Liver, biliary, and pancreas

Raised plasma concentrations of 3-methoxy-4-hydroxyphenylethyleneglycol in cirrhotic patients with or without hepatic encephalopathy

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SUMMARY We measured the plasma concentration of a centrally derived noradrenaline (NA) metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), in 20 cirrhotic patients (eight with (group A) and 12 without (group B) hepatic encephalopathy (HE)) and in 14 age matched healthy subjects to study if the central NA metabolism would be altered in liver cirrhosis patients, particularly in those with HE. The mean (SEM) plasma MHPG concentrations in the patient groups, group A (74.9 (8.6) pmol/l) and B (54.8 (7.2) pmol/l), were significantly ($p < 0.01$) greater than in the control group (22.3 (2.0) pmol/l), and that in group A was significantly ($p < 0.05$) greater than in group B. The plasma concentration of MHPG observed in these study subjects ($n = 34$) correlated ($r_s = 0.77$, $p < 0.01$) more strongly with the ratio of plasma catecholamine precursor amino acids (tryptophan and phenylalanine) to other neutral amino acids (tryptophan, leucine, isoleucine, and valine) known to compete with catecholamine precursor amino acids for uptake into the brain than with plasma concentration of tyrosine plus phenylalanine alone ($r_s = 0.63$, $p < 0.01$). In addition, the mean plasma MHPG concentrations measured in another group of eight cirrhotic patients (group C) during HE (79.3 (10.6) pmol/l) was significantly ($p < 0.01$) greater than that measured after the recovery from HE (47.2 (5.2) pmol/l). The results suggest that the central NA metabolism may be altered in patients with liver cirrhosis, particularly in those with HE, and that the derangement in the central NA metabolism may be associated not only with an increase in plasma catecholamine precursor amino acids but also with a decrease in branched chain amino acids.

Although the pathogenesis of hepatic encephalopathy (HE) remains enigmatic, lines of evidence suggest that a derangement in the metabolism of central monoaminergic neurotransmitters – for example, noradrenaline (NA), dopamine, serotonin, may play an important role in the development of HE.¹⁻³ Previous studies have shown that metabolites of these monoamine neurotransmitters were grossly raised in the brain and cerebrospinal fluids of animals and humans during HE.⁴⁻⁸ A possible derangement in the central monoamine metabolism during HE may be associated with an augmented transport of

their precursor amino acids – that is, tryptophan, phenylalanine, tryptophan, into the brain. Previous studies have shown that concentrations of these aromatic amino acids (AAA) were substantially raised not only in plasma but also in the cerebrospinal fluid in patients with HE.^{6,9} To our knowledge, however, it remains unclear if a certain index of the central monoamine metabolism – for example, plasma or cerebrospinal fluid concentrations of centrally derived monoamine metabolites, obtained from cirrhotic patients with HE would significantly differ as compared with those from cirrhotic patients without HE and from normal healthy subjects.

Inasmuch as neutral amino acids – for example, AAA and branched chain amino acids (BCAA), are known to share a common transport system at the

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blood brain barrier (BBB), brain uptake of individual amino acids would depend not only on the plasma concentration of the respective amino acid but also on those of competing amino acids.¹⁰ Accordingly, a characteristic plasma amino acid imbalance – that is, high in AAA concentrations and low in BCAA concentrations, which has been observed in previous studies^{9 11–13} in patients with liver cirrhosis, may be in favour of a facilitated transport of plasma AAA across the BBB in these patients. No attempt has been made, however, to ask if such an amino acid imbalance in plasma would correlate with a certain index of the central monoamine metabolism in patients with liver cirrhosis, particularly in those with HE.

Direct assessment of the central monoamine metabolism in man is not feasible for ethical reasons. In recent studies, therefore, an attempt has been made to study if plasma concentrations of centrally derived monoamine metabolites, such as, MHPG and homovanillic acid, would be associated with therapeutic responses of certain psychiatric diseases^{14–16} and the prognosis of patients with subarachnoid haemorrhage.^{17 18} To our knowledge, however, no studies have utilised this approach to address the question if the central monoaminergic metabolism would be altered in cirrhotic patients with or without HE as compared with healthy subjects. In this context, our study aims were set (1) to probe the central NA metabolism in cirrhotic patients with or without HE by measuring plasma concentrations of a centrally derived NA metabolite, MHPG; (2) to determine if the plasma amino acid imbalance observed in the cirrhotic patients would be associated with the possibly deranged central NA metabolism; and (3) to ask if plasma MHPG concentrations during and after the recovery from HE would differ within the same cirrhotic patients.

