Metabolic Effects of Epinephrine in the Perfused Rat Heart

II. Control Steps of Glucose and Glycogen Metabolism

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SUMMARY

This paper describes the kinetics of the changes in the levels of the glycolytic intermediates, phosphorylase *a*, cyclic AMP, creatine phosphate, the adenine nucleotides, and inorganic phosphate in isolated perfused rat hearts following the administration of epinephrine.

Cyclic AMP, ADP, AMP, creatine and inorganic phosphate, all increased monotonically from the onset of the contractile force increase. Cyclic AMP levels reached peak values after about 10 sec, and maximum values of ADP, AMP, creatine, and inorganic phosphate were observed after about 25 sec. The increases of ADP and AMP were stoichiometrically accounted for by a fall in ATP, and the increases of creatine and inorganic phosphate by a fall in creatine phosphate. Phosphorylase *a* activity increased by 60% and reached a broad peak after 20–30 seconds. This increase occurred a few seconds after the onset of the inotropic response. The tissue levels of all the glycolytic intermediates also increased transiently, there being a 10–15 sec delay relative to the inotropic response. These results demonstrate the transient effect of cyclic AMP on the phosphorylase system, and the temporal separation of the inotropic and glycogenolytic effects of epinephrine.

Control sites in the utilization of glucose and glycogen have been identified at the steps of glucose penetration into the cell, hexokinase, phosphofructokinase, and at an enzymic site between glyceraldehyde-3-P and 3-P glyceric acid. The rate of intracellular glucose and glycogen utilization appears to be controlled principally by the activities of phosphorylase and phosphofructokinase. Measurement of the complete pattern of glycolytic intermediates during the period of rapid glycogenolysis induced by epinephrine has permitted an analysis of the control exerted at the phosphofructokinase site due to changes in the tissue content of adenine nucleotides, inorganic phosphate, and cyclic AMP.

INTRODUCTION

It has previously been shown that epinephrine increases the glucose uptake of the rat heart perfused with and without insulin (1), and also increases the level of glucose-6-P to a value well above the *in vitro* inhibitory constant for hexokinase (2). These results contrast with those obtained when epinephrine is added *in vitro* to incubated rat diaphragms (3) or to anesthetized rats *in vivo*, and the diaphragm and skeletal muscle subsequently analyzed for intracellular glucose and glucose-6-P (4, 5). Kipnis *et al.* (5) observed a simultaneous accumulation of glucose and glucose-6-P in muscle tissues of the rat after epinephrine treatment, and they interpreted these findings as indicating a decreased rate of glucose phosphorylation due to glucose-6-P inhibition of hexokinase.

A possible reason for the apparently different metabolic response of cardiac and skeletal muscle may be that while the

perfused heart preparation is performing contractile work, the incubated skeletal muscle and diaphragm preparations are quiescent. It is possible, therefore, that glucose uptake may be increased when work is being done by heart muscle because of an activation of one or more rate-controlling steps in the glycolytic sequence. Phosphofructokinase is one possible site for this type of feedback control. Its activity can be greatly modified by changes in the adenine nucleotides and inorganic phosphate (6), and it has also been shown to exert a controlling influence on the rate of glycolysis in rat heart under a variety of metabolic conditions (7–9).

In the present study, a detailed kinetic investigation of metabolite changes following epinephrine administration to the perfused rat heart is reported. Control sites in the glycolytic pathway have been identified, and an attempt has been made to determine the nature and degree of control at these sites by the measurement of changes in the levels of key intermediates in the heart during and after the period of rapid glycogenolysis. In this manner, it has been possible to establish whether evidence for control at enzymic sites obtained from *in vitro* studies are consistent with metabolite changes observed in the intact tissue. Preliminary accounts of part of this work have been published (2, 10, 11).

MATERIALS AND METHODS

Male Sprague-Dawley or Wistar rats weighing between 220 and 280 g were used. All animals had unrestricted access to food and water. Techniques used for heart perfusion, isotopic techniques, and most of the analytical procedures for the estimation of metabolic intermediates are fully described elsewhere (1, 9, 12). Inorganic phosphate was determined in fresh perchloric extracts by a minor modification of the method of Wahler and Wollenberger (13), and the optical density of the phosphomolybdate complex in isopropyl acetate was measured at 310 mμ in a Zeiss spectrophotometer.

Analytical determinations reported in Tables 1 and 2 and Figs. 2, 4, and 5 were made using spectrophotometric assay procedures, and fluorometric methods (9, 14) were employed for most of the other analyses. Cyclic AMP[1] was assayed by the method of Posner *et al.* (15).

RESULTS

Effects of Epinephrine on Glucose-6-^{14}C Metabolism

The results of an experiment, in which epinephrine (final concentration 0.2 μg/ml) was added after 30 min to hearts perfused with medium containing glucose-6-^{14}C and 5×10^{-5} M EDTA, are summarized in Fig. 1. Between 15 and 60 min of perfusion, the rates of glucose uptake, counts removed from the medium, and $^{14}CO_2$ production were linear in control hearts but were markedly stimulated by epinephrine, the results being similar to those previously reported with uniformly labeled glucose-^{14}C (1). Glycogen served as the precursor

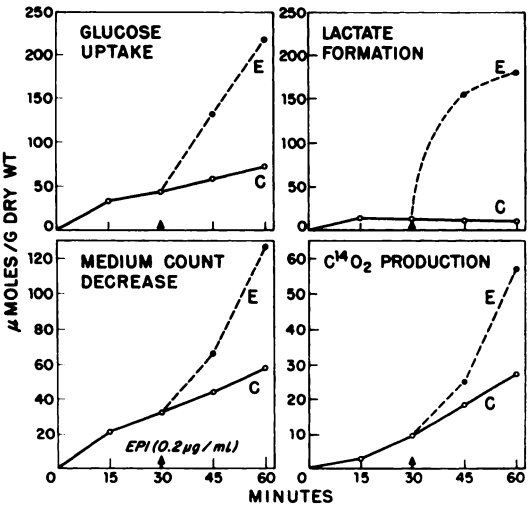

FIG. 1. *Effect of epinephrine on the uptake and metabolism of glucose-6-^{14}C*

The hearts were perfused with 20 ml medium containing 5 mM glucose, 5×10^{-5} M EDTA, and 0.1 μC/ml glucose-6-^{14}C. Epinephrine (E) was added after 30 min to give a final concentration of 0.2 μg/ml in the circulating medium. Each point represents the mean with 2–4 hearts in each group.

