The Stimulation of Hepatic Gluconeogenesis by Acetoacetate Precursors

A ROLE FOR THE MONOCARBOXYLATE TRANSLATOR*

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The regulation of the gluconeogenic pathway from the 3-carbon precursors pyruvate, lactate, and alanine was investigated in the isolated perfused rat liver. Using pyruvate (<1 mM), lactate, or alanine as the gluconeogenic precursor, infusion of the acetoacetate precursors oleate, acetate, or β-hydroxybutyrate stimulated the rate of glucose production and, in the case of pyruvate (<1 mM), the rate of pyruvate decarboxylation. α-Cyanocinnamate, an inhibitor of the monocarboxylate transporter, prevented the stimulation of pyruvate decarboxylation and glucose production due to acetate infusion. With lactate as the gluconeogenic precursor, acetate infusion in the presence of l-carnitine stimulated the rate of gluconeogenesis (100%) and ketogenesis (60%) without altering the tissue acetyl-CoA level usually considered a requisite for the stimulation of gluconeogenesis by fatty acids. Hence, our studies suggest that gluconeogenesis from pyruvate or other substrates which are converted to pyruvate prior to glucose synthesis may be limited or controlled by the rate of entry of pyruvate into the mitochondrial compartment on the monocarboxylate transporter.

The rate of pyruvate decarboxylation in the isolated perfused rat liver is stimulated by infusion of precursors of acetoacetate provided that the perfusate pyruvate concentration is less than 1 mM (1–4). It has been our contention that at low (probably physiological) concentrations of pyruvate, a situation in which nearly all of the pyruvate transport across the mitochondrial membrane occurs via the monocarboxylate transporter, intramitochondrially generated acetoacetate may effectively replace hydroxyl ions as the counter ion for pyruvate transport on the monocarboxylate transporter. Acetoacetate has been shown to be transported in exchange for pyruvate via the monocarboxylate transporter in mitochondrial systems (5, 6). It has been demonstrated that the rates of pyruvate transport and pyruvate decarboxylation are stimulated in the presence of an acetoacetate precursor in isolated rat liver mitochondria (2). Furthermore, the decarboxylation of the branched chain α-keto acid, α-ketosiovalerate, which is transported across the mitochondrial membrane via the monocarboxylate transporter (7), is accelerated by β-hydroxybutyrate and acetate, both precursors of mitochondrial acetoacetate (8–10).

Stimulation of the rate of entry of monocarboxylates such as pyruvate into the mitochondrial compartment due to exchange with mitochondrially generated acetoacetate results in an elevated mitochondrial concentration of the imported substrate. Hence, the rate of decarboxylation of pyruvate is stimulated both by mass action and the inhibitory effect of pyruvate on the pyruvate dehydrogenase kinase, resulting in the interconversion of the enzyme complex to the active, dephospho-form (3).

If our hypothesis is correct concerning the relationship between mitochondrial pyruvate uptake and acetoacetate efflux, other metabolic processes such as gluconeogenesis which depend upon pyruvate import into the mitochondrial compartment should be affected. While the published data are not conclusive, it has been suggested (11–13) that glucagon and dibutyryl cyclic AMP stimulate the operation of the mitochondrial monocarboxylate translocase, leading to a stimulation of hepatic gluconeogenesis.

The present study was performed to ascertain whether the rate of gluconeogenesis from pyruvate or its precursors is increased under metabolic conditions in which acetoacetate precursors stimulate the rate of decarboxylation of these same substrates. In effect, we have employed the decarboxylation of [1-14C]pyruvate as a probe of the monocarboxylate transport system in the perfused rat liver in order to characterize the regulatory significance of precursor translocation in the gluconeogenic process.

MATERIALS AND METHODS

Male Sprague-Dawley rats (180–200 g) were used for all experiments. Animals were fasted for 24 h prior to surgical removal of the liver under pentobarbital anesthesia. A noncirculating liver perfusion technique using a hemoglobin-free perfusion medium was employed (14). The perfusate medium was Krebs-Henseleit bicarbonate buffer, pH 7.4, equilibrated with a mixture of oxygen to carbon dioxide (95:5%) and was maintained at 37°C. Substrates were infused into the perfusion system immediately prior to the liver. Samples of the effluent perfusate were collected at 30-s intervals for metabolite analyses. Aliquots (2.5 ml) of the perfusate were placed in 25-ml Erlenmeyer flasks sealed with rubber serum stoppers equipped with plastic center wells (both obtained from Kontes Glass, Vineland, NJ) containing 0.3 ml of phenylethylamine. Labeled CO2 from the perfusate samples was released by injecting 0.5 ml of 1 N HCl into the flask followed by gentle agitation for 1 h. The center wells were transferred to scintillation vials containing 10 ml of Aquasol (New England Nuclear) and counted. Knowing the quench correction and the specific radioactivity of the 1-14C-labeled pyruvate, the metabolic flux through the pyruvate dehydrogenase complex was estimated (10).

