Zeitschrift für
Ernährungswissenschaft Z Ernährungswiss 28:149–172 (1989)

Intra- and extracellular amino acid concentrations in portacaval-shunted rabbits. Role of hyperammonemia and effects of branched-chain amino acid-enriched parenteral nutrition*)

H. Leweling, U. Staedt, J.-P. Striebel, R. Zeitz, and E. Holm

Dept. of Pathophysiology, Medical Clinic I, Mannheim, and Institute of Anesthesiology Mannheim, University of Heidelberg, FRG

Summary: Intra- and extracellular amino acid concentrations were measured in rabbits in order to elucidate the possible role of hyperammonemia in lowering the postabsorptive plasma levels of branched-chain amino acids (BCAA) and to assess the effects of BCAA-enriched total parenteral nutrition (TPN) on the amino acid pattern of muscle. The pathophysiological part of this paper deals with portacaval anastomosis (PCA) and is aimed at substantiating or rejecting our hypothesis that excessive ammonia – by stimulating glutamine synthesis – reduces the intracellular glutamate pool which is then restored, at least in part, by an intensified BCAA degradation. Regarding infusion therapy, we were mainly interested in whether an amino acid solution adapted to the metabolism in liver cirrhosis causes an accumulation of BCAA in muscle or modifies the intracellular content of glutamate and glutamine.

Eighteen rabbits did not undergo surgery and served as controls (group A), while 30 were given a portacaval end-to-side anastomosis (group B). Two weeks after creating the PCA, venous blood samples were taken and muscle biopsies (Bergström's technique) were performed postabsorptively. An 18-h TPN was then started, the regimen administered included dextrose, fat and, in addition, either a conventional (group B 1, $n = 15$) or an adapted amino acid solution (group B 2, $n = 15$). We obtained second blood specimens and muscle biopsies at the end of the infusion period. With the control animals, the same time schedule for blood sampling and muscle biopsies was followed.

Fourteen days after the operation, the PCA rabbits displayed a mean plasma ammonia level 5.1 times higher than that measured in the controls ($p \leq 0.001$). Conventional blood chemistry did not reveal any impairment of liver cell integrity or over-all hepatic function, whereas the nutritional state of the shunted animals worsened, as indicated by body weight and biochemical variables. Since in the PCA rabbits, the total amino acid pools of muscle and plasma were seen to be increased and decreased, respectively, the results concerning the individual amino acids are given in terms of both the absolute and percentage values, the latter more often revealing high levels of statistical significance. PCA induced a marked rise in the intra- and extracellular concentrations of glutamine, while the values of glutamate and alanine showed a decline in muscle and plasma. The extracellular levels of

*) Herrn Prof. Dr. med. Karl Heinz Bässler zum 65. Geburtstag gewidmet

methionine, phenylalanine, and tyrosine were raised, while those of the BCAA were diminished. During TPN, the intracellular concentrations of glutamate and glutamine fell in the animal group B 1 but remained stable in group B 2. Only a slight and insignificant accumulation of BCAA occurred in the latter group. Secondary findings confirmed that conventional TPN aggravates the pre-existing increase in the plasma levels of methionine and phenylalanine, and that BCAA-enriched TPN results in almost normalized values of these amino acids.

In conclusion, the PCA-induced alterations in the intracellular amino acid concentrations support the above-formulated hypothesis concerning the role of hyperammonemia in lowering plasma BCAA values, the preferential maintenance of intra-, rather than extracellular BCAA levels being a well-known fact. The largely stable concentrations of BCAA in muscle during BCAA-enriched TPN may indicate in intensified utilization of these amino acids, which probably involves ammonia detoxification. The observation that this type of TPN prevented a fall in the muscular levels of glutamate and glutamine fits into this concept.

Zusammenfassung: In der vorliegenden Studie an Kaninchen wurden intra- und extrazelluläre Aminosäurenkonzentrationen gemessen, um erstens zu untersuchen, ob die postabsorptive Verminderung der verzweigtkettigen Aminosäuren (VAS) im Plasma durch die Hyperammoniämie bedingt oder mitbedingt sein dürfte, und zweitens festzustellen, wie ein total parenterales Ernährungsregime mit hohem Gehalt an VAS das muskuläre Aminosäurenmuster beeinflußt. Gegenstand des pathophysiologischen Teils der Arbeit ist der Stoffwechsel nach Anlage einer portokavalen Anastomose (PKA). Hier geht es um die Stützung oder Widerlegung unserer Hypothese, daß vermehrt vorhandenes Ammoniak durch Stimulation der Glutaminbildung den intrazellulären Glutamat-Pool verkleinert, und dies mit der Konsequenz eines forcierten Abbaus der VAS zur wenigstens partiellen Erhaltung der Glutamatkonzentration. Was die Infusionstherapie betrifft, so interessierte vorrangig, ob eine den metabolischen Besonderheiten bei Leberzirrhose angepaßte Aminosäurenlösung die VAS in der Muskulatur akkumulieren läßt und ob eine solche Lösung die intrazellulären Glutamat- bzw. Glutaminspiegel modifiziert.

Von insgesamt 48 Kaninchen dienten 18 nicht operierte als Kontrolltiere (Gruppe A), während 30 eine portokavale End-zu-Seit-Anastomose erhielten (Gruppe B). Zwei Wochen nach Herstellung der PKA wurden postabsorptiv venöse Blutproben entnommen und – nach der Methode von Bergström et al. – Muskelbiopsien durchgeführt. Unmittelbar danach begann eine 18stündige totale parenterale Ernährung (TPE). Wir applizierten als Hauptnährstoffe Glukose, Fett und ein Aminosäurengemisch, wobei das letztere entweder eine konventielle (Gruppe B 1, $n = 15$) oder eine adaptierte Zusammensetzung hatte (Gruppe B 2, $n = 15$). Gegen Ende der Infusionsphase erfolgten zum zweitenmal Blutabnahmen und Muskelbiopsien. Die Kontrolltiere unterlagen bezüglich der Blut- und Muskelproben dem gleichen Zeitplan wie die operierten.

14 Tage nach der Operation boten die Kaninchen mit PKA im Schnitt einen 5,1fach höheren Ammoniakspiegel als die Kontrolltiere ($p < 0{,}001$). Die konventionellen blutchemischen Befunde ergaben keinen Hinweis auf eine Beeinträchtigung der Leberzellintegrität oder des allgemeinen Funktionsniveaus der Leber. Demgegenüber hatte sich der Ernährungszustand nach Anlage des Shunts verschlechtert (Körpergewicht, biochemische Parameter). Da bei den Tieren mit PKA die Gesamtmenge der Aminosäuren in den Muskelzellen vermindert und im Plasma vermehrt war, werden in dieser Arbeit die Spiegel der einzelnen Aminosäuren doppelt angegeben, nämlich als Absolut- und als Prozentwerte; die prozentualen Konzentrationen zeigten häufiger als die absoluten hochsignifikante Veränderungen. Die PKA verursachte eine erhebliche Zunahme der intra- und extrazellulären Glutaminspiegel, während für Glutamat und Alanin in der Muskulatur sowie im Plasma herabgesetzte Werte resultieren. Die Spiegel von Methionin, Phenylalanin und

Tyrosin waren extrazellulär gesteigert, die der VAS verringert. Unter der TPE fielen intrazellulär die Konzentrationen von Glutamin und Glutamat in der Gruppe B 1 ab, blieben jedoch in der Gruppe B 2 konstant; die intrazellulären VAS akkumulierten in der letzten Gruppe nur sehr gering und ohne Signifikanz. Darüber hinaus bestätigten die Versuche, daß eine Standard-TPE bereits bestehende Konzentrationserhöhungen von Methionin und Phenylalanin im Plasma deutlich akzentuiert, während ein mit VAS angereichertes Infusionsregime die Spiegel dieser Aminosäuren annähernd in die Referenzbereiche bringt.

Wir meinen, daß die durch eine PKA herbeigeführte Abweichungen des intrazellulären Aminosäurenmusters die eingangs formulierte Hypothese stützen, derzufolge die Hyperammoniämie zur Senkung der Plasmaspiegel der VAS zumindest beiträgt; dabei darf die bekannte Tendenz des Organismus, eher den intra- als den extrazellulären Pool der VAS aufrechtzuerhalten, nicht außer acht bleiben. Die bei TPE mit hohem Angebot an VAS weitgehend unbeeinflußten Konzentrationen derselben in der Muskulatur sprechen für eine intensivierte Nutzung dieser Aminosäuren, unter anderem wohl zum Zweck der Ammoniakentgiftung. Im übrigen paßt die Beobachtung, daß eine Abnahme der intrazellulären Spiegel von Glutamat und Glutamin durch die adaptierte TPE verhindert werden konnte, in das vorgetragene Konzept.

