Metabolism of valine and 3-methyl-2-oxobutanoate by the isolated perfused rat kidney

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(Received 8 May 1984/Accepted 1 August 1984)

1. Metabolism of branched-chain amino and 2-oxo acids was studied in the isolated perfused kidney. 2. Significant amounts of 2-oxo acids were released by perfused kidney with all concentrations of amino acids tested (0.1–1.0 mM each), despite the high activity of branched-chain 2-oxo acid dehydrogenase in kidney. 3. As perfusate valine concentration was increased from 0.2 to 1.0 mM, [1-¹⁴C]valine transamination (2-oxo acid oxidized + released) increased roughly linearly; [1-¹⁴C]valine oxidation, however, increased exponentially. 4. Increasing perfusate concentration of 3-methyl-2-oxo[1-¹⁴C]butanoate from 0 to 1.0 mM resulted in a linear increase in the rate of its oxidation and a rise in perfusate valine concentration; at the same time significant decreases occurred in perfusate isoleucine and leucine concentrations, with corresponding increases in rates of release of their respective 2-oxo acids. 5. Comparison of rates of oxidation of [1-¹⁴C]valine and 3-methyl-2-oxo[1-¹⁴C]butanoate suggests that 2-oxo acid arising from [1-¹⁴C]valine transamination has freer access to the 2-oxo acid dehydrogenase than has the 2-oxo acid from the perfusate. 6. The observations indicate that, when branched-chain amino and 2-oxo acids are present in perfusate at near-physiological concentrations, rates of transamination of the amino and 2-oxo acids by isolated perfused kidney are greater than rates of oxidation.

The first two enzymes in the branched-chain amino acid-degrading pathway, branched-chain amino acid aminotransferase and branched-chain 2-oxo acid dehydrogenase, have been found in all tissues examined (Wohlhueter & Harper, 1970; Ichihara *et al.*, 1973, 1975a; Shinnick & Harper, 1976). Kidney, because of its relatively high activities of both these enzymes (Wohlhueter & Harper, 1970; Shinnick & Harper, 1976), is assumed to be a highly active site of branched-chain amino acid oxidation. Observations on branched-chain amino acid degradation by kidney slices support this assumption (Dawson *et al.*, 1967; Dawson & Hird, 1968; Goldberg & Odessey, 1972).

Subsequent research has demonstrated that branched-chain 2-oxo acid dehydrogenase, including that of rat kidney (Odessey, 1980; Hughes & Halestrap, 1981; Lau *et al.*, 1981; Gillim *et al.*, 1983), is regulated by a phosphorylation (inactivation)/dephosphorylation (activation) system. On the other hand, branched-chain amino acid aminotransferase is not subject to complex regula-

tion. The rate of transamination, which determines the rate of supply of substrate for the dehydrogenase, appears to depend primarily on the concentration of enzyme and on the relative concentrations of substrates: [branched-chain amino acids]/[2-oxo acid] and [α -oxoglutarate]/[glutamate] (Krebs & Lund, 1977). As the activities of both branched-chain amino acid aminotransferase (Ichihara *et al.*, 1975a; Kadowaki & Knox, 1982) and branched-chain 2-oxo acid dehydrogenase are high in kidney, and as the activation state of the dehydrogenase is high *in vivo* in relation to that of other organs (Lau *et al.*, 1981; Gillim *et al.*, 1983; Wagenmakers *et al.*, 1984), the rate of catabolism of branched-chain amino acids in kidney would appear to depend mainly on substrate concentration.

The objective of the present study was (1) to investigate quantitatively, in the isolated perfused kidney, the metabolism of [1-¹⁴C]valine and 3-methyl-2-oxo[1-¹⁴C]butanoate and (2) to obtain information on the influence of substrate concentration on the rates of metabolism of valine and its

2-oxo acid in this system. The isolated perfused rat kidney was selected as a model system that would be expected to maintain transport relationships of metabolites across plasma and mitochondrial membranes, amino-acceptor and amino-donor concentrations, and interactions between cytosolic and mitochondrial enzymes, in a state resembling closely that existing *in vivo*. It was further assumed that observations on the metabolism of [1-¹⁴C]-valine and 3-methyl-2-oxo[1-¹⁴C]butanoate would be indicative of metabolism of branched-chain amino acids generally.

