

Plasma amino acids in four models of experimental liver injury in rats

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Summary. We studied the plasma amino acid profiles in four models of hepatic injury in rats. In partially hepatectomized rats (65% of liver was removed) we observed significant increase of aromatic amino acids (AAA; i.e. tyrosine and phenylalanine), taurine, aspartate, threonine, serine, asparagine, methionine, ornithine and histidine. Branched-chain amino acids (BCAA; i.e. valine, leucine and isoleucine) concentrations were unchanged. In ischemic and carbon tetrachloride acute liver damage we observed extreme elevation of most of amino acids (BCAA included) and very low concentration of arginine. In carbon tetrachloride induced liver cirrhosis we observed increased levels of AAA, aspartate, asparagine, methionine, ornithine and histidine and decrease of BCAA, threonine and cystine. BCAA/AAA ratio decreased significantly in partially hepatectomized and cirrhotic rats and was unchanged in ischemic and acute carbon tetrachloride liver damage. We conclude that a high increase of most of amino acids is characteristic of fulminant hepatic necrosis; decreased BCAA/AAA ratio is characteristic of liver cirrhosis; and decrease of BCAA/AAA ratio may not be used as an indicator of the severity of hepatic parenchymal damage.

Keywords: Amino acids – Liver – Hepatic failure – Cirrhosis – Branched-chain amino acids – Aromatic amino acids – Arginine

Abbreviations: BCAA branched-chain amino acids (i.e. valine, leucine and isoleucine); AAA aromatic amino acids (i.e. tyrosine and phenylalanine)

Introduction

In the 1950's, characteristic changes in plasma amino acids including decrease of branched-chain amino acids (BCAA; i.e. valine, leucine and isoleucine) and increase of aromatic amino acids (AAA; i.e. tyrosine and phenylalanine) and methionine in liver disease were described (Flock et al., 1951; Iber et al.,

1957). In 1975 Fischer et al. (1975) reported that the BCAA/AAA molar ratio was significantly reduced in animals and patients with hepatic encephalopathy and hypothesized that hepatic encephalopathy might in fact result from changes in the plasma concentrations of amino acids making up this ratio. Since that time, a number of papers have been devoted to seek the relationship between the plasma amino acid pattern primarily BCAA/AAA ratio and the grade of hepatic encephalopathy or liver damage and to the therapeutic effect of BCAA in liver disease.

Azuma et al. (1989) published a decrease of BCAA in serum of patients with acute hepatitis, chronic active hepatitis, and both compensated and decompensated liver cirrhosis. Changes were in parallel with the severity of liver damage and therefore he considered the BCAA/tyrosine ratio to be a good indicator of the severity of hepatic parenchymal damage. Similarly Campollo et al. (1992) concluded that BCAA/AAA ratio is a good index for the assessment of liver impairment in cirrhotics and could be used to help determine the best time for the transplant.

Unfortunately, there is an increasing evidence that the decrease of BCAA/AAA ratio has not such implication as declared by a number of papers. Cascino and co-workers (1978) confirmed the presence of high levels of AAA and low levels of BCAA in cirrhotic patients, however the magnitude of these changes was not correlated either with the degree of hepatic encephalopathy or with the presence of surgical anastomosis. In retrospective analysis of Shiels et al. (1985) the plasma molar ratio of BCAA to AAA did not reflect the degree of hepatocellular inflammation or indicate disease outcome. Also Fischer (1982) concluded that BCAA/AAA ratio could be not a numerical indicator of the grade of hepatic encephalopathy.

One of the problems in the field of amino acid metabolism in liver disease is a wide spectrum of forms of liver injury and their effect on plasma amino acids. It is well known that fulminant liver failure is associated with a marked increase in plasma amino acids, with resulting aminoaciduria, and that in cirrhosis there is an increase in aromatic amino acids and a reduction in BCAA. However, studies on the changes in a wide range of amino acids and on the significance of BCAA/AAA ratio have given conflicting results (Record et al., 1976; Holmin et al., 1983; Shi and Chang 1984; Zoli et al., 1981; Shiels et al., 1985). In view of this current controversy, the present study has been carried out in order to furnish data about amino acid pattern, primarily of BCAA and AAA in various liver injury.

