Alterations in Plasma and CSF Amino Acids, Amines and Metabolites in Hepatic Coma

ARLAN R. SMITH, M.D., PH.D.,* FILIPPO ROSSI-FANELLI, M.D., VINCENZO ZIPARO, M.D., J. HOWARD JAMES, B.S., BERNICE A. PERELLE, B.S., JOSEF E. FISCHER, M.D.

From the Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

The dog with an end-to-side portacaval shunt (PCS) has been extensively used as a model to investigate hepatic encephalopathy (HE) as it demonstrates a plasma amino acid pattern similar to patients with chronic liver disease. In adult mongrel dogs, the effect of PCS on plasma and CSF amino acids, octopamine (OCT), phenylethanolamine (PEA) and CSF 5-hydroxyindolacetic acid (5-HIAA), were studied. Moreover, the effect of correction of plasma amino acids by infusional techniques was investigated.

Tyrosine, tryptophan and phenylalanine levels increased dramatically during the development of HE in plasma and CSF, while valine, leucine and isoleucine decreased in plasma only, but CSF levels remained stable. Plasma and CSF octopamine and phenylethanolamine and CSF 5-HIAA increased markedly as clinical features in the dogs' behavior, characteristic of hepatic encephalopathy occurred, including hypersalivation, ataxia, flapping tremor, somnolence and finally coma. Once in coma, the dogs were infused with an amino acid mixture (F080) calculated to normalize the plasma amino acid pattern. After one to eight hours, the dogs began to awake. Simultaneously, blood, and CSF aromatic amino acids returned to their control values, as did OCT, PEA and CSF 5-HIAA. If F080 infusion was stopped, biochemical alterations would appear within one week, again accompanied by clinical hepatic encephalopathy.

The results indicate that the altered levels of aromatic and branched chain amino acids, octopamine and PEA in plasma and CSF correlate well with the development of HE and that correction of the plasma amino acid abnormalities improves encephalopathy simultaneously with correction of neurotransmitter derangements in CSF.

MANY THEORIES HAVE BEEN PROPOSED for the pathogenesis of hepatic encephalopathy, including toxicity of ammonia,[32] short chain fatty acids[21] and methionine.[25] Studies from this and other laboratories demonstrated that a characteristic amino acid pattern occurs in patients with advanced cirrhosis of the liver,[10] which includes increased plasma aromatic amino acids, tyrosine (TYR), phenylalanine (PHE), methionine (MET) and free tryptophan (FTRP), and a decrease in the branched chain amino acids, valine (VAL), leucine (LEU) and isoleucine (ILE). A similar pattern is also observed in experimental animal models with liver disease in several species, for example, rat[11] and dog.[9] In animals with hepatic encephalopathy following end-to-side portacaval shunt, when the plasma amino acid pattern was corrected by infusion of a special synthetic amino acid solution (F080),[9] the animals awoke. This solution contains lower amounts of aromatic amino acids and higher amounts of branched chain amino acids than the available standard hyperalimentation solutions.[10]

Due to the competition among aromatic amino acids (TRP, TYR and PHE) and the three branched chain aliphatic amino acids (VAL, LEU and ILE)[23,24] for transport across the blood brain barrier, increased transport of TRP, TYR and PHE into the brain may be expected to occur during encephalopathy, secondary to decreased plasma concentrations of LEU, ILE and VAL. The aromatic amino acids TRP, TYR and PHE are precursors for the physiological biogenic amine neurotransmitters, but may also give rise to other compounds which may affect normal neurotransmitter metabolism. The increase of aromatic amine precursors in the brain coincides with gross modifications in the brain amine content,[8] for instance, decreased norepinephrine,[6] increased levels of serotonin.[3] Increased concentrations of octopamine (OCT), a "false" or coneurotransmitter, have also been observed in plasma, brain and urine during encephalopathy in rats, dogs and humans.[8,11,15,17,28] Furthermore, increased amounts of CSF 5-HIAA have been measured in hepatic failure in both animals and man.[13,26]

In all the above studies however, no data are avail-

* Current address: Department of Surgery, Ziekenhuis St. Annadal, Maastricht, The Netherlands.

Supported in part by USPHS Grant #'s AM-15347 and AM-19124.

Reprint requests: Josef E. Fischer, M.D., Hyperalimentation Unit, Massachusetts General Hospital, Boston, Massachusetts 02114.

Submitted for publication: June 8, 1977.

0003-4932-78-0300-0343-0085

able concerning simultaneous measurements of amino acids and neurotransmitter metabolites in plasma and cerebrospinal fluid. In this paper we will present data concerning amino acids, octopamine and phenylethanolamine (PEA) (another "false" or coneurotransmitter)[14] levels in both plasma and CSF, as well as CSF 5-HIAA levels during evolution and therapy of hepatic encephalopathy.

Materials and Methods

Animal Experimental Procedure

Four adult mongrel dogs, specific pathogen free, weighing between 18–22kg, were housed in individual metabolic cages. Animals were fed with dog chow and received tap water *ad libitum*. A three week observational period was used as a control. Blood and CSF were collected under light suritol anesthesia after an overnight fast on a weekly basis for three weeks. Amino acid analysis, octopamine and phenylethanolamine determinations were carried out on both blood and CSF, and 5-hydroxyindolacetic acid, a metabolite of serotonin was measured in the CSF.

After three weeks of base line observation, end-to-side portacaval shunts were performed in dogs, after a 24 hour fast, under suritol anesthesia using sterile technique. A midline longitudinal incision was used. Portal vein and inferior vena cava were exposed and the portal vein was ligated. An anastomosis of the vena cava above the highest pancreatic duodenal branch was done, so there was no residual vascularization of the stump of the portal vein. Penicillin and streptomycin were administered for 48 hours and the animals were given intravenous fluids for the first 24 hours. Thereafter, they were maintained on the standard kennel ration and water *ad libitum*.

