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Research Article

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Evidence for Central Hypertyraminemia in Hepatic Encephalopathy

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ABSTRACT In mongrel dogs, the effect of end-to-side portacaval shunt on plasma, cerebrospinal fluid (CSF) and brain tyramine, tyrosine, dopamine, norepinephrine, and epinephrine were studied. It was found that the level of tyramine in plasma, CSF, and selected brain regions increased steadily after the construction of the shunts. These elevations became more pronounced when the dogs manifested symptoms of hepatic encephalopathy. In postshunted dogs with stage II and III hepatic encephalopathy, tyramine concentration in corpus striatum ($1,312 \pm 371$), hypothalamus (400 ± 67.0), and midbrain (660 ± 78.7 ng/g) was significantly ($P < 0.05$) higher than the level in dogs with stage 0 and I hepatic encephalopathy and sham-operated dogs serving as controls (corpus striatum, 831 ± 140 ; hypothalamus, