Metabolism of valine in rat skeletal muscle mitochondria

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Metabolism of physiological concentrations of valine was studied in mitochondria from mixed skeletal muscle and isolated soleus muscle to extend knowledge of the mechanism by which the leucine-induced branched-chain amino acid antagonism depresses valine and isoleucine concentrations in plasma and tissues.

Measurements were made of flux of valine through muscle branched-chain aminotransferase and branched-chain keto acid dehydrogenase (BCKD) and reamination of α-ketoisocaproate (KIC).

Concentrations of KIC from 0.05–1.0 mM stimulated transamination of valine. Low concentrations of KIC stimulated valine oxidation in both preparations. However, concentrations above 0.5 mM diminished the rates of CO_2 evolution from valine.

Inclusion in the assay medium of graded levels of leucine (as a potential source of KIC) depressed valine oxidation through competitive inhibition with valine for transamination in muscle mitochondria. Increasing the BCKD capacity of in vitro preparations by including liver and muscle mitochondria together did not alleviate this inhibition.

In contrast, valine oxidation by soleus muscle was stimulated by both leucine and KIC. Addition of [1-^{14}C] KIC in the medium resulted in stimulation of KIC oxidation in both intact and detergent-treated mitochondria. Addition of 0.2 mM valine increased the reamination of KIC to leucine. This can explain in part the high plasma and tissue leucine values, and the decrease in valine, and probably isoleucine, concentrations in animals fed with high leucine diets. (J. Nutr. Biochem. 4:681–689, 1993.)

Keywords: valine; leucine; antagonism; α-ketoisocaproate; branched-chain aminotransferase; muscle mitochondria

Introduction

A high dietary level of leucine suppresses growth of rats fed a low protein diet. This effect, which is largely overcome when supplements of both isoleucine and valine are provided, is attributed to a leucine:isoleucine, valine antagonism.[1] Plasma[2–4] and muscle[5] concentrations of leucine and α-ketoisocaproate (KIC) increase in animals consuming a high leucine diet, whereas pools of isoleucine, valine, α-keto-β-methylvalerate (KMV), and α-ketoisovalerate (KIV) are depleted.[2,4] Observations in vivo,[2] in vitro,[6] in isolated muscle,[7] and in adipose tissue[8] support the conclusion that a high leucine intake stimulates valine and isoleucine oxidation, producing depletion of isoleucine and valine pools in rats[1] and humans.[9]

The branched-chain amino acids (BCAA) leucine, valine, and isoleucine are the only essential amino acids that are degraded in significant amounts by extrahepatic tissues, including skeletal muscle. The first step in the catabolic pathway of BCAA is transamination catalyzed by branched-chain aminotransferase (BCAT). BCAT is widely distributed among tissues, with high activity in the heart and kidney and lowest activity in the liver.[1,10,11] Based on tissue mass, muscle probably has the highest total activity. The second step is an irreversible oxidative decarboxylation of the transamination products, the branched-chain α-keto acids (BCKA), catalyzed by the branched-chain-α-ketoacid dehydrogenase enzyme complex (BCKD). Activity of this enzyme is regulated by a phosphorylation-dephosphorylation mechanism. The keto acid of leucine, α-ketoisocaproate, inhibits

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the kinase and, thereby, reduces phosphorylation and inactivation of BCKD.[12] In studies with kidney mitochondria, addition of KIC to the incubation medium stimulated valine oxidation.[13]

The purpose of the present study was to extend knowledge of the mechanism by which the leucine-induced branched-chain amino acid antagonism depresses plasma and tissue valine and isoleucine concentrations, and examine how skeletal muscle, which contains the largest proportion of BCAT, contributes to overall BCAA metabolism. The BCAA degrading capabilities of disrupted and intact muscle mitochondria and intact muscle in vitro were investigated. Although regulation of BCKD has been studied extensively, little is known about the quantitative importance of the mitochondrial branched-chain aminotransferase of muscle in the catabolism of BCAA. Relationships among physiological concentrations of different substrates were examined.

Methods and materials

Preparation of mitochondria

Skeletal muscle mitochondria were prepared from the hind leg muscle of male Sprague-Dawley rats (150–250 g) by the method described by Hutson[14] with minor modifications. The final pellet was resuspended in assay medium containing 154 mM mannitol; 49 mM sucrose; 79 mM HEPES; 25 mM KH_2PO_4; 2 mM $MgCl_2$; 1 mM EGTA; 8 mM Na_2CO_3; 0.25 mM dithiothreitol; and was adjusted to pH 8.6 at 37° C. The yield was 5–6 mg of mitochondrial protein/g of muscle. Liver mitochondria were isolated as described by Miller.[15] The final mitochondrial pellet was dispersed in assay medium to give a protein concentration of 5–7 mg/mL.

Measurement of amino acid transamination and oxidative decarboxylation in isolated mitochondria

The pH optimum of BCAT isozymes isolated from rat and pig tissues is 8.6,[16] and uptake of branched chain α-ketoacids by rat heart mitochondria is influenced by the pH of the medium.[17] Rates of transamination and oxidative decarboxylation of several concentrations of valine by intact muscle mitochondria were therefore measured at both pH 7.4 and 8.6 in preliminary experiments. V_{max} of BCAT at pH 8.6 was 2.3 fold that at pH 7.4, but the rate of oxidation of valine was not affected. All experiments with isolated mitochondria were therefore carried out at pH 8.6 to ensure maximal BCAT activity.

