Evidence for the compartmentation of pyruvate metabolism in perfused rat skeletal muscle

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In rat hindlimbs perfused with [1-¹⁴C]pyruvate and 5 mM-dichloroacetate, the calculated apparent rate of pyruvate decarboxylation was decreased with increasing perfusate pyruvate concentrations. However, in the absence of dichloroacetate the apparent rate of decarboxylation increased under these conditions. Dichloroacetate enhanced [1-¹⁴C]pyruvate uptake, but decreased the specific radioactivity of effluent lactate. Glycogen metabolism remained unaffected. The results were not consistent with a common pyruvate pool, but provide evidence for the compartmentation of pyruvate metabolism.

Dichloroacetate treatment of man and experimental animals causes marked changes in carbohydrate, lipid and amino acid metabolism (Crabb *et al.*, 1981). It is now generally accepted that most effects, but obviously not all, e.g. the decrease of leucine oxidation in rat skeletal muscle (Goodman *et al.*, 1978; Partridge *et al.*, 1983), are due to the inhibition of the pyruvate dehydrogenase kinase (Whitehouse & Randle, 1973; for a review see Wieland, 1983).

During studies on the effect of saturating dichloroacetate concentration (5 mM; cf. Partridge *et al.*, 1983) on pyruvate metabolism in isolated rat hindlimbs perfused with physiological [1-¹⁴C]pyruvate concentrations, we observed a 4-fold increase in ¹⁴CO₂ release and a decrease in lactate output. However, in spite of enhanced [1-¹⁴C]pyruvate uptake, the incorporation of label into lactate decreased significantly. This was not obviously attributable to increased intracellular dilution of label, since glycogen breakdown has been reported to be unaffected by dichloroacetate in rat liver and muscle (Crabb *et al.*, 1976; Schneider *et al.*, 1981).

The present study was undertaken to elucidate the mechanism of the dichloroacetate-induced decrease in lactate specific radioactivity. The results provide evidence for the compartmentation of pyruvate metabolism in dichloroacetate-stimulated perfused rat skeletal muscle.

Experimental

Hindlimbs of fed rats (Wistar strain; 180–200 g body wt.) were prepared essentially as described by Ruderman *et al.* (1971). Non-recirculating per-

fusion with a medium containing 0.15 mM-sodium pyruvate was performed as described previously (Schadewaldt *et al.*, 1983a). Under standard perfusion conditions, tracer doses of [1-¹⁴C]pyruvate (750–775 MBq/mmol) were infused to give final arterial specific radioactivities of 1.3–1.5 Bq/nmol. Glycogen was labelled with [U-¹⁴C]glucose (9.44 GBq/mmol) *in vivo*. At 4 h before perfusion, platinum electrodes were placed at the sciatic nerve under hexobarbital anaesthesia, and exercise was induced by electric square-wave stimulation (1 s⁻¹, 2 V, 1 ms duration) for 15 min. Subsequently 2 ml of [U-¹⁴C]glucose solution (0.42 M, 0.92 MBq/mmol) was injected intraperitoneally. Glycogen content in freeze-clamped gastrocnemius muscles was increased from 1.1 ± 0.2 mg/g muscle wet wt. after stimulation to 4.0 ± 0.3 mg/g muscle wet wt. (*n* = 4) at the beginning of perfusion.

Lactate (Gutmann & Wahlefeld, 1974), pyruvate (Czok & Lamprecht, 1974) and glycogen (Seifter *et al.*, 1950) were determined by spectrophotometric methods. ¹⁴CO₂ production was measured as described previously (Schadewaldt *et al.*, 1983a). ¹⁴CO₂ release was calculated from the ¹⁴CO₂ production on the basis of the specific radioactivity of [1-¹⁴C]pyruvate in the perfusate. Likewise, the apparent pyruvate decarboxylation rate was calculated on the basis of the specific radioactivity of lactate in the effluent. The label incorporated into lactate was calculated from the arterio-venous difference of label in CO₂ and pyruvate. The radioactivity in these metabolites was determined simultaneously (Odessey & Goldberg, 1979). Samples were incubated with an equal volume of 2 M-citric acid/3 M-H₂O₂ solution and ¹⁴CO₂ was

