Acute Effects of Decreased Glutamine Supply on Protein and Amino Acid Metabolism in Hepatic Tissue: A Study Using Isolated Perfused Rat Liver

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Glutamine deficiency, a common finding in severe illness, has a negative influence on immune status, protein metabolism, and disease outcome. In several studies, a close relationship between glutamine, branched-chain amino acid (BCAA), and protein metabolism was demonstrated. The aim of the present study was to investigate the effect of glutamine deficiency on amino acid and protein metabolism in hepatic tissue using a model of isolated perfused rat liver (IPRL). Parameters of protein metabolism and amino acid metabolism were measured using both recirculation and single pass technique with L-[1-^{14}C]leucine and [1-^{14}C]ketoisocaproate (KIC) as a tracer. Glutamine concentration in perfusion solution was 0.5 mmol/L in control and 0 mmol/L in the glutamine-deficient group. The net release of glutamine (about 11 μmol/g/h) and higher net uptake of most of the amino acids was observed in the glutamine-deficient group. There was an insignificant effect of lack of glutamine on hepatic protein synthesis, proteolysis, and the release of urea. However, significantly lower release of proteins by the liver perfused with glutamine-deficient solution was observed. The lack of glutamine in perfusion solution caused a significant decrease in leucine oxidation (6.66 ± 1.04 *v* 13.67 ± 2.38, μmol/g dry liver/h, $P < .05$) and an increase in KIC oxidation (163.7 ± 16.5 *v* 92.0 ± 12.9 μmL/g dry liver/h, $P < .05$). We conclude that decreased delivery of glutamine to hepatic tissue activates glutamine synthesis, decreases resynthesis of essential BCAA from branched-chain keto acids (BCKA), increases catabolism of BCKA, and has an insignificant effect on protein turnover in hepatic tissue.

GLUTAMINE IS THE most abundant free amino acid in the body fluids. It is a conditionally essential amino acid acting as a "nitrogen shuttle" among organs and as a major end product of ammonia-trapping pathways in the liver. It is an important fuel for enterocytes and immune cells, a substrate for protein synthesis and gluconeogenesis, the most important precursor for ammoniagenesis in the kidney, and a precursor for the synthesis of nucleotides.[1-3]

The main source of circulating glutamine is skeletal muscle, where the most important donor of nitrogen for glutamine synthesis is undoubtedly branched-chain amino acids (BCAA; valine, leucine, and isoleucine).[4,5] The liver can be considered as an organ that adjusts blood plasma glutamine to the physiologic values because of the high activities of both glutamine synthetase (EC 6.3.1.2.) and glutaminase (EC 6.3.1.2.). Under normal conditions, the liver is a rather glutamine-consuming than glutamine-releasing tissue. The liver production of glutamine helps to fulfill the requirements of the body in starvation or when protein intake is insufficient.[6,7] However, the exact role of the liver in maintenance and regulation of glutamine concentration in plasma is still uncertain.

Glutamine synthesis and catabolism are markedly altered in severe illness, eg, in sepsis, trauma, and burns. Despite an increase in glutamine synthesis in skeletal muscle, the intracellular pool of glutamine becomes depleted owing to its accelerated utilization in visceral tissues.[8] Glutamine deficiency in severe illness undoubtedly has a negative effect on immune status, protein balance, and disease outcome and is a rationale for the use of glutamine in nutritional therapy.[3,9]

In a recent review, we summarized experimental evidence of a close relationship in glutamine, BCAA, and protein metabolism and hypothesized that glutamine administration may prevent the loss of essential BCAA and improve protein balance.[10] The speculation was supported mainly by the well-known fact that BCAA is an important substrate and regulator in pathways of protein metabolism,[11] an important source of nitrogen for glutamine synthesis in skeletal muscle,[4,5] and that glutamine and/or alanyl-glutamine infusions decrease breakdown of body proteins and BCAA catabolism in postabsorptive control, endotoxemic, and irradiated rats.[12,13] It was suggested that the favorable effect of glutamine administration on protein balance is related to decreased utilization of BCAA in pathways of glutamine synthesis in muscle and/or to enhanced resynthesis of BCAA from their ketoanalogues in liver. To estimate the second possibility, we investigated the effect of liver perfusion by solution containing glutamine at a physiologic level and by solution without glutamine on protein and amino acid metabolism using the isolated perfused rat liver (IPRL) technique.

