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Role of tryptophan in the elevated serotonin-turnover in hepatic encephalopathy

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Summary. The increase of the brain levels of 5-hydroxyindoleacetic acid (5-HIAA) in hepatic encephalopathy (HE) suggests an increased turnover of serotonin (5-HT). To study the role of tryptophan on the increased brain 5-HT metabolism in HE, we attempted to monitor brain levels of tryptophan in rats with thioacetamide-induced acute liver failure by intravenous infusion of branched-chain amino acids (BCAA). The effect of this treatment on 5-HT synthesis and metabolism was investigated in five brain areas. BCAA-infusions (1 and 2 gm/kg/24 h) increased the ratio BCAA/aromatic amino acids in plasma two- and fourfold, respectively, and lowered both plasma and brain levels of tryptophan. At the higher BCAA-dose all parameters suggesting an altered brain 5-HT metabolism (increased brain levels of 5-HT and 5-HIAA, increased 5-HIAA/5-HT ratio) were almost completely normalized. These results provide further evidence for the role of tryptophan in the elevation of brain 5-HT metabolism and for a potential role of BCAA in the treatment of HE.

Keywords: Hepatic encephalopathy, tryptophan, serotonin, branched-chain amino acids, thioacetamide, liver failure.

Introduction

Several lines of evidence point to an altered central serotonergic neurotransmission in hepatic encephalopathy (HE). The most consistent finding both in humans with acute or chronic liver failure as well as in experimental models of HE is the increase of the levels of 5-hydroxyindole-3-acetic acid (5-HIAA) in most brain areas examined (Jellinger et al., 1978; Al Mardini et al., 1993). In contrast, most other neurochemical data are conflicting – both normal

or increased levels of 5-HT, normal or increased 5-HT turnover rates (Yurdaydin et al., 1990; Bengtsson et al., 1991), and normal or decreased 5-HT receptors in various brain areas were reported (Kienzl et al., 1984; Bugge et al., 1989; Bengtsson et al., 1989; Rao et al., 1994). Some of these conflicting data can be explained by the different methods and animal models used. More recent evidence obtained in microdialysis-studies indicates that the increased extracellular concentrations of 5-HIAA and the increased 5-HIAA/5-HT ratio in experimental HE apparently is not related to an increase in neuronal release of 5-HT as demonstrated in neocortex (Bergqvist et al., 1995). Thus, it is still unknown whether HE is associated with a functional alteration of central serotonergic neurotransmission or not. Neurobehavioural experiments indicate, that 5-HT_{1A} receptor agonists ameliorate HE, while serotonin reuptake inhibitors worsen HE (Ferenci et al., 1994a; Herneth et al., 1995; Yurdaydin et al., 1996).

The increase in 5-HIAA is possibly a consequence of increased precursor availability. Increased brain tryptophan levels in HE were reported by several investigators (Ono et al., 1978; Jellinger et al., 1978; Ferenci, 1994b; Bengtsson et al., 1991; Basile et al., 1995). In normal rats it is well established that exogenous tryptophan loading (up to 180 mg/kg) produces a dose-dependent increase in the synthesis, storage and intraneuronal metabolism of 5-HT in hypothalamus or striatum (Lookingland et al., 1986; During et al., 1989). In vitro it was demonstrated that 5-HT release varies with brain tryptophan levels and that the magnitude of the tryptophan effect on 5-HT release is unrelated to neuronal firing frequency (Schaechter and Wurtman, 1990). However, the metabolism of tryptophan is rather complex, and 5-HT is neither the major nor the only neuroactive tryptophan metabolite. Decarboxylation to 5-HT is not a quantitatively important pathway even after large tryptophan loads accounting for no more than 1% of the metabolism of the loading dose (Young et al., 1978). Besides 5-HT an enhanced production of the excitotoxic quinolinic acid, a metabolite of the kynurenin pathway of tryptophan has been reported after tryptophan load (up to 200 fold increase) and in HE (Moroni et al., 1986a,b; During et al., 1989). A correlation between plasma and brain quinolinic acid levels and severity of acute HE has been established in various animal models (Basile et al., 1995), whereas chronic HE is not associated with increased brain extracellular quinolinic acid concentrations (Bergqvist et al., 1996a).

