The Effect of Propionate on the Metabolism of Pyruvate and Lactate in the Perfused Rat Liver

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1. Rates of gluconeogenesis in the perfused rat liver from propionate, L-lactate, pyruvate and the combination of propionate with either lactate or pyruvate were measured. Less than additive rates were obtained with either propionate plus lactate or propionate plus pyruvate. 2. The uptake of pyruvate plus lactate from the perfusion medium was decreased more seriously when propionate was present with lactate than with pyruvate. 3. The use of [2-¹⁴C]pyruvate in the presence of propionate showed that the decreased disappearance of pyruvate plus lactate did not result in their formation from propionate. 4. The addition of sodium butyrate to the perfusion medium caused an inhibition of gluconeogenesis from propionate and stimulated gluconeogenesis and uptake of pyruvate and lactate. 5. The observations are consistent with there being a sparing effect of propionate on lactate and pyruvate metabolism.

It has been shown in the lactating cow that butyric acid has a sparing effect on pyruvate metabolism in the intact animal (Black *et al.*, 1966). This short-chain fatty acid inhibits the oxidation but stimulates the carboxylation of pyruvate. The stimulatory effect of butyrate on gluconeogenesis from pyruvate and lactate has also been demonstrated in the perfused rat liver (Ross *et al.*, 1967). The observed effects of butyrate on pyruvate metabolism in the mammalian liver are considered to be mediated through its oxidative metabolite acetyl-CoA, which is both an inhibitor of pyruvate dehydrogenase (Garland & Randle, 1964) and an activator of pyruvate carboxylase (Utter & Keech, 1963). Since propionyl-CoA has also been shown to exert a similar effect on these two enzymes (Bremer, 1969; Scrutton & Utter, 1967), one might expect propionate to affect pyruvate metabolism in a manner similar to that of butyrate. However, an examination of reports in the literature showed apparent disagreement with the above speculation. Although glucogenic propionate administered to normal rats caused only a slight increase in blood glucose, blood lactate concentration was not altered (Williams *et al.*, 1971). Propionate given to vitamin B₁₂-deficient animals increased blood glucose slightly but the concentration of lactate in the circulation was increased significantly. Oberholzer *et al.* (1967) reported that administration of propionate to patients with congenital deficiency of methylmalonyl-CoA mutase resulted in hypoglycaemia. Therefore it appears that propionate or its metabolites has a similar effect to butyrate in sparing pyruvate oxidation but, unlike butyrate, it does not stimulate gluconeogenesis from lactate or pyruvate. As in-

dicated in these reports, the latter metabolic effect of propionate might be attributed to its metabolic intermediate methylmalonyl-CoA.

This paper describes an investigation of effects of propionate on the oxidation and carboxylation of pyruvate in the perfused rat liver.

Materials and Methods

Male Sprague-Dawley rats weighing 120–160g were starved for 48h. The technique for liver perfusion *in situ* described by Hems *et al.* (1966) was followed. The perfusion medium (100ml) contained 12ml of washed whole cells of aged human blood, 2.6g of bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo., U.S.A.), 1.3ml of 1M NaHCO₃ soln., 0.05ml of heparin (1000 v.s.p. units/ml; Invenex, San Francisco, Calif., U.S.A.) and 86.7ml of Krebs–Ringer bicarbonate solution (Krebs & Henseleit, 1932). When non-radioactive substrates were used, 0.5ml samples of perfusion medium were taken at 10min intervals and deproteinized with 2ml of 4% HClO₄. The deproteinized samples were neutralized with KOH and analysed for lactate, pyruvate (Bergmeyer, 1963) and glucose (Krebs *et al.*, 1963).

