Distribution of Branched-Chain \(\alpha\)-Keto Acid Dehydrogenases in Primate Tissues

BALWANT S. KHATRA, RAJENDER K. CHAWLA, CHARLES W. SEWELL, and DANIEL RUDMAN

From the Departments of Surgery, Medicine, Pathology, and Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322

\textbf{A B S T R A C T} The specific activity of the branched-chain \(\alpha\)-keto acid (BCKA) dehydrogenases was measured in normal tissues of the rat, monkey, and man, and in cirrhotic human liver. In the rat, specific activity of the dehydrogenases in liver, kidney, and muscle averaged 33, 26, and 0.4 \text{U/g wet tissue}, respectively; proportion of the body's content of the enzyme located in these three organs was 70, 12, and 10\%. In the monkey, specific activities in liver and kidney were only one-half to one-third as great as in the rat, whereas activity in muscle was the same; the monkey's body content of dehydrogenase was distributed 50\% in liver, 13\% in kidney, and 20\% in muscle. In man, specific activities in liver and kidney were only 1/15th to 1/25th as great as in the rat, but activity in skeletal muscle was the same. Distribution of the dehydrogenases in man was 30\% in liver, 2\% in kidneys, and 60\% in muscle.

In six patients with alcoholic cirrhosis, specific activity of the dehydrogenase in liver was reduced to 20–50\% of normal (average, 32\%). This reduction may alter the efficiency of BCKA as substitutes for branched-chain amino acids when BCKA are administered orally, but will have little influence on efficiency when they are given intravenously.

\textbf{INTRODUCTION}

Branched-chain \(\alpha\)-keto acids (BCKA; \(\alpha\)-ketoisovaleric acid, KIV; \(\alpha\)-ketoisocaproic acid, KIC; \(\alpha\)-keto-\(\beta\)-methylvaleric acid, KMV)\(^1\) can substitute for the corresponding essential branched-chain amino acids (BCAA) (valine, leucine, and isoleucine, respectively) in the diets of both rat and man (1–4). The efficiency of branched-chain and other keto acids as dietary substitutes for the corresponding essential amino acids is defined as moles of amino acid required to produce a specific nutritional effect (such as specific rate of growth or specific level of \(N\) balance)/moles of corresponding keto acid required to produce the same effect (1). Efficiency of KIV and KIC in the growing rat varies between 30 and 80\% and depends on the growth rate desired (1, 4). At maximal growth rate, efficiency is 25–30\%, so that the minimal daily requirements of KIV and KIC for maximal growth in the immature rat are three to four times greater than those of valine and leucine.

Only two metabolic pathways are available to BCKA: (a) oxidative decarboxylation followed by degradation to \(\text{CO}_2\) and water for energy purposes (5); (b) conversion via transamination into the corresponding BCAA, which can then be incorporated into tissue proteins. In the rat, oxidative decarboxylation is carried out primarily in the liver by a BCKA dehydrogenase complex specific for the three BCKA (6); transamination, however, is largely performed in kidneys and muscle (6, 7). Theoretically, efficiency of each BCKA is proportional to the ratio, velocity of transamination:velocity of oxidative decarboxylation.

The organ distribution of the BCKA dehydrogenases has so far been studied only in the rat (6). Recently, BCKA have been proposed as treatment for patients with chronic liver disease who require low-protein diets because of hyperammonemia and encephalopathy (3, 8, 9). If the dehydrogenase is primarily hepatic in man, as it is in the rat, and if the enzyme activity is adversely affected by chronic liver disease, then the efficiency of BCKA as substitutes for BCAA will be higher in cirrhotic than in normal subjects. It will then follow that the amount of BCKA required to replace the corresponding essential amino acid in the diet of cirrhotic patients will be less than in

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\(^1\) Abbreviations used in this paper: BCAA, branched-chain amino acids; BCKA, branched-chain \(\alpha\)-keto acids; KIC, \(\alpha\)-ketoisocaproic acid; KIV, \(\alpha\)-ketoisovaleric acid; KMV, \(\alpha\)-keto-\(\beta\)-methylvaleric acid.
normal individuals. Also relevant to the treatment of cirrhotic patients with exogenous BCKA is the potential neurotoxicity of these substances, as revealed by the fact that excessive levels of endogenous BCKA, resulting from hereditary absence of the BCKA dehydrogenase, cause neurologic disorders in man ("maple syrup urine disease" [10]).