Glucose in the perfusate samples was measured using the method of Bergmeyer et al. (15). Ketone bodies acetoacetate and β-hydroxybutyrate were measured by the procedures of Mellanby and William-son (16) and Williamson and Corkey (17), respectively. Lactate and pyruvate in perfusate samples were determined according to the

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procedures of Gutmann and Wahlefeld (18) and Passonneau and Lowry (19), respectively.

For each experiment, glucose production by the liver was measured in the presence and absence of various precursors in the perfusion medium. The rate of glucose output by the liver was corrected for endogenous glucose production, e.g. the rate in the absence of any gluconeogenic precursors. In this series of experiments, the endogenous rate of glucose output was 4.5 ± 0.5 (n = 15) \( \text{µmol.g}^{-1}.\text{h}^{-1} \).

Glucose production rates in the effluent perfusate were measured either at 1-min intervals or under the various steady state conditions indicated in the individual figures. Coenzyme A and various acyl-CoA derivatives were measured in neutralized perchloric acid extracts of freeze-clamped perfused livers using the high performance liquid chromatographic procedure described by DeBuyser and Olson (20).

Aquasol and phenylethylamine were obtained from New England Nuclear. [l-14C]Pyruvate was purchased from Amersham Corp. Glucose-6-phosphate dehydrogenase, hexokinase, and pyruvate were obtained from Boehringer Mannheim. Sodium acetate was obtained from Fisher. All other chemicals were of the highest purity available commercially.

RESULTS

As the perfusate pyruvate concentration is increased in a stepwise fashion in an isolated, perfused rat liver from a fasted rat, the rates of both pyruvate decarboxylation and glucose production increase in a parallel fashion as demonstrated in Fig. 1. Maximal rates of glucose output and pyruvate decarboxylation were approached only after the perfusate pyruvate concentration exceeded 20 mM. Experiments performed using livers from fed rats indicated that pyruvate decarboxylation was maximal at perfusate pyruvate concentrations of 10 mM (1).

As a starting point for our investigation of the effects of acetoacetate precursors on the supply of gluconeogenic precursors, oleate was infused into a rat liver perfused with 0.5 mM [1-14C]pyruvate (Fig. 2). Oleate addition resulted in a stimulation of the rates of [1-14C]pyruvate decarboxylation and glucose production by 33 and 120%, respectively. Concomitant with the changes in glucose production and pyruvate decarboxylation, the rate of ketogenesis was stimulated 5.5-fold, and the mitochondrial oxidation-reduction state, as measured by \([\beta\text{-hydroxybutyrylate}]/\text{acetacetate}\) ratio, increased from 0.125 in the absence of oleate to 0.37 upon oleate addition (Fig. 2). Oleate also stimulated the rate of glucose production in livers presented with gluconeogenic precursors L-lactate (180%) and L-alanine (165%) (Table I). Stimulation of the rate of ketogenesis and changes in the mitochondrial oxidation-reduction state during oleate infusion in livers perfused with either lactate or alanine were similar to those observed with pyruvate as the gluconeogenic precursor.

The effect of oleate infusion on the rates of pyruvate decarboxylation and glucose production in a liver from a 24-h fasted rat perfused with 0.5 mM [1-14C]pyruvate. The rates of pyruvate decarboxylation (O), glucose production (C), and ketogenesis were measured in the perfusate samples as described under "Materials and Methods." Rates of glucose production were corrected for endogenous glucose production from the liver in the absence of any exogenous substrates.

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TABLE I

The effect of oleate infusion on the rate of glucose production in a liver from a 24-h fasted rat perfused with either 20 mM L-lactate or 10 mM L-alanine

The perfusion experiments were conducted exactly as illustrated for pyruvate in Fig. 2 except that 20 mM L-lactate or 10 mM L-alanine replaced 0.5 mM pyruvate. The rates of glucose production, ketogenesis, and lactate and pyruvate production were measured in the perfuse samples collected under steady state conditions, i.e., between 7 and 10 min after initiation of the various substrate infusions. Glucose production rates were corrected for endogenous rates of glucose output from the livers in the absence of any exogenously added substrates. Each value is a mean ± S.D. of at least five determinations.