Valine transamination and oxidation were assayed using 400 μL of assay medium contained in a 1 × 7 cm test tube with a side arm to which a scintillation vial was attached. Muscle mitochondria were added in a volume of 100 μL containing 0.6–0.7 mg mitochondrial protein preincubated at 37° C for 30 min; 25 μL of α-ketoglutarate (αKG) or α-ketoisocaproate solution were added to provide the desired concentration of amino acceptor. To ensure maximal activities, 10 μL of a solution containing 1.9 mM NAD, 10 mM CoA, 0.2 mM thiamine pyrophosphate, and 25 μL of a fresh solution containing 1.6 mM pyridoxal phosphate were added. The reaction was initiated by adding 50 μL of 0.2 mM [1-^{14}C] valine (1500 Bq/nmol).

Treatment of mitochondria with detergent disrupts the membrane and eliminates transport barriers. In preliminary experiments, detergent treatment increased formation of KIV from valine by 4.7 fold without depressing valine oxidation. When detergent was used to examine effects of removing transport barriers, 10 μL of 0.43 mM octylphenol-poly-ethylene oxide (Nonidet P-40) was added to the assay medium.

The reaction was carried out for 10 minutes and stopped with 100 μL of 2 N H_2SO_4. The vials were shaken for 90 min to collect the $^{14}CO_2$ released in 1 ml of ethanolamine: methylcellosolve solution (1:2 (vol/vol)) present in the scintillation vials followed by addition of 10 mL of scintillation fluid. The $^{14}CO_2$ recovered during this collection period represents activity of the branched-chain α-keto acid dehydrogenase (BCKD). The trap was changed and 0.5 mL of 0.2 M $H_4Ce(SO_4)_4$ in 2 N H_2SO_4 was injected into the vial to decarboxylate chemically any α-[1-^{14}C] KIV formed that had not been oxidatively decarboxylated. The concentration of KIV in the medium was calculated from the quantity of $^{14}CO_2$ released during the second collection period. Total transamination was represented by the sum of the $^{14}CO_2$ released and the [1-^{14}C]-branched-chain α-keto acid produced. Background samples in which acid was added before the mitochondria were included with each experiment.

When [1-^{14}C] labeled branched-chain α-keto acids were used as substrate, amino acid formation was calculated from ^{14}C-label remaining in the medium after addition of 0.2 M $H_4Ce(SO_4)_4$, as described by Hutson.[15]

Rates of BCAT and BCKD reactions measured using saturating concentrations of substrates (10 mM [1-^{14}C] valine and 5 mM αKG) were linear up to 10 minutes.

Measurement of valine oxidative decarboxylation and transamination in isolated intact soleus muscle

Transamination and oxidation of valine were measured in 15–20-mg intact soleus muscles removed from 40–50-g male Sprague-Dawley rats. The conditions used were those described by Buse et al.[19] Muscles were incubated in glass flasks equipped with rubber caps and plastic center wells with 3 mL of 0.2 mM valine in Krebs-Henseleit buffer containing 5.5 mM glucose and 0.15% bovine serum albumin plus 0.3 μCi of [1-^{14}C] valine (2.07×10^9 Bq/mmol). Incubations were carried out at 37° C with continuous shaking at 70 cycles/min; flasks were pre-gassed with O_2/CO_2 (95%/5%) and the system was then closed to allow the collection of $^{14}CO_2$. After 90 min of incubation, the reaction was stopped by addition of 0.5 mL of 2.0 mM H_2SO_4. The flasks were shaken for 90 min to trap $^{14}CO_2$. Plastic center wells were replaced and α-keto acids present in the reaction medium were quantified by trapping the $^{14}CO_2$ released from [1-^{14}C] KIV during an additional 90 min of shaking after addition of 0.5 mL of 0.2 M $H_4Ce(SO_4)_4$. Then 10 mL of liquid scintillation cocktail (Biosafe, Mount Prospect, IL) was added and samples were counted with external standard for quench correction. Background samples in which acid was added before the muscle were included with each experiment. Rates of transamination and oxidation were expressed as nmol/min·g tissue.

Preparation of ^{14}C-labeled branched-chain α-ketoacids

The radioactive α-keto acids, α-ketoisocaproic and α-ketoisovaleric acid, were synthesized from their respective amino acids as described by Rudiger[20] with the modifications made by Miller.[13]

Muscle, liver, and kidney mitochondrial protein concentrations were determined according to the method of Lowry[21] with bovine serum albumin as the standard.

Chemicals and reagents

L-[1-^{14}C] valine and L-[1-^{14}C] leucine were purchased from Amersham Corp. (Arlington Heights, IL USA). Nagase was from Enzyme Development Corporation, New York, NY USA. L-amino oxidase, catalase, α-ketoisocaproic acid, and α-ketoisovaleric acid were purchased from Sigma (St. Louis, MO USA). All other reagents were obtained from commercial sources and were reagent grade.

Results

Metabolism of valine by mixed skeletal muscle mitochondria

Effect of α-ketoisocaproate (KIC) as amino-acceptor on valine catabolism. Miller and Harper[13] observed that BCAT from kidney used KIC effectively as an amino-acceptor. Transamination of 0.2 mM [1-^{14}C] valine by detergent-treated and intact skeletal mixed muscle mitochondria increased steadily with increasing concentrations of KIC as amino-acceptor (*Figure 1A*). Maximal rates were obtained with 1.0 mM KIC. BCAT activities of intact and detergent-treated mitochondria were 68- and 165-fold higher, respectively, with than without KIC. The subsequent oxidation of KIV formed during transamination of valine increased 18 fold and 10 fold in disrupted and intact mitochondria, respectively, with increasing KIC concentration up to 0.05 mM (*Figure 1B*). With 0.01 mM KIC in the medium, almost all the KIV formed was oxidized. Concentrations of KIC above 0.1 mM inhibited the oxidation of KIV, and only 1% of the KIV formed was oxidized with concentrations of 0.5 and 1.0 mM KIC. As the Km value of BCKD for KIC (Km = 15 μM) is higher than for KIV (Km = 28 μM),[1] the low oxidation of KIV with high concentrations of KIC was presumably due to competitive inhibition between KIC present in the medium and the KIV formed through transamination of valine for the BCKD. Detergent treatment of mitochondria resulted in about a three-fold increase in valine transamination and approximately a two-fold increase in oxidation of valine compared with rates for intact mitochondria.

