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Interorgan metabolism of valine*

M. E. Brosnan and **J. Letto**

Department of Biochemistry, Memorial University of Newfoundland,
St. John's, Newfoundland, Canada

Summary. Oxidation of branched-chain amino acids, leucine, isoleucine and valine, is thought to occur primarily in muscle. Theoretically, however, it is possible for valine carbon to be converted to glucose, a process which only occurs in liver and kidney. We provide evidence that valine is oxidixed to β-hydroxyisobutyrate in muscle, and that this intermediate is released from muscle and taken up by liver and kidney for subsequent conversion to glucose, thus conserving the gluconeogenic potential of valine.

Keywords: Valine – Interorgan metabolism

Valine is an essential amino acid in animals. Dietary valine enters the portal circulation, but it normally bypasses the liver [1] and proceeds to peripheral tissues for retention. In fact, when the arterial level of valine falls, liver releases this amino acid into the circulation [1]. Catabolism of valine, and the other branched-chain amino acids, isoleucine and leucine, is believed to be initiated chiefly in extrahepatic tissues [2]. However, catabolism of valine gives rise to succinyl CoA, as shown in Fig. 1. This valine carbon can be converted to glucose if the succinyl CoA can be generated in liver or kidney.

Catabolic pathway

The complete pathway for valine catabolism is given in Fig. 1, together with a reference for each of the enzymes involved, where possible. A more complete list of references is given in [13]. The first step in valine metabolism is the reversible

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Chemical reaction	Enzyme	Reference	Potential interorgan metabolite
valine			
↓ α-ketoglutarate → glutamate	branched-chain amino acid transaminase	3	
α-ketoisovalerate		4	α-ketoisovalerate
↓ CoA, CO_2; NAD → $NADH + H^+$	branched-chain keto acid dehydrogenase		
isobutyryl CoA		5	
↓ FAD → $FAD\text{-}H_2$	acyl CoA dehydrogenase		
methacrylyl CoA		6	
↓ H_2O	hydratase		
β-hydroxyisobutyryl CoA		7	
↓ CoA	deacylase		
β-hydroxyisobutyrate		8	β-hydroxyisobutyrate
↓ NAD → $NADH + H^+$	β-hydroxyisobutyrate dehydrogenase		
methylmalonic semialdehyde		9	β-amino isobutyrate (by transamination)
↓ CoA, CO_2; NAD → $NADH + H^+$	methylmalonic semialdehyde dehydrogenase		
propionyl CoA		10	
↓ CO_2; ATP → ADP + Pi	propionyl CoA carboxylase		
D-methylmalonyl CoA		11	
↓	methylmalonyl CoA racemase		
L-methylmalonyl CoA		12	
↓	methylmalonyl CoA mutase		
succinyl CoA (TCA cycle)			glutamine alanine

Fig. 1. Metabolic pathway for the catabolism of valine

transamination of valine with α-ketoglutarate to give α-ketoisovalerate and glutamate. This step is catalyzed by branched-chain amino acid transaminase [3]. The only tissue in which this step is thought to be limiting is liver [2]. Since the reaction is apparently freely reversible *in vivo*, it does not necessitate the further catabolism of valine. A low concentration (10–20 μM) of α-ketoisovalerate is present in blood (eg [14]).

The committed step in the oxidation of valine and the other branched-chain amino acids is the second enzyme, branched-chain keto acid dehydrogenase. This enzyme catalyzes the irreversible oxidative decarboxylation of α-ketoisovalerate to give isobutyryl CoA with NAD serving as hydrogen acceptor. This enzyme is subject to regulation by covalent modification [15], with the phosphorylated enzyme being less active. It is probably also sensitive to NADH/NAD^+ and/or CoA concentration of the mitochondria [15]. Branched-chain keto acid dehydrogenase has proved to be a very difficult enzyme to assay accurately in tissue homogenates [2], but intact cell preparations from most tissues, including muscle [17] and heart [18] are quite active. In fact, Kasperek et al. [19] have estimated that during exercise much of the catabolism of branched-chain keto acids could be initiated outside the liver.

Isobutyryl CoA is further oxidized to methacrylyl CoA by the flavoprotein, α-methyl branched-chain acyl CoA dehydrogenase [5]. Conversion of methacrylyl CoA to propionyl CoA is catalyzed by enzymes unique to this pathway [6–9], whereas the first three enzymes of this pathway also catalyze the conversion of isoleucine to tiglyl CoA [3–5] and the first two enzymes catalyze the conversion of leucine to isovaleryl CoA [3, 4]. It is generally assumed that the rate-limiting step for catabolism of all three branched-chain amino acids is branched-chain keto acid dehydrogenase [13]. If this were so, any methacrylyl CoA formed would be rapidly converted to succinyl CoA. Depending upon the tissue involved and the physiological situation the succinyl CoA could be anaplerotic, increasing the capacity of the TCA cycle, it could be totally oxidized to provide energy for the cell's needs or it could be converted to other amino acids (for example, glutamine or alanine in muscle), to glucose in liver or kidney, or to fatty acids.