measured as described elsewhere (Schadewaldt *et al.*, 1983a). In a number of experiments, lactate was isolated from HClO_4 extracts of the effluent. After neutralization with KOH, the amino acids were separated as described by Aronoff (1961). The neutralized anionic fraction was chromatographed on a Dowex 1 X8 column (200–400 mesh, formate form). Lactate was eluted in a sharp peak with 0.6 M-formic acid and assayed for specific radioactivity. There was a good correlation between the two methods (P. Schadewaldt, unpublished work).

The sources for the radioactive and unlabelled material were as in Schadewaldt *et al.* (1983a). Results are means $\pm$ S.D.; Student's paired *t* test was used.

Results

In rat hindlimbs perfused with 0.15 mM-[1- ^{14}C]-pyruvate as the sole substrate, addition of 5 mM-dichloroacetate enhanced pyruvate uptake from 53 ± 9 to 87 ± 9 nmol/min per g of muscle and decreased lactate release from 346 ± 75 to 259 ± 71 nmol/min per g of muscle. $^{14}\text{CO}_2$ release and apparent pyruvate decarboxylation were increased about 4-fold. The incorporation of label into lactate was decreased by 40% (cf. Fig. 1 and Table 1). All differences were highly significant ($P < 0.001$).

In gastrocnemius muscles ($n = 6$) freeze-clamped after 30 and 60 min of perfusion according to the scheme depicted in Fig. 1, phosphocreatine and adenine nucleotide contents remained essentially unchanged (cf. Goodman *et al.*, 1978). Muscle lactate (2.4 ± 0.8 $\mu\text{mol/g}$) and pyruvate (0.08 ± 0.01 $\mu\text{mol/g}$) concentrations were not significantly decreased by dichloroacetate. This is in contrast with the marked effects reported by Goodman *et al.* (1978) and Partridge *et al.* (1983) and might be due to the quite different experimental procedure used in the present study. Glycogen breakdown was not affected by dichloroacetate. In controls, glycogen breakdown was about 30 $\mu\text{g/min}$ per g of muscle. This is sufficient to account for the decrease in glycogen content from 3.76 ± 0.69 mg/g of muscle at 30 min to 2.93 ± 0.45 mg/g of muscle after 60 min of perfusion. In perfusions in the absence of [1- ^{14}C]pyruvate, the breakdown of previously labelled [^{14}C]glycogen was observed directly. The $^{14}\text{CO}_2$ release was $18 \pm 5\%$ of total radioactivity, and $21 \pm 5\%$ on infusion of dichloroacetate. The release of total radioactivity into the perfusate, however, remained unchanged. As a control, anoxia was induced by subsequent infusion of 2 mM-sodium dithionite. Thus the release of lactate was increased by 150% (889 ± 120 nmol/min per g of muscle) and the release of radioactivity by $185 \pm 86\%$ ($n = 3$).

Fig. 1. Effect of dichloroacetate on the metabolism of perfused rat hindlimb muscle

The perfusion medium contained 0.15 mM-pyruvate. Tracer doses of [1- ^{14}C]pyruvate and dichloroacetate (DCA) were added as indicated. Metabolites were measured in the effluent and calculations performed as described in the Experimental section. Effluent lactate specific radioactivity was standardized to 1 d.p.m./nmol of influent pyruvate.

Table 1. Effect of perfusate [1- ^{14}C]pyruvate concentration on the $^{14}\text{CO}_2$ release and the specific radioactivity of effluent lactate in rat hindlimb perfusions

Release rates were calculated from the $^{14}\text{CO}_2$ production before (control) and after addition of 5 mM-dichloroacetate (DCA). The specific radioactivity of lactate in the effluent was standardized to 1 kBq per nmol of influent [1- ^{14}C]pyruvate. Results are means $\pm$ S.D. for at least three experiments.