After a period of time, generally varying between four to eight weeks, animals manifested various signs of hepatic encephalopathy, as previously described.[9] When the dogs reached stage III of encephalopathy, a catheter was inserted via the left or right external jugular vein into the right atrium and tunnelled out through the back subcutaneously to exit between the shoulder blades. The position of the catheter in the right atrium was verified by x-ray. The free end of the polyethylene catheter was led subcutaneously to a point between the shoulder blades, where it was connected to a tubing in a steel speedometer cable. The tubing was connected in turn to a Holter infusion pump (NR911, pump chamber B) and infusion bottle, which was suspended from an apparatus with a counter weight, thereby allowing the animal freedom of movement as previously described. Flow rate was adjusted to between 0.6–1.2 ml/minute.

After the animals entered stage III encephalopathy, a five day infusion of physiological 5% dextrose and saline was carried out as a control. Thereafter, F080 and 23% dextrose was then infused for five days. During both of these periods, the CSF and blood samples were collected daily under light suritol anesthesia.

Control Values

Control values of plasma amino acids, octopamine, phenylethanolamine and CSF amino acids, octopamine, phenylethanolamine and 5-HIAA were obtained from ten normal dogs who were sampled under similar circumstances, and mean and SEM were calculated.

Biochemistry

Octopamine and Phenylethanolamine Assay

Blood was collected in tubes containing 50 μg pargyline as a MAO inhibitor. Plasma was separated by centrifugation at 3000 rpm, was lyophilized and stored at −20° before determination. CSF samples were analyzed without pretreatment. Octopamine and PEA were measured simultaneously by the radioenzymatic method as described by Molinoff[18] and modified by us.[27] The method is based upon the transformation of octopamine and PEA into their respective N-methyl derivates by the transmethylating enzyme, phenylethanolamine-N-methyltransferase (PNMT) purified from bovine adrenal. The methyl-group donor was C^{14} labeled S-adenosyl-methionine (SAM). The reaction was carried out at 37° and pH 8.6 in a Tris/HCl 0.1 M buffer. For the simultaneous determination of PEA and octopamine the sample was treated as described above, but both 20 ng of PEA and 10 ng of octopamine were added as an internal standard. PEA was extracted in 6 ml of 3% isoamylalcohol in Toluene; 4 ml of the aliquot was transferred to a counting vial containing 10 ml of scintillation fluid and counted. Octopamine was extracted into 6 ml of 3:2 toluene-isoamyl alcohol, and 4 ml was transferred into a counting vial containing 2 ml of fresh 3:2 toluene-isoamyl alcohol and dried overnight (at 70°C) 1 ml of ethanol and 10 ml of scintillation fluid was added and the radioactivity was counted in a scintillation photometer (PACKARD TRICARB, C^{14}, efficiency 60%). Values were expressed as ng/ml plasma or CSF.

Amino Acid Determinations

Determinations of plasma amino acids were carried out by a column chromatographic technique with a Beckman 121-MB amino acid analyzer on the supernatant of plasma which had been rendered protein-free

TABLE 1. *Tabular Summary of the Course of Four Dogs Following Portacaval Shunt and Through Encephalopathy*

Dog	Days after Shunt Encephalopathy Grade I	II	III	IV	Death	Saline Rx	F080 Rx
A	27	37	49	52	53	None	None
B	37	46	54			54–59	60–65
C	55	49,67	74			74–79	80–90
D 1	39	41	42				42–47*
2		62	67			58–63	70–80

* Oral administration.

by treatment with 4% sulfosalicylic acid. Cerebrospinal fluid samples were determined without any pretreatment. All amino acids except tryptophan were determined in this way. Values were expressed in nMol/ml. Tryptophan was determined fluorometrically according to the method of Denckla and Dewey.[5] Insufficient samples were available for free TRP determination. Plasma phenylalanine and tyrosine also were determined fluorometrically.

5-Hydroxyindolacetic Acid

5-hydroxyindolacetic acid was determined as described by the method of Curzon and Green.[4]

Statistics

Significant differences were tested with the student's t test using Yates' correction. Unless otherwise stated, statistical significance is taken at $p < 0.01$.

Results

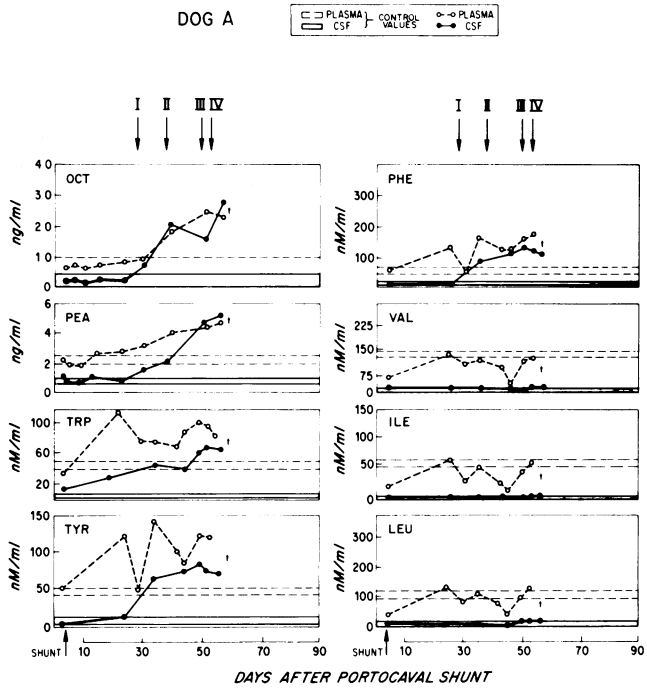
All dogs manifested hepatic encephalopathy at periods of time varying between eight to fourteen weeks, postshunt. Before passing into hepatic coma, the dogs went through three stages. Stage I was characterized by hyperactive motor movement and hypersalivation. Specific neurological symptoms, among which are ataxia, flapping tremor, narrow pupils and sometimes temporary paralysis, appear in stage II, while in stage III the dogs are asleep but still arousable and reactive to painful stimuli. During the period of encephalopathy the dogs showed a significant decrease in their weight (±20–25%) as compared with their original weight, and did not eat. Hair loss seems to be always present in this stage. Parenteral nutrition was initiated in stage III encephalopathy. The courses of the individual dogs are summarized in Table 1.