If another enzyme in the pathway distal to branched-chain keto acid dehydrogenase were less active than this supposed limiting step, then conversion of methacrylyl CoA to succinyl CoA would be incomplete and some intermediate(s) would accumulate. Two steps in this pathway involve intermediates, β-hydroxyisobutyrate and methylmalonic semialdehyde, which are not CoA derivatives and thus might be permeable to mitochondrial and plasma membranes. Such incomplete oxidation would allow catabolism of valine to be initiated in one tissue, such as muscle, and completed in another, such as liver, so that the carbon skeleton of valine would be available for gluconeogenesis. In order to determine whether the oxidation of valine in heart was complete or incomplete, hearts were perfused with α-ketoisovalerate or α-ketoisocaproate (keto-leucine, for comparison) labelled with ^{14}C in only the 1-position or

uniformly-labelled, and production of $^{14}CO_2$ was analyzed [18]. A summary of the data is given in Table 1. The ratio of $^{14}CO_2$ produced from U-^{14}C-labelled α-ketoisocaproate to that produced from 1-^{14}C-labelled keto acid was 5.2 and did not differ from the predicted value for complete oxidation. Thus the isovaleryl CoA produced from α-ketoisocaproic acid was completely oxidized in perfused heart and branched-chain keto acid dehydrogenase was the limiting step in its catabolism. In contrast, the ratio of $^{14}CO_2$ produced from differentially-labelled α-ketoisovalerate was only 1.8 compared to a theoretical ratio of 5.0. Thus oxidation of isobutyryl CoA by perfused heart was incomplete and must have resulted in the accumulation of some intermediate. Perfusate from hearts perfused with U-^{14}C-α-ketoisovalerate was analyzed for ^{14}C-labelled intermediates, focusing on those shown in Fig. 1, but including all amino acids (by amino acid analyzer) and carboxylic acids (by HPLC) [18]. Radioactivity was present in valine, formed by transamination of the keto acid, and in β-hydroxyisobutyrate only. The rate of appearance of β-hydroxyisobutyrate was 3.8 ± 0.9 μmol. min. g. dry wt. ($n = 3$ perfusions), which compares under the same conditions with a flux through branched-chain keto acid dehydrogenase of 5.5 μmol. min. g dry wt. and a rate of total oxidation of α-ketoisovalerate of approximately 1 μmol. min. g dry wt. (calculated from ref. 18). β-hydroxyisobutyrate has also been reported to accumulate after perfusion of rat hindquarter with U-^{14}C valine [17], incubation of slices of bovine mammary gland with U-^{14}C valine [20] or incubation of rat hemidiaphragms with either U-^{14}C valine or U-^{14}C α-ketoisovalerate [21].

β-hydroxyisobutyrate would be oxidized to methylmalonic semialdehyde by β-hydroxyisobutyrate dehydrogenase with NAD serving as hydrogen acceptor [8]. This is apparently a reversible reaction, at least in vitro [8]. Methylmalonic semialdehyde is oxidatively decarboxylated by methylmalonate semialdehyde dehydrogenase in a reaction analogous to several other dehydrogenases (pyruvate,

Table 1. Relative production of $^{14}CO_2$ from [U-^{14}C] and from [1-^{14}C] α-keto acids of leucine and valine by perfused hearts from rats

Substrate	$^{14}CO_2$ produced $\frac{[U\text{-}^{14}C]}{[1\text{-}^{14}C]}$	
	Measured	Theoretical
α-ketoisocaproate	5.2[a]	6.0
α-ketoisovalerate	1.8	5.0

[a] Data calculated from [24]. Hearts were perfused for 25 min. with the appropriately labelled [^{14}C] α-keto acid. Theoretical $^{14}CO_2$ release was calculated assuming complete oxidation of the carbon skeleton derived from decarboxylation of the [1-^{14}C] α-keto acid.

branched-chain keto acid) [9]. This latter step is probably irreversible; thus one of these two enzymes must be limiting for the catabolism of β-hydroxyisobutyrate in perfused heart, although neither of these enzymes has been studied in this tissue.