Key words: PCA, ammonia, amino acids, muscle, TPN

1 Introduction

The intracellular amino acid pattern of muscle in animals with chronic hyperammonemia could help either to substantiate or to reject the hypothesis that excessive ammonia contributes to or is even mainly responsible for the decrease in the plasma levels of branched-chain amino acids (BCAA) in patients suffering from liver cirrhosis (57). According to this hypothesis, hyperammonemia indirectly lowers the extracellular BCAA concentrations by stimulating glutamine synthesis; the greatly increased formation of glutamine is thought to reduce the intracellular

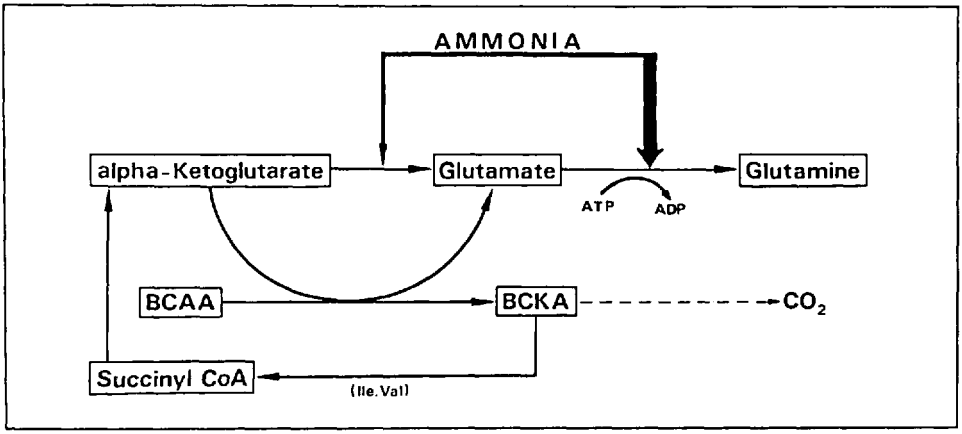

Fig. 1. Postulated influence of ammonia on the metabolism and, thereby, on the plasma levels of BCAA (for details see text).

glutamate pool, which can be restored, at least in part, by an intensified BCAA transamination (Fig. 1). Besides this transamination process, a supply – via succinate – of the alpha-ketoglutarate carbon skeleton by degradation of valine and isoleucine could, theoretically, participate in replenishing the glutamate pool (Fig. 1). The main elements of the "ammonia hypothesis" were, to our knowledge, first formulated by Hayashi and co-workers (31).

Several groups of authors consider the deficit of BCAA in plasma to be brought about by hormonal alterations, with insulin rather than glucagon playing a decisive role in reducing BCAA (50, 66, 89, 90). Arguments for and against this view have been thoroughly discussed elsewhere (39, 41). Since BCAA metabolism takes place predominantly in peripheral tissues (1, 2, 5, 27, 29, 63, 69, 90), the effects of insulin on peripheral BCAA uptake, metabolism (particularly in adipose tissue) and release (19, 24, 26, 27, 66, 72, 89, 95) could, in fact, contribute to the subnormal values of plasma BCAA in liver cirrhosis, at least in the sense of "longterm control" (90). This, however, would not exclude hyperammonemia having an additional, substantial influence on BCAA concentrations. Insofar as we are dealing with experimental portacaval anastomosis (PCA), it appears important to emphasize that an extensive portal-systemic circulation of blood hardly affects the fate or action of insulin (6, 53, 71, 88), but is, in humans as well as in animals, invariably associated with hyperammonemia (12, 15, 36, 41, 55) and also with a decline in plasma BCAA levels (36, 38, 39, 41, 46, 55). The shunt-induced changes in the ammonia and BCAA concentrations can be observed in the absence of any impairment or deterioration of hepatocellular function (13, 36, 41, 55). Fulminant hepatic failure, both in its clinical (74, 77) and experimental form (36, 37, 38, 43), is accompanied by normal or even raised BCAA values in plasma.

Apart from the pathophysiological findings described in this paper, our experiments shine some light on aspects on infusion therapy, since the PCA animals underwent total parenteral nutrition (TPN) which included either a conventional amino acid solution or an amino acid mixture adapted to the metabolism of cirrhotic patients. Whereas the effects of the so-called Hepa solutions on extracellular amino acid levels have been sufficiently recognized and reviewed (15, 16, 18, 35, 40), no information seems to be available as to whether TPN high in BCAA causes an accumulation of these amino acids in muscle or modifies the intracellular content of glutamate and glutamine.

2 Materials and methods

2.1 Animal groups and study design

Experiments were carried out on a total of 48 male Chinchilla-Bastard rabbits weighing, on average, 3.1 kg. The rabbits had been bred in the Ivanovas Institute, Kißlegg/Allgäu. They were kept in single cages. In our fully air-conditioned animal house the temperature was maintained at 21 ± 2 °C. To simulate a day/night rhythm, an automatic light-switch system was installed, with a dark period from 1800 to 0600 hrs. Altromin was given as standard diet.

Eighteen animals did not undergo surgery and served as controls (group A), while 30 were given a portacaval end-to-side anastomosis (group B). Fourteen days after

the surgical procedure, venous blood samples were taken and muscle biopsies were performed at 0800 hrs in the postabsorptive state. On the same day, at 1600 hrs, an 18-h TPN was started, the nutritional regimen including either a conventional amino acid solution (group B 1, n = 15) or an amino acid mixture adapted to the metabolism typical of liver cirrhosis (group B 2, n = 15). We obtained second blood specimens and muscle biopsies at the end of the infusion period. The animals were then sacrificed by means of a narcotic drug. In the control rabbits, the same time schedule as above was followed for blood sampling and muscle biopsies.

2.2 Portacaval shunt operation

As pre-medication, azepromazine maleate was used (0.2 mg/kg i.m.). Anesthesia was started by an injection of ketamine hydrochloride into the most prominent vein of the ear (25 mg/kg) and was continued by administering pentobarbital intravenously at appropriate intervals. Normal body temperature was maintained throughout by a heating blanket. After the operation, an intravenous infusion compensated for loss of water and electrolytes.

In brief, the operation to construct an end-to-side portacaval anastomosis consisted of the following steps: aseptic laparotomy; mobilization and moistening of the stomach and parts of the bowel; preparation of the portal vein with elimination of two incoming venous tributaries; exposure of the cava inferior; ligation of the portal vein (at a distance of 1–2 cm from the liver); clamping of the caval vein; end-to-side portacaval anastomosis. After having opened the caval vein, we infused 8.4 % sodium bicarbonate, the dosage of 5–6 ml having been chosen as a result of pH determinations in preceding experiments. Following the suturing of the peritoneal cavity, the rabbits were able to breathe spontaneously. The total time required for anesthesia was 90–120 min, while the ligation of the portal and caval veins took 25–45 min. One or two days after the operation, the majority of the animals began eating almost normally. Group B animals were weighed both at the beginning of the experiments and two weeks after construction of the PCA, i.e., immediately before the onset of TPN.

2.3 Parenteral nutrition

TPN was administered as an "all-in-one system" through a catheter implanted in the central ear vein of the rabbits, while they were sitting in a pyrogen test box (Ehret Company). The regimen contained an amino acid solution (16 kcal %), 50 % dextrose (60 kcal %), a 10 % fat emulsion (Intralipid, 24 kcal %), and electrolytes (sodium chloride and potassium phosphate). The amount of calories (3.4 kcal/kg × h) as well as the glucose/fat caloric ratio were the same in all animals. However, while group B 1 rabbits were given a conventional amino acid solution (Aminosteril KE, 10 %), a modified amino acid mixture was used in group B 2 rabbits (Aminosteril Hepa, 8 %), this modified mixture being low in methionine, phenylalanine, and tryptophan but high in BCAA (42 %)[1]). The composition of the amino acid solutions can be seen from Table 1. The volumes administered were kept constant by means of a pump (Perfusor Unita[1]), Braun Melsungen, Inc.). We placed bacterial filters with pores of 0.22 μ diameter between the infusion lines and the animals.

2.4 Muscle biopsies, blood samples, and analysis of intracellular amino acids

To prepare the rabbits for the muscle biopsies, the same pre-medication and anesthetic agents were chosen as for the shunt operations. After shaving the lateral thigh, a skin incision was made and a specimen was obtained from m. quadriceps femoris using the biopsy needle developed by Bergström et al. (4). Thereafter, a

[1]) Both amino acid solutions produced by Fresenius AG, Oberursel, FRG.

Table 1. Composition of the two amino acid (AA) solutions (grams/1,000 ml) given as part of TPN to the PCA rabbits.

	Conventional AA Solution, 10 %	Modified AA Solution, 8 %
Essential amino acids		
Isoleucine	5.00	13.00
Leucine	7.40	16.36
Lysine	6.60	8.60
Methionine	4.30	1.38
Phenylalanine	5.10	1.10
Threonine	4.40	5.50
Tryptophan	2.00	0.88
Valine	6.20	12.60
Sum	41.00	59.42
Non-essential amino acids		
Arginine	12.00	13.40
Histidine	3.00	3.50
Cysteine	0	0.65
Tyrosine	0	0
Proline	15.00	7.16
Alanine	15.00	5.80
Glutamate	0	0
Glycine	14.00	7.28
Ornithine aspartate	0	0
Ornithine	0	0
Aspartate	0	0
Asparagine	0	0
Serine	0	2.80
Sum	59.00	40.59

blood sample was drawn through a catheter which had been placed in the inferior caval vein.