Octopamine and Phenylethanolamine in Plasma

Both octopamine and phenylethanolamine increased during the development of hepatic encephalopathy to between two and five times normal values (Figs. 1–4). Dextrose saline infusion failed to reverse coma or measured blood or CSF values (Figs. 1–4), but F080 infusions returned amino acids and other determinations to normal value and even to somewhat below this value as the dogs awoke. Normal values of OCT and PEA were, respectively, 0.69 ng/ml ± 0.04 and 2.6 ng/ml ± 0.14 (Figs. 1–4, Table 2). When F080 infusion was stopped, octopamine and PEA returned to their high levels as before the F080 treatment and the dogs became encephalopathic again.

Octopamine and PEA Levels in the CSF

Normal values of octopamine and PEA in the CSF were lower than in plasma: 0.25 ng/ml ± 0.01 and 0.9 ng/ml ± 0.12, respectively. Both OCT and PEA increased during the development of hepatic encephalo-



FIGS. 1–3. The alterations of amino acids, octopamine and phenylethanolamine in the individual dogs during the development of hepatic encephalopathy. Amino acids are expressed in nM/ml, octopamine and phenylethanolamine in ng/ml. Small but gradual changes occur in the levels of the AAA and the BCAA in the plasma during the development of HE; however, as the animal becomes comatose, a spike-like phenomena could be observed. In the CSF only the AAA showed significant changes, while BCAA remain relatively constant. Octopamine and phenylethanolamine levels in blood and CSF show small alterations without coma in the dog. Once in coma, however, (stage III) dogs will die unless treated (Fig. 1). From these graphs it is clear that both TYR and PHE increases in plasma and CSF are followed by an increase of respectively, octopamine and phenylethanolamine (Control values:white area—plasma; grey area—CSF. Open circles—plasma; solid circles—CSF). Abbreviations: I: Stage I; II: Stage II; III: Stage III F080: Administration of F080 is started SAL: 0.9% NaCl saline solution is started.

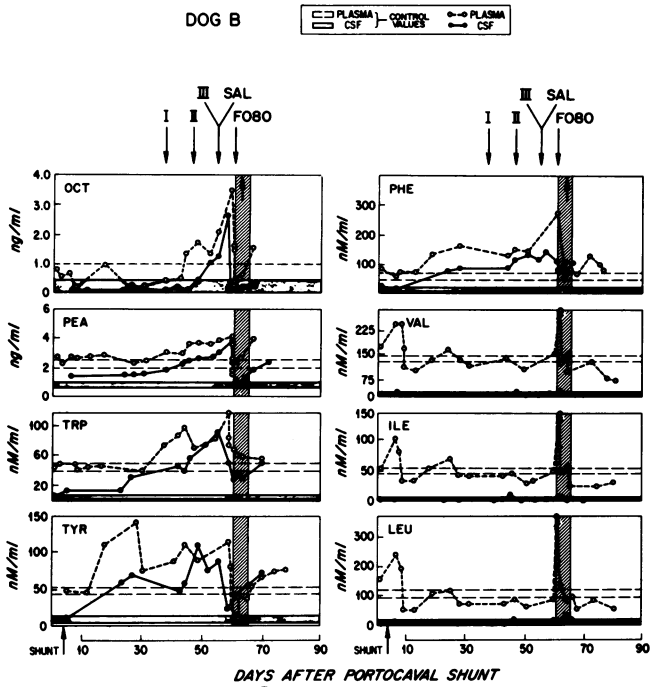


FIG. 2. See legend for Figures 1–3.

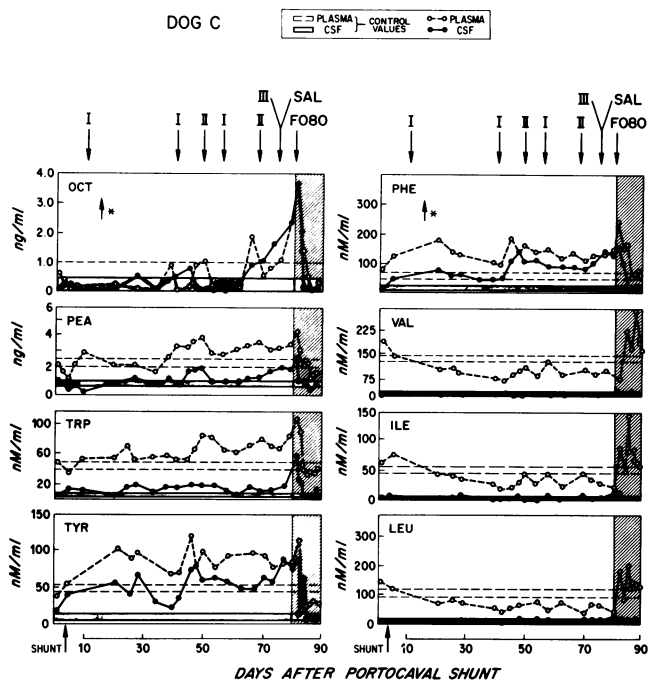


FIG. 3. See legend for Figures 1–3.

pathy, in the CSF to about five to six fold of their original value (Figs. 1–4); plasma and CSF tended to increase at the same time. PEA however, appeared to become elevated earlier than OCT, although they reach their maximum level at the same time (Figs. 1–4). While absolute values of OCT and PEA in the CSF in stage III hepatic coma are a bit lower than the plasma levels at the same time, the relative increase in the CSF (six to ten fold) is significantly higher than in the plasma (Table 2). After F080 administration both OCT and PEA returned to their original values within 24 hours, while the dogs awoke and behaved relatively normally. If F080 infusion was stopped, OCT and PEA levels rose again to their high preinfusion levels within 2 weeks and the dogs became encephalopathic again (Fig. 4).

