Studies on the Regulation of the Branched Chain α-Keto Acid Dehydrogenase in the Perfused Rat Liver*

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The regulation of the branched chain α-keto acid dehydrogenase multienzyme complex was investigated in the isolated, perfused rat liver. The metabolic flux through the branched chain α-keto acid dehydrogenase was monitored by measuring the production of $^{14}$CO$_2$ from infused 1-1$^{14}$C-labeled branched chain α-keto acid substrates. The demonstration of α-keto-1$^{14}$Cisocaproate exceeded that of α-keto-1$^{14}$Cisovalerate at all concentrations of the substrates infused. Co-infusion of either α-ketoisovalerate or α-keto-β-methylvalerate inhibited the rate of α-keto-1$^{14}$Cisocaproate decarboxylation. The rate of α-keto-1$^{14}$Cisovalerate decarboxylation was enhanced during coinfusion of t(-)-carnitine, while α-keto-1$^{14}$Cisocaproate decarboxylation was unaffected. The presence of pyruvate in the perfusion medium resulted in an inhibition of the flux through the branched chain complex with either α-ketoisocaproate or α-ketoisovalerate as the substrate. dl-β-hydroxybutyrate infusion inhibited α-keto-1$^{14}$Cisocaproate decarboxylation by 18% but resulted in nearly a 100% stimulation of α-keto-1$^{14}$Cisovalerate decarboxylation.

The evidence presented indicates that (a) the metabolic flux through the branched chain α-keto acid dehydrogenase complex can be monitored effectively in a continuous fashion in the perfused liver by following the release of $^{14}$CO$_2$ from infused 1$^{14}$C-labeled substrates and (b) the changes observed in the metabolic flux through the branched chain complex during coinfusion of alternative substrates and other compounds may be entirely different depending upon which branched chain α-keto acid substrate is utilized to monitor this reaction.

It has been established that during starvation the circulating blood levels of the branched chain amino acids, leucine, isoleucine, and valine increase transiently both in humans and in experimental animals (1, 2). These amino acids represent a carbon source for the generation of energy, carbohydrate (e.g. valine and isoleucine), and ketone bodies (leucine) in various tissues (3). The branched chain amino acids are transaminated with α-ketoglutarate to form the corresponding α-keto acids which subsequently undergo oxidative decarboxylation in the mitochondrial compartment by the branched chain α-keto acid dehydrogenase multienzyme complex which is an analogous enzyme complex to the pyruvate and α-ketoglutarate dehydrogenase complexes (4). The three branched chain α-

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(New England Nuclear Corp.) and counted. Knowing the correction for quenching and the specific radioactivity of the infused 14C-labeled substrates, a continuous measurement of the metabolic flux through the branched chain α-keto acid dehydrogenase complex was obtained.

Ketone bodies, β-hydroxybutyrate and acetoacetate, were measured in samples of the effluent perfusate using procedures described by Williamson and Corkey (19) and Mellanby and Williamson (20), respectively. [1-14C]Leucine, [1-14C]Valine, aquasol, and phenethylamine were purchased from New England Nuclear (Boston, MA). 1-14C-labeled α-ketoisocaproate and α-ketoisovalerate were prepared from the corresponding l-14C-labeled amino acids (leucine and valine) using procedures described by Rudiger et al. (21). Unlabeled α-ketoisocaproate, α-ketoisovalerate, α-keto-β-methylvalerate, and l-leucine were purchased from Sigma (St. Louis, MO). Pyruvate and β-hydroxybutyrate dehydrogenase were obtained from Boehringer Mannheim (Indianapolis, IN). dl-β-Hydroxybutyrate was purchased from Calbiochem (La Jolla, CA). L(-)Carnitine chloride was the generous gift of the Osaka Pharmaceutical Factory (Osaka, Japan).

All other reagents and materials were of the highest purity available from commercial sources.