For determining the concentrations of free amino acids in intracellular water of muscle, we employed the Bergström procedure (4), with chloride assessed by means of the following instruments: TTA 80, ABU 12, pH meter 26, Titrator 11; (Radiometer Company, Copenhagen).

2.5 Measurements in plasma and serum

A new reflectometric micromethod, the "Ammonia Checker System" (Kyoto Daiichi Kagaku, Japan) was modified in our department (60, 96) and used for measuring plasma ammonia. As described elsewhere, we found coefficients of variation ranging from 4.0 % to 6.8 % in serial analyses (60). To prepare the measurement of free amino acid levels in venous plasma, blood samples were deproteinized with sulfosalicylic acid and deep frozen. Analyses were done on a Biotronic LC 5001 instrument (93).

Apart from plasma ammonia, conventional blood chemistry comprised GPT, GOT, lactate dehydrogenase, albumin, prothrombin time (Quick's test), triglycerides, cholesterol, bilirubin, alkaline phosphatase, gamma-GT, glucose, urea, and creatinine. The Institute of Clinical Chemistry, Mannheim, carried out the analyses

Table 2. Standard biochemical variables (mean ± SD) measured in the rabbits without PCA (controls) and in those with PCA two weeks after starting the experiments.

Variables	Group A Controls $\bar{x} \pm s$	Group B PCA $\bar{x} \pm s$
Ammonia, µg/l	73 ± 36	375 ± 125
GPT, U/l	28 ± 10	34 ± 15
GOT, U/l	8 ± 8	11 ± 8
LDH, U/l	151 ± 106	70 ± 40
Albumin, g/l	38 ± 3	35 ± 3
Quick's test, %	58 ± 30	72 ± 23
Triglycerides, mg/dl	132 ± 68	96 ± 45
Cholesterol, mg/dl	41 ± 25	118 ± 62
Bilirubin, mg/dl	0.10 ± 0	0.12 ± 0
Alkaline phosphatase, U/l	100 ± 64	54 ± 17
gamma-GT, U/l	3.2 ± 1	3.4 ± 2
Glucose, mg/dl	152 ± 30	143 ± 17
Urea, mg/dl	38 ± 13	41 ± 17
Creatinine, mg/dl	1.2 ± 0	1.4 ± 0

using standard procedures. Blood coagulation is faster in the rabbit than in man. Therefore, blood conservation for the purpose of determining the prothrombin time had to be modified (0.4 ml of citrate, 1.0 ml of 0.9 % NaCl, 0.6 ml of blood).

2.6 Statistics

Error probabilities for inter-group differences concerning plasma ammonia as well as the amino acid values in the compartments investigated were calculated by means of the Wilcoxon, Mann and Whitney U-test. This test was also employed for estimating significant differences between the effects of the two amino acid solutions infused in PCA rabbits.

3 Results

3.1 Changes in body weight and in standard biochemistry after the shunt operation

Initial body weight of the rabbits forming group B was 3.21 ± 0.17 kg (mean ± SD). Two weeks later, it was found to be reduced to 2.70 ± 0.27 kg. This means an average weight loss of 1.2 ± 0.49 % per day.

In Table 2, the animal groups A and B are compared with regard to standard biochemical variables obtained two weeks after starting the experiments. At that time, the PCA rabbits displayed a mean plasma ammonia level 5.1-times higher than that of the rabbits without PCA ($p < 0.001$). The transaminases had mean activities within the reference ranges of the controls. Lactate dehydrogenase was even lower in group B than in group A. Considering the parameters of hepatic function, the

Table 3. Postabsorptive pool sizes of total, essential and non-essential amino acids (μmol/l, mean ± SEM) in plasma (P) and muscle (M) of the rabbits without PCA (controls) and of those with PCA. */** $p \leq 0.05/0.01$ vs controls.

Groups of amino acids (AA)		Controls	PCA
Total AA	P	2,933 ± 139	3,376 ± 88*
	M	16,998 ± 3,355	14,213 ± 1,102
Essential AA	P	773 ± 32	797 ± 44
	M	2,149 ± 687	1,853 ± 228
Non-essential AA	P	2,160 ± 121	2,579 ± 66**
	M	14,849 ± 2,724	12,360 ± 930

prothrombin time (Quick's test), bilirubin, alkaline phosphatase, and gamma-GT did not indicate any liver cell insufficiency at all. By contrast, the values of those two variables (albumin, triglycerides) which depend on both hepatic function and the nutritional state proved to be moderately decreased. The concentrations of glucose, urea, and creatinine did not significantly differ between the animal groups.

3.2 Changes in intra- and extracellular amino acid levels after the shunt operation

As shown in Table 3, the total plasma amino acid pool was found to be significantly augmented in the PCA rabbits, this being due to a significant rise in the plasma levels of non-essential amino acids. The total amount of essential amino acids measured in plasma remained almost unaffected. In intracellular water of muscle, the concentrations of total and non-essential amino acids had changed in directions which were opposite to those seen in plasma. However, the essential amino acids were also reduced. Our findings pertaining to the three groups of muscle amino acids did not reach statistical significance, since the intracellular amino acid levels varied much more than the values in plasma. In view of the differences between the animal groups A and B regarding the amino acid pool sizes depicted in Table 3, we felt that the results concerning individual amino acids should be given in terms of both the absolute and the percentage values.

The key observations with respect to amino acid groups and individual amino acids are presented in Fig. 2. Percentages of the total amino acid concentrations measured in plasma and muscle are indicated. As to the essential amino acids as a group, the post-shunt stability of their absolute amount in plasma (Table 3) has already been mentioned; however, the percentage amount was significantly diminished (Fig. 2). In addition, the amino acids directly related to ammonia metabolism showed significant deviations from the reference values. Thus, the intra- and extracellular concentrations of glutamine were markedly increased in the PCA rabbits (Fig. 2). By contrast, alanine exhibited lowered levels in muscle as well as in plasma (Fig. 2). In muscle, there was also a reduction in the level of glutamate (Fig. 2).

Table 4 indicates the absolute and percentage concentrations of nine amino acids measured in the rabbits without and in those with PCA in

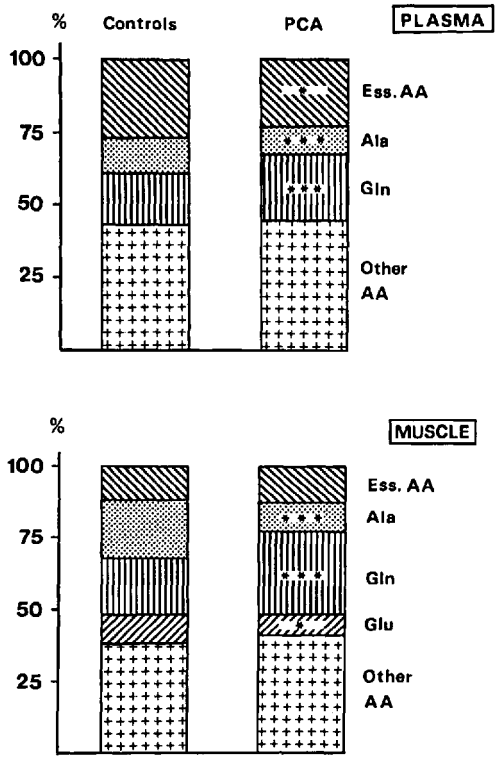

Fig. 2. Postabsorptive extra- and intracellular percentage concentrations (mean values) of amino acid groups and selected individual amino acids in rabbits without PCA (controls) and in those with PCA. */** p = 0.05/0.001 vs control.

plasma as well as in muscle. After creation of the PCA, the levels of methionine, while hardly being influenced in muscle, were moderately and significantly raised in plasma. Phenylalanine and tyrosine showed a pronounced increase in both compartments, with significancies resulting only for the extracellular space. As to the elevated concentrations of the three amino acids mentioned, the error probabilities obtained were lower for the absolute than for the percentage values (Table 4).

The opposite holds true for the deviations in the quantities of valine, leucine, and isoleucine in plasma, in that the observed decrease of these amino acids became significant, particularly in terms of the percentages. In muscle, valine, and isoleucine were diminished, while leucine tended to be augmented; however, none of the differences between control and PCA rabbits regarding intracellular BCAA concentrations proved to be significant (Table 4).

The information on glutamate, glutamine, and alanine given in Fig. 2 is supplemented in Table 4 which, in contrast to Fig. 2, also presents the absolute concentrations and the standard errors. The PC-shunted rabbits displayed a rise in the intra- and extracellular glutamine levels; this applies to the absolute and percentage values, although the deviations of

Table 4. Postoperative absolute and percentage concentrations of selected amino acids (mean ± SEM) in plasma (P) and muscle (M) of the rabbits without PCA (controls) and of those with PCA. */**/*** p = 0.05/0.01/0.001 vs controls.