To establish whether stimulation of valine oxidation by low concentrations of KIC was specific for this keto acid, KIV was used as amino-acceptor. Transamination was similar with the two keto acids, although the Km of BCAT for KIC is lower (0.05 mM) than for KIV (0.10 mM), indicating that KIC is a better amino-acceptor than KIV.

Reamination of [1-^{14}C] KIC. As valine oxidation was inhibited by concentrations of KIC higher than 0.1 mM, the results raised a question as to whether the excess of KIC that was not oxidized due to competition with KIV for the low BCKD activity in muscle mitochondria would be reaminated. To answer this question, rates of transamination of 0.2 mM valine were measured using graded levels of [1-^{14}C] KIC as amino-acceptor; rates of KIC oxidation and reamination of KIC to leucine were also measured. A small amount of transamination of [1-^{14}C] KIC to leucine occurred with detergent-treated mitochondria without addition of any amino donor (*Figure 1C*). This can be explained by the presence of small amounts of other branched-chain amino acids or glutamic acid in the mitochondria serving as amino donors. Addition of 0.2 mM valine resulted in a four-fold increase in the rate of transamination of 1.0 mM [1-^{14}C] KIC to leucine (*Figure 1C*).

Oxidation of [1-^{14}C] KIC by detergent-treated mitochondria was linear up to 0.05 mM [1-^{14}C] KIC and increased with increasing levels of KIC in the medium. Maximal rates were obtained with 1.0 mM KIC. Addition of 0.2 mM valine to the medium did not significantly affect the rate of oxidation of [1-^{14}C] KIC (*Figure 1D*). From these results it appears that, although low concentrations of KIC stimulate oxidation of valine, when KIC concentration is increased above about 0.1 mM, it is oxidized rapidly and KIV oxidation is depressed (*Figure 1B*). Also, as the concentration of KIC is increased above 0.05 mM, KIC is readily reaminated to form leucine. These results may, in part, explain the high leucine levels and increase the valine oxidation observed in rats fed a low protein diet with a high content of leucine.

Effect of leucine on transamination and oxidation of valine by skeletal muscle mitochondria

Because leucine can serve as a source of KIC, which is considered to be involved in the physiological regulation of BCKD activity, rates of transamination and oxidation of 0.2 mM [1-^{14}C] valine were measured in intact and disrupted mitochondria or a homogenate of skeletal muscle in the presence of graded levels of leucine with 0.1 mM KG as amino acceptor. Transamination and oxidation of valine with or without leucine were negligible in the absence of an amino acceptor.

The conversion of [1-^{14}C] valine to KIV by both mitochondria and homogenates was inhibited progressively as the concentration of leucine in the medium was increased (*Figure 2A*). The Ki value (Ki = 0.036 mM) was calculated from the slope-intercept ratio of the double reciprocal plot 1/Vo-V versus 1/[Leu] (Vo = rate of valine transamination in the absence of leucine and V = rate of valine transamination at each respective level of leucine). Inhibition of this type could be explained by either competition between leucine and valine for BCAT or preferential transamination of leucine, as the Km of BCAT for valine (1.30 mM) is higher than for leucine (0.40 mM). Similar results were obtained when isoleucine was included in the reaction mixture in place of leucine.

Valine oxidation was also inhibited when leucine was included in the reaction mixture (*Figure 2B*). A high rate of formation and oxidation of KIC could account for this inhibition of oxidation of KIV. Similar results were obtained with a reaction mixture containing both muscle and liver intact mitochondria, which gave a preparation with high BCAT and BCKD activities (data not shown).

These observations indicate that valine and leucine compete with each other as substrates for BCAT when leucine concentration is 0.1 mM or greater. This was demonstrated clearly when valine transamination was