Methods

STUDY SUBJECTS

Twenty eight patients with liver cirrhosis, having been verified by biopsy specimen and clear cut clinical and laboratory findings, and 14 age matched normal healthy subjects were enrolled in the present study. Clinical characteristics of patients pertinent to the present study are summarised in Tables 1 and 2. Patients with liver cirrhosis consisted of three groups according to the presence (group A and C, n=eight each) and absence (group B, n=12) of HE. The grade of HE in the group A and C patients was determined by two independent observers (HE and NU) according to the Parsons-Smith's criteria.¹⁹ The grades of HE observed in the majority of the group A and C patients were low and their liver function was

relatively stable (Tables 1 and 2) and no life threatening complications such as severe bacterial infection and/or concurrent variceal bleeding, were present. The control subjects were judged to be normal with a history taking and physical examination carried out by one of the authors (HE) and with appropriate biochemical tests. Informed consent was obtained before the study from each of the healthy subjects and group B patients. For the group A and C patients, the informed consent was obtained from the next of kin. Patients manifesting signs of hepatorenal syndrome assessed according to the established criteria at the symposium held in Sassari²⁰ were excluded from the study. The study was approved by the Institutional Ethics Committee and performed according to the principles of the Declaration of Helsinki.²¹

STUDY DESIGN AND MEASUREMENTS

The study protocol consisted of two parts. The first part was designed to determine if (1) there would be any significant differences in plasma MHPG concentrations among cirrhotic patients with (group A) and without (group B) HE and healthy controls, and (2) plasma MHPG concentrations in these patients and controls would be not only a reflection of plasma NA precursor amino acid concentrations but also that of other neutral amino acids known to share a common transport system across the blood brain barrier.¹⁰ In the second part of the study, plasma MHPG concentrations were measured twice in the same cirrhotic patients (group C) during and after the recovery from HE to ask if plasma MHPG concentrations in these patients would show any intraindividual difference in relation to the improvement of their mental status.

The group B patients received a hospital diet containing 75 g protein, 345 g carbohydrates, and 25 g fat daily. Total caloric intake was adjusted to 1900 kcal/day during hospitalisation. The group A and C patients were prohibited from oral nutritional intake until their neurological manifestations disappeared. The treatment of HE for the group C patients consisted of the elimination of precipitating factors for HE, the restriction or reduction of dietary protein intake, and/or the administration of lactulose. A BCAA-enriched amino acid solution with glucose was given to the patients who were unable to tolerate sufficient protein intake to prevent a long standing negative nitrogen balance. Plasma MHPG concentrations were measured twice – that is, during and after the recovery from HE, without changing the individually required treatment schedule or under the same therapeutic background.

For each cirrhotic patient the routine laboratory examinations and coagulation tests were done. For plasma ammonia measurements, blood samples

Table 1 Clinical characteristics and laboratory data of patients with liver cirrhosis and healthy control subjects

Subjects	Age (yr)	Sex	Albumin g/l	Total bilirubin μmol/l	Prothrombin time (s)	Ammonia μmol/l	Grade of hepatic encephalopathy	Aetiology
Liver cirrhosis with HE (group A, n=8)	45	M	(35–50)‡	(3–17)	(10.1–11.1)	(18–50)	II	Alcoholic
	63	M	30	50	12.6	41	II	Cryptogenic
	73	M	36	31	12.1	77	III	Posttransfusional
	67	M	28	74	15.2	60	II	Cryptogenic
	70	M	29	39	13.8	74	II	Cryptogenic
	72	F	30	41	11.9	95	II	Posttransfusional
	59	M	28	34	12.0	109	III	Alcoholic
	62	F	27	43	13.6	77	II	Posttransfusional
Liver cirrhosis without HE (group B, n=12)	64 (4)	M:6 F:2	30 (1)*	42 (5)*†	13.1 (0.4)	75 (7)		
	71	F	28	29	13.6	43	O	Posttransfusional
	50	M	33	31	13.0	35	O	Alcoholic
	50	M	29	31	13.0	18	O	Alcoholic
	74	M	34	48	11.9	55	O	Alcoholic
	81	M	33	12	12.0	92	O	Cryptogenic
	41	M	26	48	13.5	63	O	Alcoholic
	67	M	32	19	11.6	79	O	Posttransfusional
	63	F	36	31	15.2	48	O	Cryptogenic
	52	M	35	26	13.8	113	O	Cryptogenic
	72	M	27	48	16.3	85	O	Cryptogenic
	53	M	33	35	12.8	43	O	Alcoholic
Normal healthy controls (n=14)	65	F	29	30	12.4	29	O	Posttransfusional
	62 (4)	M:9 F:3	31 (1)*	32 (3)*	13.3 (0.4)	59 (8)		

Data are mean values (SEM). *Statistically significant at $p<0.01$ compared with the control subjects; †Statistically significant at $p<0.05$ between groups A and B; ‡Normal reference values in the Department of Clinical Chemistry, National Medical Center, Tokyo.

