Effect of Hyperammonemia on Leucine and Protein Metabolism in Rats

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The cause of muscle wasting and decreased plasma levels of branched chain amino acids (BCAA), valine, leucine, and isoleucine in liver cirrhosis is obscure. Here we have evaluated the effect of hyperammonemia. Rats were infused with either an ammonium acetate/bicarbonate mixture, a sodium acetate/bicarbonate mixture, or saline for 320 minutes. The parameters of leucine and protein metabolism were evaluated in the whole body and in several tissues using a primed constant intravenous infusion of L-[1-^{14}C]leucine. Ammonium infusion caused an increase in ammonia and glutamine levels in plasma, a decrease in BCAA and alanine in plasma and skeletal muscle, a significant decrease in whole-body proteolysis and protein synthesis, and an increase in leucine oxidized fraction. A significant decrease in protein synthesis after ammonium infusion was observed in skeletal muscle while a nonsignificant effect was observed in liver, gut, heart, spleen, and kidneys. We conclude that the decrease in plasma BCAA after ammonia infusion is associated with decreased proteolysis and increased leucine oxidized fraction.

IN THE 1950s, it was established that the plasma concentrations of the branched-chain amino acids (BCAA), valine, leucine, and isoleucine are markedly reduced in cirrhotic patients.[1,2] The BCAA exert a specific regulatory effect on the rates of protein degradation and synthesis, primarily in muscle.[3,4] In addition, the BCAA may influence the rate of synthesis of some neurotransmitters because BCAA are transported into the brain via the same carrier that transports aromatic amino acids.[5] These metabolic properties of BCAA are the rationale for the suggestion that the decrease in plasma BCAA levels in liver cirrhosis is implicated in the development of hepatic encephalopathy and skeletal muscle wasting and thus the rationale for the use of BCAA in liver disease.[6-8]

The pathogenesis of the decreased levels of BCAA in liver cirrhosis is not clear, although several factors have been proposed as the cause of the decreased plasma BCAA levels.[9] The main factors considered are insulin and ammonia.[10-14] However, there are several objections, which significantly restrict the theory of insulin being responsible for the deficiency of BCAA in liver cirrhosis.[15,16] It appears that the principal etiologic factor in decreasing BCAA levels in liver cirrhosis is ammonia. Several studies have shown that hyperammonemia decreases plasma BCAA levels and have shown an inverse relationship between the plasma ammonia and BCAA concentrations.[17,18] In addition, it was shown that plasma BCAA levels were 30% lower when they were infused with ammonia than when infused alone.[19]

The decrease in plasma BCAA concentrations may result from a decrease in their rate of appearance in body fluids or from an increase in their rate of disappearance. In our previous studies, we showed a significant decreased in proteolysis and protein synthesis and an increase in leucine oxidized fraction in cirrhotic and partially hepatectomized rats.[20,21] Analogous data about the effect of hyperammonemia on leucine and protein turnover, which could significantly support the ''ammonia hypothesis,'' are missing. Therefore, the main objective of this study was to evaluate the effect of hyperammonemia on protein turnover and leucine oxidation.

MATERIALS AND METHODS

Animals

Male Wistar rats (Velaz, Prague, Czech Republic) were housed in standardized cages in quarters with controlled temperature and 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 was purchased from Amersham (Buckinghamshire, UK), [^{14}C]bicarbonate was obtained from Du Pont-NEN (Bad Homburg, Germany). Leucine, Folin-Ciocalteu phenol reagent and albumin were purchased from Sigma Chemical (St Louis, MO). Hyamine hydroxide was obtained from Packard Instrument (Meriden, CT). The remaining chemicals were obtained from Lachema (Brno, Czech Republic).