Amino Acids in the Plasma

During the development of hepatic encephalopathy, amino acids (AA) show a characteristic pattern seen in earlier investigations.[9,10] The aromatic amino acids (AAA), (TYR, TRP, PHE) increased while the BCAA (VAL, ILE, LEU) decreased. Interestingly, methionine decreased in concentration (Figs. 1–4, Table 2). After the administration of F080, "normalization" of both AAA and BCAA occurred (Figs. 1–4, Table 1), while methionine did not change significantly and stayed at a fairly constant level (Fig. 5). Glutamic and aspartic acid tended to follow the same pattern as the aromatic amino acids: increased during the

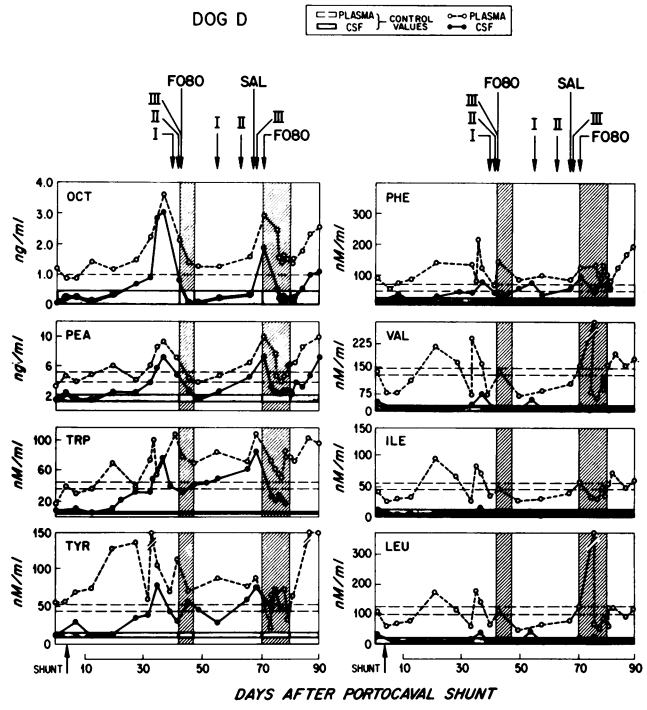


FIG. 4. 42 days after PCS this dog manifested grade II-III encephalopathy at which time F080 was administered orally (1000 ml/day) for five days. Other food intake was allowed. The dog recovered and all values decreased to control levels. F080 was stopped and after ten days the dog manifested grade III encephalopathy. F080 was administered intravenously. Normal behavior occurred after 24 hours (Control values: white area—plasma; Grey area—CSF. Open circles—plasma; Solid circles—CSF). Abbreviations: I: Stage I; II: Stage II; III: Stage III F080: Administration of F080 is started SAL: 0.9% NaCl saline solution is started.

TABLE 2.

	Before PCS	Hepatic Coma	After F080
Octopamine	0.69 ± 0.04 x	4.8 ± 0.40*	0.4 ± 0.03
OCT	0.25 ± 0.01 xx	3.5 ± 0.27*	0.5 ± 0.02
Phenyleth-anolamine (PEA)	2.6 ± 0.14	3.9 ± 0.36*	3.9 ± 0.25
	0.9 ± 0.12	3.2 ± 0.25*	0.7 ± 0.9
TRP	47.8 ± 4.50	115.6 ± 9.8*	50.4 ± 5.4
	17.4 ± 1.80	90.5 ± 7.3*	10.6 ± 1.2*
TYR	47.2 ± 6.48	106.7 ± 8.5*	40.8 ± 2.4
	9.4 ± 0.75	98.3 ± 15.6*	17.5 ± 6.1
PHE	60.2 ± 7.32	200.6 ± 12.5*	75.4 ± 6.1
	11.2 ± 0.96	119.5 ± 17.19*	22.7 ± 9.5
ASP Ac	18.7 ± 0.67	17.2 ± 0.48	12.7 ± 1.3
	3.5 ± 0.01	44.5 ± 11.33*	9.5 ± 1.6*
Glut. Ac	42.7 ± 3.87	121.5 ± 21.0*	103 ± 34.1*
	O.S.	O.S.	O.S.
VAL	145.6 ± 12.15	99.2 ± 28.5*	143.9 ± 8.4
	4.5 ± 0.12	22.1 ± 13.5*	6.0 ± 3.3
ILE	54.7 ± 4.52	33.4 ± 3.2*	6.0 ± 5.2
	6.8 ± 0.50	7.3 ± 0.1	5.9 ± 0.5
LEU	111.1 ± 9.89	57.9 ± 5.98*	106.1 ± 7.3
	18.3 ± 1.20	24.4 ± 5.81	23.9 ± 0.69
THR	143.5 ± 16.8	87.4 ± 18.45*	135.7 ± 17.6
	25.5 ± 2.5	32.15 ± 16.6	18.9 ± 4.27
PRO	159.9 ± 13.7	85.7 ± 22.4*	150.5 ± 15.3
	—	—	—
ALA	362.4 ± 35.0	244.4 ± 95.0	406 ± 83.9*
	7.52 ± 0.36	40.4 ± 24.5*	29.9 ± 15.9*
LYS	122.6 ± 4.8	108.4 ± 28.8	190.5 ± 40.5*
	42.3 ± 3.6	63.7 ± 10.0*	34.6 ± 2.5*
GLY	240.1 ± 16.5	285.0 ± 39.57	260.5 ± 25.3
	8.7 ± 0.5	14.4 ± 3.66*	7.60 ± 0.7
METH	49.7 ± 4.39	59.09 ± 5.81	41.0 ± 2.23
	8.5 ± 0.42	25.6 ± 3.35*	10.6 ± 1.0
ARG	120.1 ± 13.9	145 ± 20.6	112.5 ± 10.1
	47.1 ± 4.19	35.6 ± 2.0	32.5 ± 6.8*
TAUR	73.5 ± 8.0	92.3 ± 4.9*	70.0 ± 5.3
	2.46 ± 0.5	2.62 ± 1.2	2.3 ± 0.6
CITR	73.0 ± 6.5	14.5 ± 6.87*	18.6 ± 1.8*
	4.0 ± 2.5	5.0 ± 0.5	4.8 ± 0.5
HIS	73.0 ± 10.9	112.1 ± 18.0*	90.5 ± 7.3*
	12.6 ± 0.83	55.0 ± 2.3*	28.7 ± 1.5*
SER	140.3 ± 18.95	189.7 ± 12.4*	150.5 ± 16.3
	22.0 ± 0.82	73.6 ± 13.5*	44.2 ± 16.7*