Material and methods

Male Wistar rats weighing between 200–220 g were obtained from VELAZ, Prague, CZ. Rats were housed in standardized plexy-glass cages in quarters with a controlled temperature, 12 h light-dark cycle and received Velaz-Altromin 1320 (VELAZ, Prague, CZ) Laboratory Chow and drinking water ad libitum before the experiment.

To assess the effect of pathogenesis of liver injury on the plasma amino acid pattern four models of liver injury were studied. Experiments were performed separately as follows:

(a) Partial hepatectomy

Partial hepatectomy (median and left lateral lobes were removed) was carried out under light diethyl ether narcosis according to Higgins and Anderson (1931). Control rats underwent sham operation. Rats were sacrificed 24 h following surgery.

(b) Liver ischemia

Liver ischemia was performed in sodium pentobarbital narcosis (45 mg/kg b.w. i.p.) by clamping of the portal venous and hepatic arterial blood supply to the median and left lateral lobes (Frederiks et al., 1982). The actual clamping was performed by means of a microvascular clip and the abdomen was temporarily closed during 2 hours of ischemia. Immediately after loosening the clip, a colour change of liver tissue was observed. In sham operated animals hepatic ischemia was induced only for a period of 5 seconds. The rectal temperature of animals was $37 \pm 0.5^{\circ}\text{C}$ from the start of anesthesia till the final suture of the abdominal cavity. The rats were killed 2 h following the removal of the clip, i.e. at the time of maximum of serum ALT and AST activities (Frederiks et al., 1983).

(c) Carbon tetrachloride (CCl_4) induced acute liver damage

Carbon tetrachloride was diluted in olive oil (1:1) and administered intragastrically in a dose of 4 g of CCl_4 /kg b.w. The sham treated rats received olive oil only. The rats were killed 24 h after CCl_4 treatment.

(d) Carbon-tetrachloride induced liver cirrhosis

Carbon tetrachloride was diluted in olive oil (1:1) and administered intragastrically in a dose of 2 g of CCl_4 /kg b.w. thrice-weekly for 10 weeks. Using this scheme cirrhosis was induced in all rats used in a previous experiment (unpublished). We did not use pretreatment with cytochrome P450 inducer such as phenobarbital for an unacceptable contaminating variable. The sham treated rats received olive oil only. The rats were killed 72 h following the last CCl_4 treatment.

To exclude nutritional effects on aminocidemia, the rats were only given water during the 12 h before sacrifice by blood withdrawal in light ether narcosis. For amino acid analysis in the plasma, the sample was immediately deproteinized using sulphosalicylic acid. Individual free amino acid concentrations were measured by ion-exchange chromatography on an automatic analyzer (T339, Mikrotechna, Prague, CZ). Because tryptophan is bound to plasma proteins and it is partially lost during deproteinization with sulphosalicylic acid (Milson et al., 1979), this amino acid was not determined. Total bilirubin concentration, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using commercial kits (Lachema, Brno, CZ). The liver tissue was stained with haematoxylin-eosin for histology.

Results are presented as the mean $\pm$ S.E.M. Significance of differences of the means was checked by F-test and Student's t-test. SOLO Statistical System = 4.0 was used.

Results

*Liver damage patterns (Fig. 1 and Table 1)**(a) Partial hepatectomy*

Morphological examination of the liver of partially hepatectomized rats revealed small drop steatosis of hepatocytes. The weight of remaining liver 24 h