The purpose of the present study was to define in the rat model of thioacetamide (TAA)-induced liver failure whether the elevation in brain 5-HT metabolism in HE results from increased precursor load or possibly reflects activation of serotonergic neurons. Therefore brain levels of tryptophan in rats with liver failure were decreased by systemic application of branched-chain amino acids (BCAA) and the effect of decreased tryptophan levels on 5-HT synthesis and metabolism was investigated. We provide evidence that in the TAA-rat model the normalization of tryptophan levels in brain achieved by application of excess of BCAA is associated with a reduction of the levels of 5-HT, 5HIAA and 5-HT turnover rate to control values in various brain regions.

Materials and methods

Animal model

In the present study the model of HE in rats with TAA-induced acute liver failure was used. This model has been well characterized by clinical, light microscopic, electron microscopic and neurochemical investigations (Zimmermann et al., 1989; Yurdaydin et al., 1990). Briefly, male Sprague Dawley rats (Versuchstieranstalt Himberg, Austria) weighing 200 to 300 gm were used. Six days before the start of the experiments cannulas were implanted in the jugular vein. Liver failure was induced by oral administration of 300 mg TAA/kg body weight by gavage on two consecutive days. To prevent hypoglycemia and renal failure a supportive therapy was added, consisting of normal saline, 5% glucose and 20 mEq/liter potassium (12 ml t.i.d.). To evaluate the progression of HE rats were neurologically tested by a score based on 14 different reflexes (Zimmermann et al., 1989). Infusion-therapy was started 60 h after the first TAA-application (Püspök et al., 1995). Saline or two different concentrations of BCAA (leucine: isoleucine: valine = 2:1:1; 1 or 2 gm/kg body weight/24 h) were infused via the cannula in the jugular vein at a rate of 0.5 ml/h. At this point of time none of the animals showed overt signs of HE. Infusion-therapy was prolonged until the appearance of symptoms of HE (i.e. loss of reflexes according to Zimmermann et al., 1989), but at longest for 8 h. The infusion of 1 or 2 gm/kg/24 h BCAA results in a 3 to 4 fold and 8 to 10 fold increase of the BCA/AAA ratio in TAA treated rats, respectively (Püspök et al., 1995). Animals, which developed symptoms within 2 h after initiation of infusion-therapy, were excluded. The mean infusion time was 6.6 ± 0.5 h. At the end of the infusion period blood was drawn by cardiac puncture and rats were decapitated. The brains were rapidly removed, immediately frozen on dry ice and stored at −70°C.

Determination of 5-HT, 5-HIAA and tryptophan

Frozen brains were dissected on a cold plate (−5° to −10°C) according to the atlas of König and Klippel (1970). Frontal cortex, hippocampus, striatum, parietal cortex and hypothalamus were taken as representative brain areas from coronal slices. The brain areas from the left hemisphere were used for the determination of 5-HT and 5-HIAA, the right-sided areas were reserved for the analyses of tryptophan. Tissue samples were homogenized by ultrasonication in 20–40 volumes of 0.1 M perchloric acid containing 0.4 mM $NaHSO_3$, and the precipitated protein was removed by centrifugation at 25.000 g at 4°C for 15 min. In the supernatant 5-HT and 5-HIAA were determined by HPLC with electrochemical detection as described previously (Sperk, 1982). For the determination of tryptophan tissue samples were homogenized by ultrasonication in 10 to 20 volumes of a 40 gm/L solution of sulfosalicylic acid with 100 μm/L of β-thienylalanine added as internal standard. Plasma samples were deproteinized with sulfosalicylic acid containing β-thienylalanine as internal standard. After centrifugation, the supernatant was adjusted to pH 2.2 with LiOH (0.2 mol/L) and stored until analysis at −70°C. Amino acids were separated by HPLC and determined with ophthalaldehyde (Karner et al., 1989).

Data analyses

All data are presented as means ± SEM. For statistical analyses the one-way ANOVA followed by Newman-Keuls test was applied.

Results

Changes in 5-HT, 5-HIAA and tryptophan

The TAA-induced changes in 5-HT, 5-HIAA, tryptophan and in the molar ratio of 5-HIAA to 5-HT are summarized in Fig. 1–3 and Table 1. The present