[2-¹⁴C]Pyruvate (4.04mCi/mmol) was purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.). [2-¹⁴C]Lactate was obtained by enzymic reduction of [2-¹⁴C]pyruvate (Bergmeyer, 1963, pp. 253, 266). Since the conversion was quantitative, the specific radioactivity of the [2-¹⁴C]lactate prepared was similar to that of [2-¹⁴C]pyruvate. In experiments with ¹⁴C-labelled substrates, lactate

and pyruvate were separated from glucose with ion-exchange columns. Neutralized samples were introduced into anion-exchange columns (1cm × 6cm) packed with AG-1 (X8; 100–200 mesh; Cl[−] form; BioRad Laboratories, Richmond, Calif., U.S.A.), charged with 2M-(NH₄)₂CO₃. The eluates were concentrated by freeze-drying. The freeze-dried material was redissolved in 1.0ml of water. Samples were then counted in a Packard Tri-Carb liquid-scintillation spectrometer for radioactivity in 15ml of Bray's (1960) scintillation fluid.

Results

The rates of gluconeogenesis from 10mM-pyruvate, 10mM-L-lactate and 10mM-propionate in the perfused rat liver are shown in Table 1. Propionate, at 10mM concentration, forms glucose at a rate approx. 130% of that reported by Weidemann *et al.* (1970), who perfused with a 4mM substrate concentration. It has been demonstrated by Ross *et al.* (1967) and ourselves (Chan & Freedland, 1971) that a combination of two substrates proceeding to glucose via independent rate-limiting reactions forms glucose at a rate greater than the rates with the individual substrates. Since propionate and pyruvate (or lactate) follow different pathways of reaction before converging at the level of malate and oxaloacetate, their combination should result in a gluconeogenic rate greater than 1 μmol/min per g of liver, provided that there is no interaction of their intermediates and the transport of the carbon skeleton across the mitochondrial wall is not the rate-limiting factor in gluconeogenesis from

the two substrates. As shown in Table 1, glucose was synthesized in the presence of lactate plus propionate at a rate not significantly different from that with lactate alone ($P < 0.05$). Further, a combination of pyruvate with propionate actually resulted in a diminution of glucose formation ($P < 0.005$).

Concomitant with the decreased rate of gluconeogenesis, the presence of propionate also was associated with a decreased rate of disappearance of pyruvate plus lactate from the perfusate (Figs. 1 and 2). The rate of hepatic uptake of pyruvate plus lactate, 2.98 ± 0.08 μmol/min per g of liver in the presence of 10mM-pyruvate, decreased to 1.80 ± 0.02 when, in addition, propionate was present. The propionate effect was even more drastic when pyruvate was replaced by lactate (Fig. 2). Under these conditions lactate plus pyruvate uptake was only 25% of the rate obtained with 10mM-lactate alone.

Labelled substrates were used to test whether the apparent inhibition of substrate uptake by propionate was due to a conversion of propionate into lactate and pyruvate. The rate of gluconeogenesis in the presence of pyruvate plus propionate was 50% of that with pyruvate alone (Table 1), and the incorporation of radioactive label from [2-¹⁴C]pyruvate into glucose was decreased by 40% in the presence of propionate (Table 2). However, unlike results with non-radioactive substrate, the rate of disappearance of [2-¹⁴C]pyruvate from the perfusate was only slightly decreased by the presence of propionate. Consistent with the results obtained with non-radioactive substrates, both the incorporation of

Table 1. *Effect of propionate and butyrate on gluconeogenesis from various combinations of substrates*

Gluconeogenic rate is expressed as μmol of glucose formed/min per g of liver wet wt. during the first 40 min after addition of substrate. All values are averages of four to ten animals ± S.E.M., with the number of observations in parentheses. All substrates were at 10mM concentration, except butyrate, which was at 2mM. The ratio of lactate + pyruvate/net glucose formed was obtained by dividing the rate of disappearance of lactate + pyruvate (μmol/min per g of liver) from the perfusion medium by the difference observed between gluconeogenic rate from a substrate and the rate observed in the absence of that substrate.

