The activation of pyruvate dehydrogenase in the perfused rat heart by adrenaline and other inotropic agents

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Adrenaline resulted in a reversible 4-fold increase in the amount of pyruvate dehydrogenase in its active non-phosphorylated form in the perfused rat heart within 1 min. The increase was less in extent in hearts from starved or diabetic rats or in hearts from control rats oxidizing acetate, unless pyruvate was added to the perfusion medium. Increases could also be induced by other inotropic agents, supporting the hypothesis that increases in cytoplasmic Ca^{2+} can be relayed into mitochondria and influence oxidative metabolism.

Perfusion of rat hearts with medium containing adrenaline results in a marked increase in contractile force within 20 s (Williamson & Jamieson, 1966; England, 1975; England *et al.*, 1979). The enhanced rates of respiration are supported, at least in part, by the increased utilization of glucose (Williamson, 1964, 1966, 1975). This could be brought about by an increase in the proportion of the pyruvate dehydrogenase complex in its active non-phosphorylated form (PDH_a). In fact, we would predict such an increase, since the inotropic effect of adrenaline is achieved through an elevation of cytoplasmic Ca^{2+} concentration (see Williamson, 1975), and we have shown recently, using intact rat heart mitochondria, that increases in the extra-mitochondrial concentration of Ca^{2+} in the physiological range (10 nM–10 μM) lead to parallel increases in the amount of PDH_a within the mitochondria through stimulation of the Ca^{2+} -sensitive pyruvate dehydrogenase phosphate phosphatase (Denton *et al.*, 1980). However, such an effect of adrenaline on the rat heart would be in marked contrast with the effects of adrenaline on rat epididymal white adipose tissue, where the hormone decreases the amount of the active form of the complex (Coore *et al.*, 1971; Martin *et al.*, 1972). In both the rat heart and epididymal adipose tissue the effects of adrenaline are predominantly via β -type receptors (Westermann & Stock, 1970; Williamson, 1975).

We therefore examined the effects of adrenaline and other inotropic agents on the amount of PDH_a in the perfused rat heart. While the present paper was in preparation, Hiraoka *et al.* (1980) reported

that adrenaline and isoprenaline reversibly increased the conversion of [$1\text{-}^{14}\text{C}$]pyruvate into CO_2 and also the amount of PDH_a in rat hearts perfused with medium containing pyruvate (0.5 mM) with or without β -hydroxybutyrate.

Experimental

Hearts from male Wistar rats (250–300 g) were perfused by the Langendorff technique (drip-through without recirculation) with gassed (O_2/CO_2 , 19:1) bicarbonate-buffered medium (Krebs & Henseleit, 1932). Unless otherwise stated, this medium contained 1.25 mM- CaCl_2 and 1 mM- KH_2PO_4 , and the perfusion pressure was 7 kPa. Details of additions of oxidizable substrates and hormones to the perfusion media are indicated in Table and Figure legends. Extraction and assay of the amounts of 'initial' and 'total' pyruvate dehydrogenase activities from the freeze-clamped tissues were as described by Whitehouse *et al.* (1974). Results are given as the percentage of total pyruvate dehydrogenase activity existing as PDH_a (as in Hems *et al.*, 1978). Total activity was not appreciably changed in any of the conditions tested and ranged from 2 to 4 units/g wet wt. (where a unit is the amount of enzyme catalysing the conversion of pyruvate into acetyl-CoA at the rate of 1 $\mu\text{mol}/\text{min}$ at 30°C), as found previously (Kerbey *et al.*, 1976).

Alloxan and isoprenaline were from Kodak, Kirkby, Liverpool, U.K., glucagon was from Calbiochem, Bishops Cleeve, Herts. CM23 3AL, U.K., and insulin was a gift from Boots Pure Drug Co., Nottingham, U.K. All other chemicals and biochemicals were obtained from either Boehringer

* Abbreviation used: PDH_a , the active, non-phosphorylated form of pyruvate dehydrogenase.

Corp., Lewes, East Sussex BN7 1LG, U.K., or BDH Chemicals, Poole, Dorset BH12 4NN, U.K.