[1] The cyclic AMP measurements were done in collaboration with Dr. W. Y. Cheung.

of most of the lactate formed in the presence of epinephrine. The nonlinear increase in the rate of $^{14}CO_2$ production indicated that unlabeled pyruvate diluted the pyruvate-^{14}C pool during the first 15 min of perfusion with epinephrine. Parallel studies with glucose-1-^{14}C showed that the ratio of the specific yields (16) of $^{14}CO_2$ from glucose-6-^{14}C and glucose-1-^{14}C were not significantly different from unity, both in the presence and absence of epinephrine. Therefore, despite the elevated levels of glucose-6-P, it is unlikely that oxidation of glucose by the pentose-phosphate pathway represents a quantitatively significant contribution to the total glucose metabolism in epinephrine treated hearts. It has been shown previously (17) that insulin likewise failed to affect the specific yield of $^{14}CO_2$ from glucose-1-^{14}C and -6-^{14}C, but both insulin (17) and epinephrine (18) increased the ratio of NADPH to NADP in the rat heart.

Effect of Epinephrine on Glucose Phosphorylation

The rate of glucose phosphorylation in the heart is measured by the difference between the rate of glucose uptake and the rate of accumulation of intracellular glucose (19). Normally, free glucose cannot be detected in the perfused rat heart; all the glucose which enters the cells is rapidly phosphorylated. An increase in glucose uptake without glucose accumulation in the intracellular fluid signifies that there has been an increase in the rate of transport into the cell. When insulin is present in the perfusate, the permeability restriction is removed, and the glucose space becomes greater than the extracellular space. Under these conditions, the rate of glucose uptake is limited by the rate at which it can be phosphorylated and metabolized in the glycolytic pathway.

Table 1 shows the effect of epinephrine (0.2 μg/ml), in the presence and absence of insulin, on the intracellular water volume, and on the ratio of the glucose space to the sorbitol (extracellular) space of the heart. Epinephrine caused a transient increase of the intracellular volume, both in the presence and in the absence of insulin. In the absence of insulin, the glucose space was slightly less than the extracellular space, and epinephrine had no effect on the ratio of these quantities. On the other hand, in the presence of insulin, the glucose space was greater than the extracellular space, denoting the presence of free glucose in the

TABLE 1

Effect of epinephrine on the intracellular water volume and the ratio of the glucose space to the sorbitol space

After 15 min of preliminary perfusion with fluid containing 5 mM glucose, 5×10^{-5} M EDTA, 0.5 g/l ^{14}C-D-sorbitol (uniformly labeled), and 2 milliunits/ml insulin (if present in the later perfusion), hearts were transferred to a recirculation apparatus and perfused for the times stated with 15 ml of a similar fluid containing insulin and/or epinephrine, as noted in the Table. Values shown are the means ± standard error of the mean of 4 or more hearts.

Time of perfusion (min)	Epinephrine (0.2 μg/ml)	Intracellular water (μl per g of heart, dry wt)		Glucose space/sorbitol space (%)	
		No insulin	Insulin	No insulin	Insulin
0	−	2296 ± 40	2148 ± 32	93 ± 0.5	115 ± 3.5
1	+	2485 ± 48	2305 ± 21	97 ± 0.4	126 ± 2.6
3	+	2752 ± 56	2538 ± 47	92 ± 0.6	115 ± 1.4
5	−	2077 ± 118	2042 ± 55	90 ± 0.9	112 ± 3.2
5	+	2567 ± 25	2292 ± 24	89 ± 0.5	111 ± 1.1
10	−	2105 ± 57	1872 ± 18	90 ± 1.9	112 ± 1.1
10	+	2581 ± 28	2225 ± 59	91 ± 0.9	119 ± 4.8
30	−	2071 ± 24	1884 ± 32	91 ± 0.8	113 ± 1.3
30	+	2140 ± 47	1928 ± 26	83 ± 3.0	131 ± 2.0

cells. Epinephrine produced a small increase in the ratio of the glucose space to the extracellular space only after the first minute and at the end of 30 min.

The effect of epinephrine in the presence of insulin on the intracellular glucose content of the cells is more readily seen in Fig. 2, where the intracellular glucose, expressed in μmoles/100 g dry wt, is shown as a function of time. It may be seen that there is a significant extra accumulation of glucose above that found with insulin alone after 1 min, and after 30 min, but not after 3, 5, or 10 min of perfusion with epinephrine.

Effect of Epinephrine on Heart Rate and Contractile Force

Figure 3 shows the mean changes in the heart rate and contractile force in a series of

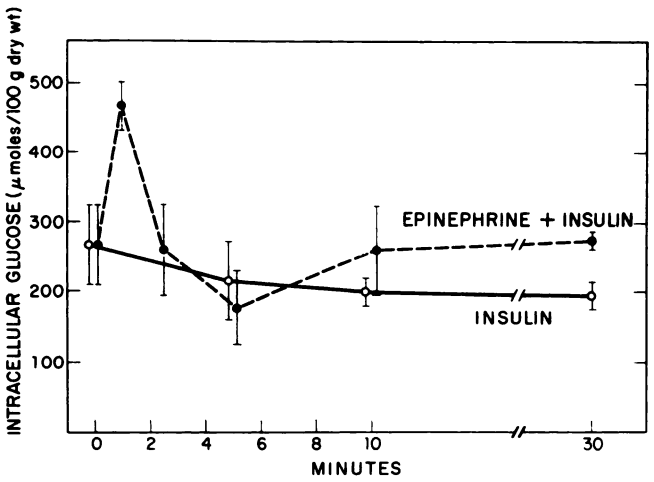

FIG. 2. *Effect of epinephrine on the intracellular glucose content*
The perfusion conditions were the same as those for Table 1.

