Biochemical Pharmacology, Vol. 16, pp. 2109–2116. Pergamon Press Ltd. 1967. Printed in Great Britain

THE EFFECT OF METABOLIC INHIBITORS ON THE RESPONSE OF THE PERFUSED RAT HEART TO EPINEPHRINE*

ROBERT S. HORN,† CARL E. ARONSON, MARILYN E. HESS‡ and NIELS HAUGAARD

Department of Pharmacology, University of Pennsylvania,
School of Medicine, Philadelphia, Pa., U.S.A.

(*Received* 7 *March* 1967; *accepted* 24 *April* 1967)

Abstract—The effects of epinephrine on contractility and metabolism were measured in the isolated, perfused rat heart. Epinephrine produced an initial increase in force of contraction followed by a lowering of tension and finally a sustained increase in contractility. When fluoroacetate or iodoacetate was present in the perfusion fluid, the mechanical response to epinephrine was markedly altered. The initial response to epinephrine was depressed in the presence of either inhibitor and the final increase in force of contraction was poorly maintained during metabolic blockade. When the perfusion fluid contained both pyruvate and iodoacetate, the mechanical response of the heart to epinephrine was restored. Results of determinations of metabolites in the myocardium demonstrated that stimulation of glycogenolysis is not essential for the contractile response to epinephrine when adequate substrate is provided for the reactions of the tricarboxylic acid cycle.

IT HAS BEEN established that in the isolated, perfused rat heart catecholamines produce an increase in the activity of the enzyme phosphorylase *a*.[1] Recently several investigators have studied the temporal relationship between the metabolic and mechanical action of epinephrine. Kinetic studies by Williamson,[2] Robison *et al.*,[3] and Øye[4] on the effects of epinephrine on the isolated heart indicate that the positive inotropic action of epinephrine precedes phosphorylase activation. The experiments presented here are concerned with the study of mechanical and metabolic responses of the isolated rat heart to epinephrine in the absence and presence of two metabolic inhibitors, iodoacetate and fluoroacetate. The results of the experiments have been reported in preliminary form.[5]

METHODS

Male rats of the Wistar strain, weighing between 180 and 250 g were killed by decapitation. After hearts were removed from the animals, they were washed free of blood in warm substrate-free Ringer–Locke solution of the following composition: 154 mM NaCl, 2·2 mM $CaCl_2$, 5·6 mM KCl, 2·4 mM $NaHCO_3$, saturated with oxygen. Each heart was blotted, weighed, and a glass cannula was inserted into the aorta. The heart was placed on a Langendorff perfusion apparatus and perfused with

* Supported by grants from the National Institutes of Health (HE-01813) and the American Heart Association.
† Supported by USPHS Training Grant 2 T1 GM 474-06.
‡ This work was done during the tenure of an Established Investigatorship of the American Heart Association.

the solution described above at 38°. A small Palmer heart clip was attached to the apex of the heart and connected to a strain gauge for recording the force of contraction with a Sanborn oscillograph. (The term "force of contraction" is used in this paper to describe the net force developed by the contracting perfused heart.) A catheter was inserted into the cannula for subsequent infusion (1 ml/min) of 0·9% NaCl or epinephrine (5 μg/ml in 0·9% NaCl). Both solutions infused were warmed to 38° during their passage through the catheter. The perfusion schedules were as follows: (A) control, 17 min with Ringer–Locke solution with an infusion of 2 ml 0·9% NaCl or epinephrine (5 μg/ml in 0·9% NaCl) during the last 2 min; (B) fluoroacetate 10 min with Ringer–Locke solution followed by 7 min with Ringer–Locke solution containing sodium fluoroacetate in a concentration of 1 mM; addition of saline or epinephrine as under A; (C) iodoacetate, 5 min with Ringer–Locke solution followed by 12 min with Ringer–Locke solution containing recrystallized sodium iodoacetate in a concentra- of 1 mM; addition of saline or epinephrine as under A; (D) iodoacetate + pyruvate, as in C, except that sodium pyruvate (5 mM) was present in the Ringer–Locke solution containing iodoacetate. The coronary effluent was collected for measurement of volume and lactic acid and glycerol concentrations.

