Plasma and Cerebrospinal Fluid Amino Acid Patterns in Hepatic Encephalopathy

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Plasma and cerebrospinal fluid amino acid levels were measured in 12 cirrhotic patients in grade 0 hepatic encephalopathy and 17 in grade 3–4 hepatic encephalopathy. In 5 of these patients amino acid determinations were performed during the evolution of the encephalopathy. No correlation was found between the degree of hepatic encephalopathy and the plasma amino acid imbalance. In the CSF of cirrhotic patients without encephalopathy, a significant increase was found in nearly all amino acids, including those known to not easily cross the blood–brain barrier; this suggests the presence of a nonspecific modification of the blood–brain barrier permeability. In patients with severe hepatic encephalopathy, the further increase only in cerebrospinal fluid aromatic amino acids and methionine levels suggests the presence of a selective stimulation of the neutral amino acid transport system across the blood–brain barrier. Finally, the good correlation between glutamine and the sum of neutral amino acids found in the cerebrospinal fluid only in the presence of encephalopathy supports the hypothesis that brain glutamine may stimulate neutral amino acid transport across the blood–brain barrier.

The characteristic plasma amino acid pattern observed in chronic liver disease, ie, high aromatic amino acids (AAA) and low branched-chain amino acids (BCAA) and the consequent brain accumulation of AAA has been implicated in the pathogenesis of hepatic encephalopathy (HE) (1, 2). Previous studies from this (3) and other laboratories (4–6), have failed to demonstrate any correlation between HE and plasma amino acid imbalance. Nevertheless, in animals with portacaval shunt, brain and cerebrospinal fluid (CSF) AAA have been reported to show a significant increase (7, 8). These findings may indicate that amino acid levels in plasma do not reflect those in the brain. On the other hand there is little doubt that CSF more closely represents brain amino acid behavior (9). Very few and somewhat conflicting data have so far been reported on CSF amino acid levels in patients with HE (10, 11). The purpose of this investigation was to study plasma and CSF amino acid patterns in cirrhotic patients with and without severe HE.

MATERIALS AND METHODS

Four groups of subjects were studied.

Group A. Ten subjects (aged between 29 and 62, 6 males and 4 females), admitted to the Division of Neurosurgery for conditions none of which induce CSF abnormalities and used for control purposes.

Group B. Twelve patients with liver cirrhosis (aged between 34 and 72, 8 males and 4 females) in grade 0 HE according to Adams and Foley's classification as reported by Fischer et al (2). Diagnosis was based on clinical, biochemical, and histological findings. Informed consent was obtained from each patient after the purpose of the study had been carefully explained.

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Group C. Seventeen patients with liver cirrhosis (aged between 24 and 80, 10 males and 7 females) in grade 3–4 HE. Diagnosis of the cirrhosis and degree of the coma were assessed as in group B. Informed consent was obtained from the next of kin.

Group D. Five patients (aged between 44 and 61, 3 males and 2 females) whose plasma and CSF samples were drawn during evolution of encephalopathy (ie, from grade 3–4 to grade 0).

Plasma and CSF samples from control subjects and group B patients were drawn after overnight fasting. Samples from group C patients were obtained at the time of diagnosis of hepatic coma and before commencing any treatment. Most of these patients were fasting at the time of the sample collection. Patients with hepatorenal syndrome were excluded from the study.

Amino Acid Determinations. Blood was drawn into heparinized tubes and then immediately centrifuged at 3000 rpm for 15 min. Thirty mg of solid sulfosalycylic acid were then added to plasma and CSF for deproteinization. The samples were centrifuged at 5000 rpm for 20 min. Hydroxyproline was added as internal standard to the supernatant to reach a final concentration of 0.50 μmol/ml. The samples were then filtered through Whatman No. 1 paper and stored at −70° C until use. One hundred microliters were analyzed in a Carlo Erba Automatic AA analyzer, model 3 A28, using lithium buffers which separate glutamine and asparagine.

Tryptophan Determination. Plasma total tryptophan was determined by the spectrophotofluorimetric method described by Duggan and Udenfriend (12). The same method was used to estimate free tryptophan in an ultrafiltrate obtained from 2 ml of plasma or CSF, centrifuged at 800*g* in an Amicon 224-CF-50 centrifuge for 45 min at room temperature (13, 14). All values were corrected for reagent blank. This method showed a recovery of 96% for total tryptophan and 88% for free tryptophan.