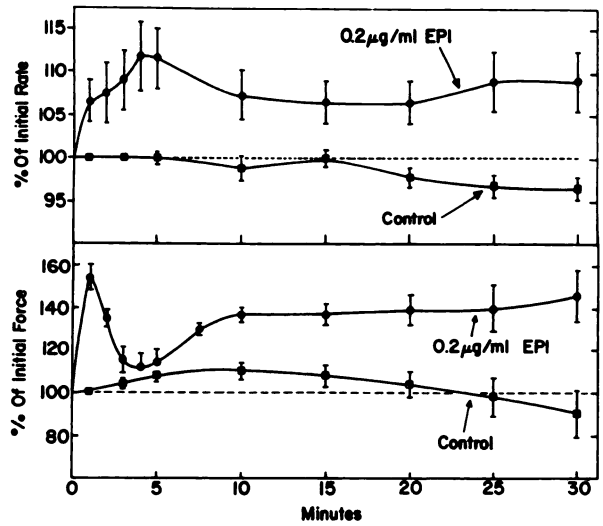

FIG. 3. *Effect of epinephrine in heart rate and force of contraction*

Hearts were perfused with 20 ml recirculating fluid containing 5 mM glucose and 5×10^{-5} M EDTA. The *vertical bars* represent two standard errors of the mean with 5 hearts in each group.

hearts after the addition of 4 μg epinephrine to 20 ml of perfusate recirculating through the hearts. Control hearts showed only small changes of heart rate or contractile force over the 30-min perfusion period. Epinephrine produced a 50% increase of contractile force after 1 min. This declined to a mean increase of 10% after 3–5 min, and then increased to a plateau value which was maintained for the duration of the experiment. The changes of heart rate in response to epinephrine were small, but consistent, and a maximum increase from a mean of 295 (range 267–315) to a mean of 331 beats/min (range 275–365) was observed after 5 min.

The size of the inotropic maximum in relation to the amplitude of the plateau depended on the epinephrine dose and the time and method of epinephrine addition. A heart, given a large dose by the injection of a small volume of concentrated solution or by continuous perfusion, responded in a manner similar to that shown in Fig. 3. Submaximal doses injected in concentrated form also produced an inotropic peak prior to the plateau, and repeated doses accentuated the effect. On the other hand, when a submaximal dose was recirculated through the heart, the force increased immediately to a plateau (20). In all hearts, a maximum increase of force was attained within 10–15 seconds. The duration of the effect depended on the dose and was prolonged by the presence of EDTA in the perfusate.

Effect of Epinephrine on Glycolytic Intermediates

Figure 4 shows that glucose-6-P and fructose-6-P increased approximately 4-fold within the first minute after perfusion with 0.2 μg/ml epinephrine, then decreased rapidly to values about twice the control. Thereafter, the levels of these intermediates gradually increased in the epinephrine-treated hearts, while in the control hearts they decreased slightly. Fructose-1,6-diP increased 11-fold within the first minute and subsequently decreased, reaching levels approximately the same as the control after 30 min. The triose phosphates followed a similar pattern, but the changes were less

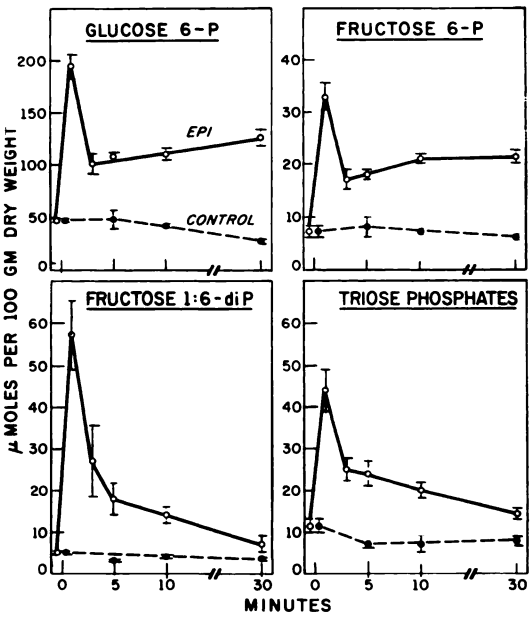

FIG. 4. *Effect of epinephrine on the levels of glycolytic intermediates*

Hearts were perfused with fluid containing 5 mM glucose and 5 × 10^{-5} M EDTA. The filled circles depict analyses of control hearts at the stated times, and the open circles represent analyses of hearts perfused in the presence of 0.2 μg/ml epinephrine. The *vertical bars* indicate two standard errors of the mean with 4–8 hearts in each group.

pronounced. The glycogen content decreased by about 50 μmoles/g dry wt to a constant level during the first 3 min of perfusion with epinephrine (1); hence, the initial large accumulation of glycolytic intermediates is undoubtedly caused by an increase in the rate of formation of hexose monophosphates from glycogen which exceeds their rate of removal by the subsequent reactions of glycolysis.

With more prolonged periods of perfusion, the elevated glucose-6-P level is probably a reflection of the increased rate of glucose phosphorylation, as indicated by the following experiment. Groups of four hearts were first depleted of glycogen by a 10-min period of anaerobic perfusion in the absence of substrate (21) and subsequently perfused aerobically for a further 10 min with medium containing 10 mM acetate or 5 mM glucose as substrate. The anaerobic

perfusion decreased the glycogen content from 119 ± 4 to 7 ± 2 μmoles/g dry wt. Upon aerobic perfusion with acetate-containing medium, epinephrine produced the usual increase in the force of contraction but no change in the mean glycogen content of hearts and only a small change in the level of glucose-6-P (from 13 ± 2 to 17 ± 3 μmoles/100 g dry wt). On the other hand, if the hearts were perfused for a similar 10-min period with medium containing glucose and epinephrine, the glycogen content remained low (8 ± 3 μmoles/g dry wt), but the glucose-6-P level increased to 126 ± 10 μmoles/100 g dry wt. The residual limit glycogen remaining after the anoxic treatment is not susceptible to further degradation by phosphorylase; hence, glucose in the medium must be the precursor of glucose-6-P in the tissue.