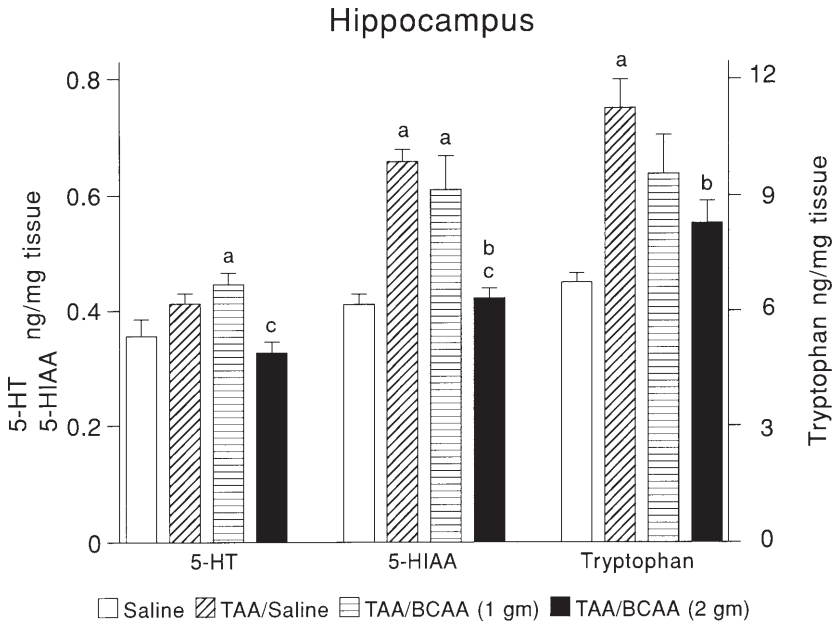

Fig. 1. Changes in the levels of 5-HT, 5-HIAA and tryptophan in the rat hippocampus in TAA-induced liver failure and in response to BCAA infusion. Saline-group: n = 6; TAA/saline-group: n = 12; TAA/BCAA (1 gm): n = 7; TAA/BCAA (2 gm): n = 7. The significance of differences between the various groups (one way ANOVA) is indicated: [a]$p < 0.05$ versus saline; [b]$p < 0.05$ versus TAA/saline; [c]$p < 0.05$ versus TAA/BCAA (1 gm)

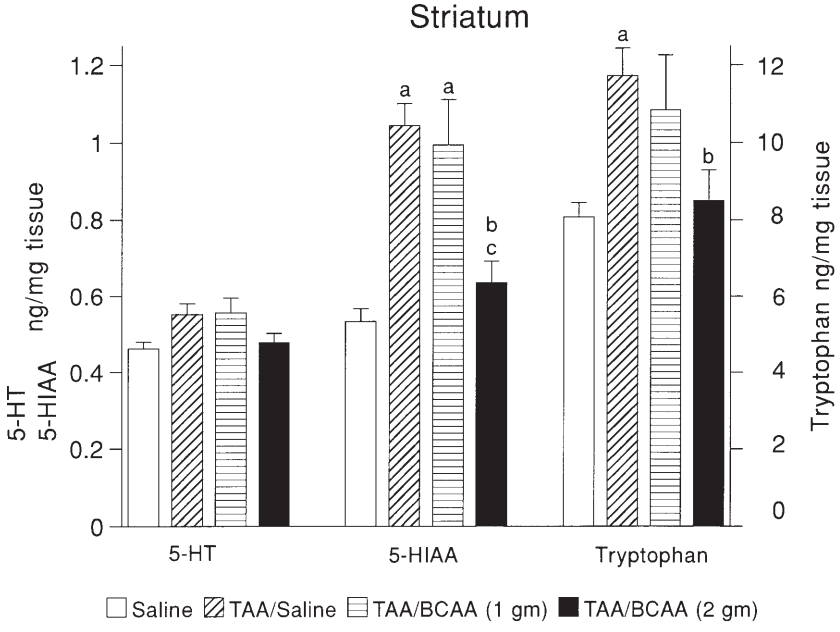

Fig. 2. Changes in the levels of 5-HT, 5-HIAA and tryptophan in the rat striatum in TAA-induced liver failure and in response to BCAA infusion. For details see legend to Fig. 1