Statistical Analysis. Student's *t* test for unpaired data was employed for the statistical analysis of the results.

RESULTS

Plasma and CSF amino acids levels in normal subjects are given in Table 1, whereas those in patients are shown in Figure 1.

Plasma Amino Acid Levels. BCAA were significantly decreased ($P < 0.05$) both in groups B and C with respect to controls (group A), without any significant difference between groups B and C. AAA were found, on the other hand, to be significantly increased ($P < 0.02$) in patients with respect to controls. Of the three AAA, only free tryptophan rose significantly ($P < 0.01$) in group C with respect to group B. Also methionine levels were significantly higher ($P < 0.02$) in patients than in controls, but no significant difference was found between groups B and C.

The remaining neutral amino acids (NAA), glutamine, taurine, threonine, serine, glycine, and alanine showed negligible modifications in the two groups of patients with respect to controls.

Within acidic amino acids, glutamic acid significantly rose in group B with respect to group A ($P < 0.005$), but no further modification was found in group C. Aspartic acid levels did not differ from controls in the two groups of patients.

CSF Amino Acids Levels. Unlike in plasma, BCAA in CSF were significantly increased in group B compared to controls ($P < 0.01$), but no further increase was observed in group C. Also AAA were significantly increased ($P < 0.02$) in group B with respect to controls but, unlike BCAA, a further significant elevation ($P < 0.02$) was found in group C with respect to group B.

Glutamine levels had the same behavior as AAA, rising from group A to group B and further in group C. Methionine rose significantly only in group C ($P < 0.02$), whereas no statistical difference was found between groups A and B.

Threonine, serine, glycine, and alanine were significantly more elevated in groups B and C vs group A ($P < 0.05$), but no variations were observed between groups B and C. Both the acidic amino acids, glutamic and aspartic acids, increased without statistical relevance in groups B and C with respect to group A.

Plasma and CSF Amino Acids Levels During Evolution of Coma. Plasma amino acid levels, with the exception of free tryptophan, displayed no significant variation when the patients regained consciousness. Conversely, modifications in CSF methionine, glutamine, and AAA paralleled the evolution of HE. In grade 3–4 HE, a significant correlation ($r = 0.82$; $P < 0.01$) was observed in the CSF between glutamine and the sum of neutral amino acids, whereas in grade 0 HE no correlation was found (Figure 2).

DISCUSSION

In recent years a great deal of attention has been devoted to the role played by plasma and brain amino acid derangements in the pathogenesis of HE (1, 2). In plasma a characteristic amino acid pattern, ie, high AAA and low BCAA levels, has constantly been found in chronic liver failure (3–5, 15). However, no correlation was demonstrated between plasma AA imbalance and HE in this (3) and other laboratories (4, 5).

On the other hand, the attempt to "normalize" the plasma AA imbalance with intravenous infusions of AA mixtures rich in (1, 16, 17), or contain-

TABLE 1. AMINO ACIDS IN FASTING PLASMA AND CSF OF CONTROLS (GROUP A)

Amino acids	*Plasma* (10 subjects)*	*CSF* (10 subjects)*
Threonine	13.66 ± 0.98	3.35 ± 0.36
Valine	27.23 ± 1.46	1.97 ± 0.21
Leucine	14.04 ± 1.08	1.88 ± 0.17
Isoleucine	6.89 ± 0.53	1.06 ± 0.26
Methionine	2.17 ± 0.16	1.81 ± 0.40
Phenylalanine	5.35 ± 0.41	1.34 ± 0.16
Lysine	18.70 ± 1.40	1.95 ± 0.24
Free tryptophan	0.43 ± 0.03	0.31 ± 0.02
Taurine	9.01 ± 1.65	0.81 ± 0.12
Aspartic acid	1.08 ± 0.51	0.40 ± 0.08
Serine	13.82 ± 0.67	3.64 ± 0.23
Glutamic acid	2.68 ± 0.36	1.95 ± 0.49
Glutamine	55.91 ± 2.88	50.99 ± 4.01
Glycine	25.19 ± 1.28	1.47 ± 0.22
Alanine	33.45 ± 3.45	3.59 ± 0.31
Tyrosine	5.64 ± 0.36	1.56 ± 0.13
Ornitine	6.43 ± 0.61	1.07 ± 0.24
Histidine	8.20 ± 0.80	1.07 ± 0.18
Arginine	7.91 ± 0.67	2.09 ± 0.51

*Results (mean ± SE) are expressed in μmol/dl.

ing exclusively (18, 19), BCAA resulted in an improvement of HE.