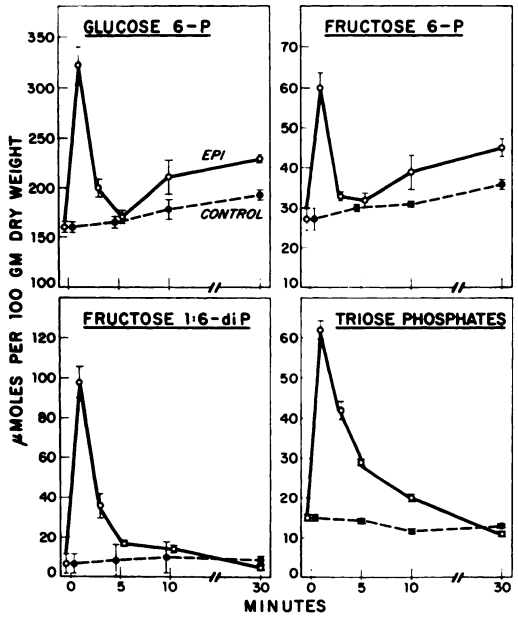

FIG. 5. *Effect of epinephrine in the presence of insulin in the levels of glycolytic intermediates*

The experimental conditions were similar to those for Fig. 4 except for the additional presence of 2×10^{-3} units/ml insulin in the perfusate of both control and epinephrine-treated hearts.

Figure 5 shows the effects of epinephrine in hearts perfused with glucose and insulin. All the measured intermediates increased maximally after 1 min of perfusion with epinephrine and decreased to values similar to the controls after 5 min. Between 5 and 30 min of perfusion with epinephrine, glucose-6-P and fructose-6-P increased by 34% and 40%, respectively, while fructose-1,6-diP and triose-P decreased by 70% and 62%, respectively. The glycogen content of hearts perfused with glucose, insulin and epinephrine decreased by 44% over the first 5 minutes of perfusion and then increased at the same rate as that of hearts perfused with glucose and insulin (1). Since the glycogen change is in the direction of a synthesis after the first 5 minutes of epinephrine action, and free glucose is present in the cell, the results show that after this initial period, the rate of glucose phosphorylation is greater than the rate of fructose-6-P phosphorylation, and identify phosphofructokinase as the rate-controlling step in the glycolytic pathway. A comparison of Figs. 4 and 5 shows that insulin alone increased the levels of glucose-6-P and fructose-6-P approximately 3-fold but had little effect on the levels of fructose 1,6-diP and the triose phosphates.

Kinetics of the Changes in the Levels of the Glycolytic Intermediates

Detailed changes in the levels of the glycolytic intermediates within the first 2 min after the injection of 1 μg epinephrine into fluid passing to hearts perfused with 10 mM glucose without recirculation, are shown in Figs. 6 and 7. Time is measured from the onset of the inotropic response. Large increases in the levels of all the intermediates occurred after 20–30 sec, but the levels subsequently declined to values which approached the controls after 2 min. From the large accumulation of glucose-1-P, it is clear that there is a massive inflow of hexose phosphates into the glycolytic system due to rapid glycogenolysis.

Analysis of the curves indicates, first, that many of the intermediates do not change appreciably until after an interval of approximately 10 sec, and second, that the accumulation-depletion curves of different groups of intermediates fall into several categories. In the experiment shown

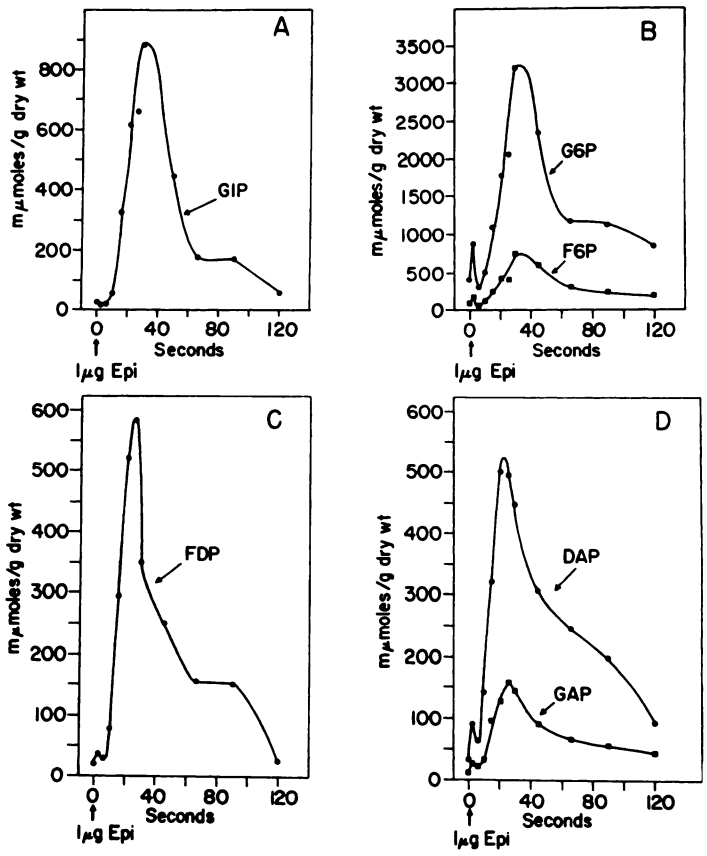

FIG. 6. *Kinetics of epinephrine effects in glycolytic intermediates*

Hearts were perfused with medium containing 10 mM glucose. Epinephrine (1 μg in 0.2 ml medium) was injected into fluid passing to the heart which was attached to a strain gauge. Zero time was measured from the start of the increased contractile response.

in Figs. 6 and 7, maximum accumulation of the hexose monophosphates was observed after 30 sec; peak values of fructose-1,6-diP and the triose phosphates occurred after 26 sec; and peak values of 3-P-glycerate, P-enol pyruvate, and pyruvate after 21 sec. Maximum values of α-glycerophosphate and lactate were observed after 45 and 30 sec, respectively. The different kinetic patterns of the changes in the intermediates allow the location of control sites at different times during the glycogenolytic flux as will be discussed in a later section.