Table 2 Clinical characteristics and laboratory data of cirrhotic patients whose plasma MHPG was measured during and after the recovery from HE (group C)

Patients	Age (yr)	Sex	Albumin g/l	Total bilirubin μmol/l	Prothrombin time (s)	Ammonia μmol/l	Grade of hepatic encephalopathy	Time required for recovery (day)	Aetiology
1–8	54	M	(35–50)†	(3–17)	(10.1–11.1)	(18–50)	II	8	Alcoholic
	58	M	30	50	12.6	56	II	10	Alcoholic
	70	F	25	43	13.6	66	II	30	Posttransfusional
	59	F	28	29	13.6	90	II	7	Posttransfusional
	73	M	36	10	12.4	86	II	13	Cryptogenic
	58	M	27	15	12.8	34	II	85	Alcoholic
	55	M	36	174	15.6	106	III	45	Alcoholic
	65	M	26	96	13.3	55	II	20	Posttransfusional
During HE	62 (2)	M:6 F:2	31 (2)	77 (5)*	13.2 (0.4)	67 (8)		–	
After recovery from HE			32 (2)	32 (7)	13.2 (0.5)	63 (9)		27 (9)	

Data are mean values (SEM). *Statistically significant at $p<0.05$ compared with that observed after recovery from HE; †Normal reference values in the Department of Clinical Chemistry, National Medical Center, Tokyo.

(2 ml) were withdrawn from an antecubital vein with a minimum of stasis into a heparinised (ammonia free) chilled glass tube and immediately centrifuged

at $2000\times g$ for five minutes at 4°C . The separated plasma was put on ice and transferred to the laboratory. Plasma ammonia was measured enzymatically

(ACMA Du Pont, Wilmington, Del) within 30 minutes from sample collection. Using this method, plasma ammonia levels in normal adults range from 18–50 $\mu\text{mol/l}$ (the Department of Clinical Chemistry, National Medical Center, Tokyo).

For plasma amino acid measurements, blood samples (5 ml each) were obtained from the antecubital vein. In the healthy group, the samples for plasma amino acid assay were obtained at 0900 h after an overnight fast. Blood samples were collected into a heparinised tube, immediately centrifuged at $600\times g$ for 10 minutes, and the separated plasma was stored at -80°C until assayed. Plasma amino acid determinations were carried out by an amino acid analyser (Model 835-50, Hitachi Ltd, Tokyo) on the supernatant of plasma, rendering it protein free by precipitation using 5% sulphosalicylic acid. The intra-assay coefficients of variation (CV) for all amino acids were less than 5% except for tryptophan (CV=10%). Plasma concentration values for tryptophan measured by this method are considered somewhat lower than those measured by other methods^{22,23} where this amino acid was separately and specifically assayed.

Plasma free MHPG was determined according to the assay method developed in this laboratory²⁴ and used for our recent clinical studies:^{17,18} briefly, blood was collected into a chilled tube containing EDTA·2Na (0.1%) and sodium metabisulphite (0.1%). Plasma was immediately separated by centrifugation at $600\times g$ for 10 minutes at 4°C , and stored at -80°C until analysed. MHPG was extracted using a small C_{18} column (Bond-Elut, Analytichem International, Harbor City, CA) under a near neutral condition. The assay was conducted by using a high performance liquid chromatography system consisting of a pump (Yanako Model L-4000W, Yanagimoto Mfg Co, Kyoto), a reversed phase column (Yanapak ODS-A) and a Model VMD-501 dual electrochemical detector with a glassy carbon electrode (Yanagimoto) set at +0.7 V against Ag/AgCl reference electrode. Vanillyl alcohol was selected as the internal standard. The intra- and interassay CVs for MHPG were less than 5%.²⁴

STATISTICAL ANALYSIS

Multiple comparisons of the mean values for laboratory data, plasma amino acid concentrations, and plasma concentrations of MHPG among group A, group B, and the control group were done using ANOVA with the least significant test. The mean values for plasma MHPG concentration and other biochemical data measured twice in the same group C patients during and after the recovery from HE were compared by using Student's *t* test or Wilcoxon's signed-rank test for paired data, where appropriate.

To ask if plasma MHPG concentrations observed in our cirrhotic patients would be associated with the biochemical tests of liver function – that is, serum albumin and total serum bilirubin, and prothrombin time, in the group A and B patients, we examined the correlation between the plasma MHPG concentrations and each of these biochemical data by using the least-squares regression analysis. Similarly, we examined the correlation between the intrapatient changes in plasma MHPG concentrations and those in the above mentioned laboratory data during and after the recovery from HE in the group C patients. To test a hypothesis that the availability of NA precursor amino acids for brain is not only a reflection of plasma tyrosine and phenylalanine concentrations but also that of other neutral amino acids known to share a common transport system across the blood brain barrier,¹⁰ we examined the correlation between the plasma concentration of MHPG and either the sum of plasma concentrations of tyrosine and phenylalanine alone or the ratio of these amino acids concentrations to other competing neutral amino acids – that is, tryptophan, leucine, isoleucine, and valine, using Spearman's rank correlation analysis. A *p* value less than 5% was considered statistically significant.