Amino acids (AA)		µmol/l		Percentage of total AA	
		Controls	PCA	Controls	PCA
Me	P	32 ± 3	49** ± 3	1.1 ± 0.1	1.4* ± 0.1
	M	64 ± 16	57 ± 10	0.4 ± 0.1	0.3 ± 0.1
Phe	P	50 ± 4	63*** ± 2	1.8 ± 0.2	1.9 ± 0.1
	M	95 ± 19	157 ± 46	0.6 ± 0.2	1.0 ± 0.2
Tyr	P	63 ± 7	84** ± 4	2.1 ± 0.2	2.6* ± 0.1
	M	97 ± 20	145 ± 32	0.7 ± 0.2	1.0 ± 0.2
Val	P	180 ± 9	161 ± 11	6.3 ± 0.5	4.7** ± 0.2
	M	544 ± 185	413 ± 97	2.9 ± 0.4	2.9 ± 0.6
Leu	P	107 ± 9	86* ± 8	3.7 ± 0.4	2.5** ± 0.2
	M	162 ± 40	187 ± 30	0.9 ± 0.1	1.3 ± 0.2
Ile	P	66 ± 4	57 ± 5	2.3 ± 0.2	1.7** ± 0.1
	M	237 ± 125	181 ± 48	1.0 ± 0.2	1.3 ± 0.3
Glu	P	30 ± 5	22 ± 1	1.0 ± 0.2	0.7 ± 0.0
	M	1509 ± 233	993* ± 116	9.6 ± 1.0	7.1* ± 0.5
Gln	P	518 ± 33	766*** ± 23	17.7 ± 0.8	22.8*** ± 0.5
	M	3339 ± 642	4301 ± 434	20.2 ± 1.7	29.0*** ± 1.5
Ala	P	345 ± 30	296 ± 14	11.8 ± 0.8	8.7*** ± 0.3
	M	3758 ± 1283	1398*** ± 112	19.5 ± 2.1	10.3*** ± 0.5

the absolute values failed to become significant for muscle. The increase of glutamine was associated with a decrease of glutamate and alanine, predominantly and most significantly in muscle (Table 4).

The intracellular absolute and percentage concentrations of other glycogenic amino acids (threonine, serine, glycine) as well as of basic amino acids (lysine, histidine, ornithine, arginine) did not significantly differ between the control and the PCA animnals.

Table 5. Plasma levels (µmol/l, mean ± SEM) of methionine and two aromatic amino acids in the rabbits without PCA (controls, only postoperative values indicated) and in those with PCA, the latter being given TPN which included either a conventional (solution 1) or a modified amino acid mixture (solution 2). The error probabilities refer to the differences between the effects of the two amino acids solutions administered.

Amino acid	Controls, No TPN	Conventional TPN		Modified TPN		p ≤
		Before	During	Before	During	
Methionine	32 ± 3	52 ± 5	116 ± 10	46 ± 4	34 ± 4	0.001
Phenylalanine	50 ± 4	64 ± 3	87 ± 4	62 ± 3	41 ± 3	0.001
Tyrosine	63 ± 7	81 ± 5	28 ± 4	88 ± 6	14 ± 4	0.05

3.3 Changes in intra- and extracellular amino acid levels during TPN in PCA rabbits: standard vs modified amino acid solution

The levels of methionine, phenylalanine, and tyrosine in intracellular water of muscle were often found to be very low or even unmeasurable. Therefore, in the therapeutic section of the present paper, we will refer only to the plasma values of these amino acids (Table 5). Animal group B 1 received the standard amino acid solution and group B 2 the modified amino acid mixture. Before TPN, both groups displayed elevated plasma concentrations of the amino acids under debate. In group B 1, TPN brought about an excessive rise in the level of methionine and a moderate increase of phenylalanine, while tyrosine was markedly diminished. By contrast, in group B 2 the values of methionine and phenylalanine approximated the reference data during TPN; tyrosine, however, was again reduced to considerably subnormal levels. Thus, leaving tyrosine out of consideration, the conventional amino acid solution strongly accentuated

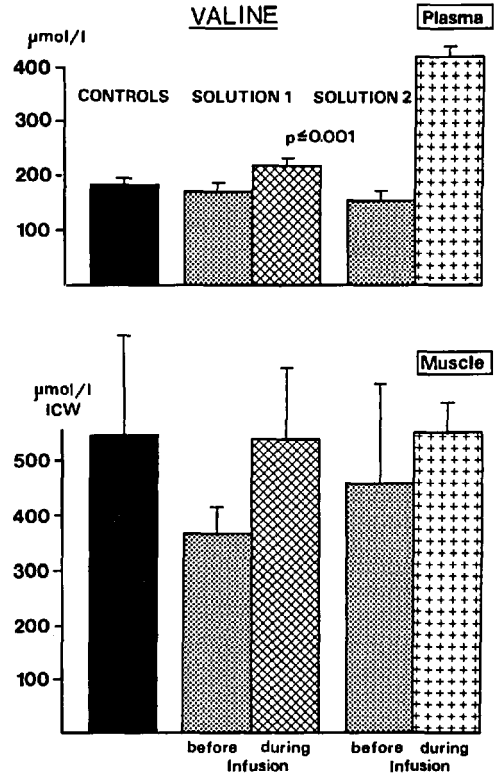

Fig. 3. Extra- and intracellular concentrations of valine (µmol/l, mean ± SEM) in rabbits without PCA (controls, only postabsorptive values indicated) and in those with PCA, the latter being given TPN which included either a conventional (solution 1) or a modified amino acid mixture (solution 2). ICW = intracellular water of muscle. The error probability refers to the difference between the effects of the two amino acid solutions administered.

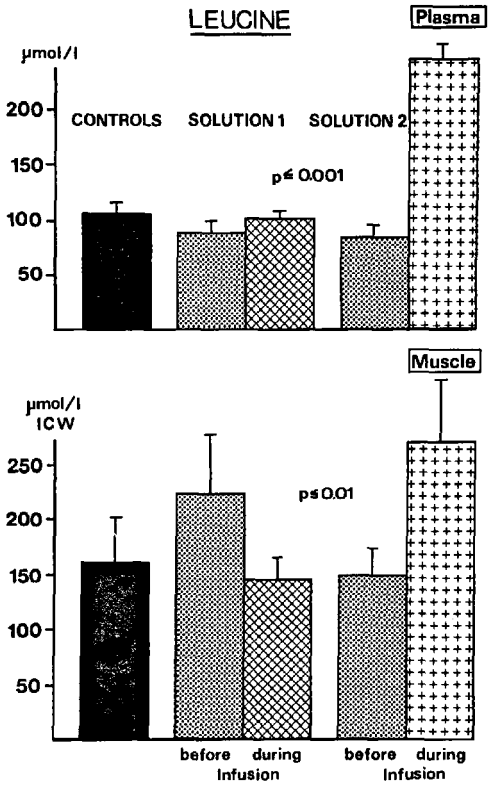

Fig. 4. Extra- and intracellular concentrations of leucine (µmol/l, mean ± SEM) in rabbits without PCA (controls, only postabsorptive values indicated) and in those with PCA, the latter being given TPN which included either a conventional (solution 1) or a modified amino acid mixture (solution 2). ICW = intracellular water of muscle. The error probabilities refer to the differences between the effects of the two amino acid solutions administered.

the already existing deviations from the reference amino acid values measured in group A rabbits, while the modified solution induced a clear-cut tendency towards normalization (Table 5).

The TPN-dependent changes in the intra- and extracellular concentrations of BCAA are demonstrated in Figs. 3–5. The pre-infusion values of valine, leucine, and isoleucine were below those of the control animals in both muscle and plasma, with the exception of leucine in muscle. The two infusion regimens significantly differed from one another regarding their influence on plasma BCAA levels, in that these levels were raised only to a slight extent by regimen 1, but excessively by regimen 2. In muscle, a normalization of valine as well as of leucine and a decrease of isoleucine were seen with the standard amino acid solution, while the modified solution normalized the concentrations of valine and isoleucine, leucine being the only BCAA which exhibited a moderate accumulation (Figs. 3–5). Thus, the adapted form of TPN greatly increased BCAA levels in plasma but did not cause an accumulation of valine and isoleucine in

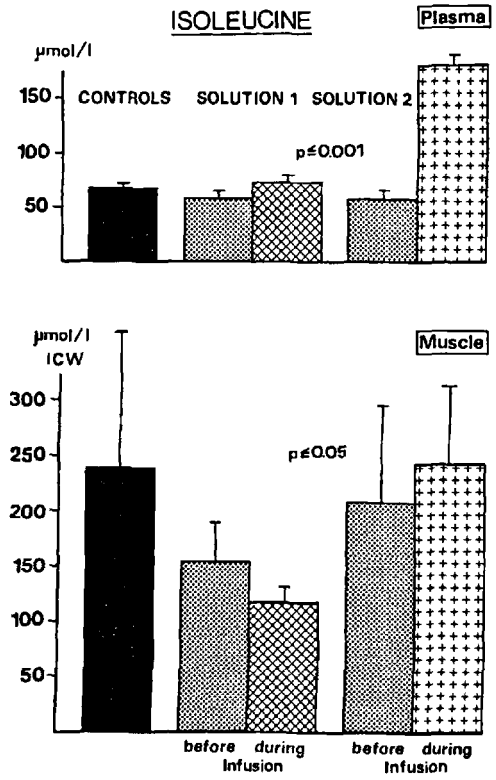

Fig. 5. Extra- and intracellular concentrations of isoleucine (µmol/l, mean ± SEM) in rabbits without PCA (controls, only postabsorptive values indicated) and in those with PCA, the latter being given TPN which included either a conventional (solution 1) or a modified amino acid mixture (solution 2). ICW = intracellular water of muscle. The error probabilities refer to the differences between the effects of the two amino acid solutions administered.

muscle; the raised intracellular level of leucine failed to become statistically significant.