RESULTS

In order to assess the validity of measuring 14CO2 production from 1-14C-labeled α-ketoisocaproate and α-ketoisovalerate as a monitor of the flux through the branched chain α-keto acid dehydrogenase reaction in the perfused rat liver, the experiment illustrated in Fig. 1A was performed. The liver from a fed rat was perfused in the presence of 1 mM unlabeled α-ketoisocaproate for a period of 15 min. At 15 min, a tracer amount of α-keto[1-14C]isocaproate was infused for 10 min and samples were collected at 30-s intervals to monitor 14CO2 production. Following the termination of the infusion of α-keto[1-14C]isocaproate, samples were collected every 12 a for 3 min and then every 30 s. The "off" or "washout" kinetics of 14CO2 production shown in Fig. 1A indicates two distinct components with approximate half-times of 27 s and 4.8 min, respectively. The rapid kinetic component with a t1/2 of 27 s accounted for 94% of the 14CO2 washed out of the liver within the initial 2½ min following the termination of the infusion of the labeled branched chain α-keto acid. This rapid kinetic component is very similar to the rapid component observed in the perfused rat heart with α-ketoisocaproate (16). In view of the fact that there are few metabolic fates for α-ketoisocaproate that would result in the rapid decarboxylation of α-ketoisocaproate, it was inferred that the rapid kinetic component observed in Fig. 1A was due to the branched chain α-keto acid dehydrogenase reaction. The slower kinetic component (i.e., t1/2 = 4.8 min) observed in the experiment shown in Fig. 1A may be the result of a variety of factors, among which are: (a) if the α-keto-[1-14C]isocaproate was not entirely labeled in the 1 position, 14C derived from the other positions would be released at a slower rate; or (b) if the infused [α-keto-[1-14C]isocaproate was transaminated to [1-14C]leucine using glutamate as the amino group donor, the 14C-labeled leucine presumably present in an intracellular pool might be expected to be metabolized at a slower rate than the infused branched chain α-keto acid. In order to examine the second of these suggestions the experiment shown in Fig. 1B was performed. A liver was perfused for 15 min with 1 mM L-leucine following which a tracer amount of [1-14C]leucine was infused during the 10-min interval indicated. Under these conditions, the metabolic flux through the branched chain α-keto acid dehydrogenase reaction was calculated as 1.2 μmol of L-leucine decarboxylated/g of liver/h. The decline in the production of 14CO2 upon withdrawal of the [1-14C]-leucine exhibited one major kinetic component which accounted for 87% of the 14CO2 "washed out" of the liver in the initial 3½ min following the cessation of the L-[1-14C]leucine infusion. The t1/2 of this kinetic component was approximately 1.3 min (Fig. 1B). Each time L-[1-14C]leucine was infused, there occurred a sharp, transient peak in the rate of 14CO2 production immediately after the infusion of the 14C-labeled substrate was initiated. Repeating this type of experiment using α-keto[1-14C]isovalerate as the substrate provided results nearly identical with those with α-keto[1-14C]isocaproate shown in Fig. 1A (results not shown).

The effect of increasing the concentration of the infused α-ketoisocaproate on the flux through the branched chain dehydrogenase reaction in the perfused liver is illustrated in Fig. 2. Increasing the concentration of α-keto[1-14C]isocaproate in the perfusate increased the rates of oxygen consumption, ketogenesis, and 14CO2 production until the branched chain α-keto acid concentration exceeded 1 mM. The metabolic flux through the branched chain α-keto acid dehydrogenase plotted as a function of infused α-keto acid concentration exhibited a near linear relationship (see inset of Fig. 2). The β-hydroxybutyrate/acetoacetate ratio in the effluent perfusate increased from values of 0.11 at 0.05 mM α-ketoisocaproate to 0.19 at 0.5 mM α-ketoisocaproate and finally to 0.22 at 5 mM α-ketoisocaproate. The overall rate of ketogenesis (i.e. β-hydroxybutyrate plus acetoacetate) upon infusion of the ketogenic branched chain α-keto acid, α-ketoisocaproate...
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FIG. 2. Effect of varying the α-keto[1-14C]isocaproate concentration on the rates of α-ketoisocaproate decarboxylation and ketogenesis in a perfused rat liver. The infused α-ketoisocaproate concentration was increased in a stepwise manner as indicated by the horizontal bars. 14CO2 production, oxygen consumption, and ketone bodies, β-hydroxybutyrate □ and acetoacetate □, were measured as described under "Materials and Methods." The inset at the top of the figure demonstrates the relationship between α-ketoisocaproate concentration in the perfusion medium and the rate of decarboxylation of this substrate.