These data, together with the findings of neurological symptoms resembling hepatic coma following intracarotid infusion of AAA in normal animals (20), support the theory that amino acids are involved in the pathogenesis of HE.

This apparent discrepancy between plasma and CSF amino acid changes is probably due to the BBB which, under normal conditions, modulates the entry into brain of many blood-borne substances. It has in fact been hypothesized that "anatomical" (21) or "functional" (22) impairments of BBB may participate in the pathogenic pathway of HE. As far as CSF or brain amino acid levels are concerned, very few and partially conflicting data have been reported either in experimental (7, 8) or in human HE (5, 10, 11). Thus definitive conclusions cannot be drawn from these findings. Data from the present study confirm that the plasma AA imbalance found in chronic liver failure is not further impaired by encephalopathy. Only free tryptophan, as previously reported by us (13, 14) and others (5, 11), increases in parallel with the severity of HE. Conversely, in CSF, cirrhotics in grade 3–4 HE showed two different and characteristic amino acid patterns:

1. CSF levels of nearly all amino acids in cirrhotics in grade 0 HE were significantly higher than normal. In particular, this increase was observed not only in AAA, which were already elevated in plasma, but also for other amino acids which, in plasma, were unchanged or even decreased, like BCAA. These findings suggest that the blood–brain barrier permeability, as already hypothesized in

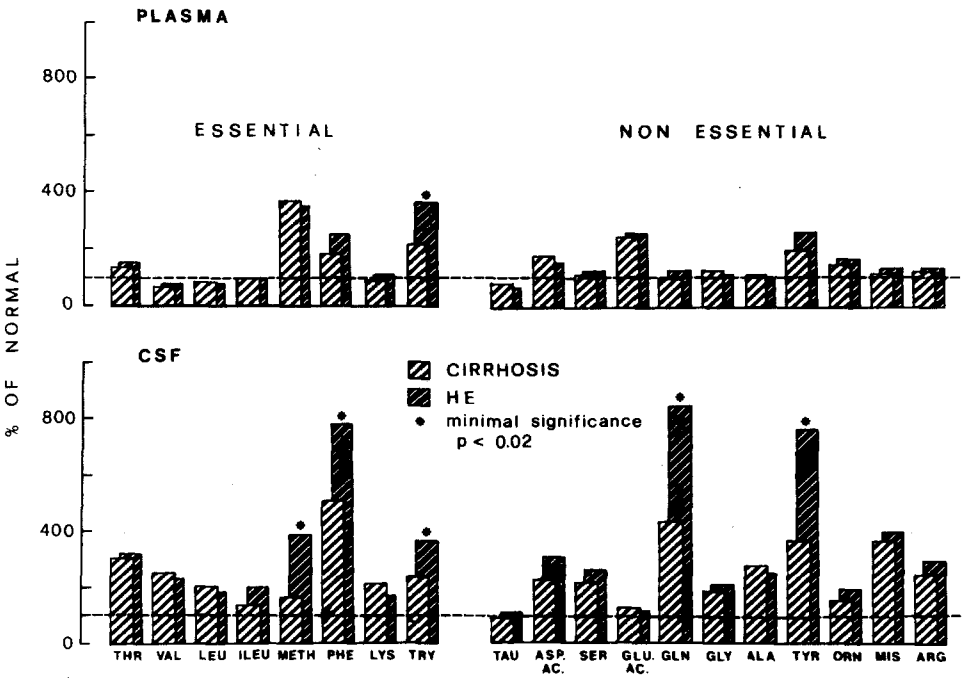

Fig 1. Plasma and CSF amino acid levels in 12 patients with liver cirrhosis in grade O HE (▨) compared with those in 17 patients in grade 3–4 HE (▩). * $P < 0.02$.

