THE REGULATION OF CARBON FLOW THROUGH PYRUVATE DEHYDROGENASE IN THE PERFUSED GUINEA PIG LIVER[1]

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SUMMARY. The relative contributions of lactate, propionate and octanoate to the acetyl CoA pool for ketone body formation in the perfused guinea pig liver was investigated with ^{14}C-labeled substrates. In the presence of octanoate, incorporation of 3-(^{14}C)-lactate or 3-(^{14}C)-propionate into acetoacetate was extremely low, amounting to only about 0.2% of the incorporation of 1-(^{14}C)-octanoate which accounted for 28% of the acetoacetate synthesized, when this labeled fatty acid was perfused together with unlabeled lactate or propionate. Incorporation of 3-(^{14}C)-lactate and 3-(^{14}C)-propionate into CO_2 was likewise very low, in the same experiments amounting only to less than 0.7% and 7.0% respectively of the 1-(^{14}C)-octanoate carbon oxidized to CO_2 in the presence of unlabeled lactate or propionate. Despite the potential availability of acetyl CoA from lactate via pyruvate; and from propionate via succinate, oxalacetate, P-enolpyruvate and pyruvate, its formation was minimal. The results indicate that pyruvate dehydrogenase is strongly inhibited during ketogenesis, when under these conditions, there is virtually complete conversion of metabolic pyruvate to glucose.

INTRODUCTION. During periods of enhanced gluconeogenesis, the liver is provided with a variety of gluconeogenic substrates such as lactate and alanine, and with fatty acids mobilized from depot stores. During gluconeogenesis, pyruvate formed from lactate or alanine is diverted from decarboxylation via pyruvate dehydrogenase toward carboxylation via pyruvate carboxylase (1). Evidence from several experimental approaches suggests that fatty acid oxidation plays a role in this process. Walter et al. (2) noted that octanoate markedly inhibited the oxidative decarboxylation of pyruvate by isolated rat liver mitochondria; and Bremer (3) found that acyl-

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carnitines inhibit the mitochondrial conversion of pyruvate to acetoacetate. That this effect was due to an inhibition of pyruvate dehydrogenase was suggested by Jagow et al. (4) who demonstrated an inhibition by palmitate of the conversion of 1-(^{14}C) pyruvate to $^{14}CO_2$. Work with purified pig heart pyruvate dehydrogenase has indicated that acetyl CoA (5), NADH (5) and ATP (6) can act as inhibitors of the enzyme.

In a previous study from this laboratory (7), we noted that when 2-(^{14}C) pyruvate was the substrate for gluconeogenesis by perfused rat liver, octanoate markedly increased ketone body formation but almost totally abolished the incorporation of label into ketone bodies. These studies, together with the results of the present report, indicate clearly that fatty acids effectively regulate the flow of pyruvate carbon during gluconeogenesis by inhibiting its conversion to acetyl CoA, thereby providing substrate for pyruvate carboxylase with consequent enhanced gluconeogenesis.

METHODS. The guinea pigs used in this study were of the Hartley strain and were fed Wayne Guinea Pig Chow supplemented with greens. The animals were fasted 48 hrs. before the experiments. The non-recycling liver perfusion technique has been described in detail in a previous publication (8). During the perfusion, we continuously monitored O_2 consumption by determining differences between the concentrations of O_2 in the fluid entering and leaving the liver, using a Clark-type oxygen electrode.

The perfusion fluid was Krebs-Ringer bicarbonate, pH 7.4, containing as substrates, when indicated, 2mM propionate and 0.2 mM sodium octanoate. In one series of experiments, 3-(^{14}C)-lactate was perfused with unlabeled octanoate, and in another, 1-(^{14}C)-octanoate with unlabeled lactate. The same arrangement was employed for combinations of 3-(^{14}C)-propionate and octanoate.