In view of these considerations, the present study was undertaken to learn the organ distribution of the BCKA dehydrogenases in primate (simian and human) organs, and to measure the specific activity of the enzyme system in normal and cirrhotic human liver.

METHODS

Animal tissues. 250-g male Sprague-Dawley rats were fed rat laboratory chow ad lib. They were sacrificed by decapitation and various organs (Table 1) were removed for study as described below. In one experiment, the organs were removed and processed within 3 min of death. In another experiment, to determine the effect of a postmortem interval on tissue BCKA dehydrogenase activity, rats were killed and kept at 5°C for 0–12 h before sections of liver, kidney, and muscle were removed and processed.

Eight normal male rhesus monkeys, 3–6 kg in weight, were sacrificed by intravenous pentobarbital. Within 15 min, sections of organs were removed and processed as below.

Antemortem human tissue. Liver biopsies were obtained at laparotomy from 12 patients whose clinical data are summarized in Table II. Six patients had alcoholic cirrhosis, and six were free of liver disease. Cirrhotic or normal condition of the liver was confirmed by microscope examination of the surgical biopsy. Criteria for cirrhosis were complete bridging between portal areas by fibrous bands and dissection of the lobule with obliteration of central veins. The six cirrhotic patients had all experienced two or more hemorrhages from esophageal varices and were undergoing shunt surgery to relieve portal hypertension. Preoperative clinical evaluation, done within 2 mo before surgery, gave the results shown in Table II. None of the cirrhotic subjects had experienced encephalopathy. For 1 wk before the operation, patients were given a diet providing each day 2,200–2,500 cal, 80–100 g of protein, 225–270 g of carbohydrate, and 100–120 g of fat. Food was withheld for 12 h before laparotomy.

The six patients with no clinical evidence of hepatic disease, and with normal liver histology, underwent laparotomy because of the conditions shown in Table II. Preoperative diet and handling of liver tissue were the same as for cirrhotic patients.

Histologically normal sections of skeletal muscle (rectus abdominis or pectoralis) were obtained from four individuals during laparotomy or radical mastectomy; these subjects were given the same diet preoperatively as specified above.

Postmortem human tissues (Table I) were obtained from 11 individuals at autopsies performed within 4 h after death from acute illnesses (myocardial infarction, trauma, or cerebrovascular accident), less than 2 days in duration. Each tissue sample for which data are presented appeared normal by gross inspection and under light microscopy.

Processing of tissues. A 0.5–1-g section of tissue was excised and chilled to 0°C. Within the next 5 min, (a) 0.1–0.2 g of the tissue was weighed, homogenized for 1 min in 0.9–1.8 ml ice cold 0.25 M sucrose with a motor-driven

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**Table 1**

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<tr>
<th>Organ</th>
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<td></td>
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<tr>
<td>Spleen</td>
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telfon pestle, and subsequently analyzed for BCKA dehydrogenase activity (see "enzyme assays" in Methods); (b) 100–500 mg was stored at −20°C for subsequent determination of DNA, total N and collagen (see "Determination of DNA, total N, and collagen" in Methods); (c) 10–20 mg of tissue was placed in 10% formalin for histologic examination.

