (10).

The principal purpose of the present work was to study the time course of the change in concentration of cyclic AMP following the injection of a single dose of epinephrine, and to compare this with the time course of the inotropic response. The activities of two enzymes known to be affected by cyclic AMP (glycogen phosphorylase and glycogen synthetase) were also measured. The results of these experiments lend support to the hypothesis that cyclic AMP is involved in the inotropic response to epinephrine.

If cyclic AMP is capable of penetrating the myocardial cell membrane without being destroyed by the phosphodiesterase, it should theoretically be possible to mimic the effect of epinephrine by perfusing the heart with cyclic AMP. Levine and Vogel (11), using unanesthetized dogs, have recently reported that the intracardiac injection of cyclic AMP is followed by increases in heart rate and cardiac output. Our attempts to obtain a similar result in the perfused rat heart, using cyclic AMP and several of its derivatives (12), were unsuccessful, leading us to carry out an experiment designed to measure the permeability of the rat heart to cyclic AMP.

MATERIALS AND METHODS

Perfusion techniques. Hearts were obtained from fed Sprague-Dawley female rats weighing 275–325 g. Rats were anesthetized with pentobarbital. Heparin (150 IU) was administered intravenously just prior to transection of the diaphragm. The heart was removed and attached to the perfusion apparatus essentially as described in an earlier paper (13). The basic perfusion medium was a modified Krebs-Ringer bicarbonate buffer containing 4.2 mM glucose plus glutamate and pyruvate at concentrations of 1.5 mM each. This was oxygenated with 95% O_2–5% CO_2 at a temperature of 37°. After the heart had begun to beat regularly the perfusion medium was changed to a heparinized solution of 0.9% NaCl at room temperature. This caused a rapid cessation of the heart beat. The pulmonary vein leading to the left atrium was then grasped with a pair of fine-tipped forceps and slipped over and tied onto a grooved perfusion cannula filled with perfusion fluid. After about 2 min the perfusion medium flowing into the aorta was changed back to the warm Krebs-Ringer buffer, and the heart was placed in a 37° chamber. The heart would then begin to beat regularly again, and as long as fluid was prevented from entering the left atrium it was defined as a nonworking heart. When buffer was allowed to flow through the atrial cannula the heart would pump this fluid through the aortic cannula to a bubble trap 70 cm above the heart, from whence it was returned to the main reservoir for recirculation. This preparation was defined as a working heart. That portion of the cardiac output which flowed through the coronary arteries and out the right atrium was returned to the main reservoir via an opening in the bottom of the heart chamber. The perfusion apparatus for these preparations has been described elsewhere (14). Pressure was measured with a Statham P 23 G transducer, and recorded by means of a Sanborn model 150 polygraph. In the present experiments the mean left atrial filling pressure was held at 12 mm Hg, while the diastolic pressure above the aortic valves was 50 mm Hg. Peak systolic pressure was taken as a measure of the contractile force. The contour of the pressure pulse of the working heart is illustrated in Fig. 1A. It will be seen that the inertia of the fluid column results in a considerable oscillation of pressure following the systolic discharge. Although of no consequence for the present experiments, this artifact can be eliminated by placing an air reservoir above the fluid leading to the pressure transducer (14).

The total volume of perfusate in the system was usually maintained at 100 ml, and drugs that were to be recirculated were added to this volume. For the experiments in which the effect of epinephrine on cyclic AMP was to be measured, recirculation was

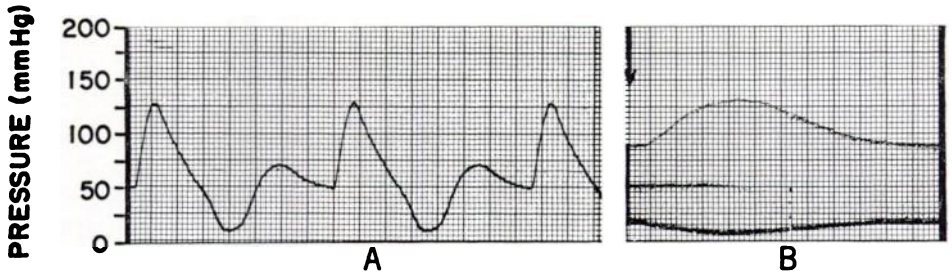

FIG. 1A. *High-speed tracing showing contour of the aortic pressure pulse of the isolated working rat heart*

Intervals between heavy lines on the abscissa represent 50 msec.