(1 mM), was 59.5 μmol·g⁻¹·h⁻¹ which is approximately 75% of the rate observed in the perfused liver from a fed rat with octanoate (0.5 mM) as the ketogenic substrate.

Infusing varying concentrations of α-keto[1-14C]isovalerate into a perfused liver from a fed rat resulted in sequential increases in the flux through the branched chain α-keto acid dehydrogenase reaction (Fig. 3) and the rate of oxygen consumption by the liver. Again the flux through the enzyme complex saturated above 1 mM α-ketoisovalerate. It is evident that the flux through the branched chain complex was greater with α-keto[1-14C]isocaproate as the infused substrate than with α-keto[1-14C]isovalerate by nearly a factor of 2 (e.g. 30 compared to 54 μmol·g⁻¹·h⁻¹). This observation stands in contrast to the situation in the perfused rat heart (16) and in rat liver mitochondria (22) where α-ketoisovalerate was a better substrate than α-ketoisocaproate at the same concentration.

In a liver perfused with α-keto[1-14C]isocaproate (1 mM), co-infusion of either unlabeled α-ketoisovalerate (1 mM) or DL-α-keto-β-methylvalerate (2 mM) resulted in a 54% and 67% decrease in the rate of 14CO2 production from the infused α-keto[1-14C]isocaproate, respectively (see Fig. 4). The rate of oxygen consumption by the liver was elevated by 10% upon infusion of either of the alternative branched chain substrates.

FIG. 3. Effect of varying the α-keto[1-14C]isovalerate concentration on the rate of α-ketoisovalerate decarboxylation in the isolated perfused rat liver. For experimental details, see legend to Fig. 1 and "Materials and Methods." The inset at the top of the figure represents the relationship between α-keto[1-14C]isovalerate concentration in the perfusion medium and the rate of α-ketoisovalerate decarboxylation.

FIG. 4. Effect of α-ketoisovalerate and DL-α-keto-β-methylvalerate co-infusion on the rate of α-keto[1-14C]isocaproate decarboxylation in a perfused rat liver. The various branched chain α-keto acids were infused into the liver as indicated by the horizontal bars. 14CO2 production and oxygen consumption by the liver were monitored as described under "Materials and Methods."
Conflicting evidence has been published concerning the effects of L(-)-carnitine on the decarboxylation of branched chain α-keto acids. Although carnitine has been reported not to affect the rate of α-ketoisocaproate oxidation in liver (23) and muscle mitochondria (24), information from other laboratories indicates that L(-)-carnitine significantly stimulates oxidation of leucine, valine, and α-ketoisocaproate in rat skeletal muscle mitochondria (25) and the rate of α-ketoisocaproate decarboxylation in rat liver homogenate and mitochondria (26, 27). The effect of L(-)-carnitine on the decarboxylation of 1-14C-labeled α-ketoisocaproate and α-ketoisovalerate in the perfused liver is illustrated in Fig. 5, A and B, respectively. With α-keto[1-14C]isocaproate as the substrate, L(-)-carnitine infusion resulted in a 13% increase in oxygen consumption by the liver with no change in the rate of α-ketoisocaproate decarboxylation. On the other hand, oxygen consumption by the liver and the decarboxylation of α-keto[1-14C]isovalerate were stimulated by approximately 11% and 60%, respectively, during the 8 min of L(-)-carnitine infusion.

We have demonstrated that pyruvate infusion into the perfused rat heart resulted in up to a 90% inhibition of the metabolic flux through the branched chain dehydrogenase reaction (16). While the exact mechanism of this pyruvate-mediated inhibition of the branched chain complex is not known, it was shown that pyruvate infusion resulted in a decrease in the extractable branched chain α-keto acid dehydrogenase activity in the perfused heart (28). The experiments depicted in Fig. 6, A and B, demonstrate the effect of pyruvate infusion (10 mM) on the flux through the branched chain dehydrogenase reaction in the perfused liver. Oxygen consumption increased by approximately 15% upon co-infusion of pyruvate and either branched chain substrate. However, pyruvate infusion inhibited the decarboxylation of α-keto[1-14C]isocaproate (1 mM) by 18% (Fig. 6A), while the decarboxylation of α-keto[1-14C]isovalerate was inhibited by 12% (Fig. 6B). In the rat heart, pyruvate infusion inhibited α-ketoisovalerate decarboxylation to a considerably greater extent than α-ketoisocaproate decarboxylation (16).