Experimental

Materials

L-[1-¹⁴C]Valine was obtained from Amersham, Arlington Heights, IL, U.S.A. 3-Methyl-2-oxo[1-¹⁴C]butanoate was synthesized as described by Rüdiger *et al.* (1972); L-amino oxidase, catalase and 3-methyl-2-oxobutanoate were purchased from Sigma. Amino acids were obtained from U.S. Biochemicals, Cleveland, OH, U.S.A. All other reagents were obtained from commercial sources and were at least reagent grade.

Male Holtzman rats (330–430 g) were used as the source of kidneys. Rats were maintained on an 18%-casein diet (Wohlhueter & Harper, 1970) with a 10h meal-feeding schedule during the 12h light cycle; the feeding period was limited to ensure the rats would be in the post-prandial state when kidneys were taken for perfusion.

Methods

The perfusion apparatus and surgical procedure were similar to those described by Nishiitsutsuji-Uwo *et al.* (1967) with modifications as described elsewhere (Hutson *et al.*, 1978; Maack, 1980) and outlined below. In order to maintain the high flow rates needed (30–40 ml/min per g), dual pumps (Master-flex, Cole-Palmer) were used. The humidified gas mixture (O₂/CO₂, 19:1) leaving the silastic tube oxygenator was diverted into two traps linked in series, each containing 5 ml of CO₂-trapping solution (methoxyethanol/ethanolamine, 2:1, v/v); the traps were changed every 10 min and the radioactivity in the solution was counted in a liquid-scintillation counter (1217 Rackbeta, LKB).

The perfusion medium was Krebs–Henseleit bicarbonate buffer (Krebs & Henseleit, 1932) containing: dialysed bovine serum albumin (Fraction V, Sigma), 7.5%; glucose, 5.5 mM; pyruvate, 0.12 mM; and all amino acids at approximately rat plasma concentrations (Hutson *et al.*, 1978; Epstein *et al.*, 1982). The individual branched-chain amino acids were increased concomitantly when different perfusate concentrations were desired; 0.1–0.2 mM

each, 0.5 mM each and 1.0 mM each. 3-Methyl-2-oxobutanoate was included in the perfusate at the final concentrations of 0.05, 0.20 and 1.0 mM, and the concentrations of the branched-chain amino acids were 0.1–0.2 mM each. [1-¹⁴C]Valine or 3-methyl-2-oxo[1-¹⁴C]butanoate were added to the reservoir after a 3 min wash-out period. The final perfusate volume was typically 140 ml.

Perfusate samples (3.5 ml) were taken every 30 min for analyses of branched-chain 2-oxo acids (Hutson *et al.*, 1978; Cree *et al.*, 1979), amino acids (Beckman Amino Acid Autoanalyzer 119CL) and for radioactivity determinations (Hutson *et al.*, 1978). Kidneys were freeze-clamped at various times during the perfusion, powdered, and 1 g of the powdered tissue was homogenized in 6.1% (w/v) sulphosalicyclic acid and centrifuged (27 000 g for 15 min); the resultant supernatant was used for amino acid, radioactivity or branched-chain 2-oxo acid analyses. To assay branched-chain 2-oxo acid dehydrogenase activity, a 10% (w/v) kidney-cortex homogenate was prepared immediately (without freeze-clamping) from control (non-perfused) kidneys, or from kidneys perfused with 0.1–0.2 mM or 1.0 mM branched-chain amino acids for 90 min.

Mitochondria were prepared from kidney cortex by the method of Johnson & Lardy (1967). The homogenization medium contained 210 mM-mannitol, 60 mM-sucrose, 10 mM-KCl, 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], 0.25 mM-dithiothreitol, 0.1 mM-EGTA and 0.5% bovine serum albumin, adjusted to pH 7.4 at 4°C. In experiments designed to determine the degree of activation of the 2-oxo acid dehydrogenase from mitochondria, 25 mM-NaF was included in both the homogenization and assay media to inhibit phosphatase activity. Homogenates were assayed for branched-chain 2-oxo acid dehydrogenase activity (Wohlhueter & Harper, 1970) by using 300 µl of a medium containing 154 mM-mannitol, 49 mM-sucrose, 79 mM-Hepes, 25 mM-KH₂PO₄, 2 mM-MgCl₂, 0.3 mM-EGTA, 8 mM-Na₂CO₃ and 0.83 mM-3-methyl-2-oxo[1-¹⁴C]butanoate, adjusted to pH 7.4 at 37°C. 2-Oxo acid dehydrogenase activity was measured in isolated mitochondria as described above, except that Triton X-100, NAD⁺ and CoA were added at final concentrations of 0.5% (v/v), 1.9 mM and 1.0 mM respectively in the assay medium. The reaction was initiated by addition of 100 µl of homogenate or mitochondria and allowed to proceed for 5 min at 37°C. Samples were run in triplicate.