The concentrations of glutamate and glutamine in muscle and plasma observed during the two types of TPN largely paralleled one another (Figs. 6 and 7). Whereas the concentrations in plasma remained virtually unchanged with both amino acid solutions, there were differences regarding the amounts in muscle. Under the influence of the conventional amino acid solution, the intracellular levels of glutamate and glutamine declined. In contrast, the administration of the adapted amino acid mixture was associated with stable intracellular concentrations (Figs. 6 and 7).

4 Discussion

4.1 Liver function and nutritional state

The portacaval shunt operation conducted in rabbits had little, if any influence upon the cellular integrity of the liver; there was no rise in the

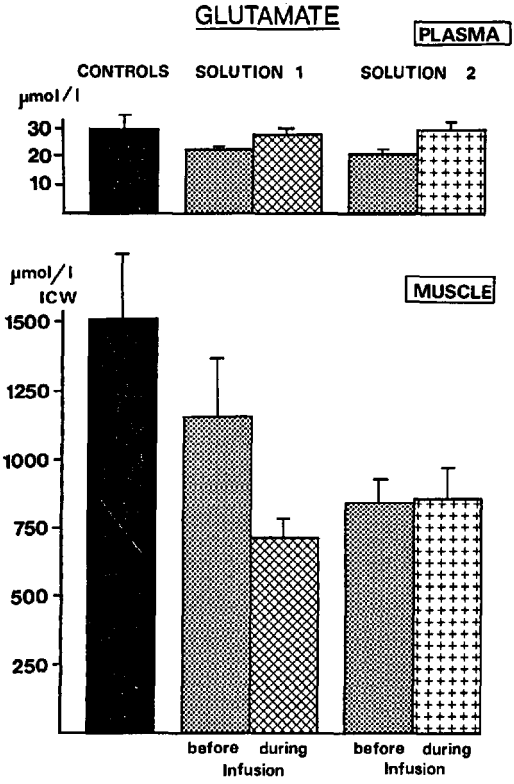

Fig. 6. Extra- and intracellular concentrations of glutamate (µmol/l, mean ± SEM) in rabbits without PCA (controls, only postabsorptive values indicated) and in those with PCA, the latter being given TPN which included either a conventional (solution 1) or a modified amino acid mixture (solution 2). ICW = intracellular water of muscle.

transaminases or in the lactic dehydrogenase activity. Those conventional variables which are indicative of hepatic function, while being largely independent of the nutritional state, i.e., the prothrombin time, bilirubin, alkaline phosphatase, and gamma-GT did not reveal a functional impairment of the liver. Several groups or authors have observed deviations of transaminases, bilirubin, and alkaline phosphatase in shunted animals, particularly in monkeys and – with some exceptions – in dogs (3, 9, 15, 73, 85). Such findings are clearly at variance with ours, probably due to biological pecularities of the species used and to differences in the surgical procedures. However, it has to be considered that the PCA rabbits showed raised plasma concentrations of methionine, phenylalanine, and tyrosine. There is little doubt that, in the postabsorptive state, the levels of these amino acids depend primarily on the functional capacity of the liver, since their catabolism takes place almost exclusively in this organ (3, 38, 63).

Following portacaval shunt operations in various species of animals, a significant rise in the concentration of methionine was established in

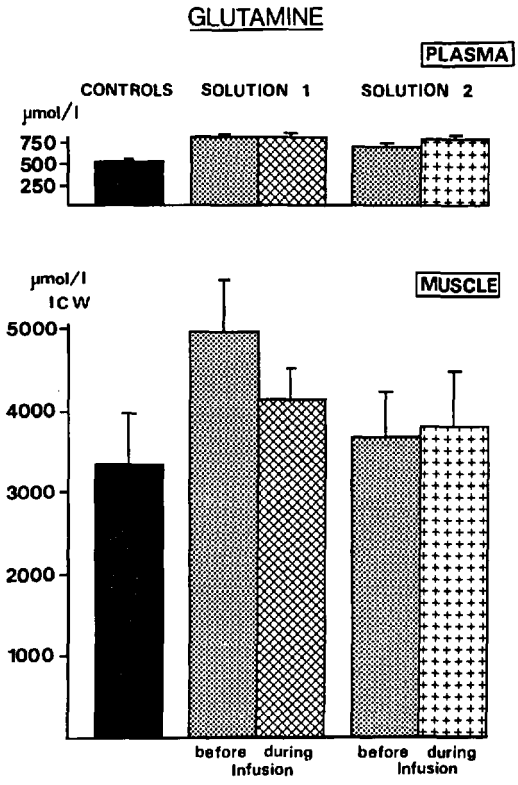

Fig. 6. Extra- and intracellular concentrations of glutamate (µmol/l, mean ± SEM) in rabbits without PCA (controls, only postabsorptive values indicated) and in those with PCA, the latter being given TPN which included either a conventional (solution 1) or a modified amino acid mixture (solution 2). ICW = intracellular water of muscle.

some studies (15, 86), but not found in others (3, 11, 47, 87). In contrast, elevated values of phenylalanine and tyrosine were always noted (3, 11, 14, 15, 47–49, 76, 87). Obviously, a shunt-induced liver cell damage of medium severity increased the concentrations of methionine, phenylalanine, and tyrosine, while a very slight lesion of the liver only raised the concentrations of the aromatic amino acids. One could speculate that portal-systemic shunting per se influences the plasma levels of phenylalanine and tyrosine; however, this certainly does not happen. Cats which had microspheres injected into the portal vein developed portal hypertension; the plasma levels of phenylalanine and tyrosine remained unchanged in these cats (38).

Furthermore, in patients with idiopathic portal hypertension and spontaneous portal-systemic shunting normal concentrations of aromatic amino acids were found (46). In cirrhotic patterns, the determination by hepatic function of aromatic amino acid concentrations in plasma became evident from close correlations between the sum of these amino acids and various liver function tests (32). Apart from liver cell damage, catabolic

states frequently present in advanced liver disease are throught to contribute to increased concentrations of the amino acids under consideration (50, 76, 91).

Our PCA rabbits were similar to shunted animals in other studies in that they showed a reduction in the values of serum albumin and triglycerides (9, 15, 73). With regard to the almost normal functional capacity of the liver in the shunted rabbits, these changes are best explained by a worsening of the nutritional state. After the shunt operation, the rabbits lost weight, as did monkeys, dogs, cats, and rats (3, 9, 15, 41, 44, 56, 73, 83, 87). It is beyond the scope of the present paper to discuss the question of whether the PCA-induced derangement in amino acid patterns contributes to malnutrition.

4.2 Role of hyperammonemia in lowering plasma BCAA levels

It has repeatedly been shown in studies on animals (31, 52, 57, 75, 84, 94) as well as on humans (54) that administering "sufficient" amounts of ammonia salts decreases the plasma concentrations of BCAA. The present experiments were aimed at elucidating possible mechanisms of this effect of hyperammonemia. The intracellular amino acid levels assessed in the PC-shunted rabbits support the hypothesis formulated in the introduction. Increased values of glutamine and decreased values of glutamate as well as of alanine were found to be associated with a deficit of BCAA in plasma, the reduction in the alanine level being explained by diminished availability of glutamate for transamination with pyruvate (7). The objection could be raised that a simultaneous and more significant decline in the intracellular BCAA concentrations should have been expected. Most probably, the organism attempts to maintain constant intracellular rather than extracellular BCAA levels. Such preferential maintenance of muscle BCAA as against plasma BCAA would be in keeping with the observation that, in cirrhotic patients without and with oral BCAA supplementation, the muscle/plasma gradient of BCAA is increased (65). The "altered transcellular distribution" has been ascribed to a change in BCAA transport across the cell membrane (65). In this context it is also of interest that, in patients with liver cirrhosis (70, 78) as well as in animals given a PCA (38, 39), reduced BCAA concentrations were not consistently ascertained in muscle, while being a regular finding in plasma.

Regarding the metabolic sequence outlined in Fig. 1, the literature offers overwhelming evidence in support of the individual steps suggested. In both humans (61) and experimental animals with hyperammonemia (34) muscle takes up a large proportion of the arterial ammonia, although this process can, because of muscle wasting, be reduced in cirrhotic patients (62). Increased plasma levels of ammonia have been noted to be accompanied by hyperglutaminemia as well as by hypoalaninemia (31, 75). These changes in amino acid concentrations obviously result from a rise and a decline in the peripheral output of glutamine and alanine, respectively. Such alterations of glutamine and alanine release have, in fact, been established in both cirrhotic patients (22, 31, 40, 45, 58, 67) and PC-shunted cats (40, 58).