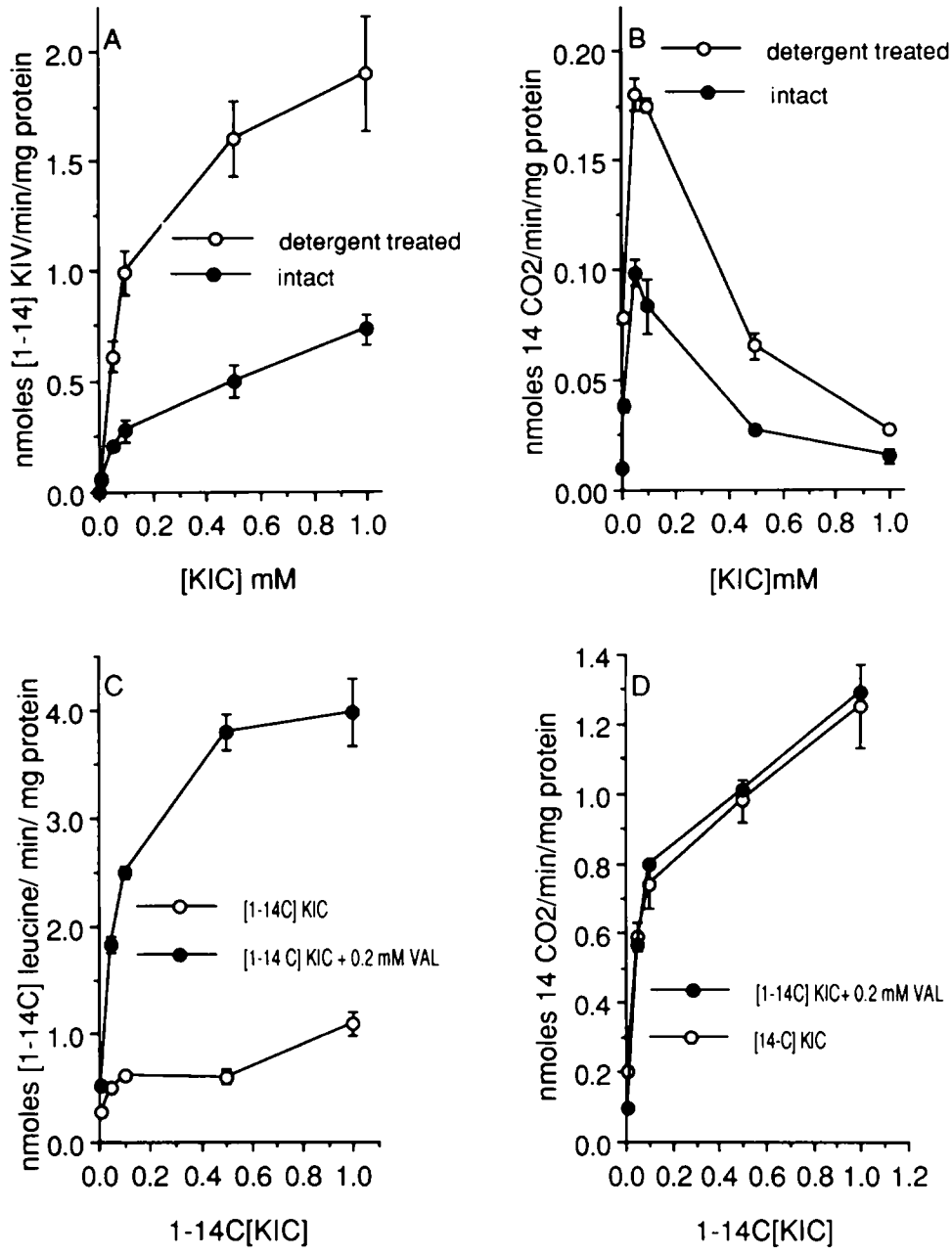

Figure 1 Effect of KIC on the rates of (A) accumulation of [1-^{14}C] KIV in the medium, and (B) oxidation of 0.2 mM [1-^{14}C] valine by intact and detergent-treated muscle mitochondria. Values are mean ± SEM; n = 3. Effect of 0.2 mM valine on the rates of (C) [1-^{14}C] leucine formation, and (D) KIC oxidation by detergent-treated muscle mitochondria in the presence of graded levels of [1-^{14}C] KIC. Values are mean + SEM; n = 6. Incubations were done at 37°C for 30 min in buffer, pH 8.6, supplemented with cofactors. See Methods and Materials.

measured in the presence of a single physiological concentration of leucine resembling that in muscle with graded levels of KG to maintain an adequate supply of amino acceptor (*Figure 3A*). Oxidation of valine was severely depressed by the added leucine (*Figure 3B*), presumably because the KIC formed was competing with KIV for BCKD. Inhibition of valine transamination and oxidation by leucine was also demonstrated in kidney mitochondria (data not shown), indicating that the effect of leucine is not unique for muscle. In contrast to the marked depressions of valine transamination by leucine, physiological concentrations (0.1–0.5 mM) of phenylalanine (which is not a substrate for BCAT but which competes with leucine for transport in most biological systems) had little effect on valine transamination (data not shown). High phenylalanine (1.0 mM) depressed valine transamination about 28% compared with the 70% depression caused by 1.0 mM leucine. Valine oxidation was not depressed even by the high concentration of phenylalanine. Although transport sys-