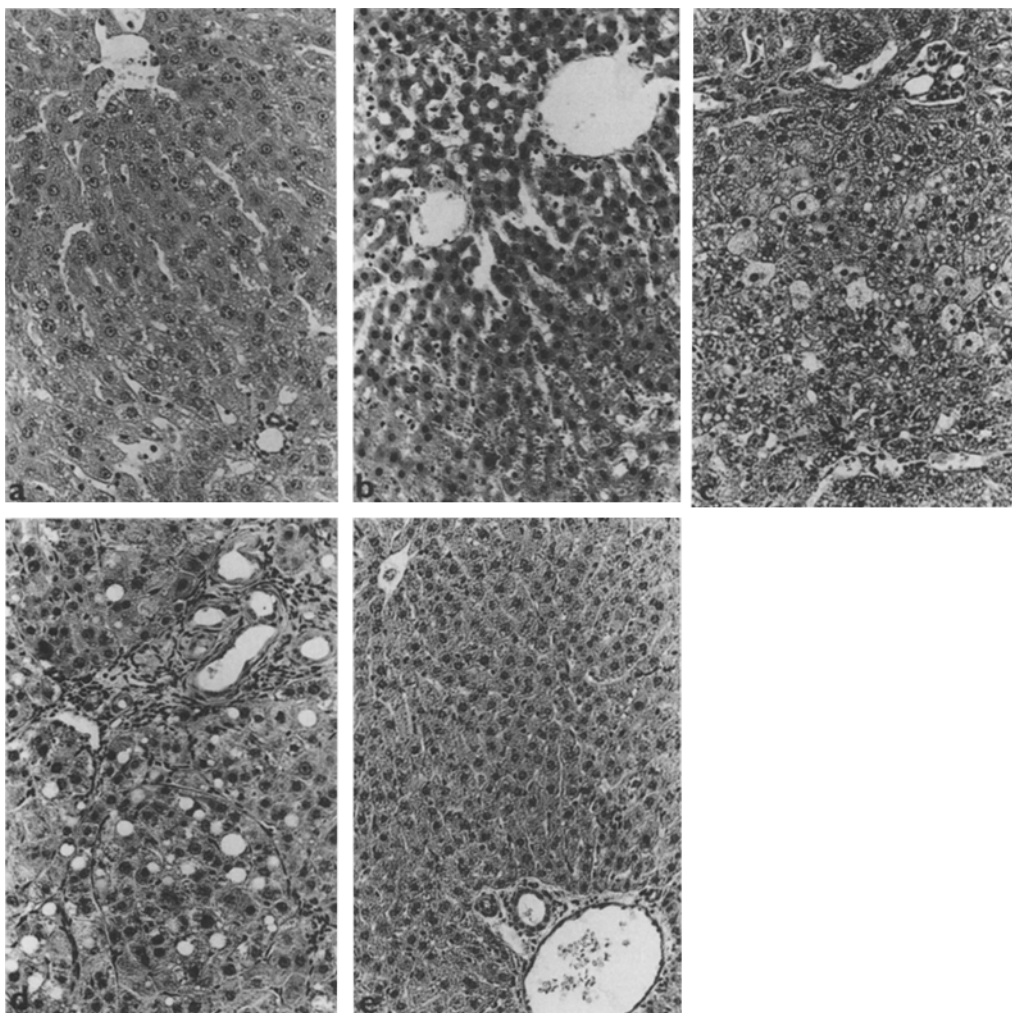


Fig. 1. Histological examination. **a** Partial hepatectomy – microvesicular steatosis of hepatocytes in peripheral region of liver lobules, H&E $\times 160$. **b** Liver ischemia – distinct eosinophilic degeneration of liver cells in the periphery and midzone of lobules, dilatation of sinusoids pericentrally especially, H&E $\times 160$. **c** Acute CCl_4 damage – pericentral necrosis of liver cells, microvesicular steatosis peripherally, steatosis and hydropic degeneration of hepatocytes in the midzone, H&E $\times 160$. **d** CCl_4 induced liver cirrhosis – micronodular regeneration, fibrosis, macrovesicular steatosis and bile duct proliferation, H&E $\times 160$, **e** Intact liver parenchyma, H&E $\times 160$

after partial hepatectomy was about 37% of sham operated rats. Modest increase of ALT and AST activities and unchanged total bilirubin concentration were observed.

(b) Ischemic liver damage

Distinct eosinophilia of hepatocytes as an early sign of future necrosis and dilatation of liver sinusoids were observed two hours following the end of

Table 1. Liver weight, plasma ALT and AST activities and bilirubin concentrations in blood plasma 24 h after partial hepatectomy, 2 h after liver ischemia, 24 h after CCl₄ induced acute liver damage or in CCl₄ induced liver cirrhosis