The ureter of perfused kidneys was cannulated only in the initial experiments so urine could be collected to determine creatinine (0.13–0.15 ml/min per g) and inulin (0.68–1.0 ml/min per g) clearance and Na⁺ reabsorption (80–90%).

Results

Release of branched-chain 2-oxo acids

Branched-chain 2-oxo acids formed in the initial transamination reaction can be either oxidized or released from tissues (Harper *et al.*, 1984). As a portion of the 2-oxo acids formed is released into the medium during perfusion of the isolated eviscerated rat hindquarter (Hutson *et al.*, 1978, 1980), the possibility that this might also occur during perfusion of the kidney was examined. With all concentrations of branched-chain amino acids tested (0.1–0.2, 0.5 and 1.0 mM), branched-chain 2-oxo acids were released into the perfusate. The rate of release of 3-methyl-2-oxobutanoate, 3-methyl-2-oxopentanoate and 4-methyl-2-oxopentanoate increased with increasing perfusate branched-chain amino acid concentrations up to 1.0 mM (Fig. 1). The rate of release was highest for 4-methyl-2-oxopentanoate, intermediate for 3-methyl-2-oxopentanoate, and lowest for 3-methyl-2-oxobutanoate; the rates were proportional to the K_m values of branched-chain amino acid aminotransferase for the respective amino acids (Ichihara *et al.*, 1975b). The rates of release of branched-chain 2-oxo acids per g of tissue were several-fold higher than those observed with the perfused-hindquarter preparation (Hutson *et al.*, 1978, 1980), reflecting, presumably, the particularly high branched-chain amino acid aminotransferase activity of kidney. The rates of release of branched-chain 2-oxo acids were constant throughout the 90 min of perfusion with 0.5 mM and 1.0 mM branched-chain amino acids, whereas with 0.1–0.2 mM branched-chain amino acids the rates tended to decrease slightly after 45 min.

Oxidation of [1-¹⁴C]valine

As an indicator of the rate of branched-chain amino acid oxidation, the rate of decarboxylation

of valine was determined by measuring the amount of ¹⁴CO₂ released from L-[1-¹⁴C]valine throughout the perfusion. Rates of oxidation with the various concentrations of valine tested are shown in Table 1. The rate of oxidation of L-[1-¹⁴C]valine increased disproportionately as perfusate valine

Fig. 1. Effect of substrate concentration on the rates of release of branched-chain 2-oxo acids by isolated perfused rat kidney

Equimolar concentrations of branched-chain amino acids were added to the perfusate as shown above. Kidneys were perfused for 90 min as described in the Experimental section. The rates of release of 4-methyl-2-oxopentanoate (■), 3-methyl-2-oxopentanoate (▲) and 3-methyl-2-oxobutanoate (●) were determined by measuring the increases in 2-oxo acid concentrations in perfusate samples, taken every 30 min as described in the Experimental section. The values are shown as means $\pm$ S.E.M. for 9–18 separate observations.

Table 1. Effect of increasing L-valine concentration on rates of oxidation and transamination of L-[1-¹⁴C]valine by isolated perfused kidney

L-Valine, L-leucine and L-isoleucine were included in the perfusate in equimolar concentrations. L-[1-¹⁴C]Valine was added to the reservoir after a 3 min washout period and ¹⁴CO₂ was collected throughout the 90 min kidney perfusions as described in the Experimental section. Perfusate L-[1-¹⁴C]valine specific radioactivity was used to calculate the rate of oxidation from 20 until 90 min of perfusion. The rate of transamination of L-[1-¹⁴C]valine was estimated as the sum of L-[1-¹⁴C]valine oxidized and 3-methyl-2-oxo[1-¹⁴C]butanoate released; tissue concentration of 3-methyl-2-oxobutanoate was <8 nmol/g, so the tissue pool should not have affected this estimation. The rates of oxidation are expressed as means $\pm$ S.E.M. for six to nine separate observations.