The effects of fatty acids and ketone bodies on the decarboxylation of the branched chain amino acids and α-keto acids have been investigated in various tissues with somewhat conflicting results. While Odyssey and Goldberg (10) observed an inhibition of [1-14C]leucine decarboxylation in rat diaphragm, Buse et al. (11) demonstrated that octanoate caused a stimulation in the rate of decarboxylation of branched chain amino acids in the diaphragm and a recirculating heart perfusion system. Additionally, stimulation of valine oxidation by octanoate and palmitate in the perfused rat hindquarter has been reported (29). Our data in the nonrecirculating heart perfusion indicate that octanoate, β-hydroxybutyrate, and acetocetate all inhibit strongly branched chain α-keto acid decarboxylation (16). Crabb and Harris (30) also demonstrated a decreased rate of L-[1-14C]leucine decarboxylation in hepatocytes upon addition of either oleate or β-hydroxybutyrate.

The effects of infused β-hydroxybutyrate on the metabolic flux through the branched chain α-keto acid dehydrogenase reaction in the perfused liver with α-keto[1-14C]isocaproat or α-keto[1-14C]isovalerate are depicted in Fig. 7, A and B, respectively. Co-infusion of β-hydroxybutyrate and either α-ketoisocaproat or α-ketoisovalerate resulted in an 18% increase in oxygen consumption over the rates observed with the branched chain substrates alone. However, while β-hydroxybutyrate infusion resulted in a 12% inhibition of the decarboxylation of α-keto[1-14C]isocaproat (Fig. 7A), the decarboxylation of α-keto[1-14C]isovalerate was stimulated greatly (i.e. 100%) (Fig. 7B). The stimulation of flux through the branched chain α-keto acid dehydrogenase reaction with α-ketoisovalerate as the substrate was reversed completely.
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**Fig. 6.** The effect of pyruvate co-infusion on the rates of α-keto[1-14C]isocaproate (A) and α-keto[1-14C]isovalerate (B) decarboxylation. In each experiment, pyruvate was co-infused for a period of 8 min as indicated by the horizontal bars. 14CO₂ production and oxygen consumption by the livers were measured as described under "Materials and Methods."

**Fig. 7.** Effect of (DL)-β-hydroxybutyrate co-infusion on the rates of α-keto[1-14C]isocaproate (A) and α-keto[1-14C]isovalerate decarboxylation (B). (DL)-β-Hydroxybutyrate was co-infused into livers for periods of 8 min as indicated by the horizontal bars. For experimental details, see "Materials and Methods."
when β-hydroxybutyrate was withdrawn from the perfusion system. Increasing the perfusion concentration of α-keto[1-14C]isocaproate to 5 mm did not change the stimulatory effect of β-hydroxybutyrate on α-ketoisovalerate decarboxylation (data not shown).

**DISCUSSION**

The primary purpose of the present study was to investigate the regulation of the branched chain α-keto acid dehydrogenase complex in the isolated perfused rat liver. Nearly all of the information available in the literature concerning the regulation of this multienzyme complex in liver has been obtained using homogenates (26, 27), isolated mitochondria (27), or isolated hepatocyte (31 33). The obvious advantage of using the perfused liver is that the regulation of the enzyme complex may be studied in a metabolic system under nearly in vivo conditions.