The concentrations of glucose (9), acetoacetate (10), and β-hydroxybutyrate (10) were determined on the effluent perfusion medium, and the radioactivity in (^{14}C)-acetoacetate was estimated by a slight modification of

the procedure of Reichard et al. (11). Since the β-hydroxybutyrate represents less than 20 percent of the total ketone bodies produced by guinea pig liver (12), only the specific activity of acetoacetate was determined. The $^{14}CO_2$ output of the liver was measured on a 5 ml sample of perfusion medium, collected from the cannula leaving the liver, to minimize diffusion of gas. The liquid was transferred to a 25 ml Erlenmeyer flask sealed with a plasma stopper equipped with a plastic hanging bucket (Kontes Glass, Vineland, N.J.). The medium was acidified by the introduction of 1 ml 2 N H_2SO_4 through the plasma stopper, and the $^{14}CO_2$ collected in hyamine hydroxide contained in the plastic bucket. After a 40 min period for diffusion and absorption of CO_2, the bucket was removed, the contents dissolved in liquid scintillation fluid, and the radioactivity determined.

MATERIALS. NAD^+, NADH and β-hydroxybutyrate dehydrogenase were purchased from Boehringer Mannheim Corp., New York. The Fermcozyme used for the analysis of

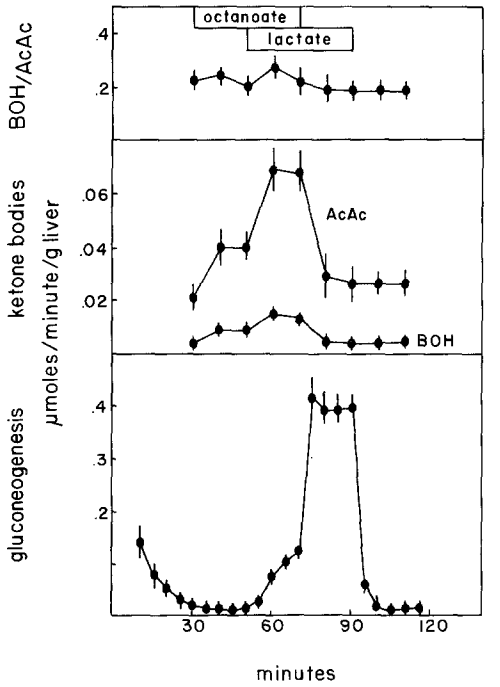

Fig. 1. The time course of glucose and ketone body synthesis in guinea pig liver perfused with octanoate and lactate. Each point is the mean ± SEM (vertical bars thru points) for 4 animals.

glucose was from Fermcolab, Chicago. 3-(^{14}C)-L-lactate (1-5 mCi/mmole), 1-(^{14}C)-sodium octanoate (1-5 mCi/mmole) and 3-(^{14}C)-sodium propionate (2-10 mCi/mmole) were obtained from New England Nuclear Corp., Boston.

RESULTS. The experimental design for this study (to be reported elsewhere in complete form) is indicated in Figure 1. After a 30-min perfusion without substrate, during which glucose production dropped nearly to zero, octanoate at a concentration of 0.2 mM was added; resulting in a doubling of ketone body production, but with no change in the β-hydroxybutyrate-acetoacetate ratio and no increase in gluconeogenesis. In contrast with rat liver, acetoacetate was the predominant ketone body, accounting for 80-90% of the total. At the 50th minute, 2mM lactate was added, and from then until the 70th minute, both substrates were perfused. During this period, there was a moderate increase of gluconeogenesis, and a sharp increase in ketogenesis, again without change in the ketone body ratio, owing to an increased O_2 uptake, concomitant with lactate addition. After 70 minutes, octanoate perfusion was discontinued and lactate alone was perfused up to the 90th minute, following which perfusion was continued for 30 minutes longer without substrate. Correspondingly, after the 70th minute ketone body production dropped to the pre-perfusion level while gluconeogenesis increased sharply; then with removal of substrate, ketone body and glucose production both returned to the original levels. An essentially similar pattern was observed when propionate was used instead of lactate in similar experiments.