| Condition                                      | Glucose (μmol·hr⁻¹·g⁻¹ wet wt⁻¹) | β-Hydroxybutyrate | Acetoacetate | Lactate | Pyruvate | Lactate/pyruvate | β-Hydroxybutyrate/acetoacetate |
|------------------------------------------------|----------------------------------|-------------------|--------------|---------|----------|-----------------|-----------------------------|
| L-Lactate, 20 mM, 10 min                        | 19.8 ± 0.8                       | 0.9 ± 0.1         | 8.0 ± 1.0    | 104.9 ± 5.3 | 0.12     |                  |                             |
| L-Lactate, 20 mM, 10 min, then L-lactate, 20 mM, 10 min, plus oleate, 0.1 mM, 20 min | 55.6 ± 3.8                       | 13.4 ± 1.3        | 17.9 ± 1.2   | 98.8 ± 6.0 | 0.75     |                  |                             |
| L-Alanine, 10 mM, 10 min                        | 9.0 ± 0.5                        | 1.0 ± 0.8         | 7.1 ± 0.95   | 30.3 ± 2.0 | 22.5 ± 1.5 | 1.4             | 0.14                        |
| L-Alanine, 10 mM, 10 min, then L-alanine, 10 mM, 10 min, plus oleate, 0.1 mM, 10 min | 23.8 ± 2.0                       | 12.1 ± 2.0        | 31.3 ± 2.0   | 25.1 ± 2.2 | 8.9 ± 1.2 | 2.8             | 0.39                        |

FIG. 3. Effect of acetate and DL-β-hydroxybutyrate on the rates of pyruvate decarboxylation and glucose production in isolated perfused rat livers from 24-h fasted rats. In separate experiments, livers were perfused with 0.5 mM [1-¹⁴C]pyruvate and acetate or β-hydroxybutyrate was co-infused for 10-min periods indicated by horizontal bars. Perfusate samples were analyzed for ¹⁴CO₂ production, glucose production, and ketone bodies as described under "Materials and Methods." Glucose production rates were corrected for endogenous glucose production from the liver in the absence of any exogenous substrates.

example, prevents the stimulation of pyruvate decarboxylation by acetoacetate precursors (1, 3, 8). In order to assess the effects of acetate on glucose production in a rat liver perfused with a low pyruvate concentration in which the pyruvate dehydrogenase complex was maximally active, dichloroacetate, a potent inhibitor of the pyruvate dehydrogenase kinase (21), was employed to allow the interconversion of nearly all of the enzyme complex to the active dephospho-form. When dichloroacetate was infused into a liver perfused with 0.5 mM pyruvate, the rate of pyruvate decarboxylation was stimulated by 45% over control rates. The rate of glucose production...
from pyruvate was not affected significantly by dichloacetate infusion. Addition of acetate in the presence of dichloacetate resulted in an acceleration of the rate of glucose production by 25% (results not shown).

Moreover, when livers were perfused with high perfusate pyruvate concentrations (e.g., 5 mM), acetate infusion into the liver did not affect either the rate of pyruvate decarboxylation or the rate of glucose production (data not shown). Under these perfusion conditions, pyruvate transport via the monocarboxylate translocator was not in any way rate-limiting, and the pyruvate dehydrogenase enzyme complex was largely (80%) converted to its active form.

In order to demonstrate the involvement of the mitochondrial monocarboxylate translocator in the stimulation of glucose production during infusion of acetoacetate precursors into livers perfused at low (e.g., <1 mM) pyruvate concentrations, a-cyanocinnamate, an inhibitor of the mitochondrial monocarboxylate translocator, was infused to inhibit the transport of pyruvate across the mitochondrial membrane (3) (Fig. 5). In the presence of a-cyanocinnamate, infusion of [1-\(^{14}\)C]pyruvate at 5 mM resulted in a rate of pyruvate decarboxylation equivalent to that observed with 0.5 mM pyruvate in the absence of a-cyanocinnamate (e.g., see Fig. 3). Since a-cyanocinnamate (3 mM) inhibits the mitochondrial monocarboxylate translocator in excess of 95% (3), it was inferred that the intramitochondrial pyruvate concentration due mainly to passive diffusion of pyruvate (5 mM) in the perfusion medium across the mitochondrial membrane in the presence of a-cyanocinnamate was equivalent to that observed in the absence of the inhibitor at a perfusate pyruvate concentration of 0.5 mM. Upon acetate addition, the rate of ketogenesis was elevated, but the mitochondrial oxidation-reduction state as measured by the \([\beta\text{-hydroxybutyrate}]/[\text{acetacetate}]\) ratio in the effluent perfusate was unaffected. In spite of the stimulated rate of ketogenesis during acetate infusion, a-cyanocinnamate prevented a significant perturbation in the rates of either pyruvate decarboxylation or glucose production.