Perfusate L-valine concn. (mM)	Rate of L-[1- ¹⁴ C]valine oxidation (nmol/min per g wet wt.)	Rate of L-[1- ¹⁴ C]valine transamination (nmol/min per g wet wt.)	Percentage of L-valine transaminated going to oxidation
0.2	1.6 $\pm$ 0.2	7.0	23
0.5	7.5 $\pm$ 1.1	16.4	45
1.0	27.8 $\pm$ 4.5	40	70

Table 2. Effect of increasing 3-methyl-2-oxobutanoate concentration on rates of oxidation and utilization of 3-methyl-2-oxo[1-¹⁴C]butanoate by isolated perfused kidney

Perfusate concentrations of L-valine, L-isoleucine and L-leucine in these perfusions were 0.20, 0.1 and 0.15 mM respectively. All other additions were as described in the Experimental section. 3-Methyl-2-oxo[1-¹⁴C]butanoate specific radioactivity was used to calculate the rate of oxidation from 20 until 90 min of perfusion. The perfusate concentration of 3-methyl-2-oxobutanoate was measured every 30 min and the disappearance of the 2-oxo acid with time was estimated as the utilization rate. The tissue pool of 3-methyl-2-oxobutanoate contributed less than 3% of the total utilization. The rates of oxidation and utilization are expressed as means $\pm$ S.E.M. for three separate observations.

Perfusate 3-methyl-2-oxobutanoate concn. (mM)	Rate of 3-methyl-2-oxo[1- ¹⁴ C]butanoate oxidation (nmol/min per g wet wt.)	Rate of 3-methyl-2-oxo[1- ¹⁴ C]butanoate utilization (nmol/min per g wet wt.)	Percentage of 3-methyl-2-oxobutanoate going to oxidation
0.05	12.4 $\pm$ 1.2	34.5 $\pm$ 1.7	36
0.20	68.6 $\pm$ 4.4	82.1 $\pm$ 6.9	84
1.0	275 $\pm$ 60	337.6 $\pm$ 16.3	82

concentration was increased, e.g. the oxidation rate increased 20-fold with a 5-fold increase in substrate concentration from 0.2 to 1.0 mM. The oxidation rate with each concentration of valine was constant after 20 min of perfusion.

With all valine concentrations tested, the specific radioactivity of perfusate L-[1-¹⁴C]valine reached steady state after 20 min and remained constant throughout the perfusion. The specific-radioactivity values for tissue L-[1-¹⁴C]valine and perfusate 3-methyl-2-oxo[1-¹⁴C]butanoate were always slightly less than that of perfusate L-[1-¹⁴C]valine (approx. 80–90%), most probably because of dilution of the intracellular pool with valine released through tissue proteolysis. These specific-radioactivity values nevertheless remained constant from 20 until 90 min of perfusion. The specific-radioactivity values for tissue 3-methyl-2-oxo[1-¹⁴C]butanoate were unreliable because 3-methyl-2-oxobutanoate concentrations were extremely low in the perfused tissue, typically less than 2 nmol/g of kidney. Perfusate L-[1-¹⁴C]valine specific-radioactivity values after 20 min were used in calculations to provide a conservative but reliable estimate of rates of valine oxidation for the period 20–90 min of perfusion.

The values for branched-chain 2-oxo acid and valine oxidation can be used to estimate the rate of valine transamination (rates of 3-methyl-2-oxobutanoate released + oxidized) by perfused kidney. The rate of valine transamination increased linearly with increasing branched-chain amino acid concentrations in the perfusate (Table 1). Not only did the rates of oxidation and transamination of valine increase with increasing perfusate valine concentration, but the percentage of the transaminated valine that was oxidized also increased, as shown in Table 1. Only when perfusate valine concentration was greater than 0.5 mM did the proportion of 3-methyl-2-oxobutanoate oxidized

equal or exceed that released into the medium by the perfused kidney.