Experimental Design

A polyethylene cannula was inserted into the jugular vein under light diethyl ether narcosis 24 hours before the beginning of the experiment to exclude the effect of stress from surgery. To exclude nutritional effects, all rats were fasted overnight before the experiment. The second day, between 7 and 8 AM, each animal was placed in a glass metabolic cage and infused with an ammonium acetate/bicarbonate mixture, the dosage of each salt being 1.4 mmol/kg/hour.[18] The control animals were treated with sodium acetate/bicarbonate mixture (the amount of each salt was again 1.4 mmol/kg/h) or with saline solution. After 2 hours, the infused solutions were enriched with L-[1-^{14}C]leucine (1.9 μCi/mL). A priming dose of 0.7 mL (ie, 1.33 μCi of L-[1-^{14}C]leucine) was followed by a constant infusion at a rate 0.36 mL/h for 200 minutes. The rats were killed by exsanguination via the abdominal aorta exactly at 321 minutes from the beginning of the infusion. Afterwards the liver, gastrocnemius muscle, spleen, kidney, small intestine, colon, and heart were quickly removed and immediately frozen in liquid nitrogen.

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Supported by the Internal Grant Agency of Charles University (Grant No. 276/98C) and the Internal Grant Agency of Ministry of Health of the Czech Republic (Grant No. 3772-3).

Submitted November 10, 1999; accepted April 9, 2000.

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0026-0495/00/4910-0022$10.00/0

doi: 10.1053/meta.2000.9531

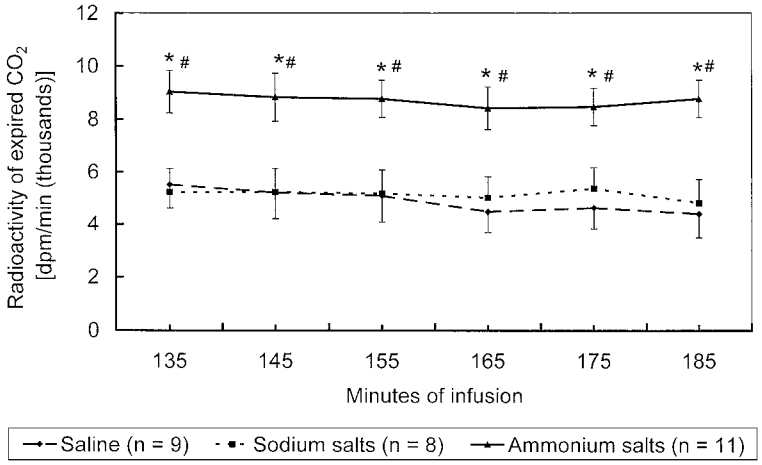

Fig 1. Recovery of labeled CO_2 at expired air of rats during continuous infusion of L-[1-^{14}C]leucine. Means ± SE, ANOVA, and Bonferroni test. *$P < .05$ *v* saline; #$P < .05$ *v* sodium salts.

Parameters of Leucine Metabolism

The parameters of whole body leucine metabolism were evaluated at steady-state conditions by the procedure described in detail previously.[22] The expired CO_2 was trapped at 10-minute intervals between 125 and 185 minutes of infusion by monoethanolamine. The average value of 6 measurements of $^{14}CO_2$ radioactivity in expired air at steady-state condition was used for calculations of the leucine oxidation rate (Fig 1). The $^{14}CO_2$ recovery factor (FR) was about 90% in both control and experimental animals. Leucine specific activity, turnover, and decarboxylation rates were calculated by the following formulas:

$$\underset{(\text{dpm}\cdot\mu\text{mol}^{-1})}{\text{Specific activity } (SA_{Leu})} = \frac{\text{Leu radioactivity (dpm}\cdot\text{mL}^{-1})}{\text{Leu concentration } (\mu\text{mol}\cdot\text{mL}^{-1})}$$

$$\underset{(\mu\text{mol}\cdot\text{h}^{-1})}{\text{Turnover rate } (Q_{Leu})} = \frac{\text{infusion rate (dpm}\cdot\text{h}^{-1})}{SA_{Leu} \text{ in plasma (dpm}\cdot\mu\text{mol}^{-1})}$$

$$\underset{(\mu\text{mol}\cdot\text{h}^{-1})}{\text{Decarboxylation rate } (D_{Leu})} = \frac{^{14}CO_2 \text{ production rate (dpm}\cdot\text{h}^{-1})}{SA_{Leu} \text{ in plasma (dpm}\cdot\mu\text{mol}^{-1}) \times FR}$$