Reagents for enzyme assays. The reagents for enzyme assays include: L-[1-14C]valine, L-[1-14C]leucine, hydroxide of Hyamine, and Omnifluor [blend of 98% PPO and 2% p-bis(O-methylstyril) benzene] (New England Nuclear, Boston, Mass.); L-[1-14C]isoleucine (Calatonic, Los Angeles, Calif.) NAD, coenzyme A, EDTA, DNA, L-hydroxyproline, acetylacetone, sodium thiosulfate, chloramine-T, dimethylamino-benzaldehyde, Tris, catalase (Sigma Chemical Co., St. Louis, Mo.); Dowex 50 WX8 (The Dow Chemical Co., Midland, Mich.); L-amino acid oxidase (Worthington Biochemical Corp., Freehold, N. J.); methyl red, bromocresol green, and β-nitrophenylhydrazine (Eastman Kodak Co., Rochester, N. Y.).

[1-14C]BCKA were prepared as described by Elsas et al. (11) and originally reported by Meister (12) and Rüdiger et al. (13).

Enzyme assays. Activity of BCKA dehydrogenases was measured at 37°C with KIC, KIV, and KMV as substrates, according to Wohlhueter and Harper (6) with slight modifications as described elsewhere.2

Preliminary experiments, described under Results, showed that the reaction medium of Wohlhueter and Harper (6) was optimal for activity of human and monkey branched-chain dehydrogenase as well as for the rat enzyme system, and that for tissue homogenates of all three species the reaction followed zero order kinetics. Fresh liver tissue from a normal 200-g male Sprague-Dawley rat raised on Purina rat chow (Ralston Purina Co., St. Louis, Mo.) ad lib. was analyzed for BCKA dehydrogenase activity simultaneously with each sample of human liver; the specific activity of the rat liver was always within the range shown in Table II.

One unit of activity was defined as formation of 1 μmol of 14CO2 in 1 h at 37°C under the assay conditions employed. Specific activity of the liver sample was calculated as: units enzyme activity/gram wet tissue, units enzyme activity/gram wet liver.

total N of tissue, units enzyme activity/gram noncollagen N of tissue, and units enzyme activity/gram DNA of tissue.

_Determination of DNA, total N, and collagen._ Frozen tissue was homogenized in cold water and the homogenate was lyophilized. 5–10 mg of lyophilized powder was analyzed for DNA according to the method of Martin et al. (14). 5–10 mg of the powder was digested in concentrated H$_2$SO$_4$, and analyzed with 2 ml 6 N HCl in a sealed ampule under vacuum at 110°C for 40 h; the hydrolysate was analyzed for hydroxyproline and thereby, collagen (15).

**RESULTS**

_Preliminary experiments on assay conditions_ (Fig. 1)

The composition of the complete assay medium of Wohlhueter and Harper (6), slightly modified is detailed in Tables A and B. Complete medium was altered by the series of omissions and additions shown in this table, and the effect of these changes upon the branched-chain dehydrogenase activity of liver and muscle homogenate from rat, monkey, and man was examined. KIC and KIV were both employed as substrates. In general agreement with Wohlhueter and Harper (6), the activity of the rat enzyme was significantly ($P < 0.05$) inhibited by these modifications: omission of NAD, coenzyme A, MgCl$_2$, CaCl$_2$, or catalase; replacement of Na$^+$ by K$^+$, K$^+$ by Na$^+$, PO$_4^-$ by Tris, or Cl$^-$ by SO$_4^-$.$^*$ On the other hand, these changes in medium did not influence the rat enzyme activity significantly ($P < 0.05$): doubling concentration of NAD or thiamine pyrophosphate; addition of lipoate; replacement of Mg$^{++}$ by Ca$^{++}$ or Mn$^{++}$. The branched-chain dehydrogenase activity of liver and muscle from man reacted in a similar way as the rat enzyme to these alterations in medium composition.

The pH profile for rat, monkey, and human dehydrogenase activity, KIC serving as substrate, revealed pH optimum was 6.8–7.0 in each instance; likewise, temperature optimum was between 34°C and 40°C for all three species.