MATERIALS AND METHODS

Animals

Male Wistar rats (Velaz, Prague, Czech Republic) were housed in standardized cages in quarters with controlled temperature, a 12-hour light-dark cycle, and received Velaz-Altromin 1320 laboratory chow and drinking water ad libitum. All procedures involving animals were performed according to guidelines set by the Institutional Animal Use and Care Committee of Charles University.

Materials

L-[1-^{14}C]leucine and α-keto[1-^{14}C]isocaproate were purchased from Amersham (Buckinghamshire, UK), [^{14}C]bicarbonate was obtained from Du Pont-NEN (Bad Homburg, Germany). Leucine and the sodium salt of α-ketoisocaproate (KIC) were purchased from Sigma Chemical (St Louis, MO). Amino acid solution Aminoplasmal 15 was

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obtained from B. Braun Medicals (Melsungen, Germany). Hyamine hydroxide was from Packard Instrument (Meriden, CT). The remaining chemicals were purchased from Lachema (Brno, Czech Republic).

Experimental Design

Overnight fasted rats were anesthetized with sodium pentobarbital (35 mg/kg body weight [bw] intraperitoneally) and the livers prepared for perfusion as previously described in detail.[14] Briefly, after laparotomy, the bile duct was cannulated and 1,000 IU/kg heparin was injected into the saphenous vein. The portal vein was then cannulated with a polyethylene catheter (ID 1.5 mm) and the hepatic artery was ligated. During portal perfusion with Krebs-Henseleit solution (20°C) the liver was quickly removed. The perfusion was performed at 37°C in a thermostatically controlled cabinet. A peristaltic pump took the perfusate from the reservoir through an oxygenator and a bubble trap to the liver. Flow rates were maintained at 3.5 mL/g/min. Viability of the perfused livers was monitored by their appearance, concentration of minerals and hepatic enzymes in perfusate, by the stability of the bile flow, oxygen consumption, and the dry/wet ratio of hepatic tissue. Tissue dry/wet weight ratio indicates the degree of tissue edema. The dry liver weight was determined from the weights of hepatic tissue specimens immediately after perfusion and after desiccation at 80°C for 48 hours using following formula: $D = (W \cdot d)/w$ where W is the weight of the wet liver immediately after perfusion, d is the weight of dry liver specimen, and w is the weight of wet liver specimen.

The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (equilibrated with 95% O_2/5% CO_2 at 37°C), 6 mmol/L glucose and amino acids at about the normal plasma concentration in rats, pH 7.4. Glutamine concentration was 0.5 mmol/L in controls and 0 mmol/L in the glutamine-deficient group. The pH of perfusion medium was monitored by sampling at 15 minutes and at the end of perfusion. We did not observe changes of more than 0.1 pH, and there were no significant differences between the control and experimental groups. Before starting the experimental protocol, livers were perfused with tracer-free perfusion medium for a period of 15 minutes to ensure stabilization of the liver and washout of endogenous hormones. Two separate studies were performed.

Study 1. In the first study we analyzed, using the recirculation technique (30 minutes), the effect of glutamine deficiency on protein synthesis, proteolysis, and the net uptake or release of individual amino acids. Proteolysis was estimated as [^{3}H] leucine release from the liver of rats labeled in vivo by an intraperitoneal injection of 4,5-[^{3}H] leucine (0.8 mCi/kg bw) 6 hours before the perfusion experiment. Protein synthesis was measured by determining incorporation of [1-^{14}C]leucine into liver proteins (16 μCi/L was added to the perfusate at the beginnig of the recirculation). Protein concentrations in the liver and perfusate were measured according to Lowry et al.[15] Amino acid exchanges were calculated using following formula:

$$E = (C_{t16} - C_{t46}) \cdot V/(W_{dr} \cdot t)$$

where C_{t16} and C_{t46} are amino acid concentrations at the end and at the beginning of the recirculation phase of the study, V is the total volume of perfusate in liters, t is the duration of recirculation in hours, and W_{dry} is the liver dry weight in grams. Results are expressed as μmol/g dry liver/h. Negative values mean net amino acid uptake and positive values indicate net release.