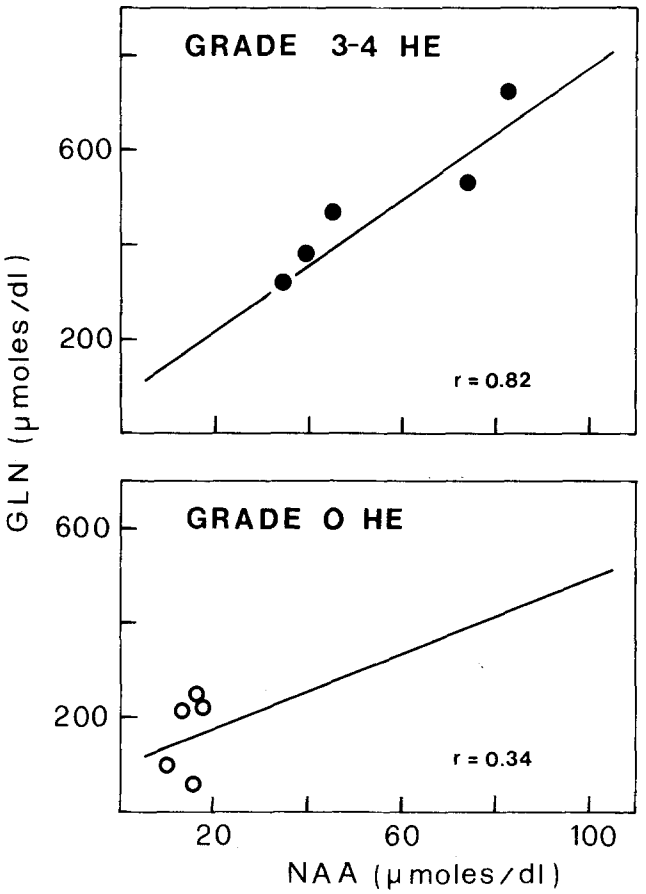

Fig. 2. Linear regression coefficient between glutamine (GLN) and sum of the following neutral amino acids (NAA): valine, leucine, isoleucine, methionine, phenylalanine, free tryptophan, tyrosine, in CSF of five cirrhotic patients during the evolution of their coma from grade 3–4 HE to grade O HE.

preliminary observations (23), may be nonspecifically altered. In fact glycine, alanine, serine, and threonine, which from *in vivo* experiments are known not to easily cross the BBB and are consequently called low-uptake amino acids (24), were greatly increased in CSF despite their normal plasma levels. Moreover, the increased brain "sensitivity" hypothesized in cirrhotic patients may be explained by this altered BBB permeability (25).

2. In patients in grade 3–4 HE, there was a significant increase in the CSF of only phenylalanine, tyrosine, tryptophan, methionine, and glutamine. This increase is not in keeping with Ono's observations (11), but it is in agreement with those of Watanabe (26). The rise in AAA might result, as recently suggested (22, 27), from a selective stimulation of the transport system from neutral amino acids across the BBB. Since the affinity for this transport system of AAA, BCAA, and methionine is similar (24, 28), brain levels of these amino acids, in the presence of a stimulated transport activity, would reflect their concentrations in plasma. According to James et al (27), the rise of brain glutamine, consequent to hyperammonemia, would increase glutamine efflux from the brain, and in turn, stimulate blood–brain entry of neutral amino acids. This hypothesis, confirmed also *in vitro* using isolated brain capillaries (29–31), is based on the strong correlation between glutamine and neutral amino acids in the brains of shunted rats. In the five patients in whom the evolution of encephalopathy was followed, this correlation was lacking in the normal mental state but became extremely significant when encephalopathy supervened.

In conclusion, in contrast with plasma, a clear parallelism was found between CSF amino acid levels and mental state. The CSF amino acid pattern in chronic liver disease is probably due to a

nonspecific alteration of the BBB permeability, which may account for the increased "brain sensitivity." Finally, when encephalopathy supervenes, the selective increase of aromatic amino acids and methionine may be due to a specific stimulation of their transport system across the BBB, probably consequent to the rise of brain glutamine.

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