Whole body leucine metabolism was considered to take place within a common metabolic pool, represented by free plasma leucine. Because exogenous leucine intake (E) was 0 in our protocol, leucine turnover (Q) estimates the leucine released from protein, ie, the protein breakdown (B) as described by the equation: Q = In + D = B + E. Using this formula, rates of leucine incorporation into protein (In), the oxidized fraction of leucine (OF = D · 100/Q) and the fraction of leucine incorporated into protein (IF = In · 100/Q) were calculated.

Tissue Protein Synthesis and Protein Content

The samples for measurement of protein synthesis were processed as described elsewhere.[20] The fractional protein synthesis rates were calculated by using the equation derived by Garlick et al[23] and expressed as the fraction of protein mass renewed each day, in percent per day (Ks). Protein content was measured according to Lowry.[24]

Other Techniques

Amino acid concentrations in deproteinized samples of blood plasma or tissues were determined with high-performance liquid chromatography (Waters, Milford, MA). Blood ammonia and plasma levels of urea and glucose were measured using commercial tests (Boehringer, Mannheim, Germany; Elitech, Sées, France, and Lachema, Brno, Czech Republic). Serum insulin was measured with the enzyme-linked immunosorbent assay (ELISA) test (Boehringer, Mannheim, Germany). The blood pH and pCO_2 were analyzed using the automatic blood gas system AVL 995 (AVL, Graz, Austria). The radioactivity of the samples was measured with the liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, Fullerton, CA).

Statistical Analysis

Results are expressed as the mean ± SE. An analysis of variance (ANOVA) followed by a Bonferroni multiple comparison procedure or paired *t*-test were used for analysis of data. A difference was considered significant at $P < .05$ and $P < .001$, respectively. Statistical software NCSS 6.0 (NCSS, Kaysville, UT) was used for the analysis.

RESULTS

Parameters of Control and Experimental Animals

In ammonia-infused animals, significantly higher values of ammonia, urea, and glucose in blood were observed (Table 1). The increase in insulin concentration was on the margin of statistical significance. It is assumed that the increased glucose level in the ammonia-infused rats is caused by a marked increase in glucagon as others have shown.[14] There were no differences in body weight, blood pH, and pCO_2 between control and experimental animals.

Table 1. Characteristics of the Experimental Animals

	Saline (n = 9)	Sodium Salts (n = 8)	Ammonium Salts (n = 11)	ANOVA *P* Value
Body weight (g)	247 ± 4	241 ± 7	240 ± 4	.4982
Ammonia (μmol/L)	125 ± 29	147 ± 25	434 ± 47*†	.0000
pH	7.42 ± 0.03	7.36 ± 0.13	7.35 ± 0.04	.7512
pCO_2 (kPa)	4.02 ± 0.17	4.10 ± 0.25	4.66 ± 0.27	.1364
Urea (mmol/L)	4.41 ± 0.16	3.95 ± 0.10	7.21 ± 0.25*†	.0000
Glucose (mmol/L)	8.62 ± 0.50	7.55 ± 0.40	10.89 ± 0.60*†	.0005
Insulin (U/L)	57.7 ± 8.3	52.9 ± 15.7	67.8 ± 8.1	.5893

NOTE. Means ± SEM, ANOVA, and Bonferroni test.
*$P < .05$ *v* saline.
†$P < .05$ *v* sodium salts.