Study 2. In the second study we analyzed the effect of glutamine deficiency on leucine metabolism in liver using the single pass technique with α-keto[1-^{14}C]isocaproate (KIC) or L-[1-^{14}C]leucine as a tracer (Patel et al[16]). At 16 minutes (after stabilization), the perfusion medium containing [1-^{14}C]KIC (2 μCi/L) KIC, 1.26 mmol/L leucine and 1 mmol/L KIC was infused for 15 minutes (single pass no. 1). The livers were then perfused with tracer-free medium for 15 minutes. At 46 minutes, the perfusion medium containing [1-^{14}C]leucine (10 μCi/L), 1.26 mmol/L leucine and 0 mmol/L KIC was administered for the next 15 minutes (single pass no. 2). Our preliminary experiments, in agreement with other studies,[16,17] showed that 1 mmol/L KIC and 1 mmol/L leucine were necessary for the substrate to be nonlimiting during the flux study.

Samples of the effluent perfusate were collected in 20-mL flasks equipped with stoppers and center wells containing 0.4 mL methylbenzethonium hydroxide at 1-minute intervals to monitor $^{14}CO_2$ production. Labeled CO_2 in the perfusate, which was produced from 1-^{14}C-labeled substrates was released by injecting 0.5 mL 5 N sulphuric acid through the stopper into the flasks. Oxidation rates of KIC and leucine were calculated as follows:

$$O = (R \cdot F \cdot RF)/(SA \cdot W)$$

where O is the substrate oxidation (μmol of substrate oxidized/g of dry liver/h), R is the radioactivity of $^{14}CO_2$ in the effluent perfusate (dpm/mL), F is the flow rate of perfusion medium through the liver (mL/h), RF is the recovery factor, SA is the specific activity of KIC or leucine in perfusion medium (dpm/μmol), and W is the liver dry weight in grams. The recovery factor, ie, the recovery of $^{14}CO_2$, was estimated using [^{14}C]bicarbonate, which was added to perfusate instead of labeled leucine or KIC in a separate study on 3 animals of both groups. The $^{14}CO_2$ recovery was 97% ± 0.6% both in the control and the glutamine-deficient groups.

Other Techniques

Amino acid concentrations were determined with high-performance liquid chromatography (Waters, Milford, MA) after precolumn derivatization with *o*-phthaldialdehyde. The radioactivity of the samples was measured with the liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, Fullerton, CA). Oxygen consumption was calculated by determining pO_2 entering the portal vein and leaving the hepatic vein (for this purpose a cannula was inserted in hepatic vein), flow rate of perfusion medium through the liver (mL/h), and the liver dry weight assuming a O_2 solubility of 0.00141 μmol/mL/mm Hg at 37°C. The pO_2 was analyzed using the automatic gas system AVL 995 (AVL, Graz, Austria). Glucose concentration and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and gamma-glutamyl transferase (GGT) were measured using commercial tests (Boehringer, Mannheim, Germany; Lachema, Brno, Czech Republic). Na^+ and K^+ were determined using ion-selective electrodes on AVL 983-S.