OS: Off Scale.
x: Values in plasma.
xx: Values in CSF.

The mean concentrations of amino acids (±SEM) and "false neurotransmitters" (OCT and PEA) in plasma and CSF before portacaval shunt (PCS), during hepatic coma and after one day of F080 administration. Amino acids are expressed in nM/ml, FNT in ng/ml. Values statistically different from control values are marked * ($p < .01$). N = 4 unless otherwise indicated.

development of hepatic encephalopathy and decreased after F080 administration. In contrast to glutamic and aspartic acid, threonine, proline and alanine manifest the opposite patterns: decreased during coma and increasing following therapy (Table 2).

Amino Acids in the Cerebrospinal Fluid

All amino acid levels in the CSF were considerably lower than in the plasma: approximately 10–20% of the plasma values. During the development of hepatic encephalopathy only the aromatic amino acids, TYR, TRP and PHE, increased, as was observed in the plasma. The branched chain amino acids however, (VAL, LEU and ILE) showed little alteration from control values. Aspartic and glutamic acids increased as did the AAA. Interestingly, ethanolamine showed a highly significant increase during the development of hepatic encephalopathy. In contrast to the normal plasma methionine levels, CSF methionine levels were significantly increased. The AAA, glutamic and aspartic acid decreased to their control values after F080 treatment (Figs. 1–4, Table 2). The changes in the CSF amino acids occurred at the same time as did changes in the plasma amino acids.

5-Hydroxyindolacetic Acid in the Cerebrospinal Fluid (5-HIAA)

Normal 5-HIAA levels in CSF were estimated at about 30 ng/ml ± 5.0. 5-HIAA increased during the

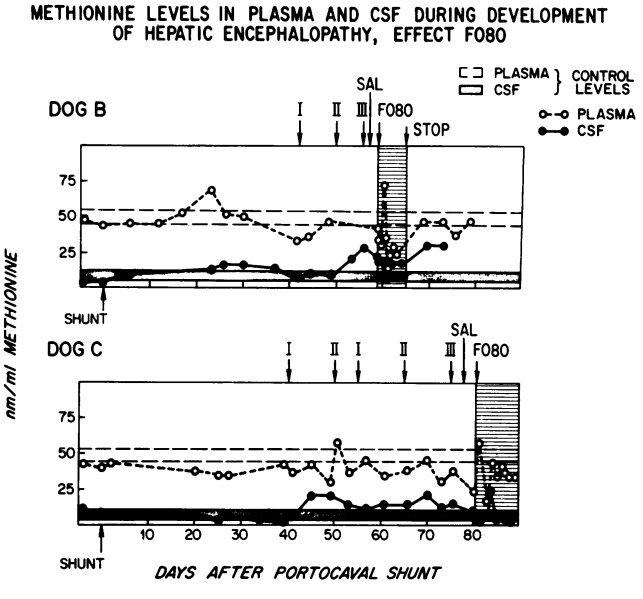


FIG. 5. Changes in methionine in plasma and CSF in 2 of the dogs during development of coma. Increased concentrations are not seen. (Control levels:white area—plasma; Grey area—CSF. Open circles—plasma; solid circles—CSF). Abbreviations: I: Stage I; II: Stage II; III: Stage III F080: Administration of F080 is started SAL: 0.9% NaCl saline solution is started.

development of hepatic coma 2–3 fold. After F080 administration, 5-HIAA levels returned to their control values (Fig. 6). A strongly positive linear correlation was observed between TRP and 5-HIAA levels in the CSF (Fig. 7).

Discussion

Numerous investigations in a variety of experimental animals as well as man, have documented changes in plasma amino acid pattern in hepatic encephalopathy superimposed on chronic hepatic disease.[9–11] These include the rather classical changes of increased aromatic amino acids, including phenylalanine, tyrosine, free tryptophan or total tryptophan, methionine and to a certain extent, aspartate and glutamate.[2] The branched chain amino acids, valine, leucine and isoleucine decrease,[16] often to levels below 50% of the normal plasma concentrations. Recent hypotheses have related the decrease in the branched chain amino acids as well as the increase in the aromatic amino acids to hormonal changes including hyperinsulinaemia and hyperglucogonemia,[19,29,30] secondary to hepatic deterioration. For obvious reasons little data is available on the changes within the CSF during development and therapy of hepatic encephalopathy. However, lumbar CSF values for both dopamine and serotonin metabolites, homovanillic acid and 5-HIAA were both seen to be elevated in a disparate group of patients studied by Knell, et al.[13] Serial changes for obvious reasons, are not reported since obtaining CSF in patients who may have difficulties with coagulation is hazardous.