Substrate	Gluconeogenic rate	Ratio
		$\frac{\text{lactate + pyruvate}}{\text{net glucose formed}}$
None	0.19 ± 0.01 (8)	—
Pyruvate	1.01 ± 0.10 (8)	3.67 ± 0.08
Lactate	1.09 ± 0.14 (10)	2.44 ± 0.10
Propionate	0.73 ± 0.09 (5)	—
Lactate + propionate	0.97 ± 0.04 (4)	0.61 ± 0.09
Pyruvate + propionate	0.50 ± 0.05 (4)	5.62 ± 0.50
Lactate + butyrate	1.63 ± 0.04 (4)	2.08 ± 0.20
Propionate + butyrate	0.51 ± 0.01 (4)	—
Lactate + propionate + butyrate	1.42 ± 0.01 (4)	1.57 ± 0.30

Fig. 1. Effect of propionate on the uptake of pyruvate plus lactate from perfusion medium containing an initial pyruvate concentration of 10 mM

All substrates were added 37 min after perfusion started. When more than one substrate was used, these were added as a mixture. Each point is an average $\pm$ S.E.M. from four animals of similar liver sizes. $\circ$, Pyruvate (10 mM); $\bullet$, pyruvate (10 mM) + propionate (10 mM).

label from [14 C]lactate into glucose and its disappearance from the perfusate were drastically decreased by the presence of propionate, more so for lactate than for pyruvate. In good agreement with the non-radioactive experiments, propionate decreased [14 C]lactate uptake to 25% of the control value, based on the disappearance of radioactivity in the lactate plus pyruvate fraction. In the presence of propionate, the radioactivity from lactate appearing in the glucose fraction was approx. 10% of the control value. More importantly, the specific radioactivity of pyruvate and lactate was only slightly altered in the presence of propionate, indicating that the conversion of propionate into pyruvate and lactate was probably insignificant.

The interaction of propionate with pyruvate at the pyruvate carboxylase level was investigated by adding butyrate to the perfusion medium. Butyrate (2 mM) stimulated gluconeogenesis from lactate ($P < 0.01$), but it inhibited gluconeogenesis from propionate ($P < 0.005$) (Table 1). The addition of butyrate to a perfusate containing lactate and propionate not only increased the gluconeogenic rate by 40% but also increased the rate of lactate uptake. The presence, however, of butyrate did not return these two processes to normal.

Fig. 2. Effect of propionate and butyrate on the uptake of lactate from perfusion medium containing an initial lactate concentration of 10 mM

All conditions are as described for Fig. 1. Δ , L-Lactate (10 mM); $\circ$, L-lactate (10 mM) + propionate (10 mM); $\bullet$, L-lactate (10 mM) + propionate (10 mM) + butyrate (2 mM).

Discussion

The hypoglycaemic effect of propionate on vitamin B₁₂-deficient patients (Oberholzer *et al.*, 1967), and its effect on lactate metabolism in normal and vitamin B₁₂-deficient rats reported by Williams *et al.* (1971), can be explained by either a significant conversion of propionate into lactate or an inhibitory effect of propionate on lactate metabolism. Likewise, the same alternatives are applicable in interpreting the effects of propionate on lactate and pyruvate metabolism that we have observed.

Significant conversion of propionate into lactate has been reported to take place in the rumen wall (Pennington & Sutherland, 1956). Such conversion would inevitably lead to a dilution of the lactate and pyruvate pool. In the present experiments the decrease in radioactivity incorporated into the glucose fraction from labelled lactate or pyruvate in the presence of propionate seems to be in agreement with such an interpretation. On the other hand, the lack of significant change in the specific radioactivities of lactate plus pyruvate at the beginning and at the end of the perfusion does not lend support to this interpretation. The decrease therefore in gluconeogenic rate, and thus the decreased rate of incorporation of radioactivity into glucose, caused by a combination of either lactate or pyruvate with propionate, must result from an inhibition of the pathway of gluconeogenesis from pyruvate. A likely site of interaction is at the pyruvate carboxylase reaction. Propionyl-CoA has been shown to be a

Table 2. *Effect of propionate on the metabolism of [2-¹⁴C]lactate and [2-¹⁴C]pyruvate in the perfused rat liver*

All substrates were added to the perfusion medium, at 10mM concentration, 37min after perfusion started. Specific radioactivities were approx. 1.5 μ Ci/mmol for pyruvate and 1 μ Ci/mmol for lactate. The procedure for separation of glucose from lactate and pyruvate is described in the Materials and Methods section. Specific radioactivity is expressed as d.p.m./ μ mol of lactate+pyruvate.

