Activation of Pyruvate Dehydrogenase in Perfused Rat Heart by Dichloroacetate

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The activity of pyruvate dehydrogenase was assayed in extracts of rat hearts perfused *in vitro* with media containing glucose and insulin $\pm$ acetate $\pm$ dichloroacetate. Dichloroacetate (100 μ M, 1 mM or 10 mM) increased the activity of pyruvate dehydrogenase in perfusions with glucose or glucose + acetate. Evidence is given that dichloroacetate may facilitate the conversion of pyruvate dehydrogenase from an inactive (phosphorylated) form into an active (dephosphorylated) form.

Lorini & Ciman (1962) showed that injection of diisopropylammonium dichloroacetate may increase the respiratory quotient in alloxan-diabetic rats, thus suggesting that this compound may stimulate glucose oxidation and inhibit fatty acid oxidation. Experiments with rat diaphragm and rat heart *in vitro* showed that sodium dichloroacetate stimulates glucose oxidation when this has been inhibited by acetate or 3-hydroxybutyrate or palmitate or by induction of alloxan-diabetes (Stacpoole & Felts, 1970, 1971; McAllister & Randle, 1970; McAllister *et al.*, 1973). Dichloroacetate inhibits oxidations of acetate, 3-hydroxybutyrate and palmitate. Stacpoole & Felts (1971) suggested that dichloroacetate may accelerate glucose oxidation in alloxan-diabetes by inhibiting fatty acid oxidation. However, measurements of metabolite concentrations in heart and diaphragm muscles with dichloroacetate are not consistent with this interpretation. In hearts from normal fed rats perfused with glucose and insulin, dichloroacetate increases the concentrations of acetyl-CoA and acetyl-carnitine. In perfusions with acetate, dichloroacetate does not lower the increased concentration of acetyl-CoA, and perfusions with [$1\text{-}^{14}\text{C}$]acetate indicated that dichloroacetate may increase the conversion of pyruvate into acetyl-CoA. These findings suggested that dichloroacetate may directly activate pyruvate oxidation, perhaps through an effect on pyruvate dehydrogenase (EC 1.2.4.1). Pyruvate dehydrogenase in heart muscle may exist in active (dephosphorylated) and inactive (phosphorylated) forms whose interconversion is catalysed by an ATPMg $^{2+}$ -requiring kinase and an Mg $^{2+}$ +Ca $^{2+}$ -requiring phosphatase (Linn *et al.*, 1969; Wieland *et al.*, 1971; Denton *et al.*, 1972). We have investigated the effects of dichloroacetate on the active and total pyruvate dehydrogenase activity in perfused rat heart.

Rat hearts from fed male albino Wistar rats were perfused for 10 min at 38°C by drip through at 8000 Pa (60 mmHg) pressure with media containing glucose

(16.5 mM) and insulin (20×10^{-3} i.u./ml) $\pm$ acetate (5 mM) $\pm$ sodium dichloroacetate (10 μ M–10 mM). Dichloroacetic acid was from BDH Chemicals Ltd., Poole, Dorset, U.K., and neutralized with NaOH before addition to perfusion medium (Krebs & Henseleit, 1932). At the end of perfusion hearts were frozen with a tissue clamp at the temperature of liquid N $_2$. The hearts were then powdered under liquid N $_2$ with a porcelain pestle and mortar. Pyruvate dehydrogenase was extracted from a weighed sample of heart powder with 100 mM-potassium phosphate buffer, pH 7, containing 10 mM-sodium pyruvate and 5 mM-EDTA (5 ml of extraction fluid/g of wet heart powder) in a Kontes tissue homogenizer. After centrifugation, active pyruvate dehydrogenase was assayed spectrophotometrically at 30°C by coupling to arylamine transacetylase under the conditions given by Coore *et al.* (1971). For assay of total pyruvate dehydrogenase a sample of extract was incubated for 20 min at 30°C with pig heart pyruvate dehydrogenase phosphate phosphatase (see Denton *et al.*, 1972) and Mg $^{2+}$ (final concn. 10 mM). Pyruvate dehydrogenase activity was then reassayed spectrophotometrically. Control experiments showed that 20 min of incubation with phosphatase was more than sufficient to effect complete conversion into the active form.

Initial control experiments showed that extracts of rat heart made with 100 mM-potassium phosphate buffer, pH 7, lose pyruvate dehydrogenase activity on incubation at 30°C. Inclusion of pyruvate (10 mM) in the extraction fluid resulted in an increase in pyruvate dehydrogenase activity on incubation at 30°C. It is known that pyruvate is an inhibitor of pyruvate dehydrogenase kinase (Linn *et al.*, 1969), but its stabilizing effect on pyruvate dehydrogenase activity in heart extracts is not necessarily due to this action. The further inclusion of EDTA (5 mM), which may be expected to inhibit pyruvate dehydrogenase phosphate phosphatase by chelation of Mg $^{2+}$ and Ca $^{2+}$, yielded extracts that showed no change in pyruvate

Table 1. *Effect of dichloroacetate on the activity of pyruvate dehydrogenase in perfused rat heart*

Hearts from normal fed rats were perfused for 10 min at 38°C with medium containing glucose (16.5 mM) and insulin (20×10^{-3} i.u./ml) $\pm$ acetate (5 mM) $\pm$ dichloroacetate (at concentrations given). Hearts were frozen with a tissue clamp, powdered, extracted and assayed for pyruvate dehydrogenase (see the text for details). Numbers of hearts analysed are shown in parentheses.