Equilibrium and Disequilibrium of the Glycolytic Reactions

Figure 8 shows the relation of the mass action ratios to the thermodynamic equilibrium constants of five 2-partner reactions and one 3-partner reaction of the glycolytic pathway during the flux increase induced by epinephrine. The results of two separate experiments are shown in the figure. These reactions are all freely reversible and are classified in the near-equilibrium group of reactions which do not exert a control in the glycolytic flux (9, 22). Despite large changes in the total content of the glycolytic intermediates during the first 2 min of epinephrine action, it is seen that the mass action ratios of the reactions remain fairly constant and are not displaced from equilibrium by more than one order of magnitude. Certain characteristic small-order deviations from equilibrium are of interest. In control hearts where glyco-

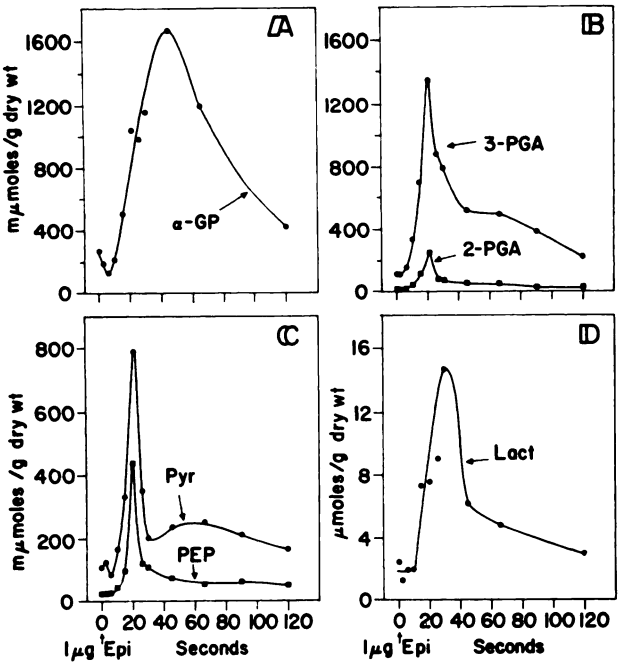

FIG. 7. *Kinetics of epinephrine effects on glycolytic intermediates*
The conditions of the experiment were the same as those for Fig. 6.

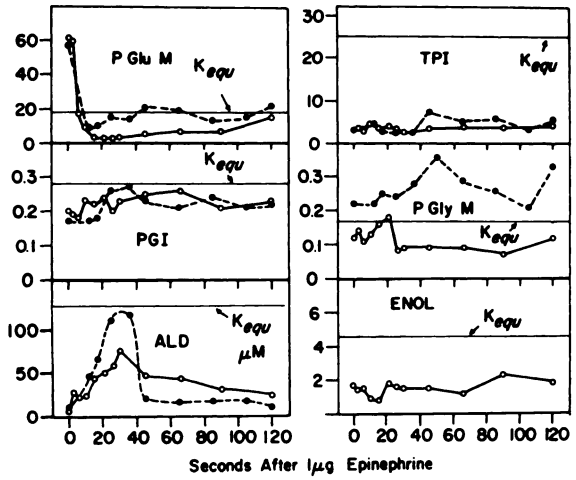

FIG. 8. *Deviation of near-equilibrium glycolytic reactions from equilibrium*
Abbreviations used are *PGluM*, phosphoglucomutase; *PGI*, phosphoglucose isomerase; *ALD*, aldolase; *TPI*, triose-phosphate isomerase; *PGlyM*, phosphoglycerate mutase; *ENOL*, enolase; K_{equ}, thermodynamic equilibrium constant of the reaction.

genolysis is negligible, the content of glucose-1-P is too small relative to the glucose-6-P level for the equilibrium proportions of the phosphoglucomutase reaction; while during active glycogenolysis, the equilibrium proportions of glucose-1-P and glucose-6-P are reached or even exceeded. Aldolase is also somewhat displaced from equilibrium in control hearts but approaches equilibrium during the period of

peak flux. Triose-P isomerase remains 4- to 6-fold displaced from equilibrium in favor of glyceraldehyde-3-P over the 2-min experimental period. The displacements of aldolase and triosephosphate isomerase from equilibrium have been noted previously (9, 22), and may be due to the spatial juxtaposition of these enzymes in the cytoplasmic structure (23).

Kinetics of the Changes in the Levels of the Adenine Nucleotides, Creatine-P, and Inorganic Phosphate

Changes in the levels of the adenine nucleotides in groups of hearts perfused continuously with 0.2 μg/ml epinephrine for times up to 30 min are shown in Table 2. There was a small, but nevertheless statistically significant, decrease of ATP after 1 min of perfusion which was reflected by a 2- to 3-fold rise in the levels of ADP and AMP. Both ADP and AMP remained elevated in these experiments for up to 10 min perfusion with epinephrine, but values after 30 min were not significantly different from the controls. Hearts perfused for 30 min in the absence of epinephrine contained 2.71 ± 0.14 μmoles/g dry wt ADP and 0.34 ± 0.03 μmoles/g dry wt AMP (4 hearts in each group), showing that the levels of the adenine nucleotides did not change appreciably during perfusion in the absence of epinephrine.

Figure 9 shows the early kinetics of the changes in the levels of the adenine nucleotides, creatine-P, creatine, and inorganic phosphate (P_i) after the addition of 1 μg epinephrine to the perfusate passing to the heart. The levels of ATP decreased monotonically over the first 20 sec and subsequently increased to values similar to the control after 2 min. The changes of ADP and AMP were approximately reciprocal to the ATP, and peak values were reached between 21 and 26 sec. However, both ADP and AMP remained slightly elevated above the controls after the 2 min of perfusion with epinephrine. The balance between the disappearance of ATP and appearance of ADP and AMP was remarkably good considering the great preponderance of ATP and the relatively small changes involved. Thus, ATP decreased maximally by 3.2 μmoles/g dry wt (14%) while ADP increased by 2.4 μmoles/g dry wt (120%) and AMP by 0.34 μmole/g dry wt (213%). There was also an approximately reciprocal relationship between the increases of P_i and creatine, and the decrease of creatine-P, the maxima or minima occurring between 20 and 30 sec. Inorganic phosphate increased by 25 μmoles/g dry wt, and creatine by 23 μmoles/g dry wt, while creatine-P decreased by 19.5 μmoles/g dry wt. As may be seen from a comparison of the values in Fig. 9 and Table 2, the effect of a

TABLE 2

Effect of epinephrine on adenine nucleotide levels in the perfused rat heart

The experimental conditions were the same as those for Table 1. Values shown are the mean ± standard error of the mean of four or more hearts.