Table 1. Parameters of Isolated Perfused Rat Liver (Study 1)

	Control (n = 6)	Glutamine Deficiency (n = 7)
Body weight (g)	237 ± 8	235 ± 7
Liver weight		
Wet (g/kg bw)	37.8 ± 1.3	36.0 ± 1.1
Dry (g/kg bw)	8.8 ± 0.1	8.8 ± 0.2
Dry/wet (%)	23.5 ± 0.7	24.4 ± 0.4
Bile flow (mg/g dry liver/h)	126 ± 15	105 ± 8
O_2 consumption (μmol/g dry liver/h)	287 ± 26	268 ± 29
Glucose (mmol/L)	6.58 ± 0.12	6.49 ± 0.01
Na^+ (mmol/L)	148 ± 1	147 ± 1
K^+ (mmol/L)	4.20 ± 0.01	4.17 ± 0.00
AST (μkat/L)	0.25 ± 0.01	0.21 ± 0.04
ALT (μkat/L)	0.22 ± 0.02	0.17 ± 0.03
LDH (μkat/L)	3.15 ± 0.20	2.77 ± 0.33
GGT (μkat/L)	0.28 ± 0.01	0.29 ± 0.01

NOTE. Mean ± SE. Mann-Whitney test.

Table 2. Parameters of Isolated Perfused Rat Liver (Study 2)

	Control (n = 9)	Glutamine Deficiency (n = 8)
Body weight (g)	280 ± 13	284 ± 14
Liver weight		
Wet (g/kg bw)	34.6 ± 2.5	33.4 ± 1.9
Dry (g/kg bw)	8.6 ± 0.3	8.2 ± 0.3
Dry/wet (%)	25.5 ± 0.9	25.0 ± 1.4
Bile flow (mg/g dry liver/h)	97 ± 8	110 ± 10
O_2 consumption (μmol/g dry liver/h)	322 ± 17	357 ± 17
AST (μkat/L)	0.13 ± 0.01	0.13 ± 0.02
ALT (μkat/L)	0.14 ± 0.01	0.14 ± 0.01
Na^+ (mmol/L)	141 ± 1	140 ± 1
K^+ (mmol/L)	5.5 ± 0.0	5.6 ± 0.1

NOTE. Mean ± SE. Mann-Whitney test.

Statistical Analysis

Results are expressed as the mean ± SE. Statistical analysis was performed using Mann-Whitney test. A difference was considered significant at $P < .05$. Statistical software NCSS 6.0 (Kaysville, UT) was used for the analysis.

RESULTS

The perfused liver had uniform color, and there was no indication of uneven flow distribution. There were no differences observed between control and glutamine-deficient groups in liver weight, the dry-to-wet liver weight ratio, the bile flow, oxygen consumption, and concentrations of glucose, hepatic enzymes, and minerals in perfusate at the end of perfusion (Tables 1 and 2).

There was an insignificant effect of lack of glutamine on hepatic protein synthesis, proteolysis, and release of urea. However, we observed significantly lower release of proteins by the liver perfused with glutamine-deficient solution (Table 3).

Liver perfusion with glutamine-deficient solution resulted in net release of glutamine and significantly higher uptake of most estimated amino acids, including aspartic acid, threonine, serine, glycine, alanine, methionine, phenylalanine, lysine, histidine, and arginine than in controls (Table 4).

The results obtained using the single pass method showed that liver perfusion with glutamine-deficient solution results in a significant decrease in leucine oxidation (Fig 1), while oxidation of KIC increases (Fig 2).

Table 4. Effect of Glutamine Deficiency on Net Amino Acid Uptake (−) or Release (+) by Isolated Perfused Rat Liver (Study 1)

