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EPINEPHRINE-MEDIATED STIMULATION OF GLUCOSE UPTAKE AND LACTATE RELEASE BY THE PERFUSED RAT HEART. EVIDENCE FOR α- AND β-ADRENERGIC MECHANISMS

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Summary : Epinephrine treatment of the perfused rat heart led to an increase in the rate of glucose uptake and lactate release as well as increases in the rate of beating and the activity ratio of phosphofructokinase. The dose of epinephrine required for half maximal increases in the rate of beating, and glucose uptake and the activity ratio of phosphofructokinase was approx.10^{-7}M. Glucose uptake, lactate release and the activity ratio of phosphofructokinase were increased by the α-agonists methoxamine and phenylephrine, and the β agonist, isoproterenol. Propranolol and phenoxybenzamine each partially blocked the stimulatory effects of epinephrine on glucose uptake and lactate production. Phenoxybenzamine blocked the stimulatory effects of methoxamine but had no effect on those produced by isoproterenol which were blocked by propranolol. It is concluded that dual α and β adrenergic control of glycolysis occurs in cardiac muscle. It is proposed that the previously reported α-adrenergic control of phosphofructokinase plays a key role in the control of heart muscle glycolysis.

Introduction

Considerable data has been accumulated which shows that catecholamines stimulate hepatic glucose output in the rat by an α-receptor mechanism, independent of changes in cyclic AMP (1-3). Although the interest in liver-specific events has grown steadily, there has been relatively little attention paid to the catecholamine-mediated biochemical events that operate in other tissues - particularly mechanisms that might be altered to make use of the increased glucose release from liver. Williamson (4,5) has shown that in the absence of exogenous lipid the isolated perfused heart responds to epinephrine by increasing glycogen breakdown and glucose uptake. Mobilization of the polysaccharide was found to be transient and within

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minutes glucose uptake was accelerated as glycogen breakdown ceased (4). Recently we have reported that epinephrine treatment of the perfused rat heart leads to the activation of phosphofructokinase by a predominantly α-adrenergic mechanism (6). This observation complemented a report by Keely et al. (7) of an α-adrenergic control process for glucose transport in heart and suggested that an α-adrenergic receptor mechanism may contribute to the control of glycolysis in this tissue. In the present work this possibility is investigated.

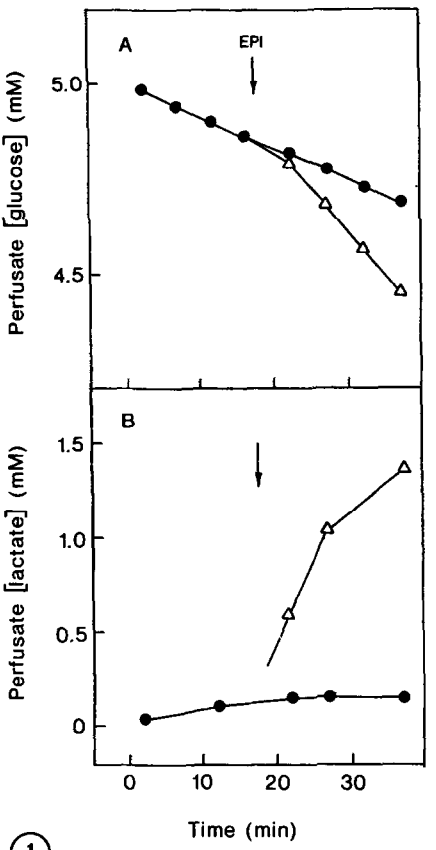
Experimental Procedures

Male rats (180 to 230 g body wt.) of the Hooded Wistar strain, maintained ad libitum on a standard diet, were used for these experiments. Hearts were perfused in the Langendorff manner using a system based on that of Williamson (4). The perfusion medium was Krebs-Ringer bicarbonate buffer containing 1.27 mM $CaCl_2$, 0.05 mM EDTA and 5 mM glucose, and the perfusion temperature was 37°C. The hearts were preperfused at a flow rate of 5-8 ml/min for 10 minutes in a non-recirculating manner prior to transfer to the recirculation apparatus. Recirculating perfusion (17 ml) was continued for a total of 38 min. Agonists and/or antagonists were added at 18 min. Samples (0.3 ml) of the recirculating buffer were removed at 5 min intervals and mixed with 0.3 ml 0.6 M perchloric acid at 0°C. The rates of glucose uptake and lactate output before (2 to 17 min) and after (22 to 37 min) agonist/antagonist addition were determined. Glucose was determined in the perchloric acid extracts (8) and lactate in the neutralized perchlorate extracts (9).