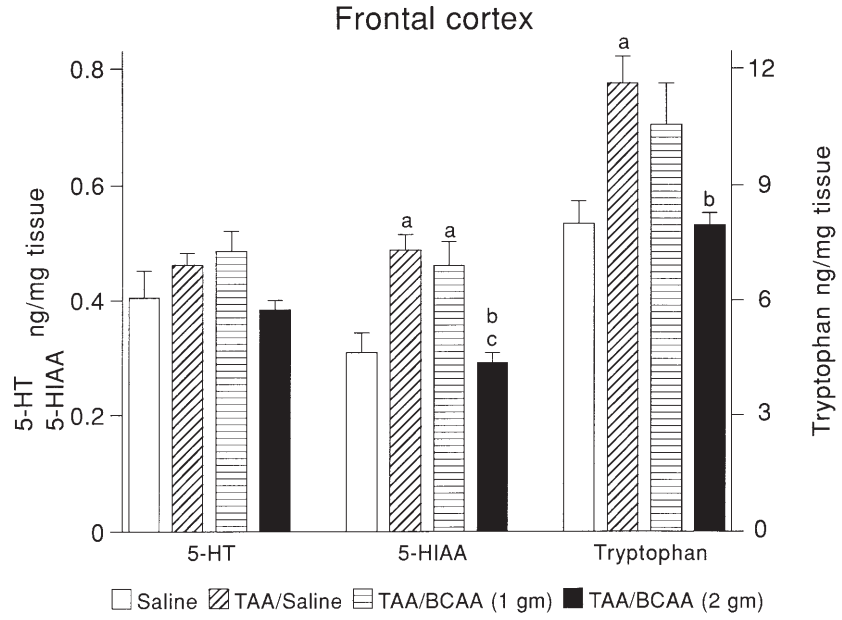

Fig. 3. Changes in the levels of 5-HT, 5-HIAA and tryptophan in the rat frontal cortex in TAA-induced liver failure and in response to BCAA infusion. For details see legend to Fig. 1

Table 1. Changes in the molar ratio of 5-HIAA/5-HT in the rat brain after TAA-induced liver failure and in response to the treatment with BCAA

Brain area	Saline (n = 6)	TAA/saline (n = 12)	TAA/BCAA (1 gm/kg/24 h) (n = 7)	TAA/BCAA (2 gm/kg/24 h) (n = 7)
hippocampus	1.112 ± 0.121	1.488 ± 0.050[a]	1.256 ± 0.080[b]	1.222 ± 0.091[b]
frontal cortex	0.727 ± 0.080	0.988 ± 0.064[a]	0.873 ± 0.038	0.704 ± 0.048[b]
striatum	1.070 ± 0.077	1.777 ± 0.117[a]	1.625 ± 0.108[a]	1.225 ± 0.092[b,c]
hypothalamus	0.870 ± 0.131	1.260 ± 0.209	1.095 ± 0.296	0.889 ± 0.212
parietal cortex	1.111 ± 0.194	1.429 ± 0.116	1.392 ± 0.157	1.211 ± 0.073

[a] $p < 0.05$ versus saline, [b] $p < 0.05$ versus TAA/saline, [c] $p < 0.05$ versus TAA/BCAA (1 gm/kg/24 h)

results demonstrate that in TAA-induced liver failure the tissue levels of 5-HIAA and the molar ratio of 5-HIAA/5-HT are uniformely increased in all regions of the rat brain investigated (Figs. 1–3). The most pronounced changes were observed in the striatum, in which the concentration of 5-HIAA increased by 95.5 ± 10.7% and the molar ratio of 5-HIAA/5-HT by a factor of 1.66 ± 0.11. The increase did not reach significance in the hypothalamus and parietal cortex (data not shown). The levels of 5-HT, in contrast, were only insignificantly elevated by maximally 20%. In addition, the TAA-

induced liver failure was associated with remarkable changes in the tissue levels of tryptophan. As compared to control rats the concentration of tryptophan increased from 6.73 ± 0.24 to 11.25 ± 0.74 ng/mg tissue ($p < 0.05$) in the hippocampus, from 8.01 ± 0.58 to 11.62 ± 0.70 ng/mg tissue ($p < 0.05$) in the frontal cortex and from 8.07 ± 0.38 to 11.73 ± 0.71 ($p < 0.05$) in the striatum.

Effect of the infusion of BCAA on brain levels of tryptophan, 5-HT and 5-HIAA

In response to the treatment with BCAA-infusion for 6.6 ± 0.5 h the elevated levels of tryptophan dose-dependently declined in all brain areas investigated (Fig. 1–3). At the higher dose range of BCAA (2 gm/kg/24 h) a return to normal levels was achieved in the striatum and frontal cortex. Only in the hippocampus the level of tryptophan remained slightly elevated (8.29 ± 0.58 vs. 6.73 ± 0.24 ng/mg tissue; $p < 0.05$). However, in this brain area the percent TAA-induced increase of tryptophan was most pronounced (to 167.2 ± 11.0% as compared to 145.1 ± 8.7 and 145.4 ± 8.8% in frontal cortex and striatum, respectively). Concomitantly, at the higher dose of BCAA the TAA-induced increase in the levels of 5-HT and 5-HIAA as well as in the molar ratio of 5-HIAA/5-HT was completely reversed (Figs. 1–3; Table 1). The normalization of the serotonergic parameters was also confirmed in the hypothalamus and parietal cortex, in which the tryptophan levels were not measured (data not shown).