Perfusate [pyruvate] (mM)	$^{14}\text{CO}_2$ release (nmol/min per g of muscle)		Sp. radioactivity of lactate (Bq/nmol)	
	Control	DCA	Control	DCA
0.15	16 ± 2	64 ± 2	189 ± 29	113 ± 15
1.5	136 ± 8	210 ± 13	580 ± 60	498 ± 66
5	293 ± 26	305 ± 19	855 ± 84	810 ± 46
10	336 ± 25	340 ± 31	978 ± 18	975 ± 72

In control and dichloroacetate perfusions with [1- ^{14}C]pyruvate the $^{14}\text{CO}_2$ release was significantly enhanced ($P < 0.005$) with increasing perfusate pyruvate concentrations (Table 1). As shown in Fig. 2, the calculated apparent pyruvate decarboxylation

Fig. 2. Effect of perfusate pyruvate concentration on apparent pyruvate decarboxylation in perfused rat hindlimbs

Rates were calculated from the $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ pyruvate on the basis of the specific radioactivity of lactate in the effluent. Data are from measurements during the isotopic and metabolic steady state of perfusion (cf. Fig. 1) before (●) and after addition of 5 mM-dichloroacetate (○). Results are means $\pm$ S.D. for at least three experiments.

rates increased in control perfusions as well, but decreased in perfusions with dichloroacetate. The main factor in the calculations which caused this apparent decrease with dichloroacetate was its effect on the incorporation of label from $[1-^{14}\text{C}]$ pyruvate into lactate. This effect was most distinct at physiological pyruvate concentrations and was not observed at high perfusate pyruvate concentrations (Table 1).

Discussion

In control perfusions no evidence for compartmentation of pyruvate metabolism was observed. At least in the lactate-free perfused rat liver (Scholz *et al.*, 1978) and in glucose- and lactate-free perfused rat hindlimbs (Schadewaldt *et al.*, 1983b), the specific radioactivity of lactate in the effluent largely reflects the intracellular dilution of influent $[1-^{14}\text{C}]$ pyruvate, and may be used to estimate the actual pyruvate decarboxylation rates in the perfused tissue.

However, in rat hindlimbs perfused with physiological $[1-^{14}\text{C}]$ pyruvate concentrations and 5 mM-dichloroacetate, the intracellular labelling of pyruvate decarboxylated by the pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.6.4.3) no

longer resembled the specific radioactivity of the pyruvate metabolized to lactate. The decrease in lactate release, the decreased incorporation of label into lactate and the opposite effect of increasing perfusate pyruvate concentrations on apparent pyruvate decarboxylation are indicative of a compartmentation in the presence of dichloroacetate.

The effects of dichloroacetate may be explained by two different types of compartmentation. If one assumes a cytosolic compartmentation of pyruvate metabolism, one pyruvate pool is primarily in connection with the mitochondrial and extracellular $[1-^{14}\text{C}]$ pyruvate. The other mainly communicates with the lactate pool and glycogen-derived pyruvate. Addition of dichloroacetate caused an enhanced flux of exogenous $[1-^{14}\text{C}]$ pyruvate through the pyruvate dehydrogenase complex, and hence a decreased flux of label into lactate. Saturating concentrations of $[1-^{14}\text{C}]$ pyruvate probably depressed the intracellular dilution and thus reconciled the labelling in both pools. An alternative explanation is based on the myofibrillar composition of the rat hindlimb. About 50% are fast-twitch red, 45% fast-twitch white and 5% slow-twitch red fibres (calculated from the data of Ariano *et al.*, 1973). If dichloroacetate primarily affected pyruvate decarboxylation in the fast-twitch red fibres, which have a high oxidative and low glycogenolytic capacity (Hintz *et al.*, 1980), this should increase $[1-^{14}\text{C}]$ pyruvate uptake and $^{14}\text{CO}_2$ release and decrease $[^{14}\text{C}]$ lactate release in a quite similar manner.

By a quite different experimental approach, Peuhkurinen *et al.* (1983) provided experimental evidence for a cytosolic compartmentation of pyruvate metabolism in the perfused rat heart. Whether this also applied to dichloroacetate-stimulated perfused rat skeletal muscle cannot be definitely deduced from the present experiments, and remains to be further investigated.

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