	Partial hepatectomy		Ischemic damage		Acute CCl ₄ damage		Liver cirrhosis	
	Sham (n = 8)	PH (n = 7)	Sham (n = 6)	Ischemia (n = 6)	Sham (n = 6)	CCl ₄ (n = 6)	Sham (n = 6)	Cirrhosis (n = 6)
liver weight (g/100 g b.w.)	3.34 ± 0.13	1.62 ± 0.09 xxx	–	–	2.89 ± 0.05	4.78 ± 0.12 xxx	2.39 ± 0.04	2.09 ± 0.16 NS
ALT (μkat/l)	0.86 ± 0.05	1.07 ± 0.06 x	1.37 ± 0.34	64.60 ± 10.78 xxx	0.61 ± 0.02	43.10 ± 2.11 xxx	0.69 ± 0.09	0.99 ± 0.08 x
AST (μkat/l)	0.98 ± 0.06	1.19 ± 0.05 x	2.41 ± 0.98	78.60 ± 7.23 xxx	1.11 ± 0.12	38.50 ± 2.4 x	0.89 ± 0.18	2.52 ± 0.58 x
Bilirubin (μmol/l)	5.02 ± 0.58	5.62 ± 0.67 NS	–	–	5.51 ± 0.52	6.07 ± 1.88 NS	6.20 ± 0.46	15.33 ± 1.72 xxx

Mean ± SEM. P compared with sham treated rats, x = P < 0.05; xx = P < 0.01; xxx = P < 0.001.

ischemia of median and left lateral lobes. ALT and AST activities in blood plasma were extremely elevated.

(c) Acute CCl₄ liver damage

Liver histology revealed hydropic degeneration and necrosis of hepatocytes with leucocytic infiltration pericentrally. Very high ALT and AST activities in blood plasma were observed.

(d) Carbon-tetrachloride induced liver cirrhosis

All rats had fully developed micronodular cirrhosis with gross ascites. The increase of plasma bilirubin and modest changes of ALT and AST activities are consistent with the observed cirrhosis, and suggest only modest necrosis or hepatocyte damage.

Plasma amino acid patterns

(a) Partial hepatectomy (Fig. 2 and Table 2)

Plasma concentrations of ten of 22 evaluated amino acids increased significantly when compared with sham operated rats. Plasma BCAA showed little or no variation. Due to an increase in AAA concentrations, BCAA/AAA ratio decreased.

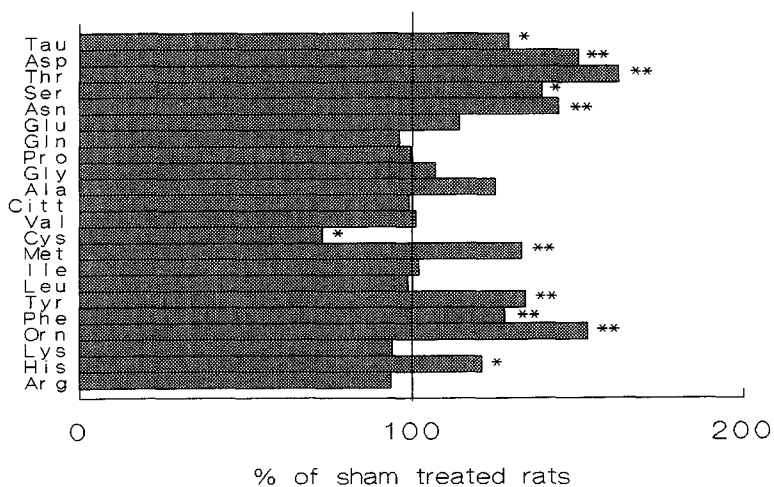


Fig. 2. Plasma amino acid concentrations 24h after partial hepatectomy (PH) or sham operation. P compared with sham treated rats. * = $P < 0.05$; ** = $P < 0.01$

(b) Liver ischemia (Fig. 3 and Table 2)

The significant increase of most of plasma amino acids was observed two hours following the end of ischemia. The only exception were decreased

Table 2. Plasma BCAA (Val, Leu, Ile) and AAA (Tyr, Phe) concentrations, and BCAA/AAA molar ratio 24 h after partial hepatectomy, 2 h after liver ischemia, 24 h after CCl₄ induced acute liver damage or in CCl₄ induced liver cirrhosis