Time of perfusion with 0.2 μg/ml epinephrine (mins)	ATP	ADP	AMP
0	20.7 ± 0.8[a]	2.67 ± 0.05	0.29 ± 0.02
1	18.1 ± 0.4[b]	4.98 ± 0.16[b]	0.80 ± 0.07[b]
5	19.6 ± 0.7	3.46 ± 0.26[b]	0.52 ± 0.05[b]
10	19.1 ± 0.4	3.48 ± 0.17[b]	0.54 ± 0.02[b]
30	19.8 ± 0.3	2.58 ± 0.18	0.39 ± 0.06

[a] Values are stated as micromoles per gram dry weight.

[b] Significantly different from control ($P < 0.01$).

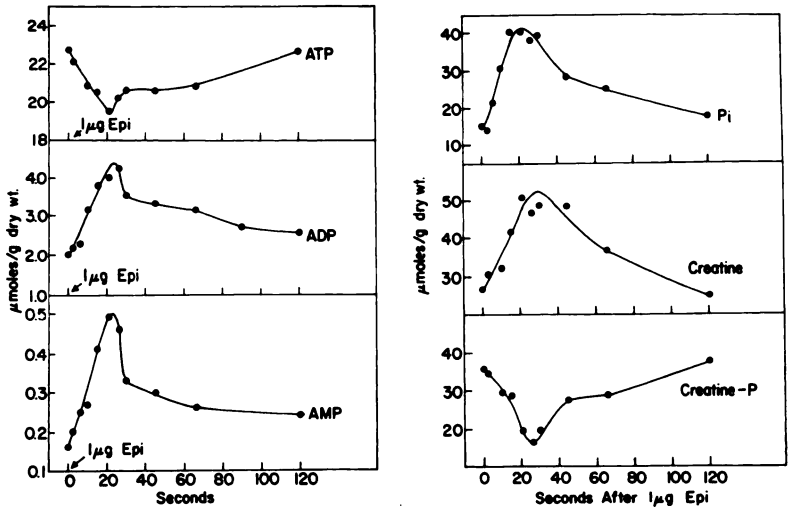

FIG. 9. *Kinetics of epinephrine effects on adenine nucleotides, creatine-P, creatine, and inorganic phosphate (P_i)*

The experimental conditions were the same as those for Fig. 6.

single dose of epinephrine passing through the coronary circulation on the adenine nucleotides was, as may be expected, considerably more evanescent than the effect produced by recirculation of perfusate containing EDTA and epinephrine. The data also show that the adenine nucleotide changes occur with the onset of the inotropic stimulus and not after a time lag as observed with the glycolytic intermediates.

Correlation of Contractile Force with Tissue Levels of Cyclic AMP and with Phosphorylase a Activity

The cause of the time lag between the inotropic response and the sharp rise in the levels of the glycolytic intermediates was investigated by measuring the levels of cyclic AMP and phosphorylase *a* after the addition of 1 μg epinephrine to a series of hearts perfused with nonrecirculating medium containing 10 mM glucose. These results are shown in Fig. 10, together with glucose-1-P values and the percentage increase of the contractile force. The glucose-1-P values shown in Fig. 10 are the same ones as those presented in Fig. 6. Zero time is taken from the time of onset of the contractile force increase. The mean level of cyclic AMP in control hearts prior to the addition of epinephrine was 1.5 mμmoles/g dry wt, and 10% of the total phosphorylase was present as phosphorylase *a* (range 8–14% in five hearts). Within 2 sec of the epinephrine effect on the contractile force, cyclic AMP levels increased 2-fold. However, phosphorylase *a* levels remained in the same range as the control hearts, with values of 14%, 14%, and 10% for times 1.5, 2, and 2.5 sec, respectively. Subsequently, the phosphorylase *a* levels increased to reach a broad peak after 20–30 seconds; the cyclic AMP level continued to rise rapidly, reached a sharp peak after 10 seconds, and abruptly decreased to a value slightly higher than the control after 30 seconds. As may be seen from Fig. 10, the rise and fall of the cyclic AMP closely followed the initial peak of the contractile force. Glucose-1-P did not begin to rise appreciably until the contractile force had reached a maximum value and the phosphorylase *a* level was between 50 and 60% of the total phosphorylase. There is thus a distinct time lag between the increases of cyclic AMP and phos-

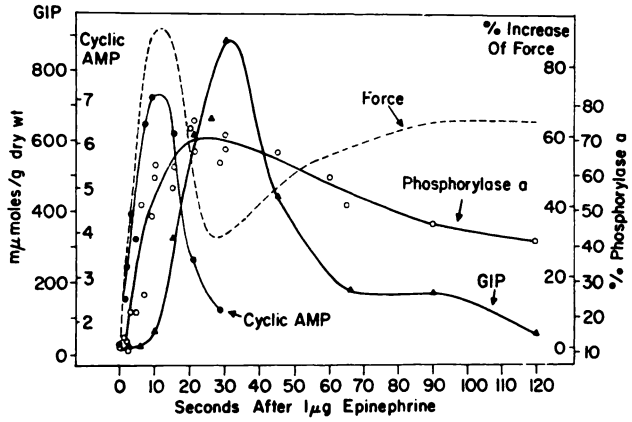

FIG. 10. *Kinetics of epinephrine effects on cyclic AMP and phosphorylase a in relation to the increment of the contractile force*

The experimental conditions were the same as those for Fig. 6.

phorylase *a*, on the one hand, and phosphorylase *a* and glucose-1-P on the other.

DISCUSSION

Control of glucose phosphorylation. It has been the purpose of this paper and the preceding paper (18) to distinguish between effects which occur immediately upon epinephrine addition and effects of a more secondary nature caused by the increased frequency and force of contraction. Although effects of epinephrine on the metabolism of cardiac muscle are generally similar to those on skeletal muscle, there are also marked differences which appear to be related to the state of mechanical activity.