Metabolism of 3-methyl-2-oxo[1-¹⁴C]butanoate

In order to investigate more adequately the capacity of the kidney for oxidation of 3-methyl-2-oxobutanoate, perfusions were done with 0.05 mM-, 0.2 mM- and 1.0 mM-3-methyl-2-oxo[1-¹⁴C]butanoate, and ¹⁴CO₂ released was trapped and measured. The decrease in perfusate 3-methyl-2-oxobutanoate concentration during the 90 min perfusions was determined to provide a measure of total 3-methyl-2-oxobutanoate utilization by kidney. The rates of oxidation and utilization of 3-methyl-2-oxobutanoate increased linearly in response to increasing perfusate 2-oxo acid concentration (Table 2). With 0.05 mM-3-methyl-2-oxobutanoate, oxidation accounted for 36% of the 3-methyl-2-oxobutanoate utilized, whereas with concentrations of 3-methyl-2-oxobutanoate of 0.2 and 1.0 mM, the percentage of 2-oxo acid being oxidized (80–85%) remained constant. The rate of 3-methyl-2-oxobutanoate oxidation observed when the kidney was perfused with 1.0 mM-3-methyl-2-oxo[1-¹⁴C]butanoate was 275 $\pm$ 60 nmol/min per g, similar to values for kidney 2-oxo acid dehydrogenase activity reported by Gillim *et al.* (1983) and Wagenmakers *et al.* (1984).

With all 3-methyl-2-oxobutanoate concentrations used, the specific radioactivity of perfusate 3-methyl-2-oxo[1-¹⁴C]butanoate reached steady state within 20 min and remained constant thereafter. Tissue 3-methyl-2-oxo[1-¹⁴C]butanoate specific radioactivity was not significantly different from that of the perfusate 2-oxo acid and remained constant throughout the perfusion. Perfusate 3-methyl-2-oxo[1-¹⁴C]butanoate specific-radioactivity values after 20 min were used in calculations of rates of 2-oxo acid oxidation for the period 20–90 min of perfusion.

Table 3. *Effect of increasing 3-methyl-2-oxobutanoate concentration on the perfusate concentrations of L-isoleucine, L-leucine and L-valine and on rates of release of their respective 2-oxo acids*

Perfusate composition was identical with that described in the legend to Table 2 and the Experimental section. Perfusate samples were taken every 30min and 2-oxo acid concentrations measured. Perfusate branched-chain amino acids were measured after 90min of perfusion. The values are expressed as means $\pm$ S.E.M. for three separate observations. * $P < 0.005$ and ** $P < 0.001$ for significance of differences from values obtained with no 2-oxo acid added.

Initial perfusate 3-methyl-2-oxobutanoate concn. (mM)	Rates of release (nmol/min per g wet wt.)		Perfusate concn. (μ M)		
	3-Methyl-2-oxopentanoate	4-Methyl-2-oxopentanoate	L-Isoleucine	L-Leucine	L-Valine
0	3.5 $\pm$ 0.5	8.1 $\pm$ 0.9	95.3 $\pm$ 0.9	144 $\pm$ 2	189 $\pm$ 31
0.05	9.2 $\pm$ 0.6**	15.6 $\pm$ 1.0**	98.0 $\pm$ 7.5	151 $\pm$ 8	162 $\pm$ 12
0.20	12.2 $\pm$ 2.1**	27.2 $\pm$ 3.6**	84.0 $\pm$ 4.2*	135 $\pm$ 7	253 $\pm$ 53*
1.0	24.4 $\pm$ 0.4**	51.8 $\pm$ 1.5**	59.3 $\pm$ 4.3**	91.7 $\pm$ 8.2**	258 $\pm$ 41*