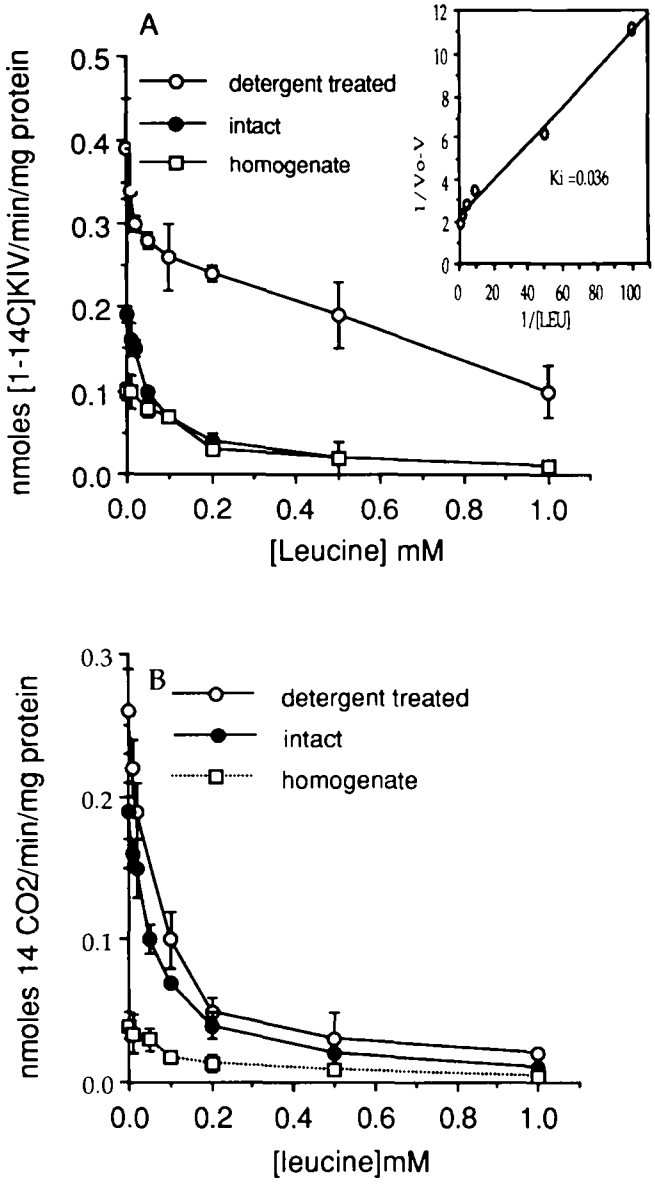

Figure 2 Effect of graded levels of leucine on the rates of (A) accumulation of [1-^{14}C] KIV, and (B) oxidation of 0.2 mM [1-^{14}C] valine by intact and detergent-treated muscle mitochondria in the presence of 0.1 mM KG. Values are mean ± SEM; n = 3.

tems for large neutral amino acids have not been characterized in mitochondria, our observations make it unlikely that inhibition of valine oxidation by low concentrations of leucine is due to depression of valine transport.

Effect of leucine and KIC concentrations on the metabolism of valine by isolated rat intact soleus muscle

The isolated intact rat soleus muscle with all BCAT activity located in the mitochondria,[22] was selected as a model system that would be expected to maintain transport relationships of metabolites across plasma and mitochondrial membranes, intracellular amino-acceptor and amino-donor concentrations, intracellular interactions between cytosolic and mitochondrial enzymes, in a state resembling more closely that existing in vivo than could be obtained with mitochondrial preparations.

For these experiments, intact soleus muscle was incubated for 90 min in buffer containing increasing concentrations of leucine and 0.2 mM [1-^{14}C] valine. KIV formation decreased sharply as leucine concentration was increased, with valine transamination being reduced by 75% when 1.0 mM of leucine was present in the medium (*Figure 4A*). A decline in KIV release was also observed by Zapalowski after inclusion of leucine in hindquarter perfusions.[6] With 0.1 mM leucine in the incubation medium, valine oxidation was 24% (1.24 ± 0.14

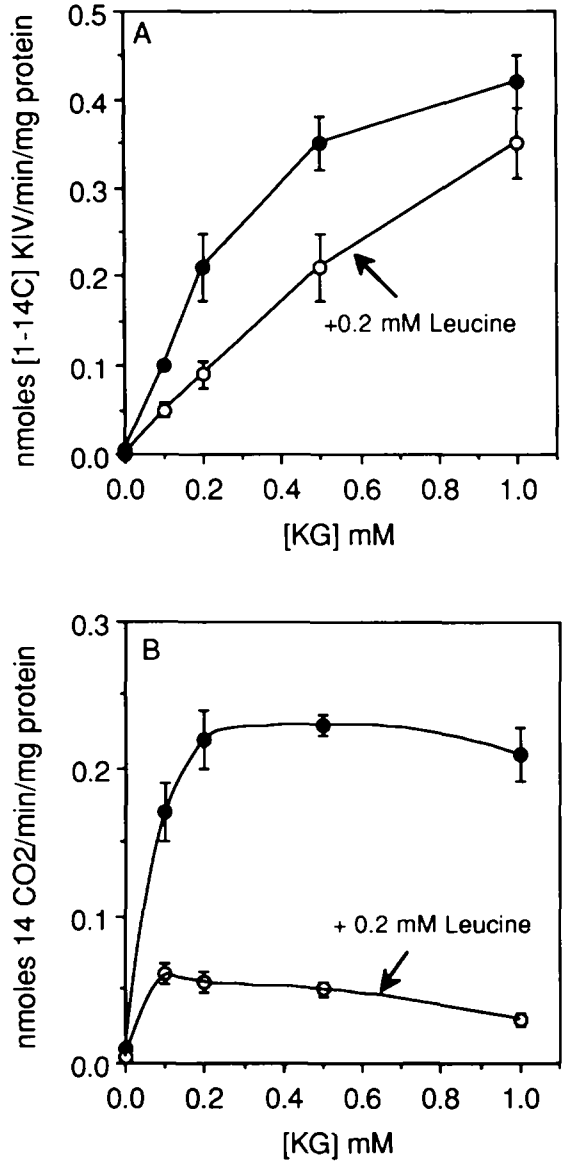

Figure 3 Effect of graded levels of α-ketoglutarate on the rates of (A) accumulation of [1-^{14}C] KIV, and (B) oxidation of 0.2 mM [1-^{14}C] valine by intact muscle mitochondria in the presence of 0.2 mM leucine. Values are mean ± SEM; n = 5. See Fig. 1 for conditions.

