Valine metabolism

Gluconeogenesis from 3-hydroxyisobutyrate

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During valine catabolism in muscle both 2-oxoisovalerate and 3-hydroxyisobutyrate can be released into the circulation. 3-Hydroxyisobutyrate is a good gluconeogenic substrate in isolated cortical tubules and hepatocytes. The maximal rate of gluconeogenesis from 3-hydroxyisobutyrate was greater than from 2-oxoisovalerate. We propose that 3-hydroxyisobutyrate is an inter-organ metabolite by which the gluconeogenic potential of valine, whose catabolism has been initiated in muscle, may be conserved.

INTRODUCTION

The branched-chain amino acids valine, isoleucine and leucine are structurally similar, but may not share the same metabolic fate. Valine and isoleucine may be converted into glucose, whereas leucine cannot. The first two enzymes in the catabolism of branched-chain amino acids are common to all three of these amino acids. The first enzyme is a reversible aminotransferase (EC 2.6.1.42) that converts the branched-chain amino acid into the branched-chain 2-oxo acid. The second enzyme is an irreversible branched-chain 2-oxo acid dehydrogenase (EC 1.2.4.4). It has been suggested that branched-chain amino acid metabolism proceeds only as far as the 2-oxo acid in muscle, and that these 2-oxo acids leave muscle and are further metabolized in liver (Wohlhueter & Harper, 1970; Shinnick & Harper, 1976; Odessey & Goldberg, 1979). This idea was originally based on the fact that, in muscle, the activity of the branched-chain amino acid aminotransferase is high relative to that of the branched-chain 2-oxo acid dehydrogenase. In liver, conversely, branched-chain 2-oxo acid dehydrogenase activity is high compared with that of the aminotransferase. Such an arrangement implies that the gluconeogenic potential of valine and isoleucine, which were deaminated in muscle, would be conserved because the irreversible branched-chain 2-oxo acid dehydrogenase would only be active in the liver. It is now apparent, however, that there can be considerable flux through the muscle branched-chain 2-oxo dehydrogenase (Spydevold, 1979; White & Brooks, 1981; Hagg *et al.*, 1982; Spydevold & Hockland, 1983). Does this mean that the gluconeogenic potential of valine and isoleucine is lost once their catabolism is initiated in muscle? Chang & Goldberg (1978) have shown that more than half of the valine and isoleucine carbon which enters the tricarboxylic acid cycle in muscle will appear in glutamine, with less than 2% appearing as alanine carbon. Under normal conditions, glutamine is utilized by intestine as its major fuel (Windmueller & Spaeth, 1980), but it can also be converted into glucose by liver and kidney (Brosnan, 1982). In addition, it has been shown in the perfused rat hindquarter that only part of the carbon from 2-oxoisovalerate actually enters the

tricarboxylic acid cycle, and that an intermediate, 3-hydroxyisobutyrate, is released (Spydevold, 1979; Lee & Davis, 1986). Mammary gland has also been shown to release 3-hydroxyisobutyrate (Wohlt *et al.*, 1977). In our laboratory we have shown that, when rat hearts are perfused with 2-oxo[U-¹⁴C]isovalerate, radiolabelled 3-hydroxyisobutyrate is released (Letto, 1986). This suggests that 3-hydroxyisobutyrate could serve as an additional inter-organ metabolite that would preserve the gluconeogenic potential of valine, provided that it can be taken up and converted into glucose by gluconeogenic tissues. The purpose of this study is to investigate whether 3-hydroxyisobutyrate is gluconeogenic in rat kidney cortical tubules and in hepatocytes, and to compare glucose production from this substrate with that from 2-oxoisovalerate and from lactate.

MATERIALS AND METHODS

Materials

3-Hydroxyisobutyrate was prepared from (s)-(+)-methyl 3-hydroxy-2-methylpropionate obtained from Aldrich Chemical Co. (Montreal, Quebec, Canada). The ester was incubated at room temperature for 1 h with 10% molar excess of NaOH and an equal volume of methanol as a co-solvent. The solvents were then removed by freeze-drying. The ¹H n.m.r. spectrum of the resulting sodium salt was identical with that of an authentic sample kindly supplied by Dr. A. P. Kozikowski, University of Pittsburgh. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Enzymes were obtained from Boehringer (Dorval, Quebec, Canada).

Animals

Male Sprague–Dawley rats (Charles River, La Prairie, Quebec, Canada) weighing 300–400 g were used in all experiments. They were allowed free access to water and Purina rat chow unless otherwise stated. Starved rats were deprived of food for 48 h. Diabetes was induced by an intravenous injection of streptozotocin (100 mg/kg body wt.) under light diethyl ether anaesthesia. Animals

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were maintained for 7 days on a daily injection (subcutaneous) of protamine–zinc insulin (3–5 units/day) adjusted so that body weight remained constant (Brosnan *et al.*, 1983). Insulin was withdrawn for 4 days before experiments.