Substrate	Perfusion no.	Glucose formation (d.p.m./min per g of liver)	Uptake of [¹⁴ C]lactate and [¹⁴ C]pyruvate (d.p.m./min per g of liver)	Specific radioactivity of lactate+pyruvate	
				At 3min	At 30min
[¹⁴ C]Lactate	1	2140	2400	1900	1870
	2	3180	3610	1999	1760
[¹⁴ C]Lactate+propionate	3	131	540	1840	1880
	4	470	913	1860	1860
[¹⁴ C]Pyruvate	5	6800	10350	2640	2270
	6	6270	10320	2590	2030
[¹⁴ C]Pyruvate+propionate	7	3690	7740	3140	2250
	8	4450	9650	2880	2100

potent inhibitor of pyruvate dehydrogenase (Bremer, 1969) and the free acid at 10mM concentration may also decrease the steady-state concentration of free CoA, thus decreasing oxidation of endogenous fatty acids. These would lead to a fall in acetyl-CoA concentrations, particularly that of the mitochondrial compartment. The result is a prevention of activation of pyruvate carboxylase, and thus a slower rate of conversion of pyruvate into glucose. Further, methylmalonyl-CoA, a metabolite of propionate, also serves to inhibit the carboxylation of pyruvate (Scrutton & Utter, 1967). These authors also demonstrated that propionyl-CoA activated pyruvate carboxylase purified from the chick liver. The inhibitory effect of propionate on gluconeogenesis from pyruvate that we observed indicates that the methylmalonyl-CoA inhibition predominates.

The tremendous decrease in both incorporation of radioactivity into glucose and uptake of lactate, caused by propionate, indicates that propionate makes a major contribution to the observed rate of glucose formation in the presence of lactate. Consistent with the above postulation, the addition of butyrate, which provides acetyl-CoA for pyruvate carboxylase activation, partially relieved both the inhibition of uptake of lactate plus pyruvate and gluconeogenesis. The C₄ fatty acid also inhibits gluconeogenesis from propionate ($P < 0.005$), possibly at the level of propionyl-CoA formation.

The less-dramatic effect of propionate on the uptake of pyruvate from 10mM-pyruvate as compared with 10mM-lactate indicates that, at such a high pyruvate concentration, pyruvate actually inhibits the metabolism of propionate, probably preventing its activation to its CoA ester by competing for free CoA. This is borne out by the results from the experiments

with radioactive substrates. The incorporation of radioactivity from [¹⁴C]pyruvate into glucose was decreased to 60% of the control value by propionate, whereas the gluconeogenic rate was half that with pyruvate alone. Therefore one may conclude that most of the glucose formed with a combination of pyruvate plus propionate was from pyruvate. Further, the uptake of the radioactive substrate was only slightly affected by propionate, indicating that pyruvate was actively oxidized.

Although our results, as well as those cited (Williams *et al.*, 1971; Oberholzer *et al.*, 1967), were obtained with monogastric animals, the effects of propionate on lactate and pyruvate metabolism, and their interpretations, are likely to be applicable to ruminants where propionate is the major gluconeogenic precursor. Peripheral blood lactate concentrations in ruminants were found to range from 0.5 to 1mM (Annison *et al.*, 1963), but little propionate was detected in circulation (Reid, 1950). This probably suggests that propionate, when transported from the rumen to the liver, is actively converted into glucose, whereas lactate is spared, by a mechanism similar to that proposed here, for use by the peripheral tissue.

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