Fig. 1 Mean plasma concentrations of MHPG obtained from cirrhotic patients with HE (group A, *n*=8), those without HE (group B, *n*=12), and healthy control subjects (*n*=14). Vertical lines indicate SEM.

Results

There were no statistically significant differences in the mean (SEM) value for age and in sex distribution among the group A and B patients and healthy controls (Table 1). The mean serum concentrations of albumin and bilirubin in group A and B were significantly ($p<0.01$) decreased and increased, respectively, as compared with those in the control group. In addition, the mean values for prothrombin time and plasma ammonia level obtained from group A and B were prolonged and raised as compared with the respective normal values (Table 1). The mean value for serum bilirubin concentration obtained from group A was significantly ($p<0.05$) greater than that from group B. Although the mean value for serum ammonia level obtained from group A was also greater than that from group B (Table 1), the difference between these patient groups reached no statistically significant level.

The mean (SEM) serum creatinine concentrations obtained from our study groups [group A, 107 (9) (range 80–115); group B, 91 (5) (range 62–141); group C during HE, 118 (13) (range 80–176); group C after the recovery from HE, 110 (12); and the age matched healthy control group, 91 (5) (range 62–124) $\mu\text{mol/l}$] were within the normal reference range in the Department of Clinical Chemistry, National Medical Center – that is, 60–120 $\mu\text{mol/l}$, and did not significantly differ among the study groups.

The mean plasma MHPG concentrations in group A (74.9 (8.6) pmol/ml) and group B (54.8 (7.2) pmol/ml ; to convert from SI unit to a traditional unit, 1 $\text{pmol/ml}=0.184 \text{ ng/ml}$) were significantly ($p<0.01$) greater than in the control group (22.3 (2.0) pmol/ml , Fig. 1). In addition, the mean plasma MHPG concentration in group A was significantly ($p<0.05$) greater than that in group B (Fig. 1).

There was no statistically significant correlation between plasma MHPG concentrations and any of the laboratory data examined in groups A and B: correlation coefficients (r) between plasma MHPG concentrations and serum albumin concentrations, total serum bilirubin concentrations, and prothrombin time were 0.046, 0.091, and -0.019 , respectively. Moreover, no significant correlation was observed between the intraindividual changes in plasma MHPG concentrations and those in any of the laboratory data during and after the recovery from HE in group C: correlation coefficients (r) for the changes in plasma MHPG *versus* those in albumin, total bilirubin, and prothrombin time were 0.209, 0.230, and -0.053 , respectively.

The mean values for plasma amino acid concentrations obtained from group A and B showed not only a significant decrease in taurine ($p<0.01$), aspartate

Table 3 Plasma amino acid concentrations obtained from cirrhotic patients with HE (group A), those without HE (group B), and normal subjects

Amino acids	Patients with liver cirrhosis		Normal controls (n=14)
	Group A (n=8)	Group B (n=12)	
		($\mu\text{mol/l}$)	
Taurine	87.1 (19.5) [†]	78.5 (7.3) [†]	167.1 (21.9)
Aspartic acid	7.7 (1.5)*	7.7 (1.4)*	22.3 (4.5)
Threonine	158.3 (21.0)	197.4 (27.1)	181.7 (9.4)
Serine	158.1 (25.7)	202.8 (21.8)	171.3 (7.6)
Asparagine	101.2 (11.5)	110.9 (13.6)	86.5 (3.2)
Glutamic acid	141.9 (17.5)	112.9 (19.9)	96.7 (10.9)
Glutamine	705.8 (87.7)	717.2 (75.9)	696.3 (28.6)
Proline	259.0 (26.8)	327.3 (40.6)	265.0 (20.8)
Glycine	273.9 (18.2)	304.7 (36.1)	308.9 (16.3)
Alanine	318.6 (29.5)* [‡]	491.1 (48.5)	456.9 (30.9)
Citrulline	62.9 (16.7)	49.1 (7.1)	43.1 (4.4)
Valine	147.1 (13.6) [†]	190.0 (19.3) [†]	266.2 (19.8)
Cystine	33.1 (16.7)	21.9 (5.5)	35.5 (5.8)
Methionine	59.1 (16.9) [†]	49.7 (5.4)*	23.7 (1.5)
Isoleucine	43.6 (4.3)*	57.0 (6.2)	76.5 (10.0)
Leucine	77.9 (8.5) [†]	91.5 (10.0)*	136.0 (15.3)
Tyrosine	116.0 (10.5) [†]	139.8 (15.7) [†]	65.9 (4.6)
Phenylalanine	98.7 (10.7) [†]	93.1 (7.4) [†]	59.9 (2.9)
Tryptophan	41.5 (4.7)	54.3 (8.4)	42.3 (2.3)
Lysine	202.7 (26.1)	272.1 (39.8)	224.1 (11.4)
Histidine	93.6 (9.5)	98.6 (15.3)	82.4 (3.7)
Arginine	166.5 (16.5)	176.6 (37.8)	119.1 (8.5)

Data are given as the mean values (SEM). *Statistically significant at $p<0.05$ as compared with the healthy control group; [†]Statistically significant at $p<0.01$ as compared with the healthy control group; [‡]Statistically significant at $p<0.01$ between groups A and B.