Isolated kidney tubules and hepatocytes

Cortical tubules from kidneys were prepared by collagenase digestion as described by Lowry & Ross (1980). Tubules (about 10 mg dry wt.) were incubated with constant shaking in stoppered 25 ml Erlenmeyer flasks for 30 min at 37 °C in a total volume of 2 ml of Krebs–Henseleit (1932) medium gassed with O₂/CO₂ (19:1). The incubations were terminated by addition of HClO₄ (final concn. 3%, w/v), extracts were neutralized with K₃PO₄ and supernatants were used for glucose assay.

Hepatocytes were prepared by collagenase perfusion of the liver as described by Krebs *et al.* (1974). Hepatocytes (about 5 mg dry wt.) were incubated in a total volume of 4 ml of Krebs–Henseleit medium gassed with O₂/CO₂ (19:1) for 60 min at 37 °C. Incubations were terminated by addition of HClO₄ (final concn. 3%). Extracts were neutralized with K₃PO₄ and assayed for glucose.

Glucose was measured by a standard enzymic assay as described by Bergmeyer *et al.* (1974). Results were corrected for glucose formed in the absence of added substrate. Viability of hepatocyte and tubule preparations was assessed by determining the latency of lactate dehydrogenase (Morrison *et al.*, 1966). In the cell preparations include in this study, lactate dehydrogenase was 96–98% latent.

RESULTS

Glucose was readily formed from 3-hydroxyisobutyrate by both hepatocytes and kidney cortical tubules. Glucose production by hepatocytes from 3-hydroxyisobutyrate and from 2-oxoisovalerate was linear for 60 min; in isolated cortical tubules glucose production was linear for at least 45 min (results not shown). Therefore we used 60 min for hepatocytes and 30 min for tubules as our standard incubation times.

The effect of substrate concentration on glucose production is shown in Fig. 1. Glucose production was maximal with 2 mM-3-hydroxyisobutyrate in both kidney tubules and hepatocytes (Fig. 1a). There was no statistically significant increase in glucose production with further increase in substrate concentration above 2 mM. Maximal rates of glucose production were observed with 0.5 mM-2-oxoisovalerate in hepatocytes and kidney tubules (Fig. 1b). With hepatocytes no further significant increase in glucose production was observed up to 5 mM-2-oxoisovalerate, whereas with tubules a significant decrease was observed above 0.5 mM substrate. Inhibition of gluconeogenesis in tubules at high concentrations of 2-oxoisovalerate has been reported by Stumpf & Kraus (1978). No glucose production was observed with valine as substrate in either preparation.

Table 1 give the rates of glucose production in isolated cortical tubules and hepatocytes prepared from rats in different physiological states. In isolated cortical tubules from fed rats, the maximal rates of gluconeogenesis from 3-hydroxyisobutyrate and lactate were similar, and these

Fig. 1. Glucose production as a function of substrate concentration in hepatocytes and isolated cortical tubules

Hepatocytes from 48 h-starved rats (●) and kidney cortical tubules from fed rats (▲) were prepared as described in the Materials and methods section. Incubations contained either 3-hydroxyisobutyrate (a) or 2-oxoisovalerate (b) as substrate. Hepatocytes were incubated for 60 min and tubules for 30 min. Data are means $\pm$ S.D. of five separate experiments for hepatocytes and three separate experiments for tubules with each substrate.

rates were higher than that from 2-oxoisovalerate. In 48 h-starved rats, glucose production from lactate increased significantly compared with fed controls, whereas the rate from 3-hydroxyisobutyrate was unchanged. Glucose production by either liver or kidney cells of diabetic rats from any of the three substrates was not significantly different ($P > 0.05$) from that observed with tissues from either fed or starved rats.

The maximal rate of glucose production from 3-hydroxyisobutyrate by hepatocytes from starved rats was about half of that from 10 mM-lactate. Diabetes did not significantly influence the rate of glucose production from any of these substrates. The maximal rate of gluconeogenesis from 3-hydroxyisobutyrate was always greater than that from 2-oxoisovalerate.

Mercaptopicolinate (0.6 mM), an inhibitor of phospho-

Table 1. Gluconeogenesis in isolated renal cortical tubules and hepatocytes

Kidney cortical tubules and hepatocytes were prepared and incubated as described in the Materials and methods section. Substrate concentrations for tubules were 2 mM-3-hydroxyisobutyrate (HIB), 0.5 mM-2-oxoisovalerate (KIV) or 5 mM-lactate, and for hepatocytes were 5 mM-3-hydroxyisobutyrate, 2 mM-2-oxoisovalerate or 10 mM-lactate. Results are expressed as μmol of glucose produced/min per g dry wt. (means $\pm$ S.D. for the numbers of experiments in parentheses). Endogenous rates of glucose production, which have been subtracted, were: 0.25 ± 0.07 , 0.74 ± 0.09 and 1.07 ± 0.05 $\mu\text{mol}/\text{min}$ per g dry wt. for tubules from fed, 48 h-starved and diabetic rats respectively; 0.58 ± 0.18 and 0.71 ± 0.04 $\mu\text{mol}/\text{min}$ per g dry wt. for hepatocytes from 48 h-starved and diabetic rats. * $P < 0.05$ compared with fed.