	Control (n = 6)	Glutamine Deficiency (n = 7)
Aspartate (93)	−0.10 ± 0.66	−4.18 ± 0.53*
Threonine (57)	−0.72 ± 0.71	−2.51 ± 0.16*
Serine (48)	0.70 ± 0.68	−1.28 ± 0.15*
Glutamate (156)	−2.80 ± 1.12	−3.88 ± 0.56
Glutamine (500 or 0)	−7.42 ± 2.30	10.91 ± 1.36*
Glycine (339)	−8.06 ± 2.02	−15.37 ± 0.77*
Alanine (444)	−3.63 ± 3.04	−27.64 ± 1.45*
Valine (108)	−0.53 ± 1.13	−2.06 ± 0.39
Methionine (53)	−1.81 ± 0.21	−2.62 ± 0.52*
Isoleucine (77)	−0.20 ± 0.95	−0.06 ± 0.69
Leucine (133)	−1.87 ± 1.01	−2.86 ± 0.45
Tyrosine (6)	−0.02 ± 0.09	0.98 ± 0.23*
Phenylalanine (52)	−0.56 ± 0.72	−2.65 ± 0.12*
Lysine (63)	−2.24 ± 0.57	−3.85 ± 0.24*
Histidine (72)	−0.52 ± 0.58	−3.73 ± 0.57*
Arginine (91)	−4.98 ± 0.73	−7.12 ± 0.42*
Derived values		
BCAA	−2.61 ± 3.08	−4.98 ± 1.33
Total AA	−34.78 ± 12.37	−67.92 ± 3.89
Total AA-Gln	−27.35 ± 10.69	−78.84 ± 4.14*

NOTE. Units are μmol/g dry liver/h. Data are given as mean ± SE. Mann-Whitney test.

Values in parentheses (1st column) indicate amino acid concentrations in perfusion solution in μmol/L at the beginning of the perfusion.

*$P < .05$ *v* control.

DISCUSSION

The perfusion of the liver by glutamine lacking solution induces a metabolic condition resulting in net glutamine synthesis to maintain its physiologic levels. Glutamine synthesis in the liver occurs predominantly in the perivenous population of hepatocytes where most of glutamine synthetase is located, while glutaminase and ureagenesis are located more periportally.[18] The direct precursors for glutamine synthesis are glutamic acid and ammonia.

We observed the net production of glutamine at a rate of about 11 μmol/g/h in liver perfused with solution without glutamine, while a net uptake of about 7 μmol/g/h was observed in controls. Activated glutamine production in liver perfused with glutamine-deficient solution was associated with

Table 3. Effect of Glutamine Deficiency on Leucine and Protein Metabolism in Isolated Perfused Rat Liver (Study 1)

	Control (n = 6)	Glutamine Deficiency (n = 7)
Urea release (μmol/g dry liver/h)	33.3 ± 14.9	35.4 ± 10.4
^{3}H Leu release into perfusate (dpm/g dry liver/h)	164,937 ± 17,935	185,364 ± 15,747
Protein release (μg/g dry liver/h)	1,174 ± 232	535 ± 77*
^{3}H Leu in released proteins		
dpm/g dry liver/h	40,154 ± 8,576	17,683 ± 3,793*
dpm/mg protein	35,516 ± 6,016	31,991 ± 4,239
^{14}C Leu in liver proteins (dpm/mg protein)	214 ± 17	279 ± 38

NOTE. Mean ± SE. Mann-Whitney test.

*$P < .05$ *v* control.