A surprising effect of increasing perfusate 3-methyl-2-oxobutanoate concentration on release of 3-methyl-2-oxopentanoate and 4-methyl-2-oxopentanoate was seen in these experiments. These release rates increased dramatically when 3-methyl-2-oxobutanoate concentration was increased (Table 3), even though the initial isoleucine and leucine concentrations were constant (0.1–0.15 mM) in all of the experiments. The rates of release of 3-methyl-2-oxopentanoate and 4-methyl-2-oxopentanoate when perfusate 3-methyl-2-oxobutanoate concentration was 1.0 mM were 24.4 ± 0.4 and 51.8 ± 1.5 nmol/min per g of kidney respectively (Table 3), whereas their release rates when perfusate isoleucine and leucine concentrations were 1.0 mM each, in the absence of added 3-methyl-2-oxobutanoate, were only 19.5 ± 1.4 and 29.5 ± 2.3 nmol/min per g of kidney (Fig. 1). This suggests that the supply of amino acceptor was limiting the flux of branched-chain amino acids through the aminotransferase and that provision of 3-methyl-2-oxobutanoate relieved this limitation. The rates of release of branched-chain 2-oxo acids with 1.0 mM 3-methyl-2-oxobutanoate in the perfusate remained high throughout the perfusion and, after 90 min, perfusate concentrations of 3-methyl-2-oxopentanoate and 4-methyl-2-oxopentanoate were 16.9 ± 0.2 and 35.9 ± 0.8 μ M respectively. These increases in perfusate 3-methyl-2-oxopentanoate and 4-methyl-2-oxopentanoate concentrations were accompanied by significant decreases in perfusate isoleucine and leucine concentrations, and a concomitant rise in perfusate valine concentration, as shown in Table 3. Conversion of 3-methyl-2-oxobutanoate into valine by the perfused kidney appeared to occur at the expense of perfusate isoleucine and leucine.

Tissue concentrations of branched-chain 2-oxo acids in kidneys from rats fed *ad libitum* or meal-

fed with an 18%-casein diet were estimated to be less than 2 nmol/g of kidney, in agreement with results reported by Livesey & Lund (1980). The tissue concentrations of branched-chain 2-oxo acids from perfused kidneys were also low; only with perfusate branched-chain amino acid concentrations of 1.0 mM could reliable values for branched-chain 2-oxo acid concentrations be obtained; 3-methyl-2-oxobutanoate, 3-methyl-2-oxopentanoate and 4-methyl-2-oxopentanoate concentrations were 7.3 ± 1.0 , 9.9 ± 0.6 and 9.9 ± 0.3 nmol/g of kidney respectively. These tissue 2-oxo acid concentrations were reached by 20 min and remained constant throughout the remainder of the perfusion.

Branched-chain 2-oxo acid dehydrogenase activity was determined in homogenates prepared from non-perfused and perfused kidneys and in mitochondria from non-perfused kidneys. The use of 25 mM-NaF to inhibit activation of the dehydrogenase by phosphatase had no effect on decarboxylation of 3-methyl-2-oxo[1- 14 C]butanoate by homogenates or solubilized mitochondria, 6.0 ± 1.5 nmol/min per mg of mitochondrial protein with NaF and 6.1 ± 0.5 without NaF. Branched-chain 2-oxo acid dehydrogenase activity from homogenates of non-perfused kidneys and those perfused for 90 min with either 0.1–0.2 mM or 1.0 mM branched-chain amino acids were also not different: 1.2 ± 0.1 , 1.1 ± 0.1 or 1.1 ± 0.1 nmol/min per mg of homogenate protein respectively. Although 2-oxo acid dehydrogenase activities determined in the presence and absence of NaF were not different, indicating, as reported previously (Odessey, 1980; Lau *et al.*, 1981; Gillim *et al.*, 1983), that the dehydrogenase of kidney is nearly fully activated *in vivo*, appropriate conditions for determining the actual state of activation of the dehydrogenase in kidney may not be adequately

Table 4. Comparison of rates of oxidation of L-[1-¹⁴C]valine and 3-methyl-2-oxo[1-¹⁴C]butanoate in relation to perfusate concentrations of 3-methyl-2-oxobutanoate

When L-valine was the radioactive tracer, L-leucine and L-isoleucine were included in the perfusate at concentrations equimolar with that of valine. When the 2-oxo acid was the radioactive tracer, the concentrations of the branched-chain amino acids were 0.1–0.2 mM each in all experiments. Specific radioactivity equilibration is discussed in the Results and Discussion sections. Perfusate 3-methyl-2-oxobutanoate concentrations were measured after 90 min of perfusion as described in the Experimental section.

Radioactive tracer and initial substrate concn. (mM)	Perfusate 3-methyl-2-oxobutanoate concn. after 90 min (μM)	Rate of oxidation (nmol/min per g wet wt.)
L-[1- ¹⁴ C]Valine	0.2	3.7
	0.5	5.4
	1.0	8.4
3-Methyl-2-oxo[1- ¹⁴ C]butanoate	0.05	28
	0.2	147
	1.0	783

defined, as Wagenmakers *et al.* (1984) have reported the enzyme in kidney to be only 50% activated.