FIG. 1B. *The effect of 20 μg of epinephrine (injected at arrow) on the pulse pressure*

Intervals between heavy lines on abscissa represent 5 sec.

prevented by discarding both the coronary and aortic effluent. Epinephrine was dissolved in a concentration of 40 μg/ml in an unbuffered solution containing sodium, potassium, and calcium in the same concentrations as the regular buffer. A volume of 0.5 ml of this mixture was injected into the tubing leading to the atrial cannula, and the hearts were frozen at various times after injection by clamping between two aluminum blocks cooled in liquid nitrogen (15). Control hearts received 0.5 ml of the unbuffered saline solution without epinephrine. Figure 1B illustrates the effect of epinephrine. Although an accurate dose-response curve was not determined, this dose of epinephrine was estimated on the basis of preliminary experiments to be submaximal. Since 20 μg might be expected to be supramaximal in a Langendorff preparation, it should be pointed out that in the working heart, as used in these experiments, an injection of 0.5 ml would be pumped through the heart in approximately 3 sec, and of this volume not more than 25% would be forced through the coronary arteries. Hearts having a cardiac output (exclusive of the coronary flow) of less than 25 ml/min were discarded. The frozen hearts were stored at —70°. Prior to assay they were powdered in a percussion mortar chilled in liquid nitrogen.

Analytical methods. Tissue levels of cyclic AMP were measured by the method of Butcher *et al.* (16), using 0.4 cm inner diameter columns. Phosphorylase activity was assayed by the method of Danforth *et al.* (17), using glycerol extraction. The results are expressed in terms of *apparent* percentage of phosphorylase a (i.e., activity in the absence of added AMP × 100/ activity in the presence of 1 mM added AMP). Glycogen synthetase activity was measured, in the presence and absence of glucose 6-phosphate, by two methods. In either case, extracts were prepared by homogenizing the frozen muscle powder in 9 volumes of extraction fluid in a glass homogenizer at ice-bath temperature. The homogenates were then centrifuged for 20 minutes at 7000 *g* in a refrigerated centrifuge, and the pellet was discarded. The first method, based on that of Leloir and Goldemberg (18), involved measurement of the amount of UDP produced from UDP-glucose during a 45-min incubation period. The extraction fluid contained 0.4 M sucrose, 5 mM EDTA, 0.1 M Tris (pH 7.6), and 0.025 M fluoride. The extract was added to start the reaction, being diluted fivefold in the process. The final reaction mixture contained the equivalent of 1 mg of tissue (wet weight), 7.5 μmoles imidazole (pH 7.6), 0.25 μmole UDP-glucose, 0.5 μmole glucose 6-phosphate (when present), and 0.4 mg glycogen in a total volume of 0.05 ml. The absence of significant quantities of glucose 6-phosphate in the reaction mixtures to which it had not been added was ascertained enzymically with glucose 6-phosphate dehydrogenase. The second method for glyco-

gen synthetase, based on that of Steiner *et al.* (19, 20), involved measurement of the amount of labeled glucose from UDP-glucose incorporated into glycogen during a 10-min incubation period. The extraction fluid contained 0.4 M sucrose and 5 mM EDTA, and this was diluted threefold in starting the reaction. The final reaction mixture contained the equivalent of 5 mg of fresh tissue, 10 μmoles glycerophosphate (pH 7.4), 0.15 μmole UDPG, 1 μmole glucose 6-phosphate (when present), and 1 mg glycogen in a total volume of 0.15 ml. Following the terminology of Friedman and Larner (21), according to which the activity in the absence of glucose 6-phosphate reflects the activity of the I form of the enzyme, while the increment in the presence of glucose 6-phosphate is due to the D form, the results were calculated in terms of "apparent % I" (activity in the absence of glucose 6-phosphate × 100/activity in the presence of glucose 6-phosphate).