The activity ratio of cardiac phosphofructokinase was determined on supernatants from freeze-clamped hearts as described previously (6). Since the hearts were derived from perfusions in which the perfusate contained 5 mM glucose, interference with the phosphofructokinase activity determination had to be assessed. The actual concentration of glucose in the frozen heart samples was 0.49±0.08 μmol/g (n = 6) and there was no significant difference between the concentration of glucose in stimulated versus non-stimulated frozen hearts. Thus on average the concentration of glucose from extracellular and intracellular sources in the phosphofructokinase assay was 6.2 μM. Concentrations of glucose up to 25 μM did not affect the apparent activity of phosphofructokinase at either 0.1 mM ATP or at 1 mM ATP when using 10 μM hexose 6-phosphate.

Results

Fig. 1 shows the time course for the effects of 10 μM epinephrine on glucose uptake and lactate release by the isolated perfused rat heart. These results are essentially the same as reported by Williamson (5). The rate of glucose uptake (Fig. 1, Panel A) remained linear for the duration of the perfusion unless epinephrine was added. The epinephrine-mediated increase in the rate of glucose uptake took 2-3 min to develop and did not reach the



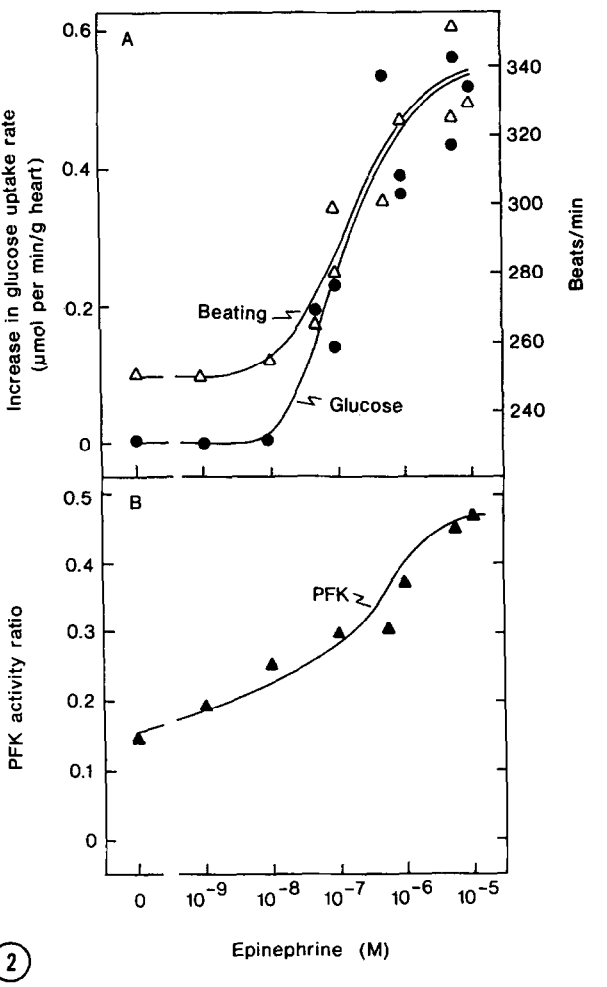


Fig. 1. Time course for the activation of glucose uptake (A) and lactate release (B) by epinephrine. After non-recirculating preperfusion for 10 min with Krebs-Ringer bicarbonate buffer containing 1.27 mM $CaCl_2$, 0.05 mM EDTA and 5 mM glucose, the isolated rat hearts were perfused in a recirculating manner for 38 min. Epinephrine (46 µl of 3 mM solution) was added at 18 min (arrow) to the perfusate immediately below the heart. This reached the heart approx. 1.5 min later. Samples of the perfusate were taken as shown. The hearts were freeze-clamped at 38 min. Representative data from four control (●) and four epinephrine treated (Δ) hearts are shown.