Since glutamine and alanine release from the peripheral tissues is known to be primarily accounted for by de novo-synthesis of these amino

acids (23, 68, 81), an increased formation of glutamine coupled with an at least relatively decreased synthesis of alanine is likely to take place in states of hyperammonemia. As demonstrated by Jaspers et al., glutamine formation in muscle appears to be preferentially regulated by the availability of ammonia (51). Chang and Goldberg showed that ammonia stimulates glutamine production because of greater conversion of glutamate to glutamine; linked with this process is a drop in alanine production (7).

In rats which had undergone ligation of the portal vein, a close correlation between peripheral ammonia uptake and glutamine release has been observed (34). Hyperammonemia activates glutamine synthetase in muscle (30, 34). As a consequence of excessive glutamine formation, the amount of glutamate in muscle tissue (7) and, likewise, in brain tissue (10, 33, 98) has been seen to be diminished. The percentage and, in part, the absolute intracellular levels of glutamine, glutamate, and alanine assessed in our PC-shunted rabbits are in excellent agreement with the findings cited. The same holds true for intracellular amino acid values observed in ammonium-infused rats (57).

Some groups of humans and animals with hyperammonemia did not display the entire pattern of increased intracellular glutamine as well as decreased glutamate and alanine (39, 70, 79). Most probably, the ammonia-induced stimulation of glutamine synthesis can be impeded by a deficiency of energy in states of malnutrition (20, 36). On the other hand, glutamine as a percentage of total glutamate plus glutamine in the cells was found to be augmented in patients with acute and chronic liver disease (80); the same occurred in cats and rats with portacaval anastomosis (36).

The suggestion that the glutamate pool is partly restored by an intensified BCAA degradation cannot be proved by the present results. However, data reported by Goldberg et al. (25) as well as by Odessey et al. (68) indicate a close relationship between BCAA degradation and glutamine synthesis. With respect to hyperammonemia, it should first be stressed that ammonia increases the activity of muscle BCAA aminotransferase (34). Secondly, a BCAA-enriched amino acid solution (not containing glutamate) raised the intracellular glutamate concentration of muscle in PC-shunted cats (39, 92). BCAA also increased the amount of glutamate in brain tissue of dogs (21) and rats (98) with hepatic failure. While there is a multitude of evidence that glutamate production from alpha-ketoglutarate utilizes BCAA-derived amino groups (8, 25, 68), the question of whether, and to what extent, the branched-chain keto acids contribute to the glutamate carbon skeleton can by no means be definitely answered and will remain out of consideration in the present paper.

4.3 Effects of BCAA-enriched parenteral nutrition

It has often been recognized that, in patients with liver cirrhosis, conventional amino acid mixtures aggravate the pre-existing derangement of the plasma amino acid pattern (17, 40, 42), this being true particularly for the elevated concentrations of methionine and phenylalanine. Such effects of standard TPN were also observed in our PCA rabbits, in spite of an only very slight degree of liver function impairment. Thus, in patients

with "mild cirrhosis", similar deviations in the amino acid levels can be expected. BCAA and BCAA-enriched amino acid solutions are known to lower the plasma levels of methionine and aromatic amino acids (16, 35, 42, 59, 97). In the PC-shunted rabbits, this influence of a modified amino acid mixture was confirmed. Since a tremendous decrease of tyrosine occurred in both group B 1 and B 2 animals, this phenomenon appears to be independent of the type of the amino acid solution administered. A TPN-induced deficit of tyrosine has been ascertained by Millikan et al. (64), as well as by Rudman et al. (82) in patients with liver cirrhosis. Peptides containing tyrosine may help to overcome this problem.

The infusion of large quantities of BCAA could be expected to cause an accumulation of BCAA in muscle. This, however, occurred only to a small extent in the rabbits with PCA. The utilization of BCAA for detoxifying ammonia is one of the possible explanations of the almost normal muscle BCAA concentrations during modified TPN.

The intracellular levels of glutamate and glutamine declined with conventional TPN, while the use of the BCAA-enriched amino acid mixture resulted in stable levels of these amino acids. In animals with fulminant hepatic failure, BCAA infusions have been shown to increase glutamate and/or glutamine in muscle (39) as well as in brain tissue (21, 98). Findings obtained in patients with severe liver disease agreed with these observations, in that a BCAA-enriched infusion regimen raised the plasma glutamine level (28). This therapeutic data does by no means prove the (pathophysiological!) "ammonia hypothesis"; it can, however, be regarded as a supporting argument.

References

1. Adibi SA (1976) Metabolism of branched-chain amino acids in altered nutrition. Metabolism 25:1287–1302
2. Adibi SA (1980) Roles of branched-chain amino acids in metabolic regulation. J Lab Clin Med 95:475–484
3. Aguirre A, Yoshimura N, Westman T, Fischer JE (1974) Plasma amino acids in dogs with two experimental forms of liver damage. J Surg Res 16:339–345
4. Bergström J, Fürst P, Norée LO, Vinnars E (1974) Intracellular free amino acid concentration in human muscle tissue. J Appl Physiol 36:693–697
5. de Boer JEG (1984) Branched chain amino acid metabolism in portal-system shunting. Proefschrift, Maastricht
6. Cavalla-Perin P, Bruno A, Nuccio P, Dallòmo AM, Fronda GR, Avagnina P, Molino G, Bozzo C, Pagano G (1986) Insulin resistance in human liver cirrhosis is not modified by porto-systemic surgical shunt. Acta Endocrinol 112:377–382
7. Chang TW, Goldberg AL (1978) The origin of alanine produced in skeletal muscle. J Biol Chem 253:3677–3684
8. Chang TW, Goldberg AL (1978) The metabolic fates of amino acids and the formation of glutamine in skeletal muscle. J Biol Chem 253:3685–3695
9. Condon RE (1971) Effect of dietary protein on symptoms and survival in dogs with an Eck fistula. Am J Surg 121:107–113
10. Cooper AJL, Plum F (1987) Biochemistry and physiology of brain ammonia. Physiol Rev 67:440–519
11. Cummings MG, Soeters PB, James JH, Keane JM, Fischer JE (1976) Regional brain indoleamine metabolism following chronic portacaval anastomosis in the rat. J Neurochem 27:501–509

12. Datta DV, Agarwalla ML, Narang APS, Samanta AKS, Chhuttani PN (1979) Ammonia tolerance test in liver disease with special reference to non-cirrhotic portal fibrosis. Indian J Med Res 70:825–832
13. Dudley FJ, Alford FP, Chisholm DJ, Findlay DM (1979) Effect of portasystemic venous shunt surgery on hyperglucagonaemia in cirrhosis: paired studies of pre- and post-shunted subjects. Gut 20:817–824
14. Faraj BA, Ali FM, Ansley JD, Malveaux EJ (1978) Decarboxylation to tyramine: an important route of tyrosine metabolism in dogs with experimental hepatic encephalopathy. Gastroenterology 75:1041–1044
15. Fischer JE, Funovics JM, Aguirre A, James JH, Keane JM, Wesdorp RIC, Yoshimura N, Westman T (1975) The role of plasma amino acids in hepatic encephalopathy. Surgery 78:276–290
16. Fischer JE, Rosen HM, Ebeid AM, James JH, Keane JM, Soeters PB (1976) The effect of normalization of plasma amino acids on hepatic encephalopathy in man. Surgery 80:77–91
17. Fischer JE, Yoshimura N, Aguirre A, James JH, Cummings MG, Abel RM, Deindoerfer F (1974) Plasma amino acids in patients with hepatic encephalopathy. Am J Surg 127:40–47
18. Freund HR, Fischer JE (1982) Our experience with FO80 in the treatment of hepatic coma in 63 patients. In: Holm E (ed) Amino acid and ammonia metabolism in hepatic failure. Witzstrock, Baden-Baden Köln New York, pp 172–183
19. Frick G, Goodman H (1980) Insulin regulation of branched chain alpha-keto acid dehydrogenase in adipose tissue. J Biol Chem 255:6186–6192
20. Fürst P (1983) Intracellular muscle free amino acids – their measurement and function. Proc Nutr Soc 42:451–462
21. Fujiwara M (1986) Role of ammonia in the pathogenesis of brain edema. Acta Med Okayama 40:313–320
22. Ganda OP, Ruderman NB (1976) Muscle nitrogen metabolism in chronic hepatic insufficiency. Metabolism 25:427–435
23. Garber AJ, Karl IE, Kipnis DM (1976) Alanine and glutamine synthesis and release from skeletal muscle. J Biol Chem 251:826–835
24. Goldberg AL, Chang TW (1978) Regulation and significance of amino acid metabolism in skeletal muscle. Fed Proc 37:2301–2307
25. Goldberg AL, Tischler ME (1981) Regulatory effects of leucine on carbohydrate and protein metabolism. In: Walser M, Williamson JR (eds) Metabolism and clinical implications of branched chain amino and ketoacids. Elsevier/North-Holland, New York Amsterdam Oxford, pp 205–216
26. Goodman HM, Blinder L, Frick GP (1984) Interaction of branched chain amino acids with lipid metabolism in adipose tissue. In: Adibi SA, Fekl W, Langenbeck U, Schauder P (eds) Branched chain amino and keto acids in health and disease. Karger, Basel, pp 20–40
27. Goodman HM, Frick GP (1981) Metabolism of branched chain amino acids in adipose tissue. In: Walser M, Williamson JR (eds) Metabolism and clinical implications of branched chain amino and ketoacids. Elsevier/North-Holland, New York Amsterdam Oxford, pp 169–180
28. Gotoh N, Musha H, Okuda K (1983) The effect of special amino acid solution (FO80) on plasma ammonia and amino acid levels in hepatic encephalopathy with hyperammonemia. Acta Hepatol Jap 24:530–536
29. Harper AE, Zapalowski C (1981) Interorgan relationships in the metabolism of the branched-chain amino and alpha-ketoacids. In: Walser M, Williamson JR (eds) Metabolism and clinical implications of branched chain amino and ketoacids. Elsevier/North-Holland, New York Amsterdam Oxford, pp 195–203
30. Haschen RJ, Neumann U, Heier B (1974) Extrahepatische Mechanismen der Ammoniakfixation. In: Imler M, Szam I (Hrsg) Neue Erkenntnisse zum Ammoniakstoffwechsel. Witzstrock, Baden-Baden Brüssel, pp 75–78