To determine the relative contributions of lactate and octanoate to ketone body formation, we measured the conversion of 3-(^{14}C)-lactate and 1-(^{14}C)-octanoate to acetoacetate by perfused guinea pig liver in identical experiments, each in the presence of the other substrate unlabeled.

The data presented in Table 1 show that in the presence of a ketogenic substrate such as octanoate, lactate or propionate contribute minimally to ketone body formation and are oxidized to CO_2 at very low rates, but are readily converted to glucose. On the basis of the aforementioned earlier

studies with mitochondria, slices or the purified pyruvate dehydrogenase, it would appear that the conversion of these 3-carbon substrates to acetyl CoA in the intact perfused liver is blocked by inhibition of this enzyme. Although the mechanism of inhibition is unknown, it may plausibly be attributed to product inhibition by a high level of acetyl CoA, and this may also be a factor in the high rate of glucogenesis through stimulation of pyruvate carboxylase.

As shown in Table 1, the rate of total ketone body formation in each of the four sets of experiments was similar, as was the rate of oxygen consumption. (^{14}C) Acetoacetate from 3-(^{14}C) lactate was approximately 0.2% of the rate of acetoacetate formation from 1-(^{14}C) octanoate, and 3-(^{14}C) propionate incorporation in acetoacetate was 0.4% of 1-(^{14}C) octanoate. Since propionate carbon cannot readily be converted to acetyl CoA except by re-cycling of P-enolpyruvate to pyruvate via pyruvate kinase, the appearance of label from propionate in acetoacetate would not normally be expected to be greater

Table 1 Relative Contributions of Lactate, Propionate and Octanoate to the Synthesis of Ketone Bodies in the Perfused Guinea Pig Liver

Process Measured	Substrates			
	1-^{14}C-Octanoate+Lactate	3-^{14}C-Lactate + Octanoate	1-^{14}C-Octanoate+Propionate	3-^{14}C-Propionate+Octanoate
	nmoles per min per g liver			
Glucose production	152±14	106±22	291±24	147±33
Total ketone bodies	982±26	847±48	889±43	794±60
Acetoacetate	764±14	678±38	689±50	596±60
O_2 consumption	2,600±80	2,350±100	2,960±100	2,890±230
Acetoacetate-^{14}C	205±13	0.38±0.07	184±21	0.76±0.08
$^{14}CO_2$ production	154±19	0.95±0.21	142±4	10.9±2.5
Specific activity of acetoacetate	0.268	0.56×10^{-3}	0.267	1.27×10^{-3}

than the labeling of acetoacetate from 3-(^{14}C)-lactate. We note in Table 1, however, that although the synthesis of acetoacetate-^{14}C from 3-(^{14}C)-propionate is low, it is double the rate with 3-(^{14}C)-lactate and octanoate. This suggests a limited re-cycling of carbon via pyruvate kinase. The oxidation of 3-(^{14}C)-lactate to CO_2 by perfused guinea pig liver is also greatly reduced by the simultaneous infusion of octanoate (Table 1).

Values are the means ± SEM for 4 experiments. Radioactivity data are expressed in natoms substrate carbon converted per min per g liver. Livers from 48-hr fasted guinea pigs were perfused 90 min as shown in Figure 1. The above parameters were measured on samples taken at 60 min, at which time both octanoate and either lactate or propionate were perfused simultaneously. The $^{14}CO_2$ has been corrected for the spontaneous decarboxylation of 1-(^{14}C)-acetoacetate which occurred during the acidification of the sample for release of $^{14}CO_2$. The specific activity of acetoacetate is expressed as (^{14}C)-acetoacetate/total acetoacetate.