	Partial hepatectomy			Ischemic damage			Acute CCl ₄ damage			Liver cirrhosis		
	Sham (n = 8)	PH (n = 7)		Sham (n = 6)	Ischemia (n = 6)		Sham (n = 6)	CCl ₄ (n = 6)		Sham (n = 6)	Cirrhosis (n = 6)	
Val ($\mu\text{mol/l}$)	157 $\pm$ 17.5	159 $\pm$ 25.6	NS	122 $\pm$ 12.4	199 $\pm$ 10.4	xx	204 $\pm$ 25.5	396 $\pm$ 38.7	xx	176 $\pm$ 16.0	116 $\pm$ 11.5	x
Leu ($\mu\text{mol/l}$)	115 $\pm$ 7.2	114 $\pm$ 9.4	NS	111 $\pm$ 10.3	178 $\pm$ 5.7	xx	185 $\pm$ 19.7	318 $\pm$ 28.0	xx	124 $\pm$ 11.0	65.8 $\pm$ 9.1	xx
Ile ($\mu\text{mol/l}$)	73.2 $\pm$ 3.3	74.8 $\pm$ 6.8	NS	59.4 $\pm$ 4.7	96.6 $\pm$ 3.8	xxx	110 $\pm$ 13.0	172 $\pm$ 13.8	xx	76.4 $\pm$ 9.2	45.1 $\pm$ 5.2	x
Tyr ($\mu\text{mol/l}$)	53.8 $\pm$ 4.6	72.0 $\pm$ 3.2	xx	37.5 $\pm$ 3.4	53.6 $\pm$ 10.9	x	65.6 $\pm$ 9.3	101 $\pm$ 14.9	NS	46.9 $\pm$ 5.9	133 $\pm$ 7.7	xxx
Phe ($\mu\text{mol/l}$)	56.5 $\pm$ 2.4	72.1 $\pm$ 3.4	xx	55.5 $\pm$ 7.7	96.8 $\pm$ 7.6	xx	39.3 $\pm$ 3.7	91.3 $\pm$ 19.9	xx	50.1 $\pm$ 8.1	74.9 $\pm$ 7.2	x
BCAA/AAA	3.17 $\pm$ 0.23	2.39 $\pm$ 0.15	x	3.22 $\pm$ 0.31	3.24 $\pm$ 0.29	NS	4.83 $\pm$ 0.48	4.83 $\pm$ 0.41	NS	3.97 $\pm$ 0.36	1.11 $\pm$ 0.13	xxx

Mean $\pm$ SEM. P compared with sham treated rats, x = P < 0.05; xx = P < 0.01; xxx = P < 0.001.

levels of cystine, histidine, and arginine. Due to parallel increases of plasma concentrations of both AAA and BCAA the BCAA/AAA ratio did not change.

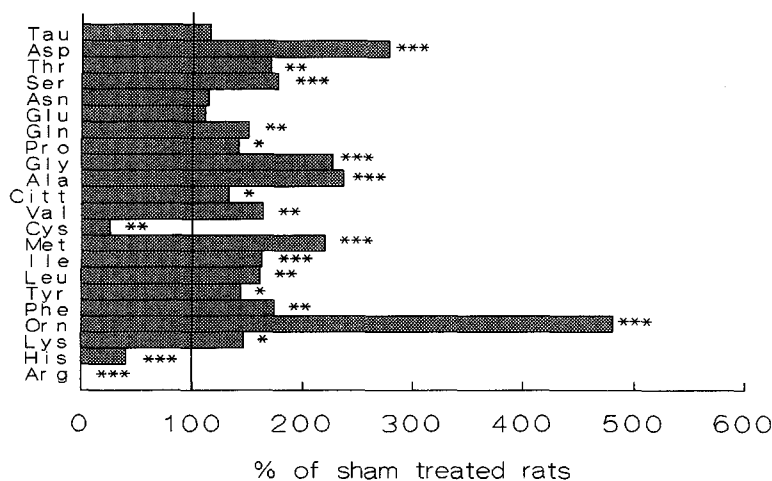


Fig. 3. Plasma amino acid concentrations 2h following the end of liver ischemia or sham operation. P compared with sham treated rats. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

(c) Acute CCl_4 liver damage (Fig. 4 and Table 2)

The amino acid pattern was similar to one in ischemic damage. We observed extreme elevation of most amino acids, profound decrease of arginine and unchanged BCAA/AAA ratio.