($p<0.05$), BCAA (valine ($p<0.01$), leucine ($p<0.01$ and 0.05), but also a significant increase in AAA (tyrosine ($p<0.01$), phenylalanine ($p<0.01$)), and in methionine ($p<0.01$ and 0.05)) as compared with those obtained from the control group, respectively (Table 3). The mean values for plasma alanine and isoleucine obtained from group A were significantly ($p<0.05$) decreased as compared with those obtained from the control group (Table 3). There were no significant differences in the mean values for plasma concentrations of any amino acids between group A and B, except for alanine of which the mean plasma concentration obtained from group A was significantly ($p<0.01$) decreased as compared with that from group B (Table 3).

Relationship between the plasma MHPG concentration and the ratio of plasma tyrosine plus phenylalanine to other competing neutral amino acids (tryptophan, leucine, isoleucine, and valine) obtained from the group A and B patients and control subjects ($n=34$) gave a correlation coefficient (r_s) of 0.77 ($p<0.01$, Fig. 2), whereas the plasma MHPG concentration correlated less strongly ($r_s=0.63$, $p<0.01$) with plasma tyrosine plus phenylalanine level.

Fig. 2 Relationship between plasma MHPG concentration and ratio of plasma catecholamine precursor amino acids [tyrosine and phenylalanine] to four competing neutral amino acids [tryptophan, leucine, isoleucine, and valine]. ● = data from cirrhotic patients with HE (group A), ▲ = data from cirrhotic patients without HE (group B), ○ = data from healthy control subjects. The least-squares regression line is: $y = 78.7x + 7.7$ ($r_s = 0.77$, $p < 0.01$).

Clinical characteristics and laboratory data obtained from the group C patients during and after the recovery from HE were largely comparable with those obtained from the group A and B patients, respectively (Tables 1 and 2). The mean periods of time required for the recovery from HE was 27 (9) days (Table 2). Among the laboratory data obtained from the group C patients during and after the recovery from HE, a significant ($p < 0.01$) difference was observed only in the mean value for total serum bilirubin concentration (Table 2). In addition, there was a significant ($p < 0.01$) difference in the mean values for plasma MHPG concentration obtained during (79.3 (10.6) pmol/l) and after the recovery from HE (47.2 (5.2) pmol/l) (Fig. 3). The mean values for plasma MHPG concentration obtained from the group C patients with and without HE were comparable to those obtained from the cirrhotic patients with (group A), and from those without HE (group B), respectively (Figs 1 and 3).

Discussion

Plasma concentrations of MHPG, a major metabolite of brain NA, may reflect a functional activity of the central noradrenergic neurones.²⁵⁻²⁹ Previous studies have shown that there is a highly significant correlation between plasma free MHPG concentrations and those in the brain in primates treated with drugs altering the central NA metabolism,²⁹ and that a centrally acting α -adrenergic agonist, clonidine, reduces MHPG in plasma³⁰ and in cerebrospinal

Fig. 3 Plasma MHPG concentrations measured twice in the same cirrhotic patients (group C) with and without HE. Vertical lines indicate SEM.

fluid³⁰ in man. Maas *et al.*²⁸ have suggested that approximately 60% of plasma MHPG is derived from the brain in man. Garfinkel *et al.*²⁵ have also agreed with this estimation based on their study using a peripheral decarboxylase inhibitor, carbidopa. Others,^{31,32} however, have claimed that the proportion of plasma MHPG deriving from the brain may be somewhat lower than the above mentioned estimation, since MHPG may be converted to vanillylmandelic acid in the peripheral tissue.³¹ Nonetheless, several clinical studies have implied that measurements of plasma concentrations of possibly centrally originating monoaminergic metabolites (MHPG and/or homovanillic acid) are useful for assessing the functional activity of the central monoaminergic neurones in patients with certain psychiatric disorders¹⁴⁻¹⁵ and in those with subarachnoid haemorrhage.^{17,18} Therefore, we accepted the hypothesis that measurements of plasma MHPG may serve as a useful index for assessing the central noradrenergic activity and used this option to search for a possible alteration in the central NA metabolism in liver cirrhosis with or without HE.