Results

Adrenaline, glucagon and isoprenaline were all found to increase the amount of PDH_a in rat hearts perfused with medium containing glucose by at least 3-fold. The time courses of the effects of saturating concentrations of these three hormones are shown in Fig. 1(a). Values of PDH_a in the absence of hormones are similar to those reported previously (Whitehouse *et al.*, 1974; Kerbey *et al.*, 1976). Maximum stimulation was apparent within 1 min with adrenaline or isoprenaline, whereas with glucagon 2–3 min was required. These time courses are somewhat slower than those of the increases in force of contraction, cyclic AMP concentration, phosphorylase kinase and phosphorylase activity (Mayer *et al.*, 1970; Williamson, 1975) and troponin-I phosphorylation (England, 1975, 1976) observed with these hormones. However, the dose-response curve for the effect of adrenaline on PDH_a (Fig. 1b) was very similar to that reported for the action of this hormone on these parameters (Williamson, 1975; England, 1975). The concentration of adrenaline required for half-maximal activation of pyruvate dehydrogenase was calculated to be $0.17 \pm 0.02 \mu\text{M}$ (value $\pm$ s.d. based on 26 different observations). We have also established that the effect of adrenaline on PDH_a in the

perfused rat heart is reversible. In these studies (results not shown), hearts received just enough adrenaline ($0.5 \mu\text{M}$) to elicit maximum stimulation at 1 min ($\text{PDH}_a = 38.9 \pm 2.8\%$ of total activity; mean $\pm$ s.e.m. for four observations), and perfusions were then continued with eight other hearts with medium containing no added adrenaline. The amount of PDH_a returned to control values (i.e. less than 10% of total activity) within 2 min. This response was again slower than the change in contractile force, which returned to control values in 10–20 s.

Comparable effects of adrenaline were observed in hearts perfused with medium containing no added substrate, 10 mM-fructose or 10 mM-glucose (Table 1). Insulin was without effect in either the absence or the presence of adrenaline. Perfusion in the presence of pyruvate or dichloroacetate results in an increase in cardiac PDH_a , presumably by inhibition of pyruvate dehydrogenase kinase (Whitehouse *et al.*, 1974; Kerbey *et al.*, 1976). An effect of adrenaline was still apparent over and above the effects of 10 mM-pyruvate or 0.1 mM-dichloroacetate (Table 1). These results suggest that the action of adrenaline is probably not via changes in pyruvate concentration in the heart.

Perfusion with medium containing acetate decreases the amount of PDH_a , probably in part via an increased intramitochondrial acetyl-CoA/CoA concentration ratio, which activates pyruvate dehydrogenase kinase (Kerbey *et al.*, 1976). At a

Fig. 1. (a) Time courses of the activation of pyruvate dehydrogenase by adrenaline, isoprenaline and glucagon in the perfused rat heart and (b) dependence of the activation on the concentration of adrenaline

Hearts were preperfused for 10 min as described in the Experimental section with medium containing 10 mM-glucose. (a) Perfusions were then commenced with the same medium plus no addition (○), $0.25 \mu\text{M}$ -glucagon (■), $5 \mu\text{M}$ -adrenaline (●), or $1 \mu\text{M}$ -DL-isoprenaline (▲), and PDH_a was determined at the times shown. (b) Perfusions were then commenced with the same medium plus various concentrations of adrenaline and PDH_a was determined after 1 min of perfusion. All results shown are the mean of at least three observations (s.e.m. values on the points given were all less than 20% of the values shown), except for isoprenaline-treated hearts (▲), where the points shown are means of two observations.

Table 1. *Effects of adrenaline, high Ca^{2+} concentration in medium and higher perfusion pressure on the activity of pyruvate dehydrogenase in hearts from normal, diabetic or starved rats perfused under a variety of different conditions*

Hearts were preperfused at 7 kPa for 10 min with bicarbonate-buffered medium with additions or changes indicated. Perfusions were then continued under the same conditions with or without adrenaline ($5 \mu\text{M}$) for 1 min before hearts were freeze-clamped and the amount of PDH_a was determined. Diabetes was induced by intravenous injection of alloxan (60 mg/kg) under light ether anaesthesia 48 h before removal of hearts for perfusion. Where appropriate, KH_2PO_4 was decreased to 0.2 mM to avoid precipitation of calcium phosphate. Results are given as means $\pm$ S.E.M. for the numbers of separate observations given in parentheses. Effect of adrenaline compared with appropriate control value by Student's *t* test: * $P < 0.01$; ** $P < 0.001$.