It must be noted that the conventional, generally accepted mechanism for explaining stimulatory effects of ketogenic substrates such as acetate, \(\beta\)-hydroxybutyrate, and oleate on the rate of hepatic gluconeogenesis is through an elevation of mitochondrial acetyl-CoA levels which should activate the pyruvate carboxylase reaction (for review see Ref. 22). The experiments depicted in Fig. 6 and Table II were performed in an attempt to minimize the potential contribution of the mitochondrial acetyl-CoA level to the gluconeogenic increase observed upon acetate and \(\beta\)-hydroxybutyrate infusion. Following stabilization of the rate of glucose production from lactate (20 mM), infusion of L-carnitine (20 mM) resulted in a modest stimulation of glucose production (40%) and an increase in the rate of acetoacetate production (54%). Infusion of DL-\(\beta\)-hydroxybutyrate (20 mM; Fig. 6, closed circles) in the presence of L-carnitine stimulated the rates of glucose and acetoacetate production by 2- and 11-fold, respectively. Similarly, acetate infusion (10 mM; Fig. 6, open circles) into livers perfused with lactate and L-carnitine stimulated the rates of glucose and acetoacetate production by 120 and 30%, respectively. Measurements of free CoASH and various appropriate acyl-CoA derivatives in extracts of perfused livers freeze-clamped under various steady state perfusion conditions described in Figs. 3 and 6 are tabulated in Table II. Infusion of \(\beta\)-hydroxybutyrate, oleate, or acetate into livers perfused with lactate increased tissue acetyl-CoA levels by 80, 70, and 22%, respectively, compared to control livers perfused in the presence of lactate alone. Inclusion of L-carnitine (a) reduced significantly the increase in tissue acetyl-CoA when \(\beta\)-hydroxybutyrate was the added acetoacetate precursor and (b) prevented completely the elevation of the acetyl-CoA level when acetate was infused. Hence, in the absence of a measurable change in the tissue acetyl-CoA level, acetate infusion...
caused nearly a doubling of the rate of glucose output by the liver (Fig. 6).

**DISCUSSION**

The present study was an attempt to provide an experimental basis for our suggestion that the translocation of monocarboxylate gluconeogenic precursors across the mitochondrial membrane may be a regulatory site in the gluconeogenic process. It is our suggestion that at low, probably physiological intracellular pyruvate concentrations, when most or all of the pyruvate transport across the mitochondrial membrane occurs via the monocarboxylate translocator (7, 24), intramitochondrially generated acetocetate exchanges for cytosolic pyruvate, resulting in an elevation of the intramitochondrial pyruvate concentration (2). An increase in the mitochondrial pyruvate concentration increases the metabolic flux through the pyruvate dehydrogenase reaction by mass action, and because pyruvate is an effective inhibitor of the pyruvate dehydrogenase kinase (23), an activation of the pyruvate dehydrogenase complex is effected (1–4, 8). If the mitochondrial pyruvate concentration is increased during a period of rapid ketogenic efflux, the rate of the pyruvate carboxylase reaction and, subsequently, the gluconeogenic pathway should be enhanced. This thesis was approached experimentally in the present study.

With pyruvate as the gluconeogenic substrate, the rate of glucose production was not maximal even when the perfusate pyruvate concentration was raised to 20 mM, indicating that the apparent $K_m$ for glucose production from pyruvate was in the millimolar range (Fig. 1). Additionally, our finding that the rate of pyruvate decarboxylation with varying perfusate pyruvate concentrations in the 24-h fasted rat was not saturated at 20 mM and the fact that pyruvate decarboxylation in the fed rat is at its maximum at 10 mM (1) indicate a difference in the regulatory characteristics of the pyruvate dehydrogenase system in these two metabolic situations. As demonstrated for the pyruvate dehydrogenase complex in heart mitochondria derived from fed and fasted rats (25, 26), this difference may be a consequence of decreased inhibition of the pyruvate dehydrogenase kinase by pyruvate in fasted rat livers. Since acetyl-CoA and NADH stimulate the pyruvate dehydrogenase kinase (27, 28) and pyruvate is an inhibitor of the kinase, it follows that in the fasted rat liver higher concentrations of pyruvate may be required to inhibit the kinase in the face of elevated acetyl-CoA/CoASH and NADH/NAD* ratios.