Recent studies by Krebs and associates (24, 25), together with earlier work quoted in these papers, have shown that epinephrine initiates glycogenolysis in skeletal and cardiac muscle by a similar mechanism. Cyclic AMP is formed from ATP by the cyclase enzyme and promotes activation of phosphorylase *b* kinase which, in turn, causes the phosphorylation of phosphorylase *b* to phosphorylase *a*. Phosphorylase *a* is the more active form of the enzyme under the conditions of low AMP and high ATP concentrations prevailing in the cell, and the rise of phosphorylase *a* is responsible for the increased rate of glycogenolysis. Glycogen metabolism interacts with glucose metabolism at the level of glucose-6-P due to this intermediate being a potent inhibitor of hexokinase (26). The rate of disposal of hexose monophosphate will thus influence the rate of glucose phosphorylation when glucose and glycogen are being metabolized simultaneously.

Unlike rat diaphragm and skeletal muscle (3–5), epinephrine increases glucose uptake in the perfused rat heart with no accumulation of intracellular glucose in the absence of insulin. These results show that glucose uptake remains limited by transport, and that transport rather than the increased level of glucose-6-P limits the rate of glucose phosphorylation. In the presence of insulin, with transport no longer limiting, epinephrine causes a further stimulation of glucose uptake over that produced by insulin alone. Since the increase in the steady state-level of intracellular glucose is small in comparison with the glucose uptake, these results indicate that glucose phosphorylation has been increased despite an increase in the concentration of glucose-6-P to above 1 mM (assuming uniform distribution in the intracellular space). This concentration is an order of magnitude greater than the K_i for inhibition of hexokinase *in vitro;* hence, it is clear that factors other than the level of glucose-6-P regulate the rate of glucose phosphorylation in the cell when the sub-

strate concentrations are above the K_m values.

Two sets of observations help explain the differences observed between the effects of epinephrine on cardiac and skeletal muscle. The first concerns a comparison between the effects of electrical stimulation and of epinephrine on isolated frog skeletal muscle (27); and the second, the finding that inorganic phosphate counteracts the inhibition of hexokinase by glucose-6-P (28). Karpatkin *et al.* (27) found that when frog muscles were stimulated anaerobically in the absence of glucose, increased phosphorylase activity resulted in a large increase of lactate formation, an increase of inorganic phosphate, and an increase of glucose-6-P. On the other hand, when epinephrine was added to nonstimulated muscles, there was a decrease of inorganic phosphate and a large increase of glucose-6-P without a corresponding increase of lactate. Fructose diphosphate showed little change with epinephrine but was markedly increased in the stimulated muscles (29). These results indicate that with stimulation there was a fairly well coordinated increase in the activities of phosphorylase and phosphofructokinase. With epinephrine, although phosphorylase was activated, phosphofructokinase activity was not increased proportionately. Thus, the key to the different behavior of the glycolytic system in the two situations seems to reside at the level of control at the phosphofructokinase site.

The increased production of inorganic phosphate during electrical stimulation of frog muscle (27) suggests that there is an imbalance between energy supply and utilization, particularly at high rates of stimulation. The ensuing decrease of ATP and corresponding increases of ADP and AMP, possibly in conjunction with pH changes in the muscle (30), would all tend to activate phosphofructokinase (6) and hence coordinate glycolytic flux with energy utilization. On the other hand, when epinephrine is added to nonstimulated muscle, there is little or no increase of energy utilization despite glycogen mobilization, and phosphofructokinase remains inactivated. Since inorganic phosphate levels fall while glucose-6-P increases (27), it is likely that hexokinase becomes fully susceptible to inhibition by glucose-6-P so that glucose uptake is inhibited. In the present experiments with rat heart, inorganic phosphate levels increase after epinephrine addition due to the greater expenditure of energy, thereby, increasing the K_i for hexokinase inhibition by glucose-6-P (28) and allowing a greater phosphorylation rate despite elevated levels of the inhibitor.

The mechanism for the facilitation of glucose transport by epinephrine in cardiac muscle is not known at present, but it appears to be related to the increased mechanical activity of the heart since it has been shown that glucose uptake may be greatly increased by increasing the work load on a working rat heart preparation (31).

Control of phosphofructokinase. Measurement of the metabolite levels in perfused rat heart reported in this paper suggests that, during the first few minutes of epinephrine action, enhanced glycogen utilization is the principal factor imposing changes on the metabolite pattern. The rapid rate of lactate production shows that the glycolytic flux is increased to such an extent that the capacity of the mitochondrial pyruvic oxidase system is exceeded, and the excess pyruvate is reduced by NADH generated at the triose dehydrogenase step. Activation of phosphofructokinase occurs partly due to the elevated fructose-6-P levels, but mainly due to changes in the concentration of the allosteric activators and inhibitors of the enzyme (6). Relative changes of these intermediates in rat hearts during the first 90 sec of epinephrine action are summarized in Fig. 11 together with the relative change of phosphofructokinase activity in hearts. This latter quantity is estimated from the mass action ratios (K_{app}) of the phosphofructokinase reaction as calculated from the measured contents of F6P, FDP, ATP, and ADP found in the tissue at the relevant times. The measured contents of the phosphofructokinase reactants and products indicate that this reaction is displaced from equilibrium in the rat heart by about five orders of magnitude; hence, a