Discussion

Despite the high activities in kidney of both branched-chain amino acid aminotransferase and 2-oxo acid dehydrogenase and the appropriate balance between their activities for efficient degradation of these amino acids (Wohlhueter & Harper, 1970; Shinnick & Harper, 1976), a substantial proportion of the 2-oxo acids formed by the isolated perfused rat kidney was released into the perfusate with all concentrations of branched-chain amino acids tested, and the rate of release increased with increasing amino acid concentrations. The 2-oxo acids present in the cytoplasm could be transported rapidly from the cell as the result of 2-oxo acid binding to albumin in the perfusate (Livesey & Lund, 1982; Nissen *et al.*, 1982). Perfusate concentrations of 2-oxo acids at the end of the 90 min perfusion with 0.5 mM or 1.0 mM branched-chain amino acids were similar to concentrations found in rat blood plasma, 0.005–0.015 mM each. *In vivo*, any branched-chain 2-oxo acids released by kidney could either circulate to the liver, where they are readily taken up and oxidized (Ichihara *et al.*, 1973; Livesey & Lund, 1980), or be reaminated by various tissues, which would conserve the indispensable branched-chain amino acid carbon skeletons (Matthews *et al.*, 1981; Nissen & Haymond, 1981).

The rate of transamination of valine (2-oxo acid released + oxidized) increased linearly with increasing perfusate concentrations of the branched-chain amino acids. However, partitioning of the valine transaminated between oxidation and 3-methyl-2-oxobutanoate accumulation changed quite dramatically as the rate of transamination

increased. With 0.2 mM-valine, only 23% of the 3-methyl-2-oxobutanoate formed was oxidized, whereas with 1.0 mM-valine, 70% was oxidized (Table 1). As a result, the rate of L-[1-¹⁴C]valine oxidation increased proportionately more in response to increasing perfusate valine concentration than would be predicted. This would be expected if branched-chain 2-oxo acid dehydrogenase were activated through the phosphorylation/dephosphorylation mechanism, but would be unlikely if the dehydrogenase is already highly activated, as observed by Gillim *et al.* (1983) and Lau *et al.* (1981). It could occur, however, if the degree of activation is only 50%, as reported by Wagenmakers *et al.* (1984). Another possible explanation is that the higher concentrations of the other two branched-chain amino acids in these experiments may have provided higher quantities of 2-oxo acids to serve as amino acceptors for valine transamination (Dawson & Hird, 1968; Table 3).

Perfusate concentrations of 0.2–1.0 mM-L-[1-¹⁴C]valine, as used in the present experiments, should be representative of valine concentrations likely to be encountered under physiological conditions (Hutson & Harper, 1981; Dixon & Harper, 1984). On the other hand, perfusate concentrations of 3-methyl-2-oxo[1-¹⁴C]butanoate of 0.05 mM, 0.20 mM and 1.0 mM are all significantly above that of 0.01 mM observed under normal physiological conditions (Hutson & Harper, 1981). It was surprising, in view of this, that the rate of oxidation with 0.05 mM-3-methyl-2-oxo[1-¹⁴C]butanoate was only slightly higher than that observed with 0.5 mM-L-[1-¹⁴C]valine and less than half that with 1.0 mM-L-[1-¹⁴C]valine, neither of which result in perfusate 2-oxo acid concentrations approaching 0.05 mM. As the specific radioactivities of 3-methyl-2-oxo[1-¹⁴C]butanoate and L-[1-¹⁴C]valine were near steady-state when L-[1-¹⁴C]valine was

included in the perfusate, and as specific radioactivities remained constant from 20 to 90 min, rates of oxidation of L-[1- 14 C]valine and 3-methyl-2-oxo[1- 14 C]butanoate (Tables 1 and 2) were compared in relation to perfusate 3-methyl-2-oxobutanoate concentrations after 90 min (Table 4). The relationships between perfusate 3-methyl-2-oxobutanoate concentration and the rates of oxidation of the two radioactive substrates were distinctly different; with 1.0 mM L-[1- 14 C]valine, perfusate 3-methyl-2-oxobutanoate concentration reached only 8.4 μ M, but the rate of oxidation of valine was double that observed for 3-methyl-2-oxo[1- 14 C]butanoate when perfusate 3-methyl-2-oxobutanoate concentration was 28 μ M. As a high proportion of branched-chain amino acid aminotransferase in kidney is mitochondrial (Kadowaki & Knox, 1982), it is possible that the 3-methyl-2-oxobutanoate arising from transamination in the mitochondrion has more ready access to 2-oxo acid dehydrogenase than does perfusate or cytosolic 3-methyl-2-oxobutanoate.