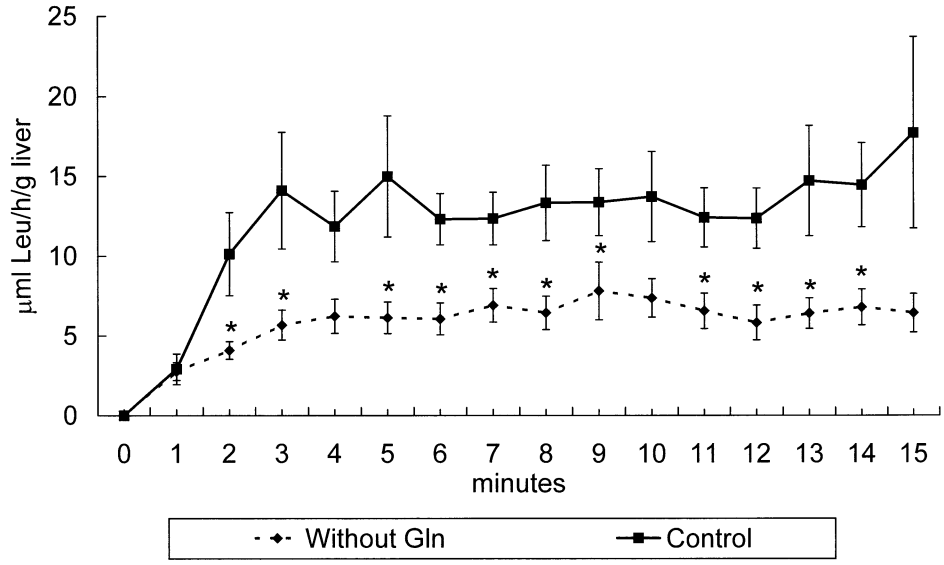

Fig 1. The effect of glutamine deficiency on leucine oxidation by isolated perfused rat liver (study 2). Mean ± SE. Mann-Whitney test. *$P < .05$ *v* control.

a higher uptake of the most of amino acids. Considering insignificant changes in protein synthesis and proteolysis, the majority of utilized amino acids serves undoubtedly as the donor of nitrogen for synthesis of glutamic acid and then glutamine. The most important is probably alanine. Its consumption by hepatic tissue was highest among all estimated amino acids (see Table 4), and a marked increase in glutamine synthesis can be observed when alanine is added to the perfusion medium.[19] It should be noted that alanine release from skeletal muscle is physiologically coupled with release of glutamine and, in addition, alanine infusion has a similar effect on protein metabolism as infusion of glutamine.[12]

We did not find significant differences in urea release between the control and glutamine-deficient group. As glutamine is a substantial source of ammonia for ureagenesis,[20,21] the insignificant difference in urea release indicates that a marked portion of synthesized glutamine was used for urea genesis, and calculated rates of glutamine production were underestimated. Unchanged rates of urea synthesis under conditions of decreased glutamine consumption were observed also in vivo. Heeneman and Deutz[22] demonstrated that methionine sulfoximine-induced decrease in arterial glutamine concentration by 50% resulted in decreased consumption of glutamine in the liver, but urea synthesis was unchanged.

Insignificant changes in hepatic protein synthesis and proteolysis indicate that the liver can maintain a constant rate of protein turnover under conditions of different delivery of glutamine. This can be facilitated by activated synthesis of glutamine in hepatic tissue. It should be noted that in terms of protein economy, a relatively short time was allowed for the

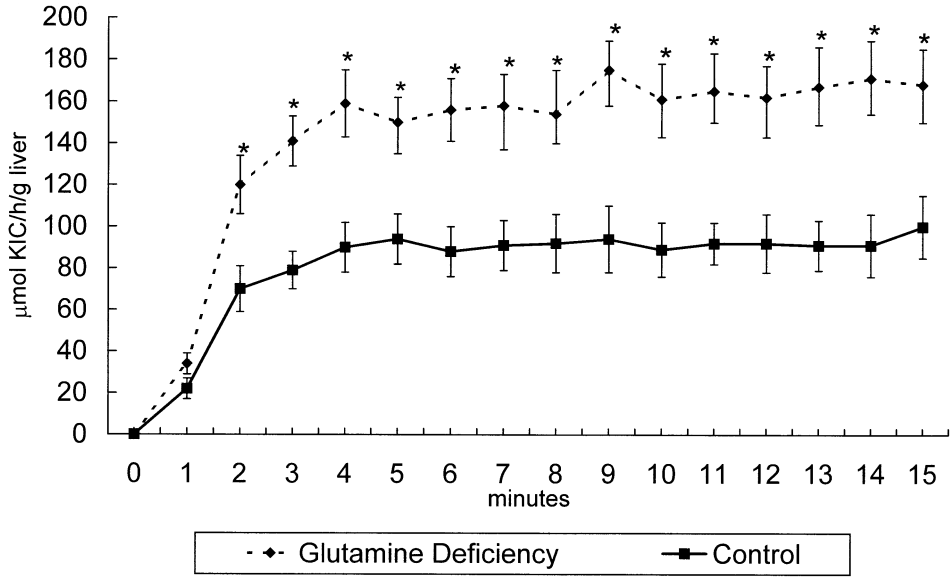

Fig 2. The effect of glutamine deficiency on KIC oxidation by isolated perfused rat liver (study 2). Mean ± SE. Mann-Whitney test. *$P < .05$ *v* control.