The present study indicates that all mitochondrial acetate-precursors, e.g. oleate, acetate, and $\beta$-hydroxybutyrate, increase the rate of glucose production by the rat liver at low ($<1$ mM) perfusate pyruvate concentrations. At higher concentrations of pyruvate in the perfusion medium, which must be considered nonphysiological, acetate infusion affected neither the rate of glucose production nor the rate of pyruvate decarboxylation. This finding is consistent with our view that at lower pyruvate concentrations the bulk of the pyruvate is transported into the mitochondrial matrix via the monocarboxylate translocator (7, 24). A rapid acceleration in the rate

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**TABLE II**

| Perfusion condition | Metabolite levels | | | | |
|---|---|---|---|---|---|
| | CoASH | Acetyl-CoA | Succinyl-CoA | Propionyl-CoA | Total CoA |
| Lactate, 20 mM, 10 min (n = 4) | 136 ± 10 | 33 ± 5 | 33 ± 7 | 21 ± 7 | 225 ± 5 |
| Lactate, 20 mM, 10 min, then lactate plus L-carnitine, 20 mM, 10 min (n = 4) | 155 ± 25 | 28 ± 4 | 39 ± 10 | 9 ± 1 | 221 ± 10 |
| Lactate, 20 mM, 10 min, then lactate plus L-carnitine, 10 min, then lactate, L-carnitine plus $\beta$-hydroxybutyrate, 20 mM, 10 min (n = 3) | 144 ± 6 | 45 ± 3 | 13 ± 10 | 11 ± 1 | 213 ± 10 |
| Lactate, 20 mM, 10 min, then lactate plus L-carnitine, 10 min, then lactate, L-carnitine plus acetate, 10 mM, 10 min (n = 3) | 153 ± 10 | 32 ± 3 | 13 ± 3 | 10 ± 2 | 207 ± 10 |
| Lactate, 20 mM, 10 min, then lactate plus $\beta$-hydroxybutyrate, 20 mM, 10 min (n = 3) | 130 ± 10 | 60 ± 5 | 16 ± 13 | 11 ± 2 | 216 ± 8 |
| Lactate, 20 mM, 10 min, then lactate plus acetate, 10 mM, 10 min (n = 3) | 137 ± 9 | 40 ± 5 | 23 ± 6 | 14 ± 2 | 214 ± 4 |
| Lactate, 20 mM, 10 min, then lactate plus oleate, 0.1 mM, 10 min (n = 3) | 109 ± 8 | 57 ± 6 | 42 ± 5 | 11 ± 3 | 218 ± 10 |
infusion of either acetate or \( \beta \)-hydroxybutyrate stimulated gluconeogenesis to a similar extent using either pyruvate or lactate as the gluconeogenic precursors. It should be noted that acetate metabolism in the perfused rat liver does not affect the mitochondrial or the cytosolic NADH/NAD\(^+\) ratio as measured by the ratios of \( \beta \)-hydroxybutyrate/acetocetate and lactate/pyruvate, respectively, in the effluent perfusate.

One of the requisites for observing the stimulation of pyruvate decarboxylation by acetocetate precursors is that the pyruvate dehydrogenase kinase/phosphatase interconversion system must not be inhibited. Hence, in the presence of dichloroacetate, an inhibitor of the pyruvate dehydrogenase kinase, the rate of pyruvate decarboxylation was not stimulated by acetocetate precursors (data not shown). Any resultant alteration (e.g. increase) in the intramitochondrial pyruvate concentration caused by an accelerated pyruvate transport should have no effect on the interconversion system in the presence of dichloroacetate. However, the rate of glucose output by the liver in the presence of dichloroacetate was increased by acetate co-infusion as would be expected if the acetocetate generated from acetate caused an enhanced exchange of pyruvate for acetocetate.

Finally, the demonstration that inhibition of the monocarboxylate translocator by \( \alpha \)-cyanocinnamate prevents the stimulation of both pyruvate decarboxylation and glucose production from pyruvate again is consistent with our proposition that a functional monocarboxylate transport is necessary to observe the putative acetocetate/pyruvate exchange transport process with the attendant acceleration of gluconeogenesis.

The implications of the suggested relationship between the ketogenic process and the gluconeogenic pathway are obvious. Under metabolic conditions, e.g. diabetes or prolonged fasting, resulting in accelerated hepatic fatty acid oxidation and a rapid ketogenic rate, gluconeogenesis from 3-carbon precursors is usually stimulated. The proposed facilitation of the rate of glucose synthesis at the level of the transport of pyruvate into the mitochondrial compartment may be considered one of a variety of regulatory alterations involved in accelerating the gluconeogenic pathway under physiological conditions.

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