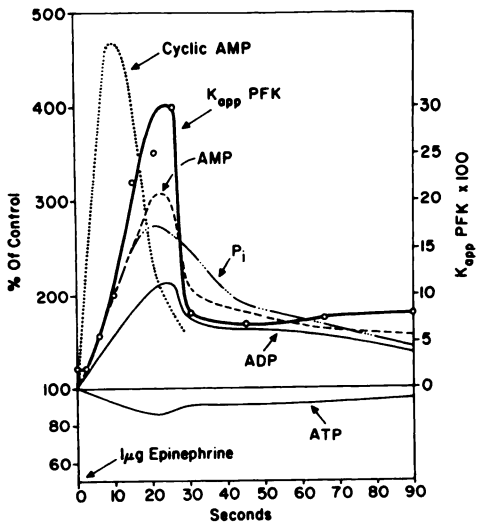

FIG. 11. *Effect of epinephrine on the percentage changes of ATP, ADP, AMP, cyclic AMP, and inorganic phosphate in relation to the mass action ratio of the phosphofructokinase reaction (K_{app} PFK)*

change of the K_{app} toward the equilibrium value (1.2×10^3) is taken as denoting an activation of the enzyme, and vice versa. It may be seen from Fig. 11 that after a short delay, the K_{app} of the phosphofructokinase reaction increases sharply to reach a peak after about 25 sec and then abruptly falls to a new steady state value four times greater than the initial. The percentage changes of AMP, ADP orthophosphate, and ATP follow the K_{app} curve fairly closely during activation and deactivation of phosphofructokinase, suggesting a good correlation between the changes of these intermediates and control at the phosphofructokinase site. On the other hand, although cyclic AMP (an additional activator of phosphofructokinase *in vitro*), increased almost 5-fold, the change occurred somewhat earlier than the activation of phosphofructokinase, suggesting that cyclic AMP may have a minor role in the modulation of phosphofructokinase activity in cardiac muscle. The fall in the K_{app}, which occurs between 26 and 30 sec after the addition of epinephrine in the experiment shown in Fig. 11, is rather more sudden than may be anticipated from the changes in AMP, ADP, and orthophosphate; and the possibility that increases in the level of citrate over this period may also contribute to stemming the flow of substrate through the phosphofructokinase reaction has been discussed recently (11).

Control at other sites. Since phosphofructokinase is the rate-controlling reaction in the linear glycolytic sequence from glucose-1-P to lactate, increased activity could result in one of two possibilities: control of flux could either be retained at phosphofructokinase or be transferred to another enzyme site further down the glycolytic pathway. If the former possibility were the case, changes in the levels of the glycolytic intermediates between fructose diphosphate and pyruvate would be expected to follow the kinetic pattern of fructose diphosphate; while in the latter case, those intermediates below the new control site would be decreasing at the same time as the intermediates above the site were increasing. The data in Figs. 6 and 7 show that over the time interval from 21 to 26 sec after the onset of the inotropic effect (these being the times at which two successive hearts were frozen), all the glycolytic intermediates up to and including glyceraldehyde-3-P increased while 3-P-glyceric acid, 2-P-glyceric acid, P-enol pyruvate, and pyruvate decreased. The latter possibility, therefore, seems to be correct, and although complete data showing the kinetic changes of all the glycolytic intermediates after epinephrine have only been presented for one experiment, qualitatively similar changes showing the same type of control transference over a limited time have been obtained in other experiments of a similar nature. After 26 seconds, fructose diphosphate levels fall, and there is a restoration of control at phosphofructokinase, as discussed above. There is thus a brief period during which evidence of control at a site between glyceraldehyde-3-P and 3-P-glyceric acid is obtained. This places it at either the triose dehydrogenase step or the triose kinase step, but since levels of 1,3-diP-glyceric acid are not available, it is not possible to distinguish directly between these two locations. A

crossover plot of the glycolytic intermediates over the two successive time intervals from 21 to 26 sec and from 26 to 30 sec, illustrating the control phenomena discussed above, has been published elsewhere (11).

Time sequence of metabolic events. In the preceding paper (18) measurements of the tissue fluorescence of the beating heart after the addition of epinephrine showed that the mean change of the pyridine nucleotide was initially toward a more oxidized state, and that subsequently there was a preponderant large cyclic change toward a more reduced state. It was proposed that the initial pyridine nucleotide oxidation represents primarily a mitochondrial response, comparable to state 4 to 3 transition in isolated mitochondria (32) as respiration was stimulated by a rise of ADP, while the later pyridine nucleotide reduction is caused by the accelerated rate of arrival of glyceraldehyde-3-P at its dehydrogenase as glycogenolysis is stimulated. The analytical results presented here support this interpretation. Thus ADP increases from the onset of the inotropic effect, whereas glycogenolysis is delayed, as shown by the kinetics of the increases of phosphorylase *a* and the glycolytic intermediates.

The temporal separation between the increased force of contraction and the rise of phosphorylase *a* is of particular interest in relation to the various theories which have been proposed to explain the inotropic effect of epinephrine (33). The present results would seem to rule out the possibilities that increases of hexosephosphates (34), high-energy phosphate compounds (33), phosphorylase *a* (35), or phosphofructokinase activity (36) bear any relation to the initiation of the increased contractile response.

The probable time sequence of metabolic events following epinephrine administration to cardiac muscle may be summarized as follows. An increased production of cyclic AMP appears to be one of the earliest events. Whether epinephrine affects the contractile mechanism directly or through the mediation of cyclic AMP must remain an open question, but the results presented here and by Cheung and Williamson (20) are consistent with the possibility (37) that the increased production of cyclic AMP is closely associated with the inotropic effect. The increased expenditure of energy needed to support the contractile response results in an energy imbalance, so that ATP levels fall and ADP levels rise. The rise of ADP is strongly buffered by interaction with the adenylate cyclase and creatine phosphokinase systems as shown by concomitant increases of AMP, creatine, and inorganic phosphate, and a decrease of creatine phosphate. These reactions in effect minimize the changes of ADP which would otherwise be much larger (38). Although it is not possible to apportion accurately the changes of ADP between mitochondrial and cytoplasmic compartments, it is likely that the changes in both compartments are in the same direction. In the mitochondria the rise of ADP is thought to lead an increased rate of respiration (32); while in the cytoplasm, phosphofructokinase is activated. These changes, together with the facilitation of glucose transport and phosphorylation, are considered secondary effects arising from the increased mechanical activity. In the beating heart, it is only after the above changes are initiated that increased levels of phosphorylase *a* and intermediary products of glycogen breakdown are observed due to time lags in the successive enzyme activation steps of the phosphorylase system.

Finally, it is interesting to note that cyclic AMP may also have an effect on cardiac lipase similar to its activation of adipose tissue lipase (39) since glycerol release is stimulated by epinephrine both in normal (1) and potassium-arrested isolated heart preparations (40).

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