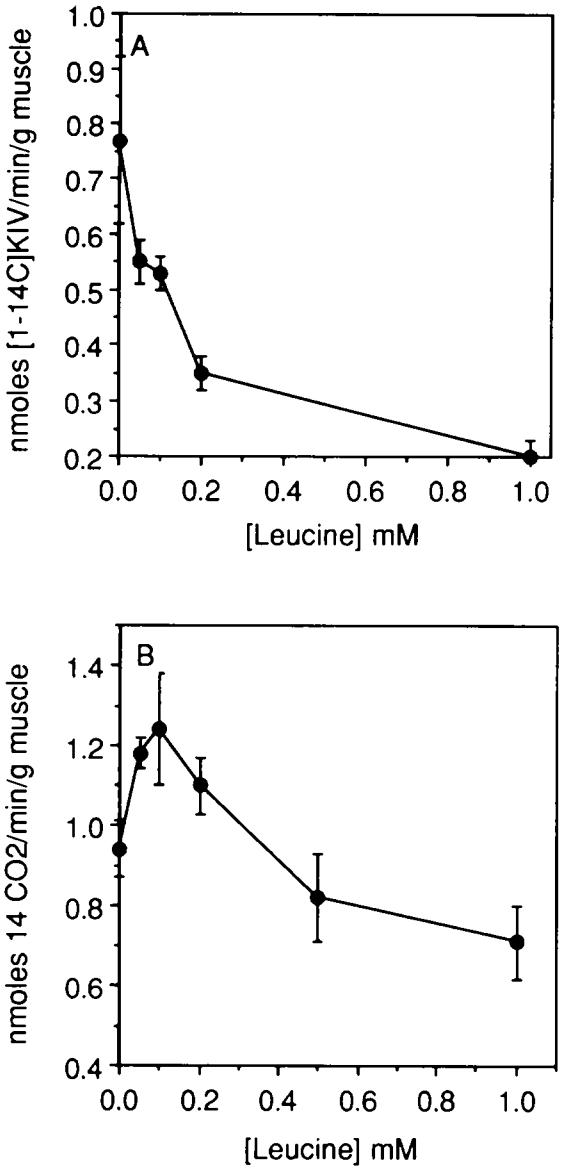

Figure 4 Effect of graded concentrations of leucine on the rates of (A) accumulation of [1-^{14}C] KIV, and (B) oxidation of 0.2 mM [1-^{14}C] valine by intact soleus muscle. Values are means ± SEM; n = 3 - 11. Muscles were incubated at 37°C for 90 min in buffer, pH 7.4. See Methods and materials

nmol · g muscle · min) above values observed without leucine (0.94 ± 0.07 nmol/g muscle · min), but concentrations of leucine above 0.1 mM substantially diminished valine oxidation (*Figure 4B*). The pattern of oxidation of valine was different from that seen in experiments with muscle mitochondria (*Figure 2B*). Oxidation of [1-^{14}C] valine by soleus muscle was stimulated by low concentrations of leucine in the range of 0.05–0.2 mM. However, soleus muscles used in this study were from rats fed a chow diet in which the extracellular and intracellular pools of most amino acids are enlarged. Intact soleus muscle oxidized 0.2 mM [1-^{14}C] valine at a rate of 0.94 ± 0.07 nmol/min · g tissue. This value is close to the range of those reported by Hutson[23] for hindquarter preparations in which 0.1-0.5 mM [1-^{14}C] leucine was oxidized at rates between 0.2 and 7.5 nmol/min · g tissue.

Oxidation and transamination of valine in intact soleus muscle were also measured with KIC, the ketoacid of leucine, as the only amino acceptor added. With increasing concentrations of KIC, the response curve for KIV formation (*Figure 5A*) was similar to that observed with intact muscle mitochondria (*Figure 1A*). Transamination of valine increased steadily with increasing concentrations of KIC between 0.01 and 1.0 mM in the medium, reaching its maximal value with between 0.2 and 0.5 mM.

Interestingly, the effect of KIC on valine oxidation was similar to that seen with muscle mitochondria (*Fig-*

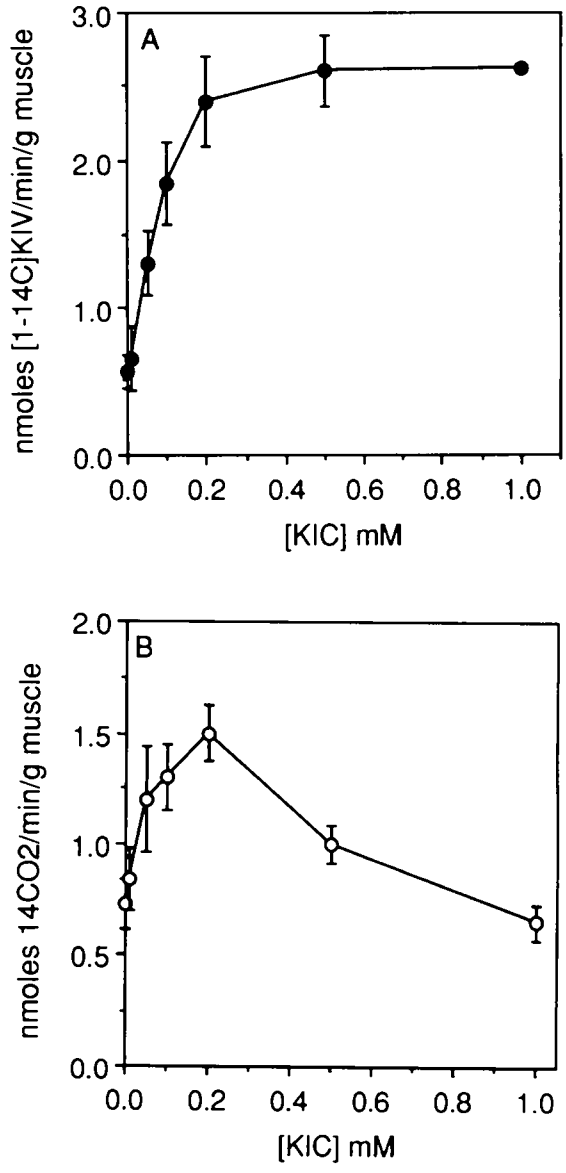

Figure 5 Effect on different concentrations of KIC on the rates of (A) accumulation of [1-^{14}C] KIV, and (B) oxidation of 0.2 mM [1-^{14}C] valine by intact soleus muscle. Values are means ± SEM; n = 3 - 4. Muscles were incubated at 37°C for 90 min in buffer, pH 7.4 See Methods and materials