At the end of the perfusion period the heart was frozen with a Wollenberger clamp[6] cooled in liquid nitrogen. The apex of the heart was chipped off, weighed, and extracted with 1 mM EDTA–20 mM NaF–50 mM Tris buffer (pH 6·8). Phosphorylase activity of the extract was determined by the method of Cori and Illingworth[7] except that the incubation temperature was increased to 37° and the extraction medium contained 0·05 M Tris buffer (pH 6·8). These modifications are similar to those made by Diamond and Brody,[8] and in our hands given somewhat lower values for phosphorylase *a* than the original method by Cori and Illingworth.[7] The remaining part of the heart was ground with frozen 0·6 N perchloric acid in a mortar cooled in liquid nitrogen. After slow thawing the resulting paste was ground with a small amount of sand. The extract was neutralized with K_2CO_3, buffered with triethanolamine (pH 7·0), and analyzed by specific enzymatic methods[9] for intermediates of glycolysis, glycerol and α-glycerophosphate, lactate, and adenine nucleotides. By keeping dilutions to a minimum and by using spectrophotometer cells which permitted determinations in 0·8-ml vol., it was possible to obtained meaningful analyses at tissue metabolite concentrations exceeding 15 mμmole/g wet wt. Concentrations below this level can be considered to be not significantly different from zero. Lactate and glycerol were also determined in the effluent by enzymatic methods.[9]

RESULTS

The effects of epinephrine on contractile force and lactate production in control hearts and in hearts treated with metabolic inhibitors are recorded in Figs. 1 and 2. In those experiments in which glycerol determinations were made on the coronary effluent, the concentration of this metabolite was below the sensitivity of the method (<2 mμ mole/ml).

The means of the observations from control experiments are recorded on the left part of Fig. 1. Epinephrine infusion produced a highly reproducible triphasic response in tension. Contractile force rose rapidly after a short lag period, then decreased below the original level, and finally increased above the control tension. There was a large increase in the output of lactate which started after the beginning of the first con-

tractile response. Infusion of saline caused a decrease in tension and did not result in any significant change in the output of lactate.

The right panel of Fig. 1 shows the results of experiments in which 1 mM fluoroacetate was present in the perfusion fluid. In the presence of this inhibitor, lactate production was markedly increased over that observed in control hearts. Epinephrine caused an enormous increase in the output of lactate, indicating that glycolysis

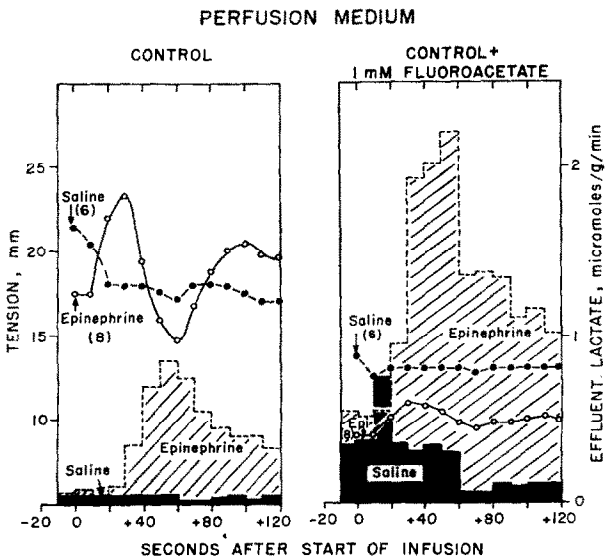

FIG. 1. The effect of epinephrine on isometric tension and lactate production of control and fluoroacetate-treated perfused rat heart. Epinephrine (5 μg/ml in 0·9% NaCl) or 0·9% NaCl was infused for 2 min at a rate of 1 ml/min. The lines represent tension developed by the heart; the histograms indicate rates of lactate output. Numbers in parentheses equal number of experiments.

proceeded rapidly and that the oxidation of pyruvate was effectively blocked. Fluoroacetate alone decreased the isometric tension during the perfusion period preceding epinephrine or saline infusion. When the catecholamine was given, there was an initial slow rise in tension, but the increase was much smaller than in control hearts. The sharp decrease in tension and the subsequent augmentation of the force of contraction were almost absent. Saline infusion caused little change in force of contraction.

The results of similar experiments with iodoacetate as the metabolic inhibitor are recorded in the left section of Fig. 2. In the presence of iodoacetate the initial systolic tension was somewhat lower than in control hearts. The infusion of epinephrine produced no increase in lactate, indicating that glycolysis was inhibited. The triphasic inotropic response to epinephrine was still present in the heart perfused with iodoacetate but the initial increase, as well as the secondary fall in tension, was less pronounced than in control hearts. The final increase in contractility was not as well maintained as in controls.