Isotope technique for distribution studies. The perfusion medium for measurement of cyclic AMP uptake contained 2.7 mM sorbitol and 0.1 mM cyclic AMP. Nonworking hearts were used for these experiments. After a 10-min washout period, the hearts were perfused for 35 min with a solution containing D-sorbitol-1-^{14}C (approximately 0.04 μC/ml) and tritiated cyclic AMP (approximately 0.05 μC/ml). Aliquots were taken at 5 and 35 min and analyzed for ^{14}C, tritium, and cyclic AMP. At the end of the perfusion period the heart was cut from the cannula into a beaker of ice-cold saline and immediately bisected with scissors. It was then blotted on filter paper and frozen in liquid nitrogen. For simultaneous measurements of ^{14}C and tritium, aliquots of frozen tissue (100–200 mg) were dried and then oxidized to CO_2 and water in a Thomas-Ogg combustion chamber. After combustion was complete the flasks were cooled on blocks of dry ice and then placed in a freezer at −20°. A volume of 6 ml of 20% ethanolamine in methanol was then injected into each flask, and the sealed flasks were allowed to stand at room temperature, with occasional swirling, for approximately 8 hr. A 3.0-ml aliquot of this mixture was then transferred to a plastic counting vial to which was added 30 ml of Bray's solution (22), and the samples were counted at 9° in a Packard Tri-Carb liquid scintillation spectrometer. The addition of the ethanolamine-methanol to Bray's solution results initially in a heterogeneous system, but this clears within 1 hr on standing. The counts per minute contributed by ^{14}C and tritium were calculated by the discriminator-ratio method (23). The specific activities of sorbitol and cyclic AMP under these conditions were approximately 1100 cpm/μmole and 60,000 cpm/μmole, respectively, and the measured radioactivity was a linear function of the nuclide concentration up to at least 5 times the concentrations encountered in this experiment. The sorbitol space was calculated as described earlier (13) on the basis of ^{14}C activity, and was assumed to be equal to the extracellular space. The cyclic AMP space was similarly calculated on the basis of both tritium activity (*apparent* cyclic AMP space, equivalent to the volume occupied by the nucleotide plus its metabolites) and enzymically measured levels in the tissue. Cyclic AMP in excess of that which could be accommodated within the sorbitol space was assumed to be in the intracellular water (total water minus extracellular water), and on this basis the intracellular concentration of cyclic AMP was calculated.

Materials. The epinephrine used was a commercial product of Parke, Davis and Company. Nethalide, 1-(2-naphthyl)-2-isopropylaminoethanol HCl, was a gift to Dr. H. C. Meng from Imperial Chemical Industries, Ltd., and *N*-isopropylmethoxamine was given to us by Dr. J. J. Burns of the Wellcome Research Laboratories. Cyclic AMP and tritiated cyclic AMP were purchased from Schwartz BioResearch, Inc. The derivatives of cyclic AMP were synthetized by Dr. Th. Posternak as part of a collaborative project (12). UDP-glucose-^{14}C was prepared by Dr. J. G. T. Sneyd according to the method of Steiner *et al.* (19, 20), and was a gift from Dr. Sneyd. D-Sorbitol-1-^{14}C was purchased from the New England Nuclear Corporation. All