Fig. 2. Dose-response curves for epinephrine-mediated changes in glucose uptake and beating rate (A) and the activity ratio of phosphofructokinase (B). Rat hearts were perfused as described in Fig. 1. The maximum beating rate is shown. For the determination of the activity of phosphofructokinase heart extracts were prepared in 100 mM Tris/HCl, pH 7.4, containing 15 mM 2-mercaptoethanol, 30 mM NaF, 0.1 mM EDTA and 3 mM NaN_3. Other details are given in the section on 'Experimental Procedures'. The values shown are the means of duplicate determinations on single hearts.

new linear rate until approx. 5 min after addition of the hormone. As previously noted by Williamson (5) the increased rate of lactate production elicited by epinephrine was not linear and possibly reflected a complex

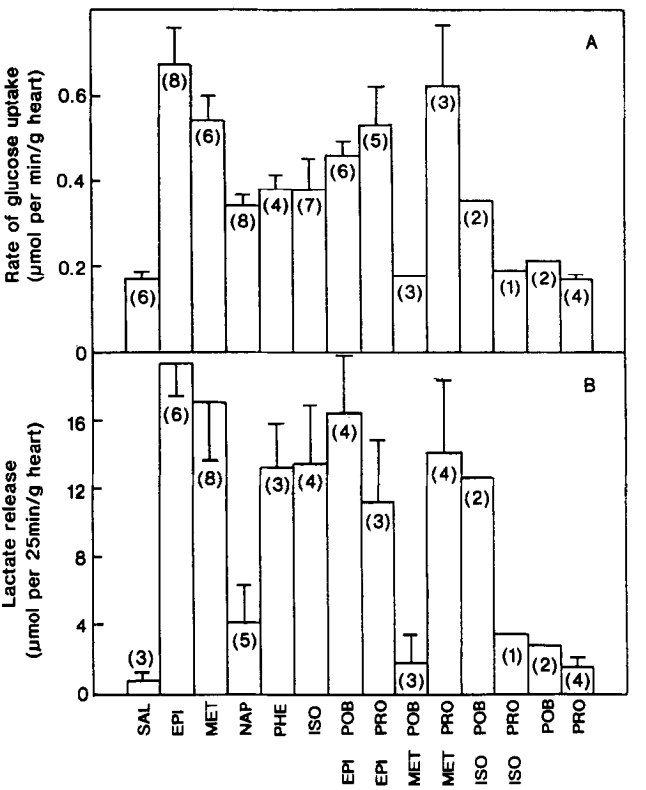


Fig. 3. Effects of α- and β-adrenergic agonists and antagonists on the rate of glucose uptake (A) and lactate release (B). Rat hearts were perfused as described in Fig. 1. The rate of glucose uptake was calculated from the perfusate concentration values determined at 5 min intervals between 22 min and 37 min. In all cases this rate was linear. Lactate determinations were conducted on perfusate samples at 7, 12, 22 and 37 min. Values are shown for the amount of lactate released between 12 and 37 min. Agonists or antagonists or a combination of the two were added at 18 min. Where appropriate means±S.E.M. have been calculated; the number of hearts is shown in parentheses. Abbreviations are saline (SAL), epinephrine (EPI), methoxamine (MET), naphazoline (NAP), phenylephrine (PHE), isoproterenol (ISO), phenoxybenzamine (POB) and propranolol (PRO).

series of events including changes in the rates of glucose uptake, glycogenolysis and pyruvate oxidation.

Dose response curves for epinephrine-mediated changes in glucose uptake, beating and the activity ratio of phosphofructokinase are shown in Fig. 2. The concentration of epinephrine required to produce half-maximal increases in glucose uptake and beating (Fig. 2, Panel A) as well as in the activity ratio of phosphofructokinase was approx. 10^{-7} M.

The relative effects of a single concentration (10 µM) of five adrenergic agonists on the rates of glucose uptake and lactate release were compared. In Fig. 3A the effects of these agonists on the rate of glucose

uptake are shown. The α-agonists, methoxamine and naphazoline, increased the rate 3.0-, 1.8-fold respectively. Agonists with both α and β activity (epinephrine and phenylephrine) also increased the rate of glucose uptake. Epinephrine produced a 3.8-fold increase and phenylephrine a 2.1-fold increase in the rate of glucose uptake. The β-agonist, isoproterenol, increased the rate 2.1-fold. Further evidence for a dual α- and β-adrenergic control of this process was obtained using the α and β blockers, phenoxybenzamine and propranolol. As shown in Fig. 3A each blocker (10 μM) partly but not totally blocked the epinephrine-mediated increase in glucose uptake. In addition phenoxybenzamine blocked the stimulatory effects of methoxamine but had no effect on those produced by isoproterenol which were blocked by propranolol.