The purpose of these experiments was using an experimental animal model manifesting an amino acid pattern similar to that seen in man with hepatic failure and encephalopathy, to correlate the changes in plasma

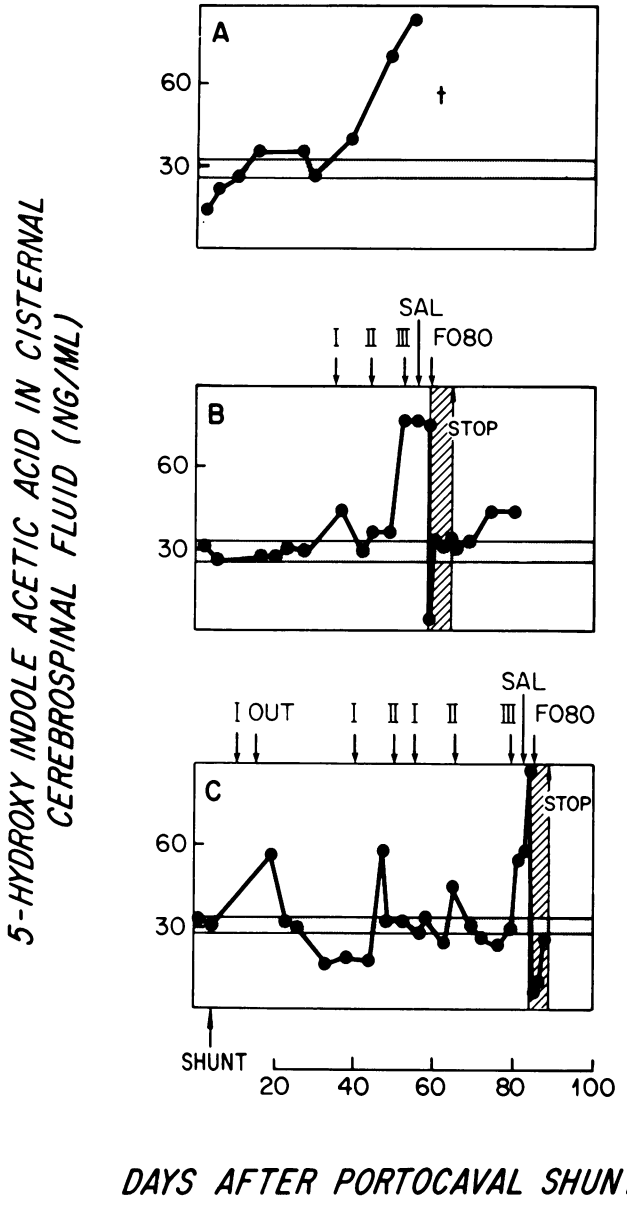


FIG. 6. Changes in 5-Hydroxy-indoleacetic acid in the CSF of dogs A, B and C. The same pattern as the aromatic amino acids can be observed. Abbreviations: I: Stage I; II: Stage II; III: Stage III F080: Administration of F080 is started SAL: 0.9% NaCl saline solution is started.

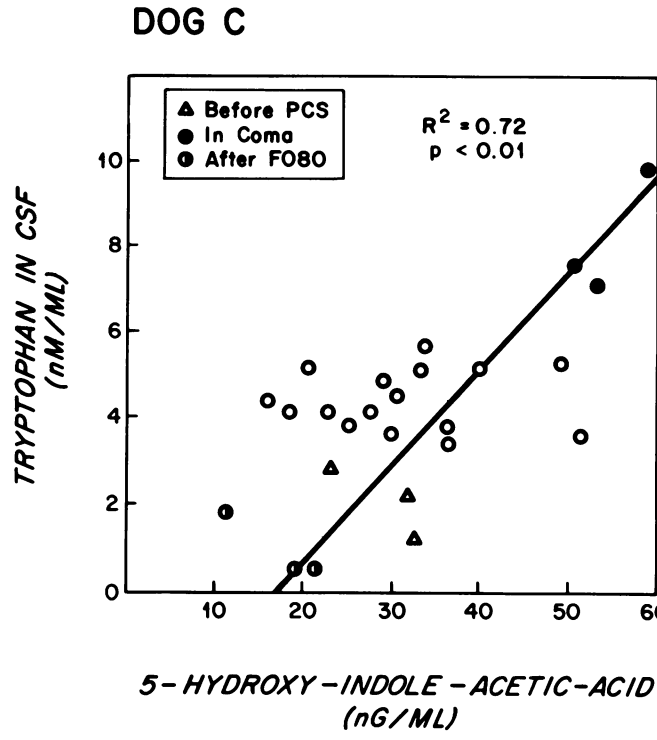


FIG. 7. Linear regression analysis of CSF TRP against CSF 5-HIAA levels. This dog is taken as an example, although its behavior is similar to other dogs. The regression eoefficient is significant. Abbreviations: I: Stage I; II: Stage II; III: Stage III F080: Administration of F080 is started SAL: 0.9% NaCl saline solution is started.

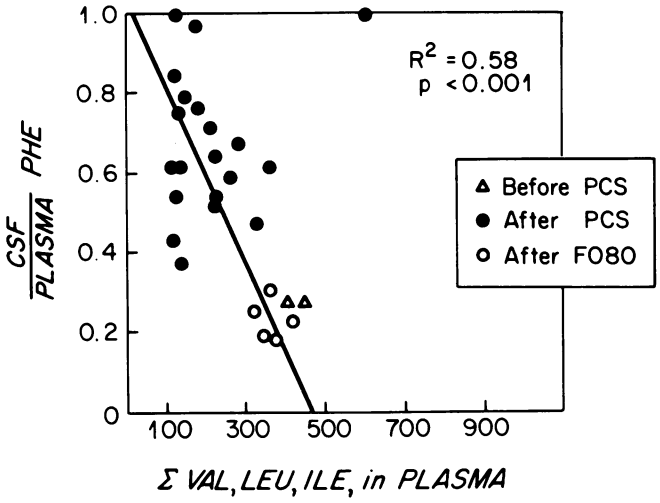


FIG. 8. Negative linear regression analysis between the ratio; CSF phenylalanine/plasma phenylalanine and the sum of plasma VAL, LEU, ILE in dog C. It suggests that the entry of PHE across the blood barrier is strongly correlated with the decrease of the branched chain amino acids in the plasma. The regression coefficient is statistically significant.