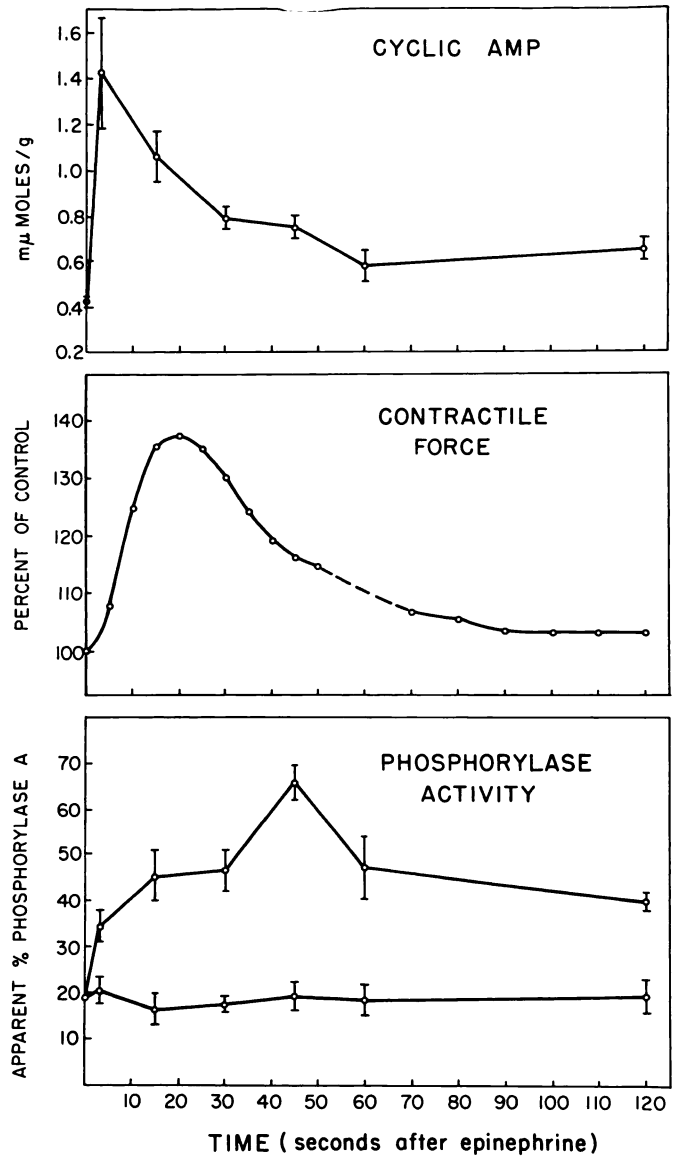

FIG. 2. *Effect of epinephrine on cyclic AMP concentration, myocardial contractility, and phosphorylase activity*

Hearts were perfused for 30 min after ventricular work had begun, and were frozen at 3, 15, 30, 45, 60, and 120 sec after the injection of 20 μg of epinephrine.

In the upper panel (cyclic AMP) the point at zero time represents the mean of 28 control hearts frozen at various times after the injection of saline. The point at 120 sec is the mean of 5 hearts, while each of the other points represents the mean of 6 hearts. The middle panel represents the average change in contractility seen in the hearts frozen at 60 and 120 sec. Peak systolic pressure prior to injection of epinephrine was taken as 100%. In the lower panel (phosphorylase activity) the value at zero time is the mean of all 36 control hearts, which are plotted individually in the lower curve (6 hearts per point). The upper curve is plotted as described for cyclic AMP. Each vertical bar represents two standard errors of the mean.

other reagents were commercial products of the highest purity available.

RESULTS

The time course of the changes in cyclic AMP concentration, contractile force, and phosphorylase activity is shown in Fig. 2. The control level of cyclic AMP was 0.42 ± 0.02 mμmole/g (wet wt.) of tissue. Within 3 sec after the injection of epinephrine this level increased nearly fourfold, and then rapidly declined. The contractile force did not reach a peak until 20 sec after the injection of epinephrine, and phosphorylase a did not reach a maximum until 45 sec. Of the three parameters shown in Fig. 2, the phosphorylase change was the longest lasting. Even after 120 sec the percentage of phosphorylase a remained close to 50% of the maximum increase seen at 45 sec.

A series of experiments were carried out to determine the effect of nethalide, an adrenergic blocking agent (24), on the cyclic AMP response (Table 1). After the heart had been working for 25 min, nethalide was added to the perfusion fluid to give a concentration of 7.5 μg/ml. This was recirculated for 5 min. Epinephrine or saline was then injected as usual, and the heart was frozen 10 sec later. Nethalide completely blocked the inotropic response to epinephrine and, as shown in Table 1, completely blocked the cyclic AMP change as well. The inhibition of phosphorylase activation by nethalide has been reported earlier (25).

TABLE 1

Effects of epinephrine and nethalide on cyclic AMP levels in heart tissue

Four groups of three hearts each were used. Nethalide and epinephrine were administered as described in the text. Results are expressed as mμmoles per gram (wet wt.) of tissue, each value representing the mean (± standard error) of three hearts.