Effect of infusion of BCAA on plasma levels of amino acids

In response to the infusion of BCAA the plasma levels of leucine, isoleucine and valine significantly increased, whereas the levels of tryptophan considerably decreased at the higher dose of BCAA. In addition, a dose-dependent increase in the ratio BCAA/aromatic amino acids (AAA) was achieved (Table 2).

Table 2. Changes in the plasma levels of BCAA, tryptophan (μmoles per liter) and in the ratio of BCAA/AAA

	TAA/saline	TAA/BCAA (1 gm/kg/24 h)	TAA/BCAA (2 gm/kg/24 h)
Leucine	264 ± 29	650 ± 114*	693 ± 110*
Isoleucine	137 ± 27	374 ± 74*	427 ± 72*
Valine	267 ± 36	809 ± 112*	860 ± 116*
Tryptophan	43 ± 4	43 ± 2	28 ± 3*
BCAA/AAA	2.3 ± 0.3	4.1 ± 0.6*	8.5 ± 0.7*

*$p < 0.05$ versus TAA/saline

Discussion

Neurochemical findings in HE are almost exclusively restricted to animal models. Most of these observations are model dependent and cannot be generalized to explain the pathophysiology of HE. With respect to changes in 5-HT and 5-HIAA the data are, however, quite reproducible, but the effects of BCAA on central 5-HT remain uncertain. The available data in experimental acute liver failure were mainly obtained in rats after total hepatectomy or liver ischemia. Both models involve surgery, anesthesia and continuous glucose administration. The absence of hepatocytes, the postoperative stress and glucose have profound effects on BCAA metabolism. Thus, data obtained in these models do not necessarily reflect the situation in fulminant hepatic failure due to hepatocellular necrosis. With this respect the TAA-model is far closer to the situation in human disease. Thus, the demonstration of an effect of BCAA on parameters of central 5-HT metabolism in this model of acute liver failure confirms and extends previous observations in other models.

The findings of this study indicate that lowering of brain tryptophan levels by BCAA infusions results in an almost complete normalization of altered brain 5-HT metabolism in HE in rats with TAA-induced acute liver failure. Similar observations were made in other experimental models of HE previously. Infusions of BCAA reduced increased brain 5-HT synthesis and turnover associated with liver failure (Bugge et al., 1987). Furthermore, our result are also in line with the finding of a normalization of 5-HT and 5-HIAA in the cerebrospinal fluid in patients with liver failure by BCAA administration (Rössle et al., 1984).

Although the link between imbalance of tryptophan metabolism and increased 5-HT synthesis and turnover appears to be well established, several open questions remain. First, a direct correlation between the normalization of tryptophan levels in brain and a decrease in 5-HT and its main metabolite has not yet been proven in HE. In the present investigation we were able to demonstrate for the first time that the reduction of elevated brain tryptophan levels induced by BCAA was strictly paralleled by a comparable decrease in the tissue levels of 5-HIAA and in the molar ratio of 5-HIAA/5-HT. The up to 1.6 fold increase in brain tryptophan in various brain regions of saline infused rats with liver failure agrees with the extent of uniform elevation of tryptophan reported in TAA-treated rats previously (Basile et al., 1995). At the higher dose range of BCAA (2 gm/kg/24 h) levels of tryptophan returned to control levels accompanied by a complete reversion of the increase in 5-HIAA and in the ratio 5-HIAA/5-HT. These data therefore indicate that the increase in 5-HT turnover results from the enhanced availability of the precursor amino acid, but that the activity of serotonergic raphe neurons per se is not elevated. Tissue levels of 5-HT are influenced by the prevailing brain tryptophan levels also under physiological conditions. In rat hypothalamic slices superfused with medium supplemented either by tryptophan or leucine both precursor-dependent elevations and reductions in brain 5-HT levels were observed, respectively (Schaechter and Wurtman, 1990). Various other

findings support the role of increased tryptophan availability as a prominent cause of increased 5-HT synthesis and turnover in HE. In the TAA-model the decrease in the molar ratio of BCAA/AAA in plasma was paralled by a corresponding increase in brain 5-HIAA (Yurdaydin et al., 1990). A decrease in the ratio BCAA/AAA was also observed in autopsied brain tissue from cirrhotic patients with HE (Bergeron et al., 1989).