amino acids, octopamine and phenylethanolamine with changes within the cerebrospinal fluid in the cisterna magna, presumably representative of the CSF circulation influenced by the midbrain as well as the brain stem regions.[20]

The results obtained in these studies, revealed typical changes of increased aromatic amino acids and "false" or coneurochemical transmitters, octopamine and phenylethanolamine within plasma and CSF which correlate well with the development of encephalopathy in these animals. In addition, within the CSF, 5-HIAA a metabolite of serotonin, a neurotransmitter thought to have an inhibitory function and which may replace norepinephrine, the putative neurotransmitter within the adrenergic system[1] increased markedly with the development of hepatic encephalopathy. Once the animals had passed through a series of changes now clearly recognizable as stages I–III of hepatic encephalopathy in the canine experimental model,[9] internal jugular lines were placed and an infusion of dextrose-saline was carried out for five days during which no improvement in encephalopathy was seen. Following this, infusion of a specific amino acid mixture rich in branched chain amino acids and poor in aromatic amino acids and calculated to normalize the plasma amino acid pattern in both experimental animals and man, was associated with awakening from hepatic encephalopathy within 24 hours and a concomitant decrease and return towards normal in the plasma and CSF, aromatic amino acids, octopamine and phenylethanolamine and CSF 5-HIAA. This to our knowledge, is the first demonstration of associated improvement in normalization of amino acid profile and neurotransmitter metabolites within the brain with amelioration and improvement in hepatic coma.

The exact mechanism by which this occurs is still not entirely clear. Decrease in CSF aromatic amino acids presumably occurs on the basis of some competition between increased plasma aromatic amino acids and the newly increased concentration of the branched chain amino acids with F080 administration as well as their effect on efflux or aromatic amino acids from muscle in a catabolic animal, a mechanism originally described by Oddessey and Goldberg.[22] After F080 decreases in octopamine, a neurotransmitter metabolite of tyrosine and phenylethanolamine, a direct metabolite of phenylalanine presumably follow as does the decreased 5-HIAA which is related to the decrease in CSF and brain tryptophan.

Of great surprise to us was the somewhat constant level of the branched chain amino acids, leucine, isoleucine and valine which were detected within the CSF despite widely fluctuating concentrations within the plasma. CSF, leucine, isoleucine and valine remained relatively constant despite profound decreases in these plasma concentrations of branched chain amino acids which tended to elevate the plasma concentration of these important energy precursors. The reason for the constancy of CSF branched chain amino acid is not clear. It is now generally accepted that during encephalopathy, branched chain amino acids decrease in plasma. To account for the decrease in branched chain amino acids, which are not metabolized in appreciable amounts in the liver, it has been suggested recently that hyperinsulinaemia, secondary to decreased hepatic insulin degradation may promote increased muscle uptake of these amino acids as well as fat.[19,29,30] The results reported herein, demonstrate that during hepatic encephalopathy, CSF levels of branched chain amino acids remain unaffected although the plasma levels are significantly decreased. It suggests that the brain will regulate the concentration of branched chain amino acids in the CNS within physiological values and that the CNS is able to take up BCAA from the plasma against a concentration gradient to assure normal levels of BCAA in the brain, possibly due to an increased activity of the carrier system at the site of the blood brain barrier. As the branched chain amino acids and the aromatic amino acids are transported across the blood brain barrier by the same carrier system,[12,23] increased BCAA transport would also result in an increased transport of the aromatic amino acids into the brain; thus TYR, PHE and TRP in-

crease in the CNS. Increased levels of the aromatic amino acids in the brain during hepatic coma have indeed been observed.[3,26,31] Supporting this concept, the ratio of phenylalanine in CSF and plasma on the one hand and the decrease of branched chain amino acids on the other, are significantly correlated (Fig. 8). Moreover, Oldendorf[23] has proposed a competition for neutral amino acid transport within the central nervous system which appears to be a leucine preferred system. The brain may prefer, as it were, a constant level of these branched chain amino acids either for energy balance, protein synthesis or perhaps even lipid synthesis. Regardless of the mechanism, the increase in branched chain amino acids in the plasma appears to coincide with the decrease in transport of aromatic amino acids within the brain as well as alterations with the neurotransmitter synthesis, especially that of octopamine, phenylethanolamine and 5-HIAA. These findings lend credence to the findings in both experimental animals and man of amelioration of hepatic coma with normalization of plasma amino acid patterns. Returning plasma amino acid patterns to normal also returns CSF (and presumably brain) amino acids to a more normal configuration.

Acknowledgments

The authors wish to thank Messrs. D. Salvucci, A. Foubert, and M. Hennessey for the skillful animal care and their assistance during the operations.