Group	Cyclic AMP (mμmoles/g)	*P*
Control	0.50 ± 0.07	—
Nethalide	0.43 ± 0.05	—
Epinephrine	1.86 ± 0.32	<0.02
Nethalide + epinephrine	0.60 ± 0.12	—

The possible effect of *N*-isopropylmethoxamine (IMA) (26) was investigated in another series of experiments (Table 2).

TABLE 2

Effect of epinephrine on cyclic AMP levels and phosphorylase activity in the presence of N-isopropylmethoxamine

Two groups of four rats each were used. *N*-Isopropylmethoxamine and epinephrine were administered as described in the text. Each value represents the mean (± standard error) of four hearts.

Parameter	Control	Epinephrine	*P*
Cyclic AMP (mμmoles/g)	0.45 ± 0.04	1.36 ± 0.14	<0.01
% Phosphorylase a	19.1 ± 3.7	49.6 ± 4.6	<0.01

This agent was of considerable interest because it is capable of blocking the epinephrine-induced rise of blood glucose, lactic acid, and free fatty acid levels in dogs, as well as the *in vitro* effect of epinephrine on rabbit liver phosphorylase, yet does not block the inotropic effect of epinephrine (26, 27). In the present work a dose of IMA of 14.5 μg/ml (5×10^{-5} M) was used. The drug was added to the perfusate after the heart had been working for 25 min, and was allowed to recirculate for an additional 5 min. Either saline or epinephrine was then injected, and the heart was frozen 10 sec later. This dose of IMA caused an average decrease in heart rate of 21% (from 193 beats/min before the addition of IMA to 152 beats/min at 3 min after it had been added). Doses much in excess of 14.5 μg/ml could not be used because of arrhythmias, together with a drastic lowering of the cardiac output. Comparison of the data in Table 2 with those in Fig. 2 shows that IMA does not reduce the epinephrine effect on cyclic AMP or phosphorylase. The inotropic response to epinephrine could actually be prolonged by

IMA. When epinephrine was recirculated at a concentration of 0.2 μg/ml, in the absence of IMA, the inotropic response was over within 2–3 min. In the presence of 14.5 μg/ml of IMA, however, the inotropic effect was maintained indefinitely. This is most likely due to blockade by IMA of the chronotropic response to epinephrine. The interval-strength relationship of the rat heart is anomalous in that an increase in rate results in a decrease in force (28).

The values for the percentage of glycogen synthetase in the I form differed somewhat depending on the method of assay, but were in neither case affected by epinephrine over a period of 2 min. The method of Leloir and Goldemberg (18) indicated that 26.9% of the synthetase was in the I form, with an average total glycogen synthetase activity of 79.2 μmoles of UDP formed per hour per gram of tissue. At no time was there a significant difference between the control hearts and those that had been exposed to epinephrine.

Attempts to elicit a positive inotropic response with either cyclic AMP itself or several of its derivatives (12) were completely negative. The dibutyryl derivative was tested in a series of thirteen working hearts. Recirculated in concentrations up to 6×10^{-4} M, it failed to alter the performance of the heart in any way. Cyclic AMP itself, the *N*-monobutyryl derivative (12), and the recently synthetized *N*-benzoyl derivative were likewise ineffective.

Since it was not known whether these compounds could penetrate the myocardial cell membranes of the rat heart, the experiments designed to measure the uptake of cyclic AMP were carried out as described in Materials and Methods. Analysis of the perfusate for ^{14}C, tritium, and cyclic AMP showed no decrease in the concentration of sorbitol or cyclic AMP during 30 min of perfusion with the radioactive buffer (Table 3). It was not possible to detect increased intracellular cyclic AMP by enzymic assay, since the average tissue concentration to be expected on the basis of the perfusate concentration (Table 3) and the sorbitol space (Table 4) was 26.7 mμmoles/g, whereas the actual measured concentration, using our standard assay (16), was 24.2 ± 0.8 mμmoles/g.