In Fig. 3B the effects of the five adrenergic agonists as well as the antagonists on lactate release by the heart are shown. The rate of lactate release was increased by epinephrine > isoproterenol = phenylephrine = methoxamine > naphazoline. The epinephrine-mediated stimulation of lactate release was inhibited to a greater extent by propranolol than by the α-antagonist, phenoxybenzamine. Overall the data imply that glucose uptake and lactate release by the perfused rat heart are controlled by both α- and β-adrenergic mechanisms. There is some suggestion that α-adrenergic mechanisms exert more control on glucose uptake than on lactate release.

Since the rate of glucose uptake remained linear throughout the 20 min perfusion regardless of the agonist present (i.e. see Fig. 1), it appeared likely that any change in the activity ratio of phosphofructokinase induced by the agonists would also be retained. Thus the effects of the five agonists on the activity ratio of phosphofructokinase were determined on the same hearts as those used for glucose uptake measurements. These were freeze-clamped at 20 min after addition of the agonists. The phosphofructokinase activity ratios were 0.20±0.08 (n=5, control hearts), 0.53±0.03 (n=8, EPI hearts), 0.55±0.07 (n=6, MET hearts), 0.20±0.02 (n=5, NAP hearts), 0.38±0.04 (n=4, PHE hearts) and 0.35±0.04 (n=5, ISO hearts).

Discussion

The present observations draw together earlier separate reports of an α-adrenergic mechanism for glucose transport (7), of an α-adrenergic mechanism for the control of phosphofructokinase (6,11) and of a component of epinephrine-mediated glucose uptake that was not blocked by β-adrenergic blockers (4). In so doing a coordinated mechanism for the α-adrenergic control of cardiac muscle glycolysis emerges. As suggested in earlier publications (6,10,11) this may complement the well-established α-adrenergic control of glucose release by the liver and thus establish a catecholamine-mediated control of a liver/heart Cori cycle.

Direct comparisons between the present data and those of our previous studies must be considered with caution. Three important changes have been introduced. These involve (i) reduction of perfusate volume, (ii) a recirculating instead of non-recirculating mode and (iii) decreased Ca^{2+} and EDTA concentrations. Thus in the present work a recirculating perfusion of small perfusate volume based on that of Williamson (4,5), and employing medium containing 1.27 mM $CaCl_2$ and 0.05 mM EDTA instead of 3.0 mM $CaCl_2$ and 0.5 mM EDTA, was used. Whereas the dose and time responses for epinephrine on glucose uptake in the present work were very similar to those obtained by Williamson (4), the dose response curves for two of the three parameters (beating rate, and phosphofructokinase) appeared to be approx. one order of magnitude less sensitive than reported using the non-recirculating system (10,11). In addition, in the present study, the activity ratio of phosphofructokinase was determined on hearts, 20 min instead of 5 min, after exposure to the catecholamine. The recirculating perfusion of small volume permits accurate assessment of the rates of glucose uptake and lactate release but does present a system in which the recirculating concentration of catecholamine becomes a function of the rate of its metabolism, oxidation, uptake etc. However, despite this limitation, two properties of the rate of glucose uptake suggest a key involvement of phosphofructokinase in controlling this process. Firstly the epinephrine-

mediated increase in the rate of glucose uptake was slower to develop (Fig. 1A) than either the change in contraction or the activation of phosphorylase (12) but was closely correlated with the activation of phosphofructokinase (6,12). Secondly, epinephrine-mediated activation of glucose uptake occurred at concentrations of the catecholamine similar to that required for activation of phosphofructokinase (Fig. 2).

In summary both α- and β-adrenergic mechanisms for the control of glucose metabolism in perfused rat heart are described. It is proposed that the α-adrenergic control of glucose transport reported by Keely et al. (7) and the α-adrenergic control of phosphofructokinase (6,11) account for α-adrenergic components of this mechanism.

The partial inhibition of the epinephrine-mediated increase in glucose uptake by propranolol indicates a separate β-receptor mediated component. Propranolol has no effect on either the epinephrine-mediated activation of 3-O-methylglucose transport (7) or phosphofructokinase (6,11). However, it remains to be examined whether this β adrenergic component accompanies the β-adrenergic control of contraction or is absent at lower, more physiological, concentrations of epinephrine when the β-adrenergic activation of phosphorylase is not apparent (11).

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