The results of experiments in which pyruvate was added to the perfusion fluid containing iodoacetate are recorded in the right-hand side of Fig. 2. The addition

of pyruvate greatly increased the output of lactate, showing that a major part of the pyruvate metabolized was converted to lactate. Pyruvate produced a startling effect on the mechanical response of the heart to epinephrine. The changes in force of contraction produced by epinephrine were qualitatively similar to those seen in control hearts, but all three phases of the epinephrine response were much more pronounced than in the absence of substrate or inhibitor.

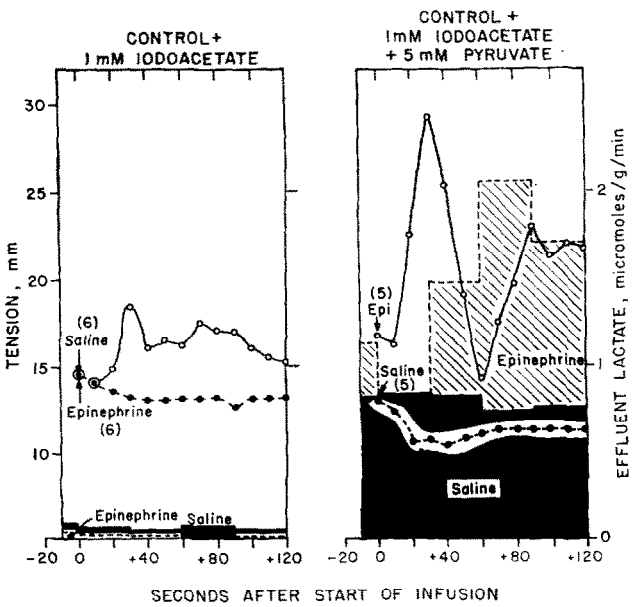

FIG. 2. The effect of epinephrine on the heart perfused with solutions containing iodoacetate or iodoacetate and pyruvate. Epinephrine (5 μg/ml in 0·9% NaCl) or 0·9% NaCl was infused for 2 min at a rate of 1 ml/min. The lines represent tension developed by the heart; the histograms indicate rates of lactate output. Numbers in parentheses equal number of experiments.

Chemical changes in the myocardium

In all of the experiments reported in Figs. 1 and 2, the hearts were frozen at the end of the perfusion period and samples of the tissue were analyzed for glycolytic metabolites and adenine nucleotides. The results of these analyses, as well as determinations of phosphorylase *a*, are recorded in Table 1.

The effect of inhibitors on myocardial metabolism

In the control hearts the concentrations of the hexose monophosphates were barely measurable by the methods used; other metabolites were present in considerable quantities. Phosphorylase *a* activity was close to values previously reported from this laboratory.[10]

When fluoroacetate was present in the perfusion fluid, there was a large increase in the concentrations of the hexose monophosphates. Fructose diphosphate levels were close to those in the control hearts, while triose phosphates declined. Since phosphorylase *a* activity was not increased after fluoroacetate, the high concentrations of hexose monophosphates without an increase in fructose diphosphate indicate