To our knowledge, the present study is the first to show that patients with liver cirrhosis, particularly

those with HE, have a significantly ($p < 0.01$) raised plasma concentration of MHPG as compared with healthy controls, and that the mean plasma MHPG concentration in patients with HE (group A) is significantly ($p < 0.05$) greater than in those without (group B) (Fig. 1). The findings obtained from this interpatient study were further validated in a prospective, inpatient study using cirrhotic patients (group C) having the impaired hepatic function similar to the group A and B patients. Based upon these findings, we are tempted to speculate that the central NA metabolism would be deranged not only in cirrhotic patients with HE but also to some extent in patients without HE whose mean plasma MHPG concentration was significantly ($p < 0.01$) greater than that in the control subjects (Fig. 1). Our data appear to be in good agreement with those of recent studies^{33,34} revealing that certain cerebral dysfunctions were detected by psychometric tests in the majority of patients with liver cirrhosis even before the first episode or between episodes of HE. The observation that there was a significant ($p < 0.01$) difference in the mean plasma MHPG concentrations measured during and after the recovery from HE in the same cirrhotic patients (Fig. 3) may suggest that a possible alteration in the central NA metabolism in these patients coincided with the development of HE. In addition, the fact that the mean plasma MHPG concentrations during and after the recovery from HE in the group C patients were fairly comparable with those in the cirrhotic patients with HE (group A) and in those without (group B), respectively (Figs 1 and 3), appears to warrant the reproducibility of our observation. Whether a possible derangement in the central NA metabolism assessed by a rise in plasma MHPG concentrations in our cirrhotic patients could be a cause for HE or a result deriving from HE should remain unanswerable from the present study.

Whether the observation that plasma MHPG was raised in cirrhotic patients can be merely attributed to the derangement in the central NA metabolism appears to be an unwarranted consideration. Inasmuch as the synthesis rate of MHPG in the brain and its clearance rate in peripheral tissues – for example, hepatic sulphotoconjugation and renal clearance, were not separately assessed in the present study, the above mentioned assumptive explanation for the underlying mechanism of the raised plasma MHPG concentrations in cirrhotic patients as compared with healthy controls must remain speculative. To our knowledge, however, there is no compelling evidence that the peripheral metabolism or disposal of MHPG is significantly impaired in patients with liver cirrhosis. Indeed, a previous study has demonstrated that the systemic clearance of NA was not significantly decreased in patients with decompensated

liver cirrhosis as compared with healthy subjects.³⁵ We observed no significant correlation between plasma MHPG concentrations and any of the biochemical tests used for assessing liver function – that is, serum albumin, total serum bilirubin, and prothrombin time, in our cirrhotic patients. In addition, the mean serum creatinine concentrations obtained from all of the study groups were within normal reference range, and did not significantly differ among the study groups.

A possibility exists that the raised plasma MHPG concentrations observed in our cirrhotic patients may not necessarily be associated with an acceleration in the central NA metabolism, but may rather implicate a depletion of NA in the brain. In these patients, an increase in brain tyrosine and phenylalanine may be directed toward the overproduction of false neurotransmitters such as, octopamine and tyramine, thereby resulting in the replacement of physiological neurotransmitters such as, NA and dopamine, with these false neurotransmitters.³⁶ Indeed, substantial rises of such false neurotransmitters have been reported in the cerebrospinal fluid³⁷ and plasma³⁸ of cirrhotic patients developing HE, whilst a significant depletion of NA in the brain of animals developing HE has been found.^{36,39} It still remains controversial, however, as to whether a similar change in the central NA concentration would occur during HE in man.^{40,41}

A possible derangement in the central NA metabolism in patients with liver cirrhosis assessed by an abnormally raised plasma MHPG concentration (Figs 1 and 3) may be related not only to an increase in plasma catecholamine precursor amino acids – that is, tyrosine and phenylalanine, but also to a decrease in plasma BCAA. The fact that the neutral amino acid transport system in the BBB has K_m values set near the physiological plasma concentrations of AAA and BCAA⁴² makes the brain uniquely sensitive to the effects of competition among these amino acids.^{10,43,44} The K_m values for AAA and BCAA obtained from the amino acid transport system in tissues other than brain (1–10 nmol/l)⁴⁵ have been shown to be about 10 times greater than those values obtained from the transport system at the BBB (0.1 to 0.5 mmol/l),⁴² suggesting that uptake of AAA and BCAA into peripheral tissues would be rather insensitive to the competition effects among these amino acids. A previous animal study⁴³ showed that the brain concentration of tyrosine is more closely correlated with the ratio of plasma tyrosine to its competing neutral amino acids including BCAA than with plasma tyrosine alone. Our observation that a possible index of the central NA metabolism, plasma MHPG concentration,^{25–29} correlated ($r_s = 0.77$, $p < 0.01$, Fig. 2) more strongly with the ratio of plasma concentration of tyrosine plus phenylalanine

to other neutral amino acids (tryptophan, leucine, isoleucine, and valine) than with plasma concentration of tyrosine plus phenylalanine alone ($r_s=0.63$, $p<0.01$) in cirrhotic and control subjects would also support the above-mentioned hypothesis.