The relation of $^{14}$CO$_2$ production from KIC by rat and human tissue homogenates to (a) concentration of tissue in assay medium and (b) duration of incubation is shown in Fig. 1A, B. CO$_2$ production was linearly related to tissue concentration in the range 10–50 mg/ml, and to duration of incubation from 10 to 40

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* Tables A and B have been deposited with the National Auxiliary Publications Service (NAPS) as NAPS document 02933. This information may be ordered from ASIS/NAPS, Microfiche Publications, 305 East 46th Street, New York 10017. Remit with order for each NAPS document number $1.50 for microfiche or $5.00 for photocopies for up to 30 pages; for each additional page over the first 30 pages, there is a 15¢ charge per page. Checks should be made payable to Microfiche Publications.

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![Figure 1](image-url)  
**Figure 1** Effect of (A) tissue concentration and (B) duration of incubation on production of $^{14}$CO$_2$ from $[1-{^4}\text{C}]$KIC by tissue homogenates of rat and man. Histologically normal human tissue obtained 2–4 h after death was used. Assay conditions are described in the text.
min. The reaction velocity for tissues of all three species increased with keto acid concentration up to 0.5 mM and remained constant between 0.5 and 8 mM (data not shown).

These observations (confirming the findings of Wendel et al. (16) with human leukocytes and fibroblasts) established the validity of using the same conditions to assay branched-chain dehydrogenase activity in homogenates of monkey and human tissues as have been used for those of the rat tissues (4, 6), namely, the complete medium described in Table B;3 tissue concentration, 20 mg/ml; duration of incubation, 20 min; temperature, 37°C.

Comparison of branched-chain dehydrogenase in rat, monkey, and man

Specific and total activities of the BCKA dehydrogenase, with each of the three BCKA as substrate, are shown in Table I. Percent distribution of the body content was calculated using the formula: organ weight × specific activity/Σ (organ weight × specific activity) × 100.

Rat. Specific activities (U/g wet wt) of the dehydrogenase ranged from: 31 to 34 in liver, 25 to 28 in kidney, 2 to 4 in heart, 2 to 4 in brain and <2 in each of the other tissues. However, when percent body distribution was calculated using the above formula, the location of the enzyme activity was 67–76% in liver, 12–13% in kidneys, 8–15% in muscle, and <2% in each of the other tissues.

Monkey. For the three substrates, the specific activities (U/g wet wt) ranged from: 9 to 14 in liver, 10 to 14 in kidney, 2 to 6 in heart, and <2 in each of the other tissues. The specific activity for muscle was similar to that of rat. The body distribution in the monkey was 48–71% hepatic, 13–18% renal, 5–16% muscular, and <5% for each of the other tissues.

Man. The corresponding series of human tissues can be obtained only at autopsy after a variable postmortem interval. Before analyzing such samples, we first studied the effect of storing rat carcass at 5°C for 0–12 h upon the specific activities of liver, kidney, and muscle (Fig. 2). In each tissue, the activity declined at an average rate of 10% every 4 h. Human muscle tissue, excised at laparotomy, lost 20% of the enzyme activity during storage at 4°C for 4 h. Accordingly, the experiments with postmortem human tissues were limited to samples obtained not more than 4 h after death. Specific activities for the three enzymes in human postmortem samples ranged between 1.7 and 2.1 in liver, 0.8 and 0.9 in kidney, and <0.6 in each of the other tissues. Specific activity in skeletal muscle was similar to that of rat and monkey. Normal human liver and normal human skeletal muscle samples analyzed immediately after excision in the operating room (Tables I and II) showed specific activities similar to those of the 2–4-h postmortem samples. Distribution of body content BCKA dehydrogenase in man was estimated as 60–62% muscular, 28–32% hepatic, 2% renal, and about 8% in other tissues.

In six cirrhotic livers biopsied at laparotomy, average specific activities with KIC, KIV, and KMV as substrates were only 20–50% as great as in normal freshly excised human liver, regardless of whether
wet weight, total N, noncollagen N, or DNA was used as unit of reference (Table II). The differences were significant at \( P < 0.05 \). Average specific activity in cirrhotic liver was 32% of normal.