The experiment illustrated in Fig. 1A indicated that more than 90% of the 14CO2 derived from the decarboxylation of infused α-keto[1-14C]isocaproate was “washed out” of the liver in the initial 2% min with a t1/2 of 27% a following cessation of the tracer infusion. The t1/2 of the kinetic component due to the pyruvate dehydrogenase reaction in the perfused liver is very similar but not identical with that calculated for the branched chain α-keto acids in the present study (e.g. compare t1/2 = 18 s for pyruvate with t1/2 = 27% for α-ketoisocaproate) (15). Also, α-keto[1-14C]isocaproate and α-keto[1-14C]isovalerate (data not presented) exhibited very similar t1/2 values for the decline of 14CO2 production following termination of the tracer infusion, suggesting a similar metabolic fate for the two branched chain α-keto acids in the perfused liver. Performing a similar type of experiment with L-[l-14C]leucine indicated that the major kinetic component had a t1/2 of 1.3 min and the rate of L-leucine decarboxylation was less than 4% of the rate of α-ketoisocaproate decarboxylation. This observation suggests that the transamination of branched chain amino acids in the liver is the rate-limiting step. Evidence for this suggestion has been provided by Ichihara et al. (7) and Shinnick and Harper (8).

Studies of the concentration dependence of the rate of decarboxylation of infused branched chain α-keto acids in the perfused liver indicated that the flux through the enzyme complex is saturated at substrate concentrations in excess of 1 mm. A noticeable difference emerges between the decarboxylation rates of 14C-labeled α-ketoisocaproate and α-ketoisovalerate in the perfused liver. At each concentration examined, the rate of α-ketoisocaproate decarboxylation exceeded the rate of α-ketoisovalerate decarboxylation. Evidence obtained in isolated liver mitochondria (22), in the perfused rat heart (16), and in a variety of other systems (5, 34, 53) has indicated that α-ketoisovalerate is decarboxylated at a more rapid rate than α-ketoisocaproate by the branched chain complex. It is possible that the various short and branched chain acyl-CoA intermediates in the pathway involved in the breakdown of α-ketoisocaproate and α-ketoisovalerate, respectively, may affect differentially the regulation of the branched chain complex in the intact liver. In the present experiments, it is suggested that acyl-CoA intermediates produced during α-ketoisovalerate oxidation may be more inhibitory to the enzyme complex than those produced during the oxidation of α-ketoisocaproate. Furthermore, because the liver is capable of rapid rates of ketogenesis using α-ketoisocaproate as the substrate, the potentially inhibitory intermediates in the metabolism of α-ketoisocaproate may not accumulate to any appreciable extent.

The rate of ketogenesis from α-ketoisocaproate (Fig. 2) exhibited saturation kinetics very similar to the kinetics of the increase in the rate of decarboxylation of α-keto[1-14C]isocaproate as the concentration of the branched chain α-keto acid was increased in the perfusion medium. Such a correlation suggests that the measurement of 14CO2 production from α-keto[1-14C]isocaproate as a monitor of metabolic flux through the branched chain complex is valid.

Studies using either partially or highly purfied preparations of the branched chain α-keto acid dehydrogenase complex have demonstrated that all three branched chain α-keto acids, α-ketoisocaproate, α-ketoisovalerate, and α-keto-β-methylvalerate, are decarboxylated oxidatively by a single multienzyme complex. The experiment illustrated in Fig. 4 indicated that co-infusion of either α-ketoisovalerate or α-keto-β-methylvalerate with α-keto[1-14C]isocaproate resulted in an increased hepatic oxygen consumption and an appreciable and reversible inhibition of the rate of 14CO2 from the labeled α-ketoisocaproate. This experimental finding may be interpreted as a competition between the unlabeled and 14C-labeled α-keto acid at the level of the branched chain enzyme complex or at the level of the mitochondrial transport of the two branched chain α-keto acids.