Source of heart	Additions or changes to preperfusion and perfusion medium	PDH_a (as % of maximum activity) in hearts perfused (1 min)	
		Without adrenaline	With $5 \mu\text{M}$ -adrenaline
Normal fed rat	None	10.7 ± 3.4 (4)	38.6 ± 4.3 (6)*
	10 mM-fructose	6.0 ± 2.3 (4)	30.3 ± 5.6 (6)*
	10 mM-glucose (as Fig. 1)	9.4 ± 1.5 (6)	38.3 ± 2.2 (6)**
	10 mM-glucose + insulin (5 munits/ml)	8.0 ± 1.4 (3)	35.9 ± 3.2 (3)*
	10 mM-glucose + 5 mM-pyruvate	37.8 ± 5.6 (4)	80.1 ± 6.1 (5)*
	10 mM-glucose + 10 mM-pyruvate	64.5 ± 4.9 (3)	100.9 ± 2.6 (5)**
	10 mM-glucose + 0.1 mM-dichloroacetate	33.8 ± 4.2 (3)	66.7 ± 5.3 (3)*
	10 mM-glucose + 1 mM-acetate	2.2 ± 0.5 (3)	9.1 ± 1.0 (3)*
	10 mM-glucose + 10 mM-acetate	0.6 ± 0.1 (3)	0.7 ± 0.2 (3)
	10 mM-glucose + 10 mM-pyruvate + 10 mM-acetate	25.0 ± 4.8 (4)	61.5 ± 5.4 (6)*
48 h-starved rat	10 mM-glucose	2.4 ± 0.6 (3)	10.5 ± 1.2 (3)*
	10 mM-glucose + 5 mM-pyruvate	4.5 ± 1.1 (3)	37.5 ± 4.2 (3)*
Alloxan-diabetic rat	10 mM-glucose	3.6 ± 1.1 (3)	15.0 ± 2.1 (3)*
	10 mM-glucose + 5 mM-pyruvate	6.2 ± 1.4 (3)	37.1 ± 4.2 (3)*
Normal fed rat	0.2 mM- KH_2PO_4 with 10 mM-glucose	7.0 ± 2.1 (3)	—
	As above, except [CaCl_2] increased to 7 mM for the 1 min perfusion period	32.5 ± 4.1 (5)	—
	10 mM-glucose (perfusion pressure 7 kPa throughout)	7.2 ± 1.3 (3)	—
	As above, except perfusion pressure increased to 18 kPa for the 1 min perfusion period	33.4 ± 2.4 (4)	—

concentration of 1 mM, the presence of acetate appreciably diminished the response to adrenaline, and at 10 mM-acetate all discernable effects of adrenaline disappeared. However, in the presence of both 10 mM-acetate and 10 mM-pyruvate, adrenaline was again effective. A similar situation was found in hearts from diabetic or starved rats perfused with medium containing glucose alone. As found in earlier studies (Kerbey *et al.*, 1976), the amount of PDH_a was diminished compared with hearts from normal fed rats perfused under the same conditions, and pyruvate (5 mM) was less effective in activating the enzyme (Table 1). The extent of the increase in PDH_a caused by adrenaline on these hearts perfused with glucose alone was small and similar to that seen with control hearts perfused with medium containing both glucose and 1 mM-acetate. This might be expected if the kinase is more active under these circumstances (Kerbey *et al.*, 1976; Baxter & Coore, 1978; Hutson *et al.*, 1978). However, if the hearts from diabetic or starved rats were perfused with medium containing both glucose and 5 mM-pyruvate, then a much larger response to adrenaline was observed. It

should be noted that the diminished response to pyruvate which was apparent in hearts from starved or diabetic rats perfused with medium containing glucose and in hearts from normal fed rats perfused with medium containing both glucose and acetate was not apparent in the presence of adrenaline.

Increases in the concentration of CaCl_2 in the perfusing medium or increases in work load also result in greater cardiac contractility, but without any change in cyclic AMP concentration (see Williamson, 1975; Randle & Tubbs, 1979). As shown in Table 1, the increases in PDH_a brought about within 1 min of increasing CaCl_2 in the medium to 7 mM or the perfusion pressure from 7 to 18 kPa were very comparable with the maximum stimulation observed with adrenaline. Illingworth & Mullings (1976) have reported previously an increase in rat heart PDH_a on increasing the work load when pyruvate was the only exogenous substrate.