		Pyruvate dehydrogenase activity (units/g dry wt. of heart)			
Perfusion	...	Active form	Total	Active form	Total
		Glucose	Glucose	Glucose + acetate	Glucose + acetate
Control		5.1 $\pm$ 0.89† (10)	24.2 $\pm$ 0.75* (10)	1.9 $\pm$ 0.25* (8)	23.5 $\pm$ 2.2† (8)
Dichloroacetate	(10 mM)	17.5 $\pm$ 1.8* (6)	19.9 $\pm$ 2.3 (6)	19.9 $\pm$ 1.4† (15)	26.6 $\pm$ 2.8 (5)
	(1 mM)	20.0 $\pm$ 0.95* (4)	27.6 $\pm$ 1.9 (4)	—	—
	(100 μ M)	15.6 $\pm$ 0.71* (4)	23.2 $\pm$ 1.9 (4)	—	—
	(10 μ M)	6.2 $\pm$ 1.0 (4)	19.2 $\pm$ 0.8 (4)	—	—

* $P < 0.01$ for difference from glucose control (active form).

† $P < 0.01$ for difference from acetate control (active form).

dehydrogenase activity on storage at 0°C (for up to 2 h) or on incubation at 30°C (for 20 min). This extraction procedure was therefore employed. Control experiments also showed that reaction velocity was proportional to extract concentration under the conditions employed (extract equivalent to 2.5–10 mg wet wt. of heart/cuvette, depending on pyruvate dehydrogenase activity, with 50 munits of arylamine transacetylase).

The results of these experiments are shown in Table 1. The active form of pyruvate dehydrogenase in glucose and insulin perfusions was 5.1 units/g dry wt. of heart and approx. 20% of total activity (at 30°C). The rate of pyruvate oxidation by hearts perfused at 38°C under these conditions is 7.2 μ mol/min per g dry wt. of heart (Neely *et al.*, 1972). The activity of pyruvate dehydrogenase measured in these experiments may thus be adequate to account for rates *in vivo* (assuming Q_{10} 2). Dichloroacetate at concentrations from 0.1 to 10 mM increased the activity of the active form of pyruvate dehydrogenase approximately threefold in perfusions with glucose and insulin. Perfusion with acetate (5 mM) lowered the activity of pyruvate dehydrogenase by approx. 60%, in confirmation of the results obtained by Wieland *et al.* (1971). With acetate, dichloroacetate produced a tenfold increase in the activity of pyruvate dehydrogenase, this being not significantly different from that achieved with dichloroacetate in glucose perfusions. The total activity of pyruvate dehydrogenase showed no significant variation with any conditions of perfusion; moreover it was not significantly greater than that of the active form in perfusions with 10 mM-dichloroacetate. The threshold concentration of dichloroacetate lay between 10 and 100 μ M.

This activation of pyruvate dehydrogenase by

dichloroacetate in the perfused heart could result from inhibition of pyruvate dehydrogenase kinase or activation of pyruvate dehydrogenase phosphate phosphatase or both. The kinase is known to be inhibited by ADP and by pyruvate, but dichloroacetate perfusion did not change the concentrations of these metabolites (McAllister *et al.*, 1973). We have found that dichloroacetate (0.1 or 1 mM) is an inhibitor of pig heart pyruvate dehydrogenase kinase in phosphate buffer, pH 7, but not in 2-(*N*-morpholino)propane-sulphonate buffer, pH 7. Pig heart pyruvate dehydrogenase was purified by the polyethylene glycol fractionation procedure of Linn *et al.* (1972). Phosphorylation of the dehydrogenase was followed during incubations with 0.1 mM-ATPMg²⁺ in 20 mM-potassium phosphate–1 mM-2-mercaptoethanol, pH 7, by the loss of pyruvate dehydrogenase activity. The latter was assayed spectrophotometrically by measurement of the initial rate of NAD⁺ reduction at 340 nm on samples of incubation mixture added to 50 mM-Tris – HCl – 0.5 mM-EDTA – 1 mM-MgCl₂ – 2-mercaptoethanol, pH 7.8, containing 1 mM-pyruvate, 0.5 mM-NAD⁺, 0.1 mM-CoA and 1 mM-thiamin pyrophosphate. In the absence of dichloroacetate the percentages of the initial pyruvate dehydrogenase activity remaining after 2, 5 and 10 min of incubation were 60 $\pm$ 1.62, 23 $\pm$ 2.55 and 14.8 $\pm$ 1.49 (means $\pm$ S.E.M. for six determinations). With 1 mM-dichloroacetate the values were 72 $\pm$ 2.85, 58 $\pm$ 4.29 and 51.5 $\pm$ 6.03 ($P < 0.01$ in each case). This finding was confirmed in further experiments, with [γ -³²P]ATP, in which it was shown that 1 mM-dichloroacetate inhibits the incorporation of ³²P into pyruvate dehydrogenase.

These studies indicate that perfusion with dichloroacetate may lead to virtually complete conversion of pyruvate dehydrogenase into an active form. The

inhibitory effect of dichloroacetate on the phosphorylation of pyruvate dehydrogenase by its intrinsic kinase may be significant in this regard, although we wish to emphasize that other mechanisms are possible at this stage. Whether or not this effect of dichloroacetate on the activity of pyruvate dehydrogenase in rat heart may explain its effects on intracellular citrate concentration (lowered), on the activity of phosphofructokinase (increased) or on the rates of oxidation of acetate, 3-hydroxybutyrate and palmitate (McAllister *et al.*, 1973) remains to be ascertained. The effect of dichloroacetate on pyruvate dehydrogenase activity and on the rate of oxidation of pyruvate in the perfused heart provides further convincing evidence for the importance of active and inactive forms of the dehydrogenase in the regulation of pyruvate oxidation in this tissue.

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