Table 2. Effect of Ammonia on Amino Acid Concentrations in Blood Plasma (μmol/L)

	Saline (n = 9)	Sodium Salts (n = 8)	Ammonium Salts (n = 11)	ANOVA *P* Value
Tau	129 ± 24	138 ± 24	150 ± 18	.7739
Asp	6 ± 1	8 ± 1	4 ± 1†	.0201
Thr	183 ± 14	144 ± 13	142 ± 7*	.0276
Ser	149 ± 10	116 ± 11	94 ± 6*	.0005
Asn	30 ± 3	32 ± 4	27 ± 2	.5014
Glu	69 ± 7	93 ± 9	85 ± 8	.1331
Gln	254 ± 7	227 ± 18	301 ± 12*†	.0010
Ala	266 ± 7	216 ± 15	198 ± 17*	.0064
ABU	12 ± 1	11 ± 1	9 ± 0*	.0374
Val	150 ± 13	149 ± 13	100 ± 5*†	.0015
Met	8 ± 1	7 ± 1	5 ± 1	.1409
Ile	71 ± 7	76 ± 8	40 ± 3*†	.0001
Leu	157 ± 13	160 ± 15	90 ± 6*†	.0001
Tyr	61 ± 5	56 ± 4	60 ± 3	.6181
Phe	48 ± 4	55 ± 5	54 ± 3	.4239
Trp	50 ± 5	55 ± 6	47 ± 3	.5067
Orn	15 ± 2	12 ± 1	12 ± 1	.4029
Lys	171 ± 12	133 ± 10	131 ± 6*	.0102
His	125 ± 10	124 ± 10	161 ± 11	.0285
Arg	76 ± 10	70 ± 9	55 ± 5	.1417
Total AA	2,030 ± 116	1,880 ± 139	1,764 ± 69	.2034
BCAA	378 ± 26	385 ± 35	230 ± 14*†	.0001
AAA	110 ± 9	111 ± 9	114 ± 5	.9090
BCAA/AAA	3.47 ± 0.09	3.44 ± 0.12	2.01 ± 0.06*†	.0000

NOTE. Means ± SEM, ANOVA, and Bonferroni test.
Abbreviation: AAA, aramatic amino acids.
*$P < .05$ *v* saline.
†$P < .05$ *v* sodium salts.

Amino Acids in Blood Plasma

Infusion of ammonium salts caused a marked decrease in plasma BCAA levels and an increase in glutamine concentration when compared with both of the control groups (Table 2).

Whole Body Leucine and Protein Metabolism

The considerable decrease in leucine turnover (in our study, this estimates proteolysis, and the rate of leucine appearance and disappearance in the plasma) and in leucine incorporation in proteins was observed in the ammonia-infused rats (Table 3). However, changes in leucine oxidation and leucine clearance were insignificant. For a marked decrease in leucine turnover, a significant increase in the fraction of oxidized leucine was observed in ammonia-infused rats despite unaltered leucine oxidation.

Protein Synthesis in Tissues

Infusion of ammonium salts caused a significant decrease in protein synthesis in skeletal muscle (Table 4). Insignificant changes were observed in plasma proteins, in liver, jejunum, colon, kidneys, spleen, and heart.

Amino Acids in Tissues

The most impressive effect of infusion of ammonium salts was observed in gastrocnemius muscle, where a significant decrease in the concentrations of leucine, valine, isoleucine, and alanine were detected, while the concentration of glutamine increased (Table 5). A significant decrease in the BCAA concentration after ammonia infusion was also observed in the heart. We did not observe marked effect of ammonia on amino acid levels in other tissues.

DISCUSSION

The results obtained in this study clearly show that hyperammonemia induces marked alterations in protein metabolism associated with a significant decrease in BCAA levels in blood plasma and skeletal muscle. Considering that the physiologic role and the main catabolic pathways of the BCAA have common features,[25] the observed changes in leucine metabolism indicate similar alterations in the metabolism of all 3 BCAA.