Increases in rat heart PDH_a were also found in intact animals injected intraperitoneally with either adrenaline (5 mg/kg in 0.5 ml of 0.9% NaCl,

for 1 min) or glucagon (0.2 mg/kg in 0.5 ml of 0.5 M-KCl, pH 10, for 15 min). Adrenaline increased PDH_a from 27.2 ± 3.3 (11) to 69.4 ± 5.2 (8)% of total activity ($P < 0.001$) and glucagon increased PDH_a from 28.1 ± 5.1 (7) to 51.3 ± 6.3 (7)% of total activity ($P < 0.05$) (results are given as means $\pm$ S.E.M. for the numbers of observations in parentheses; P values were calculated by using Student's t test).

Discussion

These studies demonstrate that adrenaline can markedly increase the amount of PDH_a in hearts from fed rats and that this occurs over the same range of concentrations of the hormone which result in changes in contractility, cyclic AMP concentration and other metabolic parameters (Mayer *et al.*, 1970; Williamson, 1975; England, 1975). Separate studies have shown that the increase in PDH_a with adrenaline does represent dephosphorylation of the α -subunits of the pyruvate dehydrogenase complex. This was accomplished by perfusing hearts with medium containing $^{32}\text{P}_i$ (as in England, 1975) and then determining the amount of ^{32}P incorporated after separating the α -subunits by a combination of immunoprecipitation and gel electrophoresis as described by Hughes *et al.* (1980) (W. A. Hughes & R. M. Denton, unpublished work).

The increase in rat heart PDH_a observed with adrenaline could be the result of a decrease in pyruvate dehydrogenase kinase activity. This could be brought about by a combination of an increased concentration of pyruvate plus increases in the NAD⁺/NADH and ADP/ATP concentration ratios in mitochondria. Some evidence for all these changes has been reported (Williamson & Jamieson, 1966; Williamson, 1966, 1975). However, in each case the increases appear to be transient and largely disappear within 1 min of the start of exposure to adrenaline, although the effects of adrenaline on contraction, substrate oxidation and PDH_a are fully maintained for at least 5 min. It therefore would appear more likely that the increase in PDH_a is brought about by the increase in the time-averaged cytoplasmic Ca²⁺ concentration being relayed into the mitochondria and activating pyruvate dehydrogenase phosphate phosphatase. This view is supported by the finding that other agents which induce greater contractility and thus presumably the time-averaged cytoplasmic Ca²⁺ concentration (namely glucagon, increased extracellular Ca²⁺ and increased work load) also increase the amount of PDH_a. Hiraoka *et al.* (1980) have also arrived at this conclusion. These workers measured the whole tissue concentrations of the principal effectors of pyruvate dehydrogenase kinase (acetyl-CoA, CoA, NAD⁺, NADH, ATP and ADP) and concluded

that the changes observed after 90 s exposure to isoprenaline were unlikely to be involved in the activation of pyruvate dehydrogenase. On the other hand, Hiraoka *et al.* (1980) found that the effects of adrenaline and isoprenaline on [^{14}C]-pyruvate oxidation and the amount of PDH_a were greatly diminished in the presence of two agents (verapamil and the ionophore A23187) which alter the normal distribution of Ca²⁺ in the perfused heart.

Since both NAD⁺:isocitrate dehydrogenase (EC 1.1.1.41) and oxoglutarate dehydrogenase show a similar sensitivity to activation by Ca²⁺ to pyruvate dehydrogenase phosphate phosphatase, we would expect that the activity of these other two intramitochondrial dehydrogenases would also be enhanced when cardiac contractility is increased (Denton & McCormack, 1980). We have pointed out that the parallel activation of the three dehydrogenases with Ca²⁺ may be an important means whereby an increased supply of reducing power can be supplied to the respiratory chain with minimum decreases occurring in the NADH/NAD⁺ and ATP/ADP concentration ratios (Denton & McCormack, 1980). Indeed, it seems reasonable to suggest that the recovery of these ratios together with the concentration of pyruvate to near control values in rat hearts in the period 30–60 s after addition of adrenaline to the perfusion medium (Williamson, 1966) may reflect the activation of the three dehydrogenases by an increase in the intramitochondrial concentration of Ca²⁺.

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