TABLE 3

Effect of perfusing rat heart for 30 min on the perfusate concentrations of sorbitol and cyclic AMP

Initial perfusate volume was 20 ml. Aliquots were removed 5 and 35 min after perfusion with the radioactive buffer had begun. These were diluted fivefold and counted after mixing 1 ml with 20 ml of Bray's solution (22). Contributions from ^{14}C and ^{3}H were calculated by the discriminator-ratio method (23). The aliquots were diluted 250-fold for measurement of cyclic AMP. Each value followed by a standard error represents the mean of four hearts.

Parameter	Time of perfusion (min)	
	5	35
^{14}C (cpm/ml)	18436 ± 8	18532 ± 50
Tritium (cpm/ml)	24513 ± 72	24590 ± 42
$^{14}C/^{3}H$ ratio	0.75	0.75
Cyclic AMP (mμmoles/ml)	92.3 ± 2.1	92.4 ± 1.4

Comparison of tissue tritium radioactivity with perfusate tritium (Table 4) indicated that the tritium space was about 357 μl/g, or about 68 μl/g greater than the sorbitol space. This would suggest that after 35 min of perfusion sufficient tritium had accumulated intracellularly to be the equivalent of 12.2 mμmoles of cyclic AMP per milliliter of intracellular water. If these tritium counts were still in the form of cyclic AMP, the enzymic assay would have been capable of detecting it. Analysis of tissue extracts by paper chromatography indicated that approximately 27% of the tritium present could be accounted for as metabolites of cyclic AMP (versus about 2% of the tritium in the perfusate). If one assumes that all the tritium which entered the cell entered in the form of cyclic AMP, and was then modified intracellularly and trapped there, the average rate of entry could be estimated to be about 0.35 mμmoles per milliliter of intracellular water per minute. Figure 2 shows that between 3 and 60 sec after epinephrine the rat heart is on the average capable of

TABLE 4

Apparent uptake and intracellular accumulation of cyclic AMP and/or its metabolites by the perfusea rat heart

Each of 4 hearts were perfused for 35 min with radioactive buffer, as described in Materials and Methods section. All values for sorbitol and cyclic AMP in this table are based on measurements of ^{14}C and ^{3}H, respectively, using the original buffer as the standard. Values followed by a standard error are the means of 4 hearts.

Parameter	Value
Tissue sorbitol (μg/g)	150 ± 3
Perfusate sorbitol (μg/ml)	521 ± 1
Sorbitol space (μl/g)[a]	289 ± 6
Apparent tissue cyclic AMP (mμmoles/g)	34.2 ± 1.9
Apparent perfusate cyclic AMP (mμmoles/ml)	95.8 ± 0.9
Apparent cyclic AMP space (μl/g)[a]	357 ± 21
Total tissue water (μl/g)	820 ± 7
Intracellular water (μl/g)	531 ± 12
Apparent intracellular cyclic AMP (mμmoles/ml)[b]	12.2 ± 2.6

[a] Space, μl/g $= \frac{\text{(tissue concentration, μg/g) (1000)}}{\text{(perfusate concentration, μg/ml)}}$

[b] Intracellular cyclic AMP, mμmoles/ml = (cyclic AMP space − sorbitol space, μl/g) times $\frac{\text{(perfusate cyclic, AMP, mμmoles/ml)}}{\text{(total water − sorbitol space, μl/g)}}$

reducing its intracellular concentration of cyclic AMP from 2.84 to 1.16 mμmoles/ml (assuming 50% of the wet weight of the tissue to be intracellular water), representing an average rate of destruction of 1.77 mμmoles/ml/min. This value is likely to be an underestimate of the true rate of destruction, since the peak concentration of cyclic AMP after epinephrine may in fact be greater than the 3-sec value plotted in Fig. 2. Even on this basis, however, it can be seen that the heart is capable of destroying cyclic AMP at a rate five times faster than the calculated rate of entry. If some of the cyclic AMP was metabolized before it entered the cells, then the rate of entry would be correspondingly less.