The results shown in this study significantly support the hypothesis that ammonia is responsible for the decrease of BCAA levels in liver disease. Marked decrease of BCAA levels was observed in hyperammonemic rats not only in blood plasma, but also in skeletal muscle. Considering the observed effect of ammonia on leucine turnover, it can be suggested that the decrease in BCAA level is caused by decreased BCAA appearance (ie, by decreased proteolysis) and not by increased BCAA disappearance (increased incorporation in body proteins and/or oxidation). In the establishment of a new equilibrium between BCAA appearance and disappearance resulting in the decrease of BCAA levels in body fluids, unequal changes in BCAA incorporation in body proteins and BCAA oxidation are undoubtedly also involved. Actually, no decrease in leucine oxidation was shown. This phenomenon is shown by a marked increase in leucine oxidized fraction in ammonia-infused rats. The results presented in this study agree with our previous studies in which we showed significant decrease in whole-body

Table 3. Effect of Ammonia on Parameters of Whole Body Leucine and Protein Metabolism

	Saline (n = 9)	Sodium Salts (n = 8)	Ammonium Salts (n = 11)	ANOVA *P* Value
Leu specific activity in plasma (dpm/μmol Leu)	59984 ± 4432	58077 ± 6937	100844 ± 9269*†	.0004
Proteolysis (μmol Leu/h/kg)	113 ± 9	129 ± 17	72 ± 6*†	.0024
Protein synthesis (μmol Leu/h/kg)	91 ± 10	102 ± 17	46 ± 4*†	.0016
Protein balance (μmol Leu/h/kg)	−22 ± 4	−26 ± 5	−26 ± 3	.6146
Leu clearance (mL/h/kg)	750 ± 65	810 ± 83	818 ± 77	.7904
Leu oxidized fraction (%)	20 ± 4	21 ± 4	36 ± 3*†	.0031

NOTE. Means ± SEM, ANOVA, and Bonferroni test.
*$P < .05$ *v* saline.
†$P < .05$ *v* sodium salts.

proteolysis and increase in leucine oxidized fraction in cirrhotic and partially hepatectomized rats.[20,21] Decreased leucine disappearance from the extracellular fluid in ammonia-infused rats (caused mainly by decreased leucine incorporation in proteins) could explain the marked increase in plasma BCAA levels after a protein meal in hyperammonemic patients,[26] despite unchanged or rather increased leucine clearance.

A further interesting observation, which indirectly supports the idea that ammonia is the responsible factor in decreasing BCAA levels in cirrhosis, is an inverse alteration in the concentrations of glutamine (increase) and alanine (decrease) in plasma and skeletal muscle. This finding is in agreement with others.[18] The cause of these interesting changes in alanine and glutamine levels is undoubtedly related to the effect of ammonia on synthesis of these amino acids. Nonhepatic tissues, predominantly skeletal muscle, can detoxify ammonia by amidation of glutamate to glutamine,[27] and it was shown that glutamine synthesis is limited by the rate of glutamate accumulation and not by the activity of glutamine synthetase.[28] The main source of nitrogen for synthesis of glutamate from α-ketoglutarate are the BCAA, and it has been suggested that hyperammonemia intensifies BCAA utilization via stimulation of glutamine synthesis.[28,29] As BCAA are known also as essential donors of nitrogen for synthesis of alanine from pyruvate in skeletal muscle, the increased demands for glutamic acid caused by hyperammonemia should decrease the alanine synthesis rate and alanine concentration both in muscle and blood. However, it should be emphasized that the necessity of BCAA for ammonia detoxification, as explained above, cannot be the crucial cause of reduced plasma BCAA levels. The principal reason is that the BCAA aminotransferase reaction, which is important for synthesis of sufficient amounts of glutamate from α-ketoglutarate, is reversible. The BCAA can be resynthesized from branched-chain ketoacids (BCKA) in several tissues of the body, particularly in the liver.[30]