ure 1B). Valine oxidation increased in the presence of 0.05–0.2 mM KIC in the medium from 0.73 ± 0.11 to 1.5 ± 0.08 nmol/min · g tissue (*Figure 5B*). It reached maximal stimulation with 0.2 mM KIC (1.5 nmol/min · g tissue). Higher concentrations of KIC (1.0 mM KIC) reduced valine oxidation, presumably because of the competition between the KIV formed and the KIC present in the medium for BCKD.

Discussion

Effects of leucine and KIC on valine metabolism in mitochondria

During the search for a metabolic explanation for the BCAA antagonism observed in vivo,[2] excessive intakes of leucine by animals or higher concentrations of leucine in the medium of intact tissue preparations were found to increase the rate of oxidation of valine.[6–8] In the present in vitro study of intact and detergent-treated muscle mitochondria and homogenates (*Figure 2*), concentrations from 0.1 to 1.0 mM leucine did not stimulate, but rather depressed both transamination and oxidation of 0.2 mM valine. The progressive decrease in valine transamination is attributed to competitive inhibition by leucine for BCAT. Similar results were obtained when graded concentrations of isoleucine were included in the medium in place of leucine. Ichihara[24] reported competitive inhibition when purified hog heart BCAT was incubated with radiolabeled leucine and cold valine or isoleucine; the formation of KIC was inhibited progressively with increasing concentrations of the other two amino acids. Inhibition of valine transamination and oxidation by leucine was observed with mitochondria from kidney in which both BCAT and BCKD activities are high and with a mixture of skeletal muscle and liver mitochondria, with high BCAT activity in the former and high BCKD activity in the latter (data not shown). These results indicated that the effect of leucine was not unique for mitochondria from skeletal muscle.

Muscle mitochondrial BCAT can also use BCKA as amino acceptors to form BCAA as in kidney mitochondria.[13] Each of the BCKA may be reaminated to its respective amino acid or be irreversibly decarboxylated (*Figure 1C and D*). The presence of increasing concentrations of KIC (0.05–1.0 mM) in the medium stimulated the rate of transamination of physiological concentrations of valine with both intact and disrupted mitochondria (*Figure 1A*). Low concentrations of KIC (0.01–0.1 mM) in the medium, similar to those found in plasma of rats fed a high leucine diet, stimulated oxidation of 0.2 mM valine by 10 and 18 fold in intact and disrupted mitochondria, respectively, over the rates observed when no amino acceptor was present. KIC may produce this effect by: (1) substituting for KG as co-substrate with valine for BCAT, thus increasing the rate of KIV formation; (2) serving as an amino acceptor in the presence of valine as amino donor so that excess KIC is reaminated to leucine (*Figure 1C*); and (3) by inhibiting BCKD kinase and thereby increasing the amount of active BCKD.[25] All of these could contribute to the depressed tissue valine pool observed as a result of leucine-isoleucine and valine antagonism induced by feeding animals a high leucine diet.[2] Recent observations in rats,[26] have demonstrated that diets containing individual branched-chain keto acids result in activation of muscle BCKD.

One would predict that if KIC were highly effective in stimulating valine oxidation, leucine as a potential source of KIC should produce similar effects. Several observations in vivo,[2] in vitro,[6] in isolated muscle,[7] and in adipose tissue[8] support this explanation for the depletion of isoleucine and valine pools seen in rats[1] and humans[9] after administration of high leucine diets. However, leucine did not stimulate, but rather competitively inhibited, valine oxidation in the present in vitro studies, presumably through competing with valine and isoleucine for transamination. Nonetheless, in detergent-treated muscle mitochondria of rats fed a 9% casein diet containing 5% leucine basal BCKD activity of muscle increased two fold (unpublished results). This is consistent with observations that KIC produced by the transamination of leucine will inhibit BCKD kinase in vitro.[25]

The high reversibility of BCAT in muscle may favor the reamination of BCKA in mitochondria depending on: (1) the rate of transport of BCKA[14,17,27] and BCAA into the mitochondrion; (2) steady state concentrations of the reactants and products of the BCAT reaction that result in either transamination of BCAA or reamination of BCKA; and (3) the activation state of BCKD. The results in *Figure 1C* illustrate the reversibility of the transamination reaction. Because the first step in BCAA catabolism, transamination of BCAA to BCKA, is reversible, the use of [1-^{14}C] valine alone supplies information on the generation of [1-^{14}C] KIV, but provides no insight into the rate of oxidation or reamination of KIC to leucine. Addition of graded levels of [1-^{14}C] KIC to the medium stimulated KIC oxidation in disrupted mitochondria (*Figure 1D*). Addition of 0.2 mM valine did not affect the rate of oxidation of KIC, but it significantly increased reamination of [1-^{14}C] KIC to leucine (*Figure 1C*). These results support the conclusion that inhibition of valine oxidation by concentrations of KIC above 0.1 mM was due to preferential oxidation of KIC.