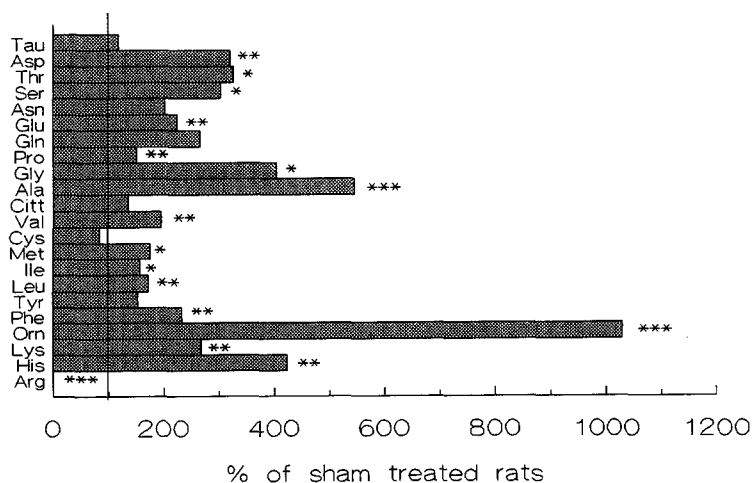


Fig. 4. Plasma amino acid concentrations 24h after CCl_4 induced acute liver damage or sham treatment. P compared with sham treated rats. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

(d) Carbon-tetrachloride induced liver cirrhosis (Fig. 5 and Table 2)

Increased AAA, methionine, ornithine, asparagine, and aspartate and decreased BCAA concentrations were observed. BCAA/AAA molar ratio decreased significantly.

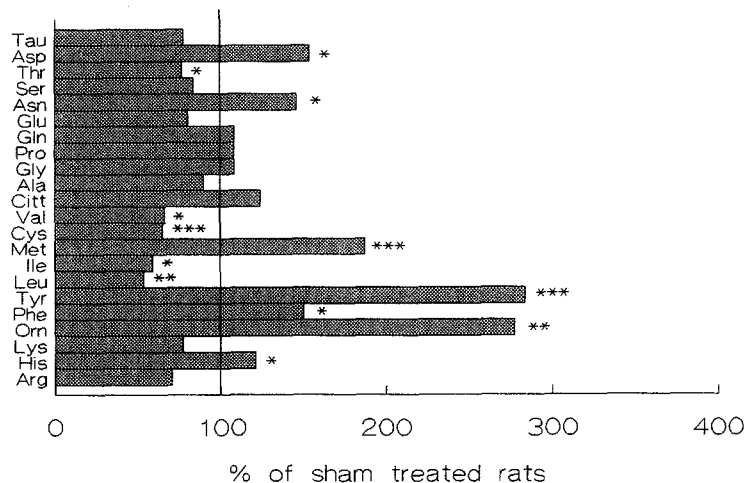


Fig. 5. Plasma amino acid concentrations in rats with liver cirrhosis induced by repeated CCl_4 treatment or sham treated rats. P compared with sham treated rats. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Discussion

The merits of our study using experimental models of liver disease as compared to clinical trials are nearly the same degree of liver injury within each group and the appropriate sham animals. Surgically induced loss of liver tissue without necroses or damage of hepatocytes was associated with a mild increase of a number of amino acids and unaffected levels of BCAA. Similar changes were observed also four and eight hours after partial hepatectomy (Holeček et al., 1991). Acute ischemic or carbon tetrachloride liver damage resulted in an extreme elevation of most of amino acids. In rat liver cirrhosis induced by carbon tetrachloride a significant increase of AAA, aspartate, asparagine, ornithine and histidine and decrease of BCAA, threonine and cystine were observed.

What are the mechanisms of amino acid changes?

From our data it is possible to speculate about the mechanisms responsible for changes of particular amino acids during liver disease as follows:

(a) Reduced utilisation

As a consequence of reduced liver cell mass or shunting an increase of amino acids metabolized primarily in the liver such as AAA, methionine and alanine

can be observed. This mechanism is involved in all experimental models used in our study.