Condition of rat	Substrate	Glucose production	
		Kidney cortex tubules	Hepatocytes
Fed	HIB	1.20 ± 0.18 (4)	—
	KIV	0.59 ± 0.10 (3)	—
	Lactate	1.42 ± 0.27 (4)	—
48 h-starved	HIB	0.99 ± 0.31 (3)	1.20 ± 0.68 (5)
	KIV	—	0.52 ± 0.14 (5)
	Lactate	2.45 ± 0.48 (3)*	2.60 ± 0.79 (3)
Diabetic	HIB	0.98 ± 0.24 (3)	1.14 ± 0.24 (4)
	KIV	—	0.44 ± 0.20 (4)
	Lactate	1.82 ± 0.10 (3)	3.80 ± 0.58 (4)

enolpyruvate carboxykinase, inhibited the production of glucose from 3-hydroxyisobutyrate in tubules (1.11 ± 0.10 versus 0.09 ± 0.04 $\mu\text{mol}/\text{min}$ per g dry wt.) and hepatocytes (1.14 ± 0.24 versus 0.43 ± 0.04 $\mu\text{mol}/\text{min}$ per g dry wt.). Thus gluconeogenesis from 3-hydroxyisobutyrate must require phosphoenolpyruvate carboxykinase, as does that from other C_4 intermediates.

DISCUSSION

Movement of 3-hydroxyisobutyrate out of muscle has been demonstrated *in vitro* (Spydevold, 1979; Lee & Davis, 1986; Letto, 1986), and the present paper demonstrates that liver and kidney cells are capable of extracting 3-hydroxyisobutyrate from the medium and converting it into glucose. Indeed, maximal rates of gluconeogenesis from 3-hydroxyisobutyrate were greater than from 2-oxoisovalerate. Studies on the inter-organ movement of 3-hydroxyisobutyrate *in vivo* have not been made. Nevertheless, 3-hydroxyisobutyrate has been reported to be a normal constituent of plasma and, indeed, is excreted in the urine of diabetics (Landaas, 1975). In normal humans, the concentration of 3-hydroxyisobutyrate in serum is 1–4 mg/l (O. A. Mamer, personal communication) ($10\text{--}45$ μM). This compares with a 2-oxoisovalerate concentration in plasma from normal humans of 8 μM (Hutson & Harper, 1981). Therefore we postulate 3-hydroxyisobutyrate to be an inter-organ metabolite in the catabolism of valine.

When valine catabolism is initiated in muscle, some of the carbon is released as 2-oxoisovalerate and some is metabolized via the branched-chain 2-oxo acid dehydrogenase and subsequent enzymes of valine catabolism. The intermediates of this pathway are all CoA derivatives until 3-hydroxyisobutyrate, which is formed by hydrolysis of 3-hydroxyisobutyryl-CoA. Removal of CoA facilitates transport through membranes, so that 3-hydroxyisobutyrate may now leave the muscle and be converted into glucose in the liver and the kidney. Such a scenario requires that 3-hydroxyisobutyrate be a good substrate for transport out of muscle mitochondria and

muscle cells on the one hand, and across the cell membranes and mitochondrial membranes of liver and kidney on the other hand. Whether this is via the well-described monocarboxylate transporter remains to be determined.

The experiments with mercaptopycolinate suggest that gluconeogenesis from 3-hydroxyisobutyrate requires phosphoenolpyruvate carboxykinase and presumably occurs via the conventional gluconeogenic pathway. The maximal rate of gluconeogenesis from 3-hydroxyisobutyrate, however, was always lower than that from lactate. In addition, the maximal rate of gluconeogenesis from 3-hydroxyisobutyrate did not increase in kidney cortical tubules during starvation, a situation in which the maximal rate of gluconeogenesis from lactate did increase, and where the activity of phosphoenolpyruvate carboxykinase has been reported to be increased (Henning *et al.*, 1966). Thus phosphoenolpyruvate carboxykinase does not exert a significant limitation on the maximal rate of gluconeogenesis from 3-hydroxyisobutyrate. There are two irreversible reactions between 3-hydroxyisobutyrate and succinyl-CoA (conversion of methylmalonyl semialdehyde into propionyl-CoA, and propionyl-CoA carboxylase), which probably determine the rate of gluconeogenesis from 3-hydroxyisobutyrate.

This work was supported by grants from the Canadian Diabetes Association and the Medical Research Council of Canada. We thank Dr. C. Jablonski, Department of Chemistry, Memorial University of Newfoundland, for carrying out the n.m.r. analysis. J.L. acknowledges the award of a graduate student bursary by Memorial University of Newfoundland.

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Received 8 July 1986/4 September 1986; accepted 2 October 1986