The mechanism by which hyperammonemia induces the observed changes in leucine and protein turnover is not clear. Studies using rat neocortical slices showed that ammonium chloride, which is used as an inhibitor of lysosomal protein degradation, was shown to also strongly inhibit protein synthesis.[31] Other speculation can be based on results of our recent study, in which alanyl-glutamine caused a significant decrease in proteolysis and decreased BCAA levels in plasma.[32] This finding led us to propose that the observed decrease in proteolysis in ammonia-infused rats can be, at least in part, related to the observed increase in glutamine levels. In addition, alanyl-glutamine caused a significant decrease in leucine oxidation and leucine oxidized fraction. These observations indicate that the catabolism of BCAA can be affected by the administration of the products of BCAA aminotransferase reaction.

We conclude that hyperammonemia induces the decrease in whole-body protein turnover and the decrease of BCAA in body fluids. We hypothesize that the decrease in BCAA levels is related to decreased proteolysis and increased BCAA oxidized fraction.

Table 4. Effect of Ammonia on Fractional Rate of Protein Synthesis in Various Tissues

	Saline (n = 9)	Sodium Salts (n = 8)	Ammonium Salts (n = 11)	ANOVA *P* Value
Blood plasma	51.1 ± 9.4	39.0 ± 6.0	52.3 ± 5.7	.3890
Liver	4.74 ± 0.97	5.19 ± 0.61	5.63 ± 0.55	.6782
Skeletal muscle	0.60 ± 0.11	0.55 ± 0.05	0.31 ± 0.02*†	.0063
Kidney	13.3 ± 0.4	16.9 ± 1.8	12.9 ± 0.9	.0416
Jejunum	14.6 ± 1.7	15.1 ± 1.6	15.2 ± 1.2	.9597
Colon	3.65 ± 0.36	3.87 ± 0.50	4.35 ± 0.50	.5332
Heart	1.77 ± 0.20	1.68 ± 0.32	1.26 ± 0.45	.1898
Spleen	7.42 ± 0.60	7.91 ± 0.53	9.33 ± 0.81	.1336

NOTE. Fractional rate of protein synthesis is expressed as the fraction of protein mass renewed each day (%/day). Means ± SEM, ANOVA, and Bonferroni test.

*$P < .05$ *v* saline.

†$P < .05$ *v* sodium salts.

Table 5. Effect of Ammonia on Amino Acid Concentrations in Tissues (μmol/g)