**DISCUSSION**

Specific activities of the rat tissues confirm those of earlier workers (6). In this species, distribution of the body’s content of the enzyme is 70% hepatic, 12% renal, 10% muscular, and 5% other organs. The hepatic- and renal-specific activities being considerably less in monkey and man, a smaller proportion of the body content of the enzyme is located in these organs. In man, only 30% is hepatic, 5% is renal, and the major portion, 60%, is in skeletal muscle.

Another difference between the three species is total body content of enzyme/kilogram of body weight: 1,410–1,610 U/kg in rat, 320–630 U/kg in monkey, and 15–170 U/kg in man.

The present findings have several implications for the use of BCKA in human subjects with nitrogen accumulation diseases: (a) the efficiency of the BCKA as substitutes for the BCAA is theoretically a function of the ratio, rate of decarboxylation:rate of transamination. In the rat, efficiency of KIV and KIC varies with the growth rate desired, but at maximal growth, is only about 30%. Since the human body content of branched-chain dehydrogenase, expressed as units/kilogram lean body mass, is only 1/30th as great as in the rat, the nutritional efficiency of BCKA in man may be considerably greater than 30%. The picture will be incomplete, however, until the specific activities of branched-chain aminotransferases in human tissues are determined. (b) The nutritional efficiency of BCKA in cirrhotic patients will probably be greater than in normal subjects for the following reasons. Assume that branched-chain aminotransferase is largely extrahepatic in man, as in the rat. Orally-ingested BCKA are delivered in normal subjects via the portal vein to the liver; only those molecules which escape decarboxylation by the hepatic branched-chain dehydrogenase can reach the extrahepatic aminotransferases. Therefore, the decline in the hepatic enzyme’s activity caused by cirrhosis (Table II) may increase the proportion of ingested BCKA which is available for transamination. Furthermore, the rate of decarboxylation of ingested BCKA in the liver will be determined not only by the enzyme’s specific activity, but also by the mass of the liver and the rate of portal blood flow. All three factors will be reduced in advanced cirrhosis. In the present series of cirrhotics, moreover, cases with advanced hepatocellular failure were excluded by the criteria for shunt surgery (17), as indicated by the only moderate accumulation of collagen and DNA (Table II) compared to autopsy specimens of cirrhotic liver (18, 19), by the absence of encephalopathy, and by the limited degree of inflammation and fat infiltration in the liver (Tables A and B). Thus we can expect an even greater than 70% reduction in specific activity of hepatic branched-chain dehydrogenase to occur in cirrhotic patients with more advanced disease than the present cases. Finally, natural or surgical portasystemic shunting in cirrhotic patients will further reduce the proportion of ingested BCKA which is catabolized by oxidative decarboxylation in the liver and increase the proportion available for transamination to BCAA in extrahepatic tissues. (c) Intravenously administered BCKA, however, can be expected to possess similar efficiency in cirrhotic and normal subjects, because the body content of BCKA dehydrogenase would be reduced only 20% by even total loss of the hepatic dehydrogenase. (d) Since the human kidneys contain <5% of the body’s branched-chain dehydrogenase, advanced renal disease will have negligible effect on the metabolic degradation of BCKA. The normal human kidney may, however, contribute more importantly to transamination of BCKA; if so, the nutritional efficiency of BCKA in uremics could be impaired.

It must be emphasized that the discussion above concerning the probable effects of hepatic and renal disease on the nutritional efficiency of BCKA administered by the oral and intravenous routes is not quantitative and attempts only to predict the direction of the anticipated changes. Rates of decarboxylation and transamination of BCKA in vivo will depend not only on enzymatic-specific activities as measured in vitro, but also on the in vivo concentrations of reactants in the enzymatic process, and on the in vivo concentrations of BCKA at the surfaces of the involved enzymes. Monitoring of blood levels of BCKA during treatment of patients will be necessary in order to quantify the effect of cirrhosis on the metabolism of these compounds.

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**REFERENCES**