We observed a differential effect of L(--)-carnitine infusion on the rate of decarboxylation of α-ketoisocaproate and α-ketoisovalerate. This effect (e.g. a stimulation of the rate of α-ketoisovalerate decarboxylation and no appreciable change in the rate of α-ketoisocaproate decarboxylation) likely is a result of the differential accumulation of acyl-CoA intermediates involved in the respective pathways for the metabolism of these two compounds in the absence and presence of L(--)-carnitine. Indeed, Solberg and Bremer (36) demonstrated in rat liver mitochondria incubated in the presence of L-[Me-3H]-carnitine and α-ketoisocaproate or α-ketoisovalerate that levels of propionylcarnitine and medium chain acylcarnitine were much greater with α-ketoisovalerate as the substrate than with α-ketoisocaproate. Hence, L(--)-carnitine may remove propionyl-CoA and other medium (branched) chain acyl-CoAs formed from α-ketoisovalerate oxidation which either may be inhibitory to the branched chain complex or may release free CoA which could accelerate the oxidation of the branched chain α-keto acid. If acyl-CoA intermediates in the pathway of α-ketoisocaproate oxidation do not accumulate or if the acyl-CoA species which accumulate are not as inhibitory to the branched chain complex, L(--)-carnitine infusion would not cause an observable effect. It is unlikely that L(--)-carnitine causes an appreciable diversion of intermediates toward acylcarnitine formation during α-ketoisocaproate oxidation as the ketogenic rate in the presence and absence of L(--)-carnitine was unchanged (data not shown).

Infusion of relatively low levels of pyruvate into the perfused rat heart resulted in a 90% inhibition of the metabolic flux through the branched chain complex using α-ketoisocaproate as the substrate. A less extensive flux inhibition was noted using α-ketoisocaproate as the substrate (16). Additionally, the extractable activity of the branched chain complex was diminished by 72% upon infusion of pyruvate into a heart which had been perfused under conditions which maximally activated the branched chain complex (28). Infusion of pyruvate into the perfused liver resulted in an inhibition of α-keto[1-14C]isocaproate decarboxylation to a greater degree than α-keto[1-14C]isovalerate decarboxylation, just the reverse situation as was observed in the heart. Whether the flux inhibition observed in the liver was the result of an inactivation of the enzyme complex or was the result of a simple competitive inhibition of the branched chain complex by pyruvate or NADH is currently under investigation.

The decarboxylation of α-keto[1-14C]isocaproate was inhibited by the co-infusion of β-hydroxybutyrate into the liver.
On the surface, this effect would seem to be a simple NADH-mediated inhibition of the enzyme complex since few other fates for β-hydroxybutyrate than oxidation to acetoacetate can occur in the liver.

However, despite the increase in the mitochondrial NADH/NAD⁺ ratio upon infusion of β-hydroxybutyrate, the rate of decarboxylation of α-keto[1-14C]isovalerate was stimulated by 100%. Stimulation of the decarboxylation of [1-14C]pyruvate in the perfused liver upon infusion of octanoate, oleate, or β-hydroxybutyrate has been reported (15, 37). It was proposed that the stimulation of pyruvate decarboxylation and the activation of the pyruvate dehydrogenase complex at low (i.e. physiological) pyruvate levels were results of an accelerated acetoacetate/pyruvate exchange across the mitochondrial membrane on the monocarboxylate transporter. Under conditions of rapid ketogenesis (i.e. rapid acetoacetate efflux), the mitochondrial pyruvate level would be elevated and the pyruvate dehydrogenase kinase would be inhibited by pyruvate leading to an interconversion of the complex by the phospho-protein phosphatase to its active form (15, 37). It was demonstrated that acetoacetate could replace OH⁻ ions as an effective antiport for pyruvate on the monocarboxylate transporter. Hence, it is likely that as α-ketoisocaprate is oxidized, acetoacetate is generated and an exchange of the branched chain α-keto acid substrate for the product, acetoacetate, is established. Also, this exchange phenomena could explain why α-ketoisocaprate decarboxylation exceeds α-ketoisovalerate decarboxylation in the liver. In the case of the stimulation of the decarboxylation of α-ketoisovalerate by β-hydroxybutyrate, there may occur a situation analogous to the case for pyruvate. As acetoacetate is produced intramitochondrially, an exchange is established between acetoacetate and α-ketoisovalerate. Thus, an acceleration of the decarboxylation of this branched chain α-keto acid occurs either by mass action (e.g. an increase in the intramitochondrial concentration of the substrate) or by virtue of the fact that the branched chain α-keto acids tend to activate or to stabilize the enzyme complex (6, 28). This scenario requires that the transport of α-ketoisovalerate is limiting the oxidative system when infused alone into the perfusion system.

REFERENCES