	Saline (n = 9)	Sodium Salts (n = 8)	Ammonium Salts (n = 11)	ANOVA *P* Value
Liver				
BCAA	1.26 ± 0.19	1.09 ± 0.09	1.20 ± 1.10	.6917
Glu	1.12 ± 0.07	0.95 ± 0.04	1.27 ± 0.11	.0628
Gln	0.82 ± 0.07	0.73 ± 0.07	0.78 ± 0.06	.6421
Ala	1.52 ± 0.13	1.45 ± 0.05	2.00 ± 0.21	.0470
Muscle				
BCAA	0.72 ± 0.06	0.66 ± 0.04	0.48 ± 0.02*†	.0007
Glu	0.84 ± 0.05	0.79 ± 0.04	0.85 ± 0.02	.5754
Gln	2.99 ± 0.17	2.36 ± 0.25	4.16 ± 0.28*†	.0001
Ala	1.40 ± 0.07	1.39 ± 0.06	1.17 ± 0.04*†	.0091
Kidney				
BCAA	5.30 ± 0.27	6.49 ± 0.48	5.55 ± 0.50	.1807
Glu	4.55 ± 0.72	4.66 ± 0.61	4.40 ± 0.68	.9647
Gln	5.80 ± 0.53	5.09 ± 0.66	5.32 ± 0.37	.6222
Ala	7.03 ± 0.72	7.85 ± 0.60	7.75 ± 0.67	.6655
Jejunum				
BCAA	1.71 ± 0.16	1.57 ± 0.15	1.51 ± 0.16	.6410
Glu	1.04 ± 0.08	1.03 ± 0.06	1.06 ± 0.07	.9566
Gln	2.53 ± 0.27	2.19 ± 0.27	2.24 ± 0.24	.6268
Ala	1.44 ± 0.11	1.50 ± 0.07	1.47 ± 0.09	.9125
Colon				
BCAA	1.20 ± 0.11	1.05 ± 0.15	1.15 ± 0.12	.7366
Glu	1.16 ± 0.03	1.23 ± 0.06	1.18 ± 0.05	.5386
Gln	4.37 ± 0.35	4.17 ± 0.31	4.40 ± 0.30	.8625
Ala	1.31 ± 0.06	1.37 ± 0.11	1.27 ± 0.07	.6657
Heart				
BCAA	0.82 ± 0.09	0.80 ± 0.09	0.55 ± 0.04*	.0206
Glu	1.26 ± 0.06	1.24 ± 0.04	1.16 ± 0.03	.2781
Gln	7.83 ± 0.63	6.79 ± 0.20	7.21 ± 0.16	.1935
Ala	1.51 ± 0.10	1.44 ± 0.04	1.26 ± 0.05*	.0369
Spleen				
BCAA	1.78 ± 0.16	1.78 ± 0.10	2.09 ± 0.12	.1599
Glu	1.31 ± 0.08	1.23 ± 0.07	1.28 ± 0.07	.7598
Gln	4.93 ± 0.47	5.34 ± 0.32	5.41 ± 0.24	.5836
Ala	1.52 ± 0.12	1.56 ± 0.08	1.57 ± 0.07	.9312

NOTE. Means ± SEM, ANOVA, and Bonferroni test.

*$P < .05$ *v* saline.

†$P < .05$ *v* sodium salts.

ACKNOWLEDGMENT

We are grateful for the technical support of I. Altmannová, J. Hofmanová, L. Kriesfalusyová, and R. Ryšavá. Many thanks to Connie Bennett for proofreading of the English grammar.