References

1. Baldessarini, R. J. and Fischer, J. E.: Serotonin Metabolism in Rat Brain After Surgical Diversion of the Portal Venous Circulation. Nature, (New Biol.) 254:25, 1973.
2. Biebuyck, J., Fischer, J. E., Dedrick, S., et al.: Brain energy intermediates in acute hepatic coma, *In* Williams, R. and Murray-Lyon, I (eds.) Artificial Liver Support, Tunbridge Wells, Kent, 1975, Pitman Medical Publishers, p. 51.
3. Cummings, M. G. and Soeters, P., James, J. H. et al.: Regional Brain Indoleamine Metabolism Following Chronic Portocaval Anastomosis in the Rat. J. Neurochem., 27:501, 1976.
4. Curzon, G. and Green, A. R.: Effects of Immobilization on Rat Liver Tryptophan Pyrrolase and brain 5-hydroxytryptamine metabolism. Am. J. Pharmacol., 37:689, 1969.
5. Denckla, W. D. and Dewey, H. K.: The Determination of Tryptophan in Plasma, Liver and Urine. J. Lab. Clin. Med., 69:160, 1967.
6. Dodsworth, J. M., James, J. H., Cummings, B. S. and Fischer, J. E.: Depletion of Brain Norepinephrine in Acute Hepatic Coma. Surgery, 75:811, 1974.
7. Fernstrom, J. D. and Wurtman, R. J.: Brain Serotonin Content: Physiological Regulation by Plasma Neutral Amino Acids. Science, 178:414, 1972.
8. Fischer, J. E. and Baldessarini, R. J.: False Neurotransmitters and Hepatic Failure. Lancet, 2:75, 1971.
9. Fischer, J. E., Rosen, H. M., Ebeid, A. M., et al.: The Role of Plasma Amino Acids in Hepatic Encephalopathy. Surgery, 78:276, 1975.
10. Fischer, J. E., Funovics, J. M., Aguirre, A., et al.: The Effect of Normalization of Plasma Amino Acids on Hepatic Encephalopathy in Man. Surgery, 80:77, 1976.
11. James, J. H., Hodgman, J. M., Funovics, J. M. and Fischer, J. E.: Alterations in Brain Octopamine and Brain Tyrosine Following Portocaval Anastomosis in Rats. J. Neurochem. 27: 223, 1976.
12. Kiely, M. and Sourkes, T. L.: Transport of L-tryptophan into Slices of Rat Cerebral Cortex. J. Neurochem. 19:2863, 1974.
13. Knell, A. J., Davidson, A. R., Williams, R., et al.: Dopamine and Serotonin Metabolism in Hepatic Encephalopathy. Br. Med. J., 1:549, 1974.
14. Kopin, I. J.: False Adrenergic Transmitters. Ann. Rev. Pharm., 8:377, 1968.
15. Lam, K. C., Tall, R. R., Goldstein, G. B. and Mistilis, S. P.: Role of False Neurotransmitter, Octopamine in the Pathogenesis of Hepatic and Renal Encephalopathy. Scand. J. Gastroenterol., 8:465, 1973.
16. Iob, V., Mattson, W. J., Jr., Sloan, M., et al.: Alterations in Plasma Free Amino Acids in Dogs with Hepatic Insufficiency. Surg. Gynecol. Obstet., 130:794, 1970.
17. Manghani, K. K., Lunzer, M. R., Billings, B. H. and Sherlock, S.: Urinary and Serum Octopamine in Patients with Portal Systemic Encephalopathy. Lancet, 15:943, 1975.
18. Molinoff, P. B., Landsberg, L. and Axelrod, J.: An Enzymatic Assay for Octopamine and Other B-hydroxylated Phenylethanolamines. J. Pharm. Exp. Ther., 170:253, 1969.
19. Munro, H. N., Fernstrom, J. D. and Wurtman, R. J.: Insulin, Plasma Amino Acid Imbalance and Hepatic Coma. Lancet, 1:722, 1975.
20. Moir, A. T. P.: Interaction in the Cerebral Metabolism of the Biogenic Amines: Effect of Intravenous Infusion of L-tryptophan on the Metabolism of Dopamine and 5-hydroxyindoles in Brain and Cerebrospinal Fluid. Br. J. Pharmacol., 43:715, 1971.
21. Muto, Y., Takahishi, Y. and Kanamura, H.: Effects of Short Chain Fatty Acids and Ions on the Electrical Activity of Neo, Paleo, and Archicortical Systems. Brain nerve, 16:608, 1964.
22. Oddessey, R., Khairallah, E. A. and Goldberg, A. L.: Origin and Possible Significance of Alanine Production by Skeletal Muscle. J. Biol. Chem., 249:7623, 1974.
23. Oldendorf, W. H.: Brain Uptake of Radio Labeled Amino Acids, Amines and Hexoses After Arterial Injection. Am. J. of Physiol., 6:1629, 1971.
24. Orlowski, M. G., Sessa, J. P. and Green, J. P.: γ-glutamyl Transpeptidase in Brain Capillaries: Possible Site of a Blood Brain Barrier for Amino Acids. Science, 184:66, 1974.
25. Phear, E. A., Rubner, B., Sherlock, S. and Summerskill, W. H. J.: Methionine Toxicity in Liver Disease and its Prevention by Chlortetracycline. Clin. Sci., 15:93, 1956.
26. Record, O. C., Buxton, R. A., Chase, G., et al.: Plasma and Brain Amino Acids in Fulminant Hepatic Failure and Their Relationship to Hepatic Encephalopathy. Eur. J. Clin. Invest., 6:387, 1976.
27. Rossi-Fanelli, F., Smith, A. R., Cangiano, C., et al.: Studies on the Simultaneous Determination of Phenylethanolamine and Octopamine in Plasma and CSF. Submitted for publication.
28. Rossi-Fanelli, F., Cangiano, C., Angelico, M., et al.: Octopamine Plasma Levels and Hepatic Encephalopathy: A Re-appraisal of the Problem. Clin. Chim. Acta, 67:255, 1976.
29. Sherwin, R., Joshi, P., Hendler, R., et al.: Hypergluconaemia in Laennec Cirrhosis: The Role of Portal-systemic Shunting. N. Engl. J. Med., 290:239, 1974.
30. Soeters, P. B. and Fischer, J. E.: Insulin Glucagon, Amino Acid Imbalance and Hepatic Encephalopathy. Lancet, 23: 880, 1976.
31. Young, S. N., Lal, S., Sourkes, T. L., et al.: Relationships Between Tryptophan in Serum and CSF, and 5-hydroxyindoleacetic Acid in CSF of Man: Effect of Cirrhosis of the Liver and Probenicid Administration. J. Neurol. Neurosurg. Psychol., 38:322, 1975.
32. Zieve, L.: Pathogenesis of Hepatic Coma. Arch. Intern. Med., 118:211, 1966.