Mitochondrial BCAT would appear to play a key role in conserving BCAA,[28] and in controlling the concentrations of BCAA and BCKA in plasma by limiting release of BCKA, which can be readily oxidized by liver.[1,29] As much as 80–90% of the BCKA arising from transamination of BCAA in tissues can be reaminated before decarboxylation occurs.[30] Walser[28] has reported that although at least half of the KIC and KIV given orally are oxidized in splanchnic organs, these keto acids also serve effectively as precursors of intracellular amino acids used for protein synthesis, particularly in brain and heart.

Effects of leucine and KIC on valine metabolism in intact soleus muscle

It was thought that studies of the catabolism of BCAA in intact soleus muscle with physiological concentrations

of BCAA should provide information that would more closely bear on the in vivo capacity of this tissue to oxidize BCAA. With intact soleus muscle (with more than 90% of BCAT activity located in mitochondria), concentrations of leucine between 0.1–1.0 mM in the medium decreased the formation of KIV from the transamination of 0.2 mM [1-^{14}C] valine. Similar results, a severe depression in the rate of valine transamination (sum of KIV formed and oxidation) and in the intracellular KIV concentration, were obtained in perfused rat hindquarter preparations.[6] The decrease in transamination of valine in the presence of leucine was unlikely to be due to competition between leucine and valine for transport across the plasma membrane of the soleus muscle. Tovar et al.[31] observed that competition for transport of BCH, a model amino acid for the L system, into soleus muscle occurred only with very high concentrations of the large neutral amino acids. Also, valine transamination by mitochondria was not depressed when physiological concentrations of phenylalanine, which competes with BCAA for transport in a variety of systems, was included in the medium.

Oxidation of valine in intact soleus muscle, unlike in muscle mitochondria, was stimulated by additions of 0.05–0.2 mM leucine. Rates were similar to those observed in studies with perfused hindquarter preparations; however, in hindquarters from rats fed a 9% casein diet the stimulation occurred with 0.8 and 1.0 mM leucine in the perfusate.[6] Soleus muscle used in the present study were from rats fed a chow diet in which both extracellular and intracellular pools of most amino acids are enlarged. Both plasma and intracellular leucine concentrations increased in direct proportion to the protein content of the diet and the activation state of BCKD complex in muscle increases in parallel with increases in plasma leucine concentration.[32] Also, valine decarboxylation by epitrochlearis muscle from rats fed a 14% protein diet increased several fold when 0.15 mM leucine was added to the incubation medium although the net rate of transamination was not stimulated,[33] and infusion of leucine (0.25 nmol/kg) into fed rats increased plasma leucine concentration by about 50% and muscle BCKD activity by four fold.[34] Stimulation of valine oxidation by physiological concentrations of leucine, as seen with intact soleus muscle, has also been observed with adipose tissue from rats fed a chow diet.[8] The stimulation of BCKD activity by leucine is attributed to inhibition of BCKD kinase by KIC derived from leucine that suppressed phosphorylation, thereby preventing inactivation of BCKD.[35,36] Taken together, these observations indicate that an increase in the degree of activation of BCKD as the result of elevated tissue concentrations of leucine can account, at least in part, for leucine stimulation of valine oxidation and depletion of valine and isoleucine pools.

Reamination of KIC to leucine (*Figure 1C*) with valine and isoleucine as aminodonors would also contribute to depletion of the tissue pools of these two amino acids. In addition, leucine may stimulate valine oxidation in the intact muscle by increasing the supply of KIC, which can serve as an amino acceptor for transamination of valine. Under usual nutritional and physiological conditions, BCAT in muscle depends primarily on KG as cosubstrate; in resting muscle, in vivo KG is considered to serve as amino acceptor for approximately 95% of the leucine transaminated.[37] The concentration of KG of 0.1–0.2 mM in resting muscle, however, is the range of the Km of BCAT; hence, an increased supply of amino acceptor should increase the rate of BCAA transamination. Observations that KIC is released into the medium during incubation of intact soleus muscle with leucine and that intracellular concentrations of KIC in the perfused hindquarter increase with increasing perfusate leucine concentration[6] indicate that transamination of leucine can readily provide KIC as an additional source of amino acceptor. Plasma KIC values of rats fed a high leucine diet range from 38–88 μM.[2,4] Concentrations of KIC of 0.05–0.2 mM, comparable to those observed in rats fed a high leucine diet, caused the greatest stimulation of CO_2 evolution from valine in soleus muscle and mitochondria (*Figures 1B and 4B*). Stimulation of valine oxidation by leucine in intact muscle, but not in muscle mitochondria, would seem to require some type of compartmentation of branched-chain keto acids in intact cells, which facilitates channeling of these acids directly to sites of transamination and oxidation.

In summary, leucine stimulation of the catabolism of valine and isoleucine and their respective keto acid provides a satisfactory explanation for depletion of valine and isoleucine pools as a result of the leucine-isoleucine and valine antagonism induced by feeding animals a low protein-high leucine diet,[1] but the mechanism by which this is accomplished is still not clear. It would appear to involve: (1) stimulation of transamination of valine and isoleucine by the KIC formed from excess leucine increasing the supply of amino acceptor; (2) an increase in the degree of activation of the BCKD complex owing to inhibition of BCKD kinase by KIC; and (3) reamination of KIC to leucine to valine, and presumably also isoleucine, serving as amino donors; and, possibly also, (4) to channeling (via compartmentation?) of branched-chain keto acids within the cell into catabolic pathways; and, in the intact organism, interorgan relationships in the metabolism of BCAA with keto acids released from muscle being oxidized by liver.

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