Harris and his co-workers [22] recently purified β-hydroxyisobutyrate dehydrogenase from rabbit liver, and they have shown that it is highly sensitive to product inhibition by NADH. If this were true of the enzyme from heart, then valine or its keto acid could be completely oxidized under conditions of energy need, but the metabolic intermediate could be released if NADH were elevated. Alternatively, heart may have very little activity of β-hydroxyisobutyrate dehydrogenase, in which case this enzyme would normally limit valine catabolism in this tissue. The same arguments would hold for methylmalonic semialdehyde dehydrogenase; however, no information is available on the activity or regulation of this enzyme for any tissue [9]. It is interesting that two of the mitochondrial enzymes which catalyze similar oxidative decarboxylation reactions, pyruvate dehydrogenase and branched-chain keto acid dehydrogenase are regulated by covalent modification [23].

If β-hydroxyisobutyrate accumulates during valine catabolism and is released from perfused tissues, it should be detected in the blood. In fact, the concentration in blood of normal fed rats is 10–45 μM [24]. Low levels can be detected in urine of normal rats, but the urinary excretion of β-hydroxyisobutyrate increases markedly during ketoacidosis from various causes [25].

When β-hydroxyisobutyrate is released by muscle, heart or other tissues, what is its fate? If the gluconeogenic potential of valine is to be realized, the intermediate must be taken up by liver and/or kidney and converted to glucose. When isolated hepatocytes or proximal kidney tubules were incubated with β-hydroxyisobutyrate, they were able to convert it to glucose (Table 2). Glucose production was inhibited by mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase, thus indicating that gluconeogenesis from β-hydroxyisobutyrate requires phosphoenolpyruvate carboxykinase, as does

Table 2. Gluconeogenesis from β-hydroxyisobutyrate in isolated renal cortical tubules and hepatocytes

	Kidney cortex tubules	Hepatocytes
β-hydroxyisobutyrate	1.11 ± 0.10	1.14 ± 0.24
β-hydroxyisobutyrate + mercaptopicolinate	0.09 ± 0.04	0.43 ± 0.04

Kidney cortical tubules and hepatocytes were prepared from fasted rats and incubated as described [24]. Concentration of β-hydroxyisobutyrate for tubules was 2 mM and for hepatocytes 5 mM; concentration of mercaptopicolinate was 0.6 mM. Results are expressed as μmol glucose produced.min.g dry wt. (mean ± S.D.).

that from other C_4 intermediates. Under the same conditions, α-ketoisovalerate was a relatively poor glucogenic substrate compared to β-hydroxyisobutyrate [24].

Model

The observation that metabolism of valine is incomplete, resulting in accumulation and release of β-hydroxyisobutyrate in heart [18] and several other tissues [17, 20, 21], coupled with the observations that β-hydroxyisobutyrate is a normal constituent of blood and a good glucogenic substrate in kidney and liver cells [24], has prompted us to propose the model shown in Fig. 2 for the metabolism of valine. Nitrogen is removed from the amino acid in peripheral tissues, such as muscle, where the activity of branched-chain amino acid transaminase is high. When the branched-chain keto acid dehydrogenase is active, for example during fasting [26], or exercise [19], α-ketoisovalerate will be irreversibly converted to isobutyryl CoA and further metabolized to β-hydroxyisobutyrate. This metabolic intermediate will then be released into the circulation, where it can be transported to the glucogenic tissues for conversion to glucose. This model would conserve the glucogenic potential of valine, although its catabolism is initiated in a non-glucogenic tissue. One might expect this multiorgan pathway to be particularly active at times when amino acids are available in excess of requirements for protein synthesis and when glucose production is required, for example, during ingestion of a high protein, low carbohydrate diet, or during fasting or exercise. This model remains to be confirmed in vivo, although each individual step has been shown to occur in intact cell preparations. In addition, studies are required on the regulation of the activity of β-hydroxyisobutyrate dehydrogenase and methylmalonic semialdehyde dehydrogenase, the reciprocal control of which is essential in non glucogenic (dehydrogenases inactive) and glucogenic (dehydrogenases active) tissues for this pathway to function.

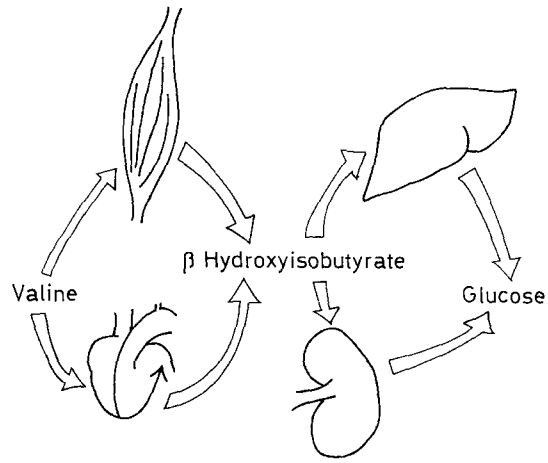

Fig. 2. Proposed model for the interorgan metabolism of valine

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Authors' address: Dr. Margaret E. Brosnan, Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9.