The observation that rates of release of 3-methyl-2-oxopentanoate and 4-methyl-2-oxopentanoate, the 2-oxo acids of isoleucine and leucine, by the kidney increased when the concentration of 3-methyl-2-oxobutanoate in the perfusate was increased, suggests that isoleucine and leucine were serving as amino donors for 3-methyl-2-oxobutanoate. If this is correct, then the estimated rate of utilization of 3-methyl-2-oxobutanoate, based on the sum of the rates of 3-methyl-2-oxo[1- 14 C]butanoate oxidation (Table 2) and release of 3-methyl-2-oxopentanoate and 4-methyl-2-oxopentanoate (Table 3), should approximately equal the overall rate of 3-methyl-2-oxobutanoate utilization. The estimated rates of utilization with perfusate 2-oxo acid concentrations of 0.05 mM, 0.2 mM and 1.0 mM, calculated in this way, are 108, 132 and 104%, respectively, of those measured directly (Table 2). The similarity of these two sets of values and the decreases in perfusate isoleucine and leucine concentrations (Table 3) support the conclusion that isoleucine and leucine donate their amino groups to 3-methyl-2-oxobutanoate in the perfused kidney. This appears true even though the tissue concentration of glutamate, the presumed major amino donor in the branched-chain amino acid aminotransferase reaction (Taylor & Jenkins, 1966; Krebs & Lund, 1977), is high ($4.6 \pm 0.7 \mu$ mol/g of kidney). Other investigators have observed that, *in vivo*, amino groups exchange readily between one branched-chain amino acid and another branched-chain 2-oxo acid (Pozefsky & Walser, 1977; Ben-Galim *et al.*, 1980; Nissen & Haymond, 1981; Matthews *et al.*, 1981; Eriksson *et al.*, 1982).

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accounted for 36%, 84% and 82% of the total 3-methyl-2-oxobutanoate utilized when concentrations of 3-methyl-2-oxobutanoate in the perfusate were respectively 0.05, 0.2 and 1.0 mM (Table 2). If the 3-methyl-2-oxobutanoate that was not accounted for by oxidation in the present study was aminated to valine (Mitch & Chan, 1978; Brand, 1981; Table 3), then the degree of partitioning between amination and oxidation should determine the efficiency with which 3-methyl-2-oxobutanoate can be used as a substitute for valine. The high proportion of this 2-oxo acid oxidized when circulating concentrations are high could explain the low efficiency of utilization of 2-oxo acids as substitutes for indispensable amino acids by mammals when the quantity included in the diet is high (Harper, 1978). Chawla & Rudman (1974) observed that efficiency of utilization of 3-methyl-2-oxobutanoate as a precursor of valine in rats ranged from 80% when its intake was low to 37% when intake was high, values similar to those that would be predicted from the kidney perfusion studies assuming that metabolism of 3-methyl-2-oxobutanoate in kidney is representative of that in other tissues.

The observations that significant amounts of 2-oxo acids are released by kidney, that 3-methyl-2-oxobutanoate arising through transamination of valine is oxidized more rapidly than 3-methyl-2-oxobutanoate included directly in the perfusate, and that provision of this 2-oxo acid increased the release of the other branched-chain 2-oxo acids by kidney, together with observations by others that the extent of reamination of branched-chain 2-oxo acids *in vivo* can be great (Nissen & Haymond, 1981; Matthews *et al.*, 1981), suggest that metabolism of valine by the perfused kidney is not regulated exclusively at the level of 2-oxo acid decarboxylation. The rate of metabolism of branched-chain amino acids in the kidney thus appears to depend, not only on the degree of activation of branched-chain 2-oxo acid dehydrogenase, but also on the flux through the aminotransferase, which supplies 2-oxo acids for oxidation, and probably also on rates of release and reamination of the 2-oxo acids formed.

This work was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and by a grant from the U.S. Public Health Service, National Institutes of Health (AM 10748).

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