liver to respond to glutamine deprivation and that different results may be obtained using a different approach. The unchanged rates in protein turnover are in discrepancy with changes in release of proteins into perfusate. We observed both decrease in protein concentration and the ^{3}H radioactivity of proteins in perfusate of the glutamine-deficient group. We assume that glutamine may act as a signal regulating the release of hepatic proteins under conditions of altered metabolic response in severe illness. This effect of glutamine can help to coordinate the hepatic response in the course of systemic inflammatory response to injury.

The findings presented in this report are in agreement with the hypothesis suggesting that glutamine administration significantly affects metabolism of BCAA. At first, decreased leucine oxidation in the glutamine-deficient group indicates decreased flux of BCAA through BCAA aminotransferase. However, as the direction of transamination under in vivo conditions depends on several factors, this observation may indicate both decreased conversion of BCAA to branched-chain keto acids (BCKA) and vice versa.

A more unambiguous interpretation can be derived from the observation of increased hepatic oxidation of KIC in the glutamine-deficient group. This finding indicates increased activity of BCKA dehydrogenase, the key enzyme in catabolism of BCAA. BCKA dehydrogenase catalyzes irreversible decarboxylation of BCKA to thioesters of coenzyme A, which then through a series of reactions, are converted to acetoacetate and acetyl-CoA (leucine), propionyl-CoA and acetyl-CoA (isoleucine) and succinyl-CoA (valine). These substances are utilized in pathways of carbohydrate and/or lipid metabolism. Because the liver lacks enzymes, which enable the conversion of ketone bodies to acetyl-CoA, acetoacetate is released together with β-hydroxybutyrate (generated from acetoacetate by β-hydroxybutyrate dehydrogenase) to the blood stream. Our finding is in agreement with the observation of a substantial reduction of BCAA production in the liver of glutamine-deficient rats.[22]

In a number of studies, it was demonstrated that the main source of BCKA for hepatic BCKA dehydrogenase is skeletal muscle.[23,24] The delivery of the BCKA to hepatic tissue increases during activated breakdown of muscle proteins or as a result of their by mouth or intravenous administration (eg, nutritional supplementation during chronic renal failure). It can be suggested that glutamine is an important source of nitrogen for conversion of BCKA to BCAA in liver. On the contrary, decreased glutamine supply results in preferential catabolism of BCKA and release of ketone bodies from the liver to the blood stream.

It can be summarized that glutamine deficiency activates glutamine synthesis, activates catabolism of BCKA (increased activity of BCKA dehydrogenase), and decreases reamination of BCKA to BCAA (decreased activity of BCAA aminotransferase) in the liver. The decreased rate of conversion of BCKA to BCAA is very likely caused by a diminution of intrahepatic glutamate, which is considered an effective amino donor for each of the BCKA.[25] Enhanced catabolism of BCAA has undoubtedly a negative effect on protein balance of the whole body and the outcome of a particular disease. The results obtained also suggest that the correction of glutamine deficiency in severe illness may significantly affect partition of BCKA between oxidation and reamination. For instance, in treatment of chronic renal failure, the conversion of administered ketoanalogues of essential amino acids to corresponding amino acids may be affected by glutamine availability.

The liver of healthy, overnight-fasted animals was used in our studies to estimate the possible effect of glutamine deprovation on hepatic protein and amino acid metabolism, particularly on catabolism of BCAA and their resynthesis from BCKA. To obtain a clear answer, it was advantageous to compare the effect of solution containing glutamine in physiologic ranges with solution without glutamine. For possible clinical application of the obtained results, the response of the liver isolated from critically ill animals and the effect of different glutamine concentrations in perfusate will be evaluated in further studies.

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