It is also possible to calculate the rate of entry on the assumption that the 2% of perfusate tritium which could not be accounted for as cyclic AMP represents material that entered the intracellular space as cyclic AMP, was metabolized there, and subsequently escaped into the external medium. The data necessary for this calculation are the apparent perfusate concentration (95.8 mμmoles/ml), the final perfusate volume (approximately 15 ml), the time of perfusion (35 min), the weight of the heart being perfused (approximately 1.2 g), and the intracellular water (531 μl/g). The rate of entry on this basis is 1.63 mμmoles/ml/min (1.28 mμmoles/ml/min plus the 0.35 mμmole/ml/min calculated previously), which is still less than the rate of destruction calculated from the data in Fig. 2. These observations would suggest that under the conditions of these experiments exogenous cyclic AMP does not enter the cells of the rat heart at a rate sufficient to build up intracellular concentrations comparable to those seen after the injection of epinephrine.

DISCUSSION

It has now been demonstrated that epinephrine causes a rapid transient increase in the concentration of cyclic AMP in the isolated perfused working rat heart. Since this change precedes the inotropic response, the data are in accord with the hypothesis that cyclic AMP is an intermediate in the inotropic effect of epinephrine. This hypothesis suggests that epinephrine must first interact with and activate the membrane adenyl cyclase system (29), resulting in an increased intracellular concentration of cyclic AMP. The cyclic AMP acts in turn to trigger another event which then leads to the increased force of contraction. This hypothesis is supported by the studies with nethalide and *N*-isopropylmethoxamine. Nethalide blocks both the cyclic AMP effect and the inotropic response, while IMA, which blocks many of the metabolic effects of epinephrine (26), blocks neither the rise in cardiac cyclic AMP nor the inotropic response. Our inability to mimic the myocardial effects of epinephrine with cyclic AMP, which appears to be possible in the unanesthetized dog (11), is explained on the basis of the

low permeability which the rat heart exhibits toward this nucleotide, together with its high phosphodiesterase activity. Whether or not conditions can be found in the perfused rat heart that will permit the intracellular accumulation of exogenous cyclic AMP and the simultaneous measurement of contractile force is a matter for future research.

Assuming that cyclic AMP is involved in the inotropic response to epinephrine, the question arises as to how it might be involved. Experiments which appeared to show a direct effect of the nucleotide on the rate of superprecipitation of actomyosin could not be confirmed (30). The earlier notion that epinephrine might act through phosphorylase activation seems to be no longer probable (31–33), and the increased oxygen consumption seen after epinephrine appears to be a result rather than the cause of the inotropic effect (34, 35). Hypotheses based on the idea that an increased availability of intracellular ATP would necessarily lead to an increased force of contraction seem unattractive in any event, since work with various muscle models (36, 37) would suggest that increasing the concentration of ATP could as easily lead to a negative inotropic effect as a positive one.

Recently, Koch-Weser *et al.* (38) have presented evidence to support their contention that epinephrine increases myocardial contractility chiefly by increasing the amount of the positive inotropic effect of activation (PIEA) produced per beat (28); epinephrine has little or no inotropic effect if the interval between beats is sufficiently prolonged (28, 38). Since it may be assumed that epinephrine stimulates the adenyl cyclase system whether the heart is beating or not (6), a reasonable suggestion would seem to be that cyclic AMP exerts its effect on an enzyme or enzyme system involved in the production of the PIEA. The chemical nature of the PIEA is presently so obscure, however, that further speculation on this point would not be warranted.

While it is well known that the amount of phosphorylase in the "a" form increases following the administration of epinephrine (39), the time course of this response has not previously been measured. It is interesting that the maximum phosphorylase response lags behind the inotropic response. Indeed it is conceivable that part of the phosphorylase effect may be due to the increased work of the heart rather than to the effect of epinephrine per se. Electrical stimulation of anaerobic skeletal muscle leads to increased levels of phosphorylase a in that tissue (17, 40, 41).

The apparent lack of effect of epinephrine on cardiac glycogen synthetase is in agreement with the recently reported work of Williams and Mayer (42). These investigators, using an open-chest rat preparation, showed that while large doses of epinephrine could cause an increase in the percentage of the enzyme in the I form, smaller doses, capable of eliciting a maximal inotropic response, had no significant effect on cardiac glycogen synthetase activity. It seems likely that other factors, including the tissue glycogen level (43), may be more important in regulating the activity of this enzyme than are transient changes in the level of cyclic AMP. An effect on glycogen synthetase would not appear to be an important component of the inotropic response to epinephrine.

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