(b) Leaking of amino acids

In our study, the leak of amino acids from dying hepatocytes to the circulation contributes to changes of plasma amino acids in ischemic and carbon tetrachloride models of acute liver damage. We observed very high concentrations of most of plasma amino acids, the only exceptions being a decrease of cystine and a vanishingly low concentration of arginine in both experimental groups, and a decrease of histidine in rats with ischemia.

(c) Impaired whole-body metabolism

Impaired whole-body metabolism caused by liver disease appears to be a combination of the nutritional and hormonal factors. A short time period seems to be enough to induce the number of metabolic reactions, e.g. increase of glucagon/insulin ratio (Leffert et al., 1975) or activation of BCAA oxidation (Holeček et al., 1993) may be observed early after partial hepatectomy. However, a long period seems to be necessary to induce changes responsible for decrease of BCAA plasma concentrations observed usually in chronic liver disease. The mechanism responsible for a decrease of BCAA has not been explained sufficiently up to the present and altered metabolism of skeletal muscle is probably involved (Rossi-Fanelli et al., 1987).

(d) Others

As a consequence of different schemes used in particular experiments, variations between the individual amino acids in the sham animals in each group were observed. Influences of nutrition, age and season were primarily involved.

What do the BCAA/AAA ratios show?

There is some agreement that due to increased AAA and decreased BCAA, the decreased BCAA/AAA molar ratio is a common finding in liver cirrhosis. Our result substantially support this statement. However, we have the following comment.

It is likely that both impaired whole-body metabolism, decreased liver cells mass, shunting past the cirrhotic liver and leaking of amino acids from dying hepatocytes to the circulation are the mechanisms responsible for increase of plasma AAA levels in all experimental groups. However, both the increase and decrease of BCAA may be associated with liver disease. The increase of BCAA levels was associated with hepatocyte necrosis, the decrease of BCAA with liver cirrhosis. For this reason we assume that sudden

increase of BCAA concentration may indicate both the improvement and impairment of liver disease and that BCAA/AAA may not be used as indicator of the severity of hepatic parenchymal damage. We assume that marked disparity of plasma amino acid patterns in acute hepatic damage and hepatic cirrhosis may explain the little success of nutritional therapy using amino acid mixtures high in BCAA in the first group of patients where BCAA/AAA ratio was not decreased.

Arginine and ornithine

One of the most striking changes due to acute ischemia or acute CCl_4 injury were profound decrease of arginine and extreme increase of ornithine concentrations. Because of very high liver arginase activity, the hepatic concentration of arginine is normally about 1/10 that present in blood (Ratner 1973). For this reason the increased levels of arginine due to release from damaged hepatocytes cannot be expected. Release of arginase from hepatocytes during hepatocellular damage into the blood stream may cause hydrolysis of plasma arginine and subsequent increase of ornithine and decrease of arginine levels. As substantial increase of ornithine was observed in all used experimental models we assume that the impaired utilisation of this amino acid in injured liver may be involved, too. Similar behavior of these amino acids was observed by Shi and Chang (1984) in galactosamine induced hepatic coma in rats.

However, the different pattern was observed in patients with fulminant hepatic failure, i.e. plasma arginine increased significantly like most of the other amino acids (Record et al., 1976; Rosen et al., 1977). This particular feature indicate that may be dangerous to extrapolate from experimental injuries in the rat to human liver disease.

Conclusions

The general conclusions of this paper are as follows:

- (a) the differences in species of liver injury account for the variation of plasma amino acid concentrations;
- (b) the reduction of liver cell mass is associated with a significant increase of AAA, methionine, taurine, aspartate, threonine, serine, asparagine, ornithine and histidine and unaffected levels of BCAA;
- (c) a very high increase in most amino acids (BCAA included) is characteristic of fulminant hepatic failure;
- (d) increased levels of AAA, methionine, ornithine, asparagine, and aspartate and a decreased BCAA/AAA ratio due to a decrease of BCAA and an increase of AAA are associated with liver cirrhosis;
- (e) a decreased BCAA/AAA ratio may not be used as an indicator of the severity of hepatic parenchymal damage (because increased BCAA levels were observed in two models of acute liver failure);

- (f) the difference of plasma arginine behaviour in acute liver damage in rats and humans indicates its different metabolism in these species following liver injury.

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