31. Hayashi M, Ohnishi H, Kawade Y, Muto Y, Takahashi Y (1981) 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
32. Herz R, Rössle M, Schulte U, Gerok W (1979) Periphere Aminosäuren-Konzentrationen bei eingeschränkter quantitativer Leberfunktion infolge Leberzirrhose. Z Gastroenterol 17:572–573
33. Hindfelt B, Plum F, Duffy TE (1977) Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. J Clin Invest 59:386–396
34. Hod G, Chaouat M, Haskel Y, Lernau OZ, Nissan S, Mayer M (1982) Ammonia uptake by skeletal muscle in the hyperammonaemic rat. Eur J Clin Invest 12:445–450
35. Holm E (1981) Parenteral nutrition in patients with hepatic failure. J Drug Res 6:41–48
36. Holm E, Behne F, Leweling H, Staedt U, Striebel J-P, Tschepe A (1986) Animal models of hepatic failure. Metabolic characteristics and effects of TPN. In: Dietze G, Grünert A, Kleinberger G, Wolfram G (eds) Clinical nutrition and metabolic research. Karger, Basel, pp 343–374
37. Holm E, Krämer W, Reith H-J, Magin T, Mezitis NHE (1977) Electrophysiological and psychometric studies in hepatic encephalopathy. In: Varró V, Bálint GA, Creutzfeldt W (eds) Current views in gastroenterology, Vol I. Budapest, pp 597–613
38. Holm E, Leweling H, Staedt U (1983) Metabolism and nutritional supply of amino acids in hepatic failure. In: Kleinberger G, Deutsch E (eds) New aspects of clinical nutrition. Karger, Basel, pp 377–399
39. Holm E, Leweling H, Staedt U, Striebel J-P, Jacob S (1984) The metabolic fate of branched-chain amino acids. In: Capocaccia L, Fischer JE, Rossi-Fanelli F (eds) Hepatic encephalopathy in chronic liver failure. Plenum Press, New York London, pp 161–181
40. Holm E, Leweling H, Staedt U, Striebel J-P, Tschepe A, Uhl W (1986) Protein- und Aminosäurenstoffwechsel bei Leberinsuffizienz – Infusionstherapeutische und diätetische Folgerungen. Verh dtsch Ges inn Med 92:685–737
41. Holm E, Striebel J-P, Kattermann R, Schick R, Staedt U (1982) Encephalopathy resulting from portacaval anastomosis in cats. Ammonia, amino acids, and cerebral electrical activity. In: Holm E (Hrsg) Aminosäuren- und Ammoniakstoffwechsel bei Leberinsuffizienz. Grundlagen und therapeutische Folgerungen. Witzstrock, Baden-Baden Köln New York, S 58–94
42. Holm E, Striebel J-P, Meisinger E, Haux P, Langhans W, Becker HD (1978) Aminosäurengemische zur parenteralen Ernährung bei Leberinsuffizienz. Infusionstherapie 5:274–292
43. Holm E, Striebel J-P, Münzenmaier R, Kattermann R (1977) Pathogenese der hepatischen Enzephalopathie. Leber Magen Darm 7:241–254
44. Holmin T, Siesjö BK (1974) The effect of portacaval anastomosis upon the energy state and upon acid-base parameters of the rat brain. J Neurochem 22:403–412
45. Imler M, Schlienger JL, Frick A, Stahl A, Chabrier G (1982) Study of muscular glutamine and alanine release in cirrhotics with hyperammonemia and in ammonium infused rats. In: Holm E (ed) Amino acid and ammonia metabolism in hepatic failure. Witzstrock, Baden-Baden Köln New York, pp 33–39
46. Iwasaki Y, Sato H, Ohkubo A, Sanjo T, Futagawa S, Sugiura M, Tsuji S (1980) Effects of spontaneous portal-systemic shunting on plasma insulin and amino acid concentrations. Gastroenterology 78:677–683
47. James JH, Escourrou J, Fischer JE (1978) Blood-brain neutral amino acid transport activity is increased after portacaval anastomosis. Science 200:1395–1397

48. James JH, Hodgman JM, Funovics JM, Fischer JE (1976) Alterations in brain octopamine and brain tyrosine following portacaval anastomosis in rats. J Neurochem 27:223–227
49. James JH, Hodgman JM, Funovics JM, Yoshimura N, Fischer JE (1976) Brain tryptophan, plasma free tryptophan and distribution of plasma neutral amino acids. Metabolism 25:471–476
50. James JH, Ziparo V, Jeppson B, Fischer JE (1979/II) Hyperammonaemia, plasma aminoacid imbalance, and blood-brain aminoacid transport: a unified theory of portal-systemic encephalopathy. Lancet, pp 772–775
51. Jaspers SR, Jacob S, Tischler ME (1986) Metabolism of amino acids by the atrophied soleus of tail-casted, suspended rats. Metabolism 35:216–223
52. Jeppsson B, von Meyenfeldt M, Cangiano C, Cardelli-Cangiano P, Edwards L, James JH, Fischer JE (1980) Plasma and CSF amino acid changes after NH_4 infusion in dogs. Gastroenterology 78:1309
53. Johnston DG, Taylor R, Alberti KGMM, Faber OK, Binder C, Whittaker J, Hammond VA, Wright R (1984) Insulin secretion and action in liver disease. In: Melchionda N, Horwitz DL, Schade DS (eds) Recent advances in obesity and diabetes research. Raven Press, New York, pp 117–127
54. Jonung T, Jeppsson B, Herlin P, Nobin A, Hultberg B, Aslund U (1988) The effect of ammonia tolerance test on the concentrations of amino acids in plasma and in CSF of patients with liver cirrhosis. In: Soeters PB, Wilson JHP, Meijer AJ, Holm E (eds) Advances in ammonia metabolism and hepatic encephalopathy. Excerpta Med Int Congr Series 761, Elsevier, Amsterdam New York Oxford, pp 177–181
55. Karavias Th (1983) Der Einfluß zweier hämodynamisch unterschiedlicher portosystemischer Anastomosen auf die hepatischen und zerebralen Funktionen der gesunden und zirrhotischen Ratte. Med Habilitationsschrift, Berlin
56. Lee S, Chandler JG, Broelsch CE, Flamond YM, Ortoff MJ (1974) Portal-systemic anastomosis in the rat. J Surg Res 17:53–73
57. Leweling H, Holm E, Staedt U, Striebel J-P, Tschepe A (1984) Intra- and extracellular amino acid concentrations in ammonium-infused rats. Evidence that hyperammonemia reduces BCAA levels. In: Kleinberger G, Ferenci P, Riederer P, Thaler H (eds) Advances in hepatic encephalopathy and urea cycle diseases. Karger, Basel, pp 552–554
58. Leweling H, Holm E, Staedt U, Tschepe A, Striebel J-P (1985) Rationale and nutritional effects of BCAA-enriched TPN regimens in patients with liver cirrhosis. In: Holm E, Kasper H (eds) Metabolism and nutrition in liver disease. MTP Press, Lancaster, pp 235–257
59. Leweling H, Knauff HG, Nitschke J, Paquet KJ (1980) Beeinflussung von zerebralem Funktionszustand und Serumaminogramm von Patienten mit Leberzirrhose durch parenterale Aminosäurenzufuhr. Infusionstherapie 7:88–94
60. Leweling H, Tschepe A, Holm E (1983) Eine neue Mikromethode zur Bestimmung der Ammoniakkonzentration im Vollblut und Plasma. Leber Magen Darm 13:160–162
61. Lockwood AH, McDonald JM, Reiman RE, Gelbard AS, Laughlin JS, Duffy TE, Plum F (1979) The dynamics of ammonia metabolism in man. J Clin Invest 63:449–460
62. Maier KP, Gerok W (1984) Hyperammonemia and hepatic encephalopathy. In: Kleinberger G, Ferenci P, Riederer P, Thaler H (eds) Advances in hepatic encephalopathy and urea cycle disease. Karger, Basel, pp 224–231
63. Miller LL (1962) The role of the liver and the non-hepatic tissues in the regulation of free amino acid levels in the blood. In: Holden JT (ed) Amino acid pools. Elsevier Publishing Co, Amsterdam London New York, pp 708–721