TABLE 1. EFFECTS OF EPINEPHRINE AND METABOLIC INHIBITORS ON GLYCOLYSIS IN THE PERFUSED RAT HEART

	Addition to perfusion medium*							
	None		Fluoroacetate		Iodoacetate		Iodoacetate + Pyruvate	
	Saline† N = 6‡	Epinephrine N = 8	Saline N = 6	Epinephrine N = 8	Saline N = 6	Epinephrine N = 6	Saline N = 5	Epinephrine N = 5
Gluose-1-P	0 ± 0§	16 ± 9	7 ± 5	41 ± 4	8 ± 5	13 ± 7	2 ± 8	3 ± 2
Gluose-6-P	6 ± 3	442 ± 126	289 ± 92	831 ± 183	64 ± 28	159 ± 46	60 ± 29	137 ± 37
Fructose-6-P	1 ± 1	48 ± 14	63 ± 12	166 ± 40	4 ± 8	16 ± 7	6 ± 5	21 ± 7
Fructose 1,6 DiP	48 ± 11	89 ± 15	60 ± 10	121 ± 16	1,880 ± 52	2,260 ± 250	128 ± 34	1,760 ± 110
Triose-P	56 ± 13	42 ± 4	11 ± 1	37 ± 4	420 ± 57	805 ± 116	159 ± 46	339 ± 56
a-Glycerol-P	44 ± 10	179 ± 54	36 ± 13	91 ± 17	141 ± 32	146 ± 10	103 ± 18	116 ± 16
Pyruvate	94 ± 16	101 ± 12	106 ± 17	110 ± 10	126 ± 36	119 ± 22	1,809 ± 392	2,320 ± 117
Lactate	709 ± 137	2,186 ± 751	1,009 ± 131	1,877 ± 287	1,580 ± 540	1,400 ± 310	1,655 ± 245	2,231 ± 297
AMP	330 ± 79	271 ± 58	318 ± 37	499 ± 52	290 ± 80	490 ± 14	331 ± 52	386 ± 23
ADP	660 ± 38	759 ± 68	967 ± 96	718 ± 104	820 ± 80	1,040 ± 60	754 ± 114	826 ± 54
ATP	3,140 ± 141	2,634 ± 202	2,910 ± 143	2,283 ± 100	3,360 ± 150	2,100 ± 150	2,734 ± 300	3,002 ± 215
Phosphorylase *a* (%)	15·6 ± 2·8	34·6 ± 1·8	16·3 ± 2·7	55·1 ± 2·9	16·8 ± 1·1	62·8 ± 3·3	12·5 ± 3·4	32·7 ± 3·0

* Fluoroacetate and iodoacetate, 1 mM; pyruvate, 5 mM.
† Infusion: saline, 0·9% NaCl; epinephrine, 5 μg/ml in 0·9% NaCl.
‡ Number of experiments.
§ Mμmole/g wet wt. ± S.E.M.

that phosphofructokinase was inhibited. The low concentration of triose phosphates is also consistent with phosphofructokinase inhibition. Lactate concentration in the tissue and especially in the coronary effluent was increased in the presence of fluoroacetate. Fluoroacetate did not alter the tissue levels of AMP or ATP, but the ADP concentration was significantly higher ($P < 0.025$) in the presence of this inhibitor than in the control experiments.

Inhibition of glyceraldehyde-3-P dehydrogenase by iodoacetate was clearly demonstrated by the large increase in fructose diphosphate, triose phosphates, hexose monophosphates, and α-glycerophosphate (Table 1). Pyruvate levels and the concentration of lactate in the coronary effluent did not differ from controls. The concentrations of adenine nucleotides and phosphorylase *a* activity were unaffected by iodoacetate.

The inclusion of pyruvate in the perfusion medium containing iodoacetate led to large changes in metabolite concentrations: hexose monophosphate levels were decreased; the high concentrations of fructose diphosphate, triose phosphates, and α-glycerophosphate were returned to control values; alterations in adenine nucleotides were not significant. In the presence of iodoacetate and exogenous pyruvate, fructose diphosphate, triose phosphate, and α-glycerophosphate concentrations were not different from those found in control hearts. As expected, tissue lactate and pyruvate as well as effluent lactate were markedly elevated. No significant alterations in adenine nucleotide levels were noted. There was a highly significant decrease in the per cent of phosphorylase in the *a* form.

Effect of epinephrine on cardiac metabolism

In the absence of inhibitors, epinephrine caused a large increase in the hexose monophosphates and lesser increases in fructose diphosphate, α-glycerophosphate, and tissue and effluent lactate. However, no appreciable changes in the concentrations of the triose phosphates were observed. These results are consistent with the finding of Williamson[11] that phosphofructokinase limits the rate of epinephrine-stimulated glycolysis in the perfused rat heart.

The concentration of pyruvate was unchanged by epinephrine while lactate production was greatly augmented by the catecholamine (see Fig. 1); phosphorylase *a* activity was approximately double that of the control. The ATP levels declined ($P < 0.05$), but AMP and ADP concentrations were not significantly changed.

When fluoroacetate was present in the medium, epinephrine produced a larger increase in phosphorylase *a* and in the concentrations of the hexose phosphates than in the control hearts. There was a decrease in ATP ($P < 0.01$) and an increase in AMP ($P < 0.025$) after administration of epinephrine.

Infusion of epinephrine to the iodoacetate-treated heart caused an increase in phosphorylase *a* activity which was much greater than in controls. Rapid glycogenolysis was reflected by an elevation in hexose phosphates and particularly in the concentrations of triose phosphates. In spite of the build-up of triose phosphates, there was no alteration in the concentration of α-glycerophosphate after epinephrine administration. This is in contrast to the observations with control and fluoroacetate-treated hearts, and is probably caused by a deficiency of NADH due to inhibition of glyceraldehyde phosphate dehydrogenase. Under the influence of epinephrine, there was a large drop ($P < 0.001$) in the level of ATP and a rise in AMP concentration

($P < 0{\cdot}05$). ADP also increased but the change was not statistically significant.