DISCUSSION. Since ketone body formation from both lactate and octanoate presumably involves a single acetyl CoA pool, the specific activity of acetoacetate will indicate the relative dilution of carbon from both sources. We have shown that the specific activity of the acetoacetate derived from 1-(^{14}C) octanoate is 500 times higher than that derived from 3-(^{14}C)-lactate. Furthermore, the specific activity of the acetoacetate from 1-(^{14}C)-octanoate remained constant despite a 2-fold increase in the synthesis of total ketone bodies. The almost total inhibition of pyruvate conversion to acetyl CoA in perfused guinea pig liver may involve factors such as a change in mitochondrial energy charge in part related to an alteration in the oxidation-reduction state of the liver metabolizing fatty acids. Such an interpretation is in accord with the studies with both the purified pyruvate dehydrogenase complex mentioned above and with the work of Walajtys et al. (13) with isolated rat liver mitochondria. In the presence of a ketogenic fatty acid, the conversion of lactate to acetoacetate or to CO_2 was almost completely inhibited despite a substantial conversion of

lactate to glucose via pyruvate carboxylase. This suggests that fatty acid oxidation can depress the entry of lactate-derived pyruvate into the tricarboxylic acid cycle, even though lactate carbon is entering the mitochondria and being carboxylated to oxalacetate.

The pyruvate dehydrogenase complex from heart, kidney and brain has been intensively studied during the past 5 years since the initial report by Linn et al. (6) that the enzyme is regulated by a phosphorylation-dephosphorylation mechanism. A number of factors have been reported to inhibit the activity of the pyruvate dehydrogenase complex by interacting at various points within the complex. ADP inhibits pyruvate dehydrogenase kinase in a manner competitive with ATP. Since pyruvate dehydrogenase is inactivated when phosphorylated, changes in the ATP/ADP ratio should regulate the rate of pyruvate conversion to acetyl CoA. Other factors such as Ca^{++}, Mg^{++}, acetyl CoA and NADH have been implicated in the regulation of the enzyme complex. Using isolated rat liver mitochondria, Walajtys et al. (13) have shown that pyruvate dehydrogenase kinase is regulated predominantly via changes in the activity of pyruvate dehydrogenase kinase, since under conditions of controlled respiration, the percentage of pyruvate dehydrogenase in the active form varied directly with the ADP concentration. Pyruvate, which can protect the pyruvate dehydrogenase complex from inactivation by inhibiting pyruvate kinase was also shown by Walajtys et al. (13) to regulate pyruvate dehydrogenase in liver mitochondria.

References

1. Teufel, H., Henahan, L. A., Shipp, J. C., Boning, S., & Wieland, O. (1967) European J. Biochem. 2, 182.
2. Walter, P., Paetkau, V., & Lardy, H. A. (1966) J. Biol. Chem. 241, 2523.
3. Bremer, J. (1966) Biochim. et Biophys. Acta 116, 1.
4. Jagow, G. V., Westermann, B., & Wieland, O. (1968) European J. Biochem. 3, 512.
5. Garland, P. B. & Randle, P. J. (1964) Biochem. J. 91, 6c.
6. Linn, T. C., Pettit, F. H., & Reed, L. J. (1969) Proc. Nat. Acad. Sci. USA 62, 234.
7. Friedmann, B., Goodman, E. H. Saunders, H. L., Kostos, V., & Weinhouse, S. (1971) Arch. Biochem. and Biophys. 143, 566.

8. Jomain-Baum, M., Garber, A. J., Farber, E. & Hanson, R. W. (1973) J. Biol. Chem. 248, 1536.
9. Hill, J. B. & Kessler, G. (1966) J. Lab. Clin. Med. 57, 970.
10. Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) Biochem. J. 82, 90.
11. Reichard, G. A., Owen, O. E., Haff, A. C., Paul, P. & Bortz, W. M. (1974) J. Clin. Invest. 53, 508.
12. Arinze, I. J., Garber, A. J. & Hanson, R. W. (1973) J. Biol. Chem. 248, 2266.
13. Walajtyz, E. I., Gottesman, P. & Williamson, J. R. (1974) J. Biol. Chem. 249, 1857.