Second, it is still a matter of debate whether increased 5-HT synthesis and turnover induced by elevated precursor levels have an impact on the neuronal release of 5-HT. In hypothalamic slices it has been demonstrated that elevation or reduction of tryptophan levels causes proportionate increases or decreases in the spontaneous and electrically evoked 5-HT release (Schaechter and Wurtman, 1990). In vivo, the systemic administration of the 5-HT precursors L-tryptophan or 5-hydroxy-L-tryptophan caused an immediate, dose-dependent release of 5-HT in rat hypothalamus and hippocampus as measured by microdialysis. This release occurred by a calcium-dependent mechanism (probably exocytosis) and was dependent on serotonergic neuronal activity and predominantly derived from 5-HT neurons (Gartside et al., 1992; Sharp et al., 1992). Other studies, however, failed to confirm an increased neuronal neocortical 5-HT release after an acute challenge with L-tryptophan in control rats as well as in portacaval shunted rats or after acute, subacute or chronic experimental portal-systemic encephalopathy (Bergqvist et al., 1995, 1996b). Recent results, however indicate an enhanced potassium-evoked neuronal release of 5-HT in neocortex of rats with experimental chronic portal-systemic encephalopathy (Bergqvist et al., 1997a). In conclusion, the available evidence is in support of enhanced 5-HT output under certain pharmacological conditions and suggests that drugs influencing 5-HT release or uptake may represent a potential hazard in patients with HE (Bergqvist et al., 1997b).

Third, alterations in tryptophan-hydroxylating activity may be involved additionally in the elevation of 5-HT synthesis. Although only a minor pathway in tryptophan catabolism, changed tryptophan hydroxylation and decarboxylation may be present in liver failure. A tryptophan load in liver failure may result in a higher increase in brain 5-HT turnover compared to controls (Bergqvist et al., 1997c). In tryptophan loaded portacaval shunted rats a supranormal tryptophan-hydroxylating activity was found, possibly resulting from the redox shift of the essential cofactor tetrahydrobiopterin into its active reduced form (Bengtsson et al., 1991). Similarly, the increased activities in HE of both MAO_A and MAO_B may increase the 5-HT turnover-rate in HE (Rao and Butterworth, 1993). Studies in transgenic animals with increased or lowered expression of tryptophanhydroxylase, which are in progress, will provide more detailed information on the role of tryptophanhydroxylase in altered 5-HT metabolism and the occurrence of HE.

A reduction of elevated tryptophan levels achieved by BCAA-treatment could also be of relevance to avoid an exaggerated production of quinolinic acid, another tryptophan metabolite, which acts as agonist on NMDA receptors. Considerable increases of quinolinic acid have been described in the brain and cerebrospinal fluid of patients with HE, and in various animal

models, which were further augmented after a tryptophan load (Moroni et al., 1986a,b; During et al., 1989; Basile et al., 1995). Accumulation of quinolinic acid may contribute to neuronal damage that can occur in chronic recurring HE. In this respect a treatment with BCAA resulting in the reduction of tryptophan levels and thus of quinolinic acid synthesis might be of additional benefit.

In conclusion, these results provide additional support for the use of BCAA in the treatment of patients with HE. In view of various conflicting data the clinical efficacy of BCAA, however, is still not proven and is a matter of an ongoing debate (Morgan, 1990; Riordan and Williams, 1997). Recent reports on beneficial clinical responses (Plauth et al., 1993; Huguchi et al., 1994; Fabbri et al., 1996; Chalasani and Gitlin, 1996) and the available experimental evidence on the efficacy of BCAA warrant carefully conducted studies to finally elucidate their role in HE. The convincing correlative demonstration of a normalization of elevated tryptophan levels in plasma and brain tissue followed by a normalization of 5-HT, 5-HIAA and 5-HT-turnover in response to BCAA in the present study might be one further step to overcome the pervasive sense of nihilism regarding the use of BCAA-based nutritional therapy for acute HE, which has been created by inconsistent clinical results (Charlton, 1996). Concerning the putative involvement of increased serotonergic function in the pathogenesis of HE, ongoing research in transgenic animals with manipulations in the serotonergic system of the brain will provide further insights.

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