When iodoacetate and pyruvate were present in the perfusion fluid, the amounts of fructose diphophate and triose phosphates in the heart were much lower than in the presence of iodoacetate alone. After epinephrine, there were large increases in the concentrations of fructose diphosphate and triose phosphates demonstrating that glyceraldehyde phosphate dehydrogenase was severely inhibited. As with iodoacetate alone, the administration of epinephrine did not increase the levels of α-glycerophosphate. Both in the absence and presence of epinephrine there was a large amount of lactate in the tissue and the effluent fluid showing that NADH was being utilized for the reduction of pyruvate. There were no significant changes in the concentrations of the adenine nucleotides on addition of epinephrine. This finding shows that in the presence of exogenous pyruvate there is adequate energy production from oxidations of the tricarboxylic acid cycle.

DISCUSSION

The experiments reported here show that metabolic inhibitors can profoundly influence the mechanical response of the isolated rat heart to epinephrine. When the catecholamine was administered, there was a triphasic change in force of contraction. This type of acute response to epinephrine has been observed by other investigators in the perfused heart,[2] the hypothermic open-chest dog[12] or the digitalized, hypothermic open-chest dog.[13] The mechanism of the alternating increase and decrease in contractility is unknown.

The initial increase in force of contraction seen after epinephrine administration begins before the rise in lactate concentration in the effluent fluid. At the time when the tension falls, lactate output of the heart is high, i.e. the rate of glycolysis is rapid. It is not certain whether the marked decrease in force of contraction is caused by alterations in rate of glycolysis or by changes in pH due to lactate accumulation.

The final increase in contractility is most likely dependent upon energy obtained from oxidation of pyruvate derived from glycogenolysis. Results of experiments with iodoacetate and fluoroacetate support this view since, in the presence of these inhibitors, the epinephrine-induced increase in contractility is poorly maintained. When iodoacetate is used, pyruvate cannot be formed and with fluoroacetate pyruvate cannot be readily oxidized.

Epinephrine produces a significant positive inotropic effect in the presence of inhibitors of glycolysis or the reactions of the tricarboxylic acid cycle. However, the response to epinephrine is diminished under these conditions. The importance of metabolic reactions for the full development of the mechanical effect of epinephrine is well demonstrated in the experiments described in Fig. 2. When pyruvate is added as a substrate to the heart inhibited by iodoacetate, the administration of epinephrine produces a mechanical response which is even greater than that seen in control hearts. A high rate of oxidative metabolism and maintenance of the cardiac ATP level (Table 1) made it possible for the heart to repond dramatically to epinephrine despite inhibition of glycolysis.

Conversion of phosphorylase *b* to phosphorylase *a* after epinephrine was observed in all experiments and was reflected in changes in glycolytic intermediates. The increase in phosphorylase *a* activity produced by epinephrine is greater in the presence of iodoacetate or fluoroacetate than in control hearts or in hearts perfused with

solutions containing both iodoacetate and pyruvate. Although the mechanism of this phenomenon is unknown, the findings suggest that a metabolic feedback mechanism is operative in the transformation of phosphorylase *b* to *a*.

It is clearly evident that in the presence of an ample supply of substrate for the tricarboxylic acid cycle an increase in phosphorylase activity and glycolysis by epinephrine is not necessary for mechanical stimulation of the heart. However, glycogenolytic reactions probably play a role in furnishing energy for maintenance of an increased force of contraction when the supply of energy from other sources is low. The conclusion that phosphorylase stimulation is not essential for the initial inotropic effect of epinephrine agrees with the findings of other investigators[2–4] that the contractile changes in the heart stimulated by epinephrine precede the activation of phosphorylase.

The mechanism of the positive inotropic action of epinephrine remains obscure. Robison *et al.*[3] have shown that with epinephrine the rise in the concentration of cyclic 3′,5′-AMP in the heart follows more closely in time the increase in contractility than does phosphorylase *a*. It is possible that cyclic 3′,5′-AMP has a specific role in the contractile process but evidence for this is still lacking. Whatever the mechanism of action of epinephrine in increasing contractility, the experiments presented here emphasize the importance of the supply of energy from metabolic reactions for the full expression of the inotropic effect of epinephrine.

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