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References

- Hoyumpa AM, Desmond PV, Avant G, Roberts RK, Schenker S. Hepatic encephalopathy. *Gastroenterology* 1979; **76**: 184-95.
- Zieve L. The mechanism of hepatic coma. *Hepatology* 1981; **4**: 360-5.
- Fraser CL, Ariefi AI. Hepatic encephalopathy. *N Engl J Med* 1985; **313**: 865-73.
- Knell AJ, Davidson AR, Williams R, Kantamaneni BD, Curzon G. Dopamine and serotonin metabolism in hepatic encephalopathy. *Br Med J* 1974; **i**: 549-51.
- Sourkes TL. Tryptophan in hepatic coma. *J Neural Transm* 1978; **14** [suppl]: 79-86.
- Smith AR, Rossi-Fanelli F, Ziparo V, James JH, Perelle BA, Fisher JE. Alterations in plasma and CSF amino acids, amines and metabolites in hepatic coma. *Ann Surg* 1978; **187**: 343-50.
- Hutson DG, Ono J, Dombro RS, Levi JU, Livingstone A, Zeppa R. A longitudinal study of tryptophan involvement in hepatic coma. *Am J Surg* 1979; **137**: 235-9.
- Borg J, Warter JM, Schlienger JL, Imler M, Marescaux C, Mack G. Neurotransmitter modifications in human cerebrospinal fluid and serum during hepatic encephalopathy. *J Neurol Sci* 1982; **57**: 343-56.
- Cascino A, Cangiano C, Fiaccadori F, et al. Plasma and cerebrospinal fluid amino acid patterns in hepatic encephalopathy. *Dig Dis Sci* 1982; **27**: 828-32.
- Oldendorf WH. Brain uptake of radiolabeled amino acid, amine, and hexoses after arterial injection. *Am J Physiol* 1971; **221**: 1629-39.
- Iber FL, Rosen H, Levenson SH, Chalmers TC. The plasma amino acids in patients with liver failure. *J Lab Clin Med* 1957; **50**: 417-25.
- Fischer JE, Yoshimura N, Aguirre A, et al. Plasma amino acids in patients with hepatic encephalopathy. Effects of amino acid infusions. *Am J Surg* 1974; **127**: 40-7.
- Rosen HM, Yoshimura N, Hodgman JM, Fisher JE. Plasma amino acid patterns in hepatic encephalopathy of differing etiology. *Gastroenterology* 1977; **72**: 483-7.
- Pickar D, Labarca R, Linnoila M, et al. Neuroleptic-induced decrease in plasma homovanillic acid and antipsychotic activity in schizophrenic patients. *Science* 1984; **225**: 954-7.
- Davis KL, Davidson M, Mohs RC, et al. Plasma homovanillic acid concentration and the severity of schizophrenic illness. *Science* 1985; **227**: 1601-2.
- Siever LJ, Davis KL. Overview: toward a dysregulation hypothesis of depression. *Am J Psychiatry* 1985; **142**: 1017-31.
- Minegishi A, Ishizaki T, Yoshida Y, Ahagon A, Shibata N, Kobayashi H. Plasma 3-methoxy-4-hydroxyphenylethyleneglycol and homovanillic acid concentrations in patients with subarachnoid hemorrhage. *Stroke* 1987; **18**: 223-8.
- Minegishi A, Ishizaki T, Yoshida Y, Ahagon A, Shibata N, Kobayashi H. Plasma monoaminergic metabolites and catecholamines in subarachnoid hemorrhage: Clinical implications. *Arch Neurol* 1987; **44**: 423-31.
- Parsons-Smith BC, Summerskill WHJ, Dawson AM, Sherlock S. The electroencephalograph in liver disease. *Lancet* 1957; **ii**: 867-71.
- Earley LE. Conclusive remarks and presentation of diagnostic criteria of the hepato-renal syndrome. In: Bartoli E, Chiandussi L, eds. *Hepatorenal syndrome*. Padova: Piccin Medical Books, 1980: 494-9.
- Council of International Organizations Med Sci/WHO. Biomedical research: a revised code of ethics (Declaration of Helsinki). *WHO Chron* 1976; **30**: 360.
- Hirayama C. Tryptophan metabolism in liver disease. *Clin Chim Acta* 1971; **41**: 275-86.
- Bloxam DL, Warren WH. Error in the determination of tryptophan by the method of Deckla and Dewey. A revised procedure. *Anal Biochem* 1974; **60**: 621-5.
- Minegishi A, Ishizaki T. Determination of free 3-methoxy-4-hydroxyphenylglycol with several other monoamine metabolites in plasma by high-performance liquid chromatography with amperometric detection. *J Chromatogr* 1984; **311**: 51-7.
- Garfinkel PE, Warsh JJ, Stancer HC, Godse DD, Brown GM, Vranic M. The effect of a peripheral decarboxylase inhibitor (carbidopa) on monoamine and neuroendocrine function in man. *Neurology* 1977; **27**: 443-7.
- Maas JW, Hattox SE, Landis DH, Roth RH. A direct method for studying 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) production by brain in awake animals. *Eur J Pharmacol* 1977; **46**: 221-8.
- DeMet EM, Halaris AE. Origin and distribution of 3-methoxy-4-hydroxyphenylglycol in body fluids. *Biochem Pharmacol* 1979; **28**: 3043-50.
- Maas JW, Hattox SE, Greene NM, Landis DH. 3-Methoxy-4-hydroxyphenylethyleneglycol production by human brain in vivo. *Science* 1979; **205**: 1026-7.
- Elsworth JD, Redmond DE Jr, Roth RH. Plasma and cerebrospinal fluid 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) as indices of brain norepinephrine metabolism in primates. *Brain Res* 1982; **235**: 115-24.
- Bertilsson L, Haglund K, Östman J, Rawlins MD, Ringberger VA, Sjöqvist F. Monoamine metabolites in cerebrospinal fluid during treatment with clonidine or alprenolol. *Eur J Clin Pharmacol* 1977; **11**: 125-8.
- Blombery PA, Kopin IJ, Gordon EK, Markey SP, Ebert MH. Conversion of MHPG to vanillylmandelic acid. *Arch Gen Psychiatry* 1980; **27**: 1095-8.
- Kopin IJ, Gordon EK, Jimerson DC, Polinsky RJ. Relationship between plasma and cerebrospinal fluid levels of 3-methoxy-4-hydroxyphenylglycol. *Science* 1983; **219**: 73-5.