REFERENCES

1. Flock EV, Mann FC, Bollman JL: Free amino acids in plasma and muscle following total removal of the liver. J Biol Chem 192:293-300, 1951
2. Iber FL, Rosen H, Levenson SM, et al: The plasma amino acids in patients with liver failure. J Lab Clin Med 50:417-25, 1957
3. Fulks RM, Li JB, Goldberg AL: Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. J Biol Chem 250:290-298, 1975
4. Buse MG, Reid SS: Leucine. A possible regulator of protein turnover in muscle. J Clin Invest 56:1250-1261, 1975
5. Oldendorf WH: Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. Am J Physiol 221:1629-1639, 1971
6. Fischer JE, Funovics J, Aguirre A, et al: The role of plasma amino acids in hepatic encephalopathy. Surgery 78:276-290, 1975
7. Fischer JE, Rosen HM, Ebeid AM, et al: The effect of normalization of plasma amino acids on hepatic encephalopathy in man. Surgery 80:77-91, 1976
8. Lautz HU, Selberg O, Korber J, et al: Protein-calorie malnutrition in liver cirrhosis. Clin Investig 70:478-486, 1992
9. Blonde-Cynober F, Aussel C, Cynober L: Abnormalities in branched-chain amino acid metabolism in cirrhosis: Influence of hormonal and nutritional factors and directions for future research. Clin Nutr 18:5-13, 1999
10. Munro H, Fernstrom D, Wurtman R: Insulin, plasma amino acid imbalance and hepatic coma. Lancet 1:722-724, 1975
11. Soeters P, Fischer J: Insulin, glucagon, amino acid imbalance and hepatic encephalopathy. Lancet 2:888-892, 1976
12. Petrides AS: Regulation by insulin of branched chain amino acid metabolism in cirrhosis. Ital J Gastroenterol 25:144-150, 1993
13. Yamato M, Muto Y, Yoshida T, et al: Clearance rate of plasma branched-chain amino acids correlates significantly with blood ammonia level in patients with liver cirrhosis. Int Hepatol Commun 3:91-96, 1995
14. Marchesini G, Zoli M, Dondi C, et al: Ammonia-induced changes in pancreatic hormones and plasma amino acids in patients with liver cirrhosis. Digest Dis Sci 27:406-412, 1982
15. Soeters PB, de Boer J: Why are plasma branched chain amino acid levels diminished in patients with liver cirrhosis, in Adibi SA, Fekl W, Langenbeck U, et al (eds): Branched Chain Amino and Keto Acids in Health and Disease. Farmington, CT, Karger, 1984, pp 483-496
16. Eriksson LS, Soderman C: Influence of beta blockade on branched chain amino acid concentrations in cirrhosis. Hepatogastroenterology 39:454-454, 1992
17. Hayashi M, Ohnishi H, Kawade Y, et al: Augmented utilization of branched-chain amino acids by skeletal muscle in decompensated liver cirrhosis in special relation to ammonia detoxication. Gastroenterol Jap 16:64-70, 1981
18. Leweling H, Breitkreutz R, Behne F, et al: Hyperammonemia-induced depletion of glutamate and branched-chain amino acids in muscle and plasma. J Hepatol 25:756-762, 1996
19. Gimmon Z, James JH, Von Meyenfeldst M, et al: Opposing effects of prolonged ammonia and branched chain amino acid infusions on the accumulation of aromatic amino acids by brain, in Walser M, Williamson JR (eds): Metabolism and Aclinical Implications of Branched Chain Amino Acids and Ketoacids, vol 18. New York, NY, Elsevier, 1981, pp 487-492
20. Holeček M, Tilšer I, Skopec F, et al: Leucine metabolism in rats with cirrhosis. J Hepatol 24:209-216, 1996
21. Holeček M, Tilšer I, Skopec F, et al: Leucine metabolism in partially hepatectomized rats. J Hepatol 26:1141-1147, 1997
22. Holeček M, Šprongl L, Skopec F, et al: Leucine metabolism in TNF-α- and endotoxin-treated rats: Contribution of hepatic tissue. Am J Physiol 273:E1052-E1058, 1997
23. Garlick PJ, Millward DJ, James WPT: The diurnal response of muscle and liver protein synthesis in vivo in meal-fed rats. Biochem J 136:935-945, 1973
24. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951
25. Harper AE, Miller RH, Block KP: Branched-chain amino acid metabolism. Annu Rev Nutr 4:409-454, 1984
26. Marchesini G, Bianchi G, Zoli M, et al: Plasma amino acid response to protein ingestion in patients with liver cirrhosis. Gastroenterology 85:283-290, 1983
27. Duda GD, Handler P: Kinetics of ammonia metabolism in vivo. J Biol Chem 232:303-314, 1958
28. Waniewski RA: Physiological levels of ammonia regulate glutamine synthesis from extracellular glutamate in astrocyte cultures. J Neurochem 58:167-174, 1992
29. Hod G, Chaouat M, Haskel Y, et al: Ammonia uptake by skeletal muscle in the hyperammonemic rat. Eur J Clin Invest 12:445-450, 1982
30. Holeček M, Šprongl L, Tichý M, et al: Leucine metabolism in rat liver after a bolus injection of endotoxin. Metabolism 47:681-685, 1998
31. Schott K, Poetter U, Neuhoff V: Ammonia inhibits protein synthesis in slices from young rat brain. J Neurochem 42:644-646, 1984
32. Holeček M, Skopec F, Skalská H, et al: Effect of alanyl-glutamine on leucine and protein metabolism in endotoxemic rats. JPEN (in press)