64. Millikan WJ, Henderson JM, Warren WD, Riepe SP, Kutner MH, Wright-Bacon L, Epstein C, Parks RB (1983) Total parenteral nutrition with FO80 in cirrhotics with subclinical encephalopathy. Ann Surg 197:294–304
65. Montanari A, Simoni T, Trifiro A, Abbiati R, Cisternin M, Novarini A (1986) Effects of oral administration of branched chain amino acids (BCAA) on their intra-extracellular distribution in liver cirrhosis (LC). Ital J Gastroenterol 18:294–295
66. Munro HN, Fernstrom JD, Wurtman RJ (1977) Plasma amino acid imbalance and hepatic coma. Klin Anästh Intensivther 13:103–112
67. Nosadini R, Avogaro A, Mollo F, Marescotti C, Tiengo A, Duner E, Merkel C, Gatta A, Zuin R, de Kreutzenberg S, Trevisan R, Crepaldi G (1984) Carbohydrate and lipid metabolism in cirrhosis. Evidence that hepatic uptake of gluconeogenic precursors and of free fatty acids depends on effective hepatic flow. J Clin Endocrinol Metab 58:1125–1132
68. Odessey R, Khairallah EA, Goldberg AL (1974) Origin and possible significance of alanine production by skeletal muscle. J Biol Chem 249:7623–7629
69. Paul HS, Adibi SA (1984) Regulation of branched chain amino acid catabolism. In: Adibi SA, Fekl W, Langenbeck U, Schauder P (eds) Branched chain amino and keto acids in health and disease. Karger, Basel, pp 182–219
70. Plauth M, Egberts E-H, Abele R, Fürst P (1986) Aminosäurenmuster im Muskel bei Leberzirrhose. Klin Wschr 64, Suppl V, 35, Abstract Nr 70
71. Porto F, Frugiuele PL, Noto A, Talarico G, Rolande G, Odoardi GB (1986) Relations between insulin resistance, portal hypertension and carbohydrate metabolism disorders in liver cirrhosis: Findings of a study by means of the glucose clamp technique. Ital J Gastroenterol 18:66–67
72. Pozefsky T, Felig P, Tobin JD, Soeldner JS, Cahill GF (1969) Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. J Clin Invest 48:2273–2282
73. Putnam CW, Porter KA, Starzl TE (1976) Hepatic encephalopathy and light and electron micrographic changes of the baboon liver after portal diversion. Ann Surg 184:155–161
74. Record C, Buxton B, Chase RA, Curzon G, Murray-Lyon IM, Williams R (1976) Plasma and brain amino acids in fulminant hepatic failure and their relationship of hepatic encephalopathy. Europ J Clin Invest 6:387–394
75. Riggio O, James JH, Peters JC, Fischer JE (1984) Influence of ammonia on leucine utilization in muscle and adipose tissue in vitro. In: Kleinberger G, Ferenci P, Riederer P, Thaler H (eds) Advances in hepatic encephalopathy and urea cycle diseases. Karger, Basel, pp 519–526
76. Rosen HM, Soeters PB, James JH, Hodgman J, Fischer JE (1978) Influences of exogenous intake and nitrogen balance on plasma and brain aromatic amino acid concentrations. Metabolism 27:393–404
77. Rosen HM, Yoshimura N, Hodgman JM, Fischer JE (1977) Plasma amino acid patterns in hepatic encephalopathy of differing etiology. Gastroenterology 72:483–487
78. Roth E (1981) Veränderungen des Proteinstoffwechsels bei Kachexie und Katabolie. In: Lochs H, Grünert A, Druml W (Hrsg) Aktuelle Probleme der klinischen Ernährung. Klin Ern 5, Zuckschwerdt, München, pp 1–13
79. Roth E, Funovics J, Karner J, Mühlbacher F, Rauhs R, Steiniger R, Huk I, Sporn P, Kleinberger G (1984) Muscle amino acid levels in paients with liver diseases. In: Kleinberger G, Ferenci P, Riederer P, Thaler H (eds) Advances in hepatic encephalopathy and urea cycle diseases. Karger, Basel, pp 527–537
80. Roth E, Mühlbacher F, Rauhs R, Huk I, Soderland K, Funovics JM (1983) Free amino acids in plasma and muscle in fulminant hepatic coma during an extracorporal liver perfusion. J Parent Ent Nutr 6:240–243

81. Ruderman NB, Berger M (1974) The formation of glutamine and alanine in skeletal muscle. J Biol Chem 249:5500–5506
82. Rudman D, Chawla RK, Bleier JC (1983) Cystine and tyrosine requirements during the nutritional repletion of cirrhotic patients. In: Blackburn GL, Grant GP, Young VR (eds) Amino acids. Metabolism and medical applications. Wright, Boston Bristol London, pp 484–496
83. Sarna GS, Bradbury MWB, Cremer JE, Lai JCK, Teal HM (1979) Brain metabolism and specific transport at the blood-brain barrier after portacaval anastomosis in the rat. Brain Res 160:69–83
84. Schäfer K, Ukida M, Steffen C, Bode C, Zelder O, Bode JC (1985) Effect of ammonia on plasma and cerebrospinal fluid amino acids in dogs with and without portacaval anastomoses. Res Exp Med 185:35–44
85. Schreiber HW (1962) Zur Pathophysiologie und Chirurgie des Pfortaderhochdrucks. Langenbecks Arch klin Chir 300:187–270
86. Smith AR, Rossi-Fanelli F, Freund H, Fischer JE (1979) Sulfur-containing amino acids in experimental hepatic coma in the dog and the monkey. Surgery 85:677–683
87. Smith AR, Rossi-Fanelli F, Ziparo V, James JH, Perelle BA, Fischer JE (1978) Alterations in plasma and CSF amino acids, amines and metabolites in hepatic coma. Ann Surg 187:343–350
88. Smith-Laing G, Sherlock S, Faber OK (1979) Effects of spontaneous portal-systemic shunting on insulin metabolism. Gastroenterology 76:685–690
89. Soeters PB, de Boer J (1984) Why are plasma branched chain amino acid levels diminished in patients with liver cirrhosis? In: Adibi SA, Fekl W, Langenbeck U, Schauder P (eds) Branched chain amino and keto acids in health and disease. Karger, Basel, pp 483–496
90. Soeters PB, de Boer JEG, Oostenbroek RJ, Janssen MA (1983) Fate of branched chain amino acids. In: Kleinberger G, Deutsch E (eds) New aspects of clinical nutrition. Karger, Basel, pp 337–345
91. Soeters PB, Fischer JE (1976/II) Insulin, glucagon, aminoacid imbalance, and hepatic encephalopathy. Lancet, pp 880–882
92. Staedt U, Uhl W, Leweling H, Holm E, Striebel J-P (1986) Einfluß der experimentellen portokavalen Anastomose (PKA) auf intra- und extrazelluläre Aminosäuren (AS)-Konzentrationen. Hyperammoniämie (HA) und Stoffwechsel der verzweigtkettigen Aminosäuren. Infusionsther klin Ern 13:30
93. Striebel J-P (1977) Plasmaaminosäurenspiegel im Postaggressionsstoffwechsel. Med Habilitationsschrift, Heidelberg-Mannheim
94. Strombeck DR, Rogers O, Stern JS (1978) Effects of intravenous ammonia infusion on plasma levels of amino acids, glucagon and insulin in dogs. Gastroenterology 74:1165
95. Tischler ME, Goldberg AL (1981) Leucine catabolism and production of glutamine and alanine by adipose tissue. In: Walser M, Williamson JR (eds) Metabolism and clinical implications of branched chain amino and ketoacids. Elsevier/North-Holland, New York Amsterdam Oxford, pp 283–288
96. Tschepe A, Leweling H, Schulze R, Ruhl M, Holm E (1985) A new micromethod for measuring the ammonia concentration in whole blood and plasma. In: Holm E, Kasper H (eds) Metabolism and nutrition in liver disease. MTP Press, Lancaster, pp 49–56
97. Wahren J, Eriksson S, Hagenfeldt L (1981) Influence of branched-chain amino acids and ketoacids on arterial concentrations and brain exchange of amino acids in man. In: Walser M, Williamson JR (eds) Metabolism and clinical implications of branched chain amino and ketoacids. Elsevier/North-Holland, New York Amsterdam Oxford, pp 471–480

98. Watanabe A, Shiota T, Takei N, Fujiwara M, Nagashima H (1986) Ammonia detoxification by accelerated oxidation of branched chain amino acids in brains of acute hepatic failure rats. Biochem Med Metabol Biol 35:367–375

Received February 24, 1989

Authors' address:

Dr. rer. physiol. H. Leweling, Department of Pathophysiology, Medical Clinic I, Theodor-Kutzer-Ufer, 6800 Mannheim 1, FRG