- 33 Layton R, Jenko P, Rudman D, Freides D. Subclinical hepatic encephalopathy: detection, prevalence, and relationship to nitrogen metabolism. *Gastroenterology* 1978; **75**: 462-9.
- 34 Schomerus W, Hamster W, Blunck H, Reinhard U, Mayer K, Dolle W. Latent portasystemic encephalopathy. I. Nature of cerebral functional defects and their effect on fitness to drive. *Dig Dis Sci* 1981; **26**: 622-30.
- 35 Nicholls KM, Shapiro MD, Van Putten VJ, *et al*. Elevated plasma norepinephrine concentrations in decompensated cirrhosis. Association with increased secretion rates, normal clearance rates, and suppressibility by central blood volume expansion. *Circ Res* 1985; **56**: 457-61.
- 36 Faraj BA, Camp VM, Ansley JD, Scott J, Ali FM, Malveaux EJ. Evidence for central hypertyraminemia in hepatic encephalopathy. *J Clin Invest* 1981; **67**: 395-402.
- 37 Fischer JE, Baldessarini RJ. False neurotransmitters and hepatic failure. *Lancet* 1971; **ii**: 75-9.
- 38 Nespoli A, Bevilacqua G, Staudacher C, Rossi N, Salerno F, Castelli MR. Pathogenesis of hepatic encephalopathy and hyperdynamic syndrome in cirrhosis. Role of false neurotransmitters. *Arch Surg* 1981; **116**: 1129-38.
- 39 Dodsworth JM, James JH, Cummings MC, Fisher JE. Depletion of brain norepinephrine in acute hepatic coma. *Surgery* 1974; **75**: 811-20.
- 40 Jellinger K, Riederer P, Kleinberger P, Wuketisch S, Kothbauer P. Brain monoamines in human hepatic encephalopathy. *Acta Neuropathol (Berl)* 1978; **43**: 63-9.
- 41 Cuilleret G, Pomier-Layrargues G, Pons F, Cadilhac J, Michel H. Changes in brain catecholamine levels in human cirrhotic hepatic encephalopathy. *Gut* 1980; **21**: 565-9.
- 42 Pardridge WM. Brain metabolism: a perspective from the blood brain barrier. *Physiol Rev* 1983; **63**: 1481-535.
- 43 Fernstrom JD, Faller DV. Neutral amino acids in the brain: changes in response to food ingestion. *J Neurochem* 1978; **30**: 1531-8.
- 44 Guroff G, Udenfriend S. Studies on aromatic amino acid uptake by rat brain in vivo. *J Neurochem* 1977; **28**: 803-11.
- 45 Pardridge WM. Regulation of amino acid availability to the brain. In: Wurtman RJ, Wurtman JJ, eds. *Nutrition and the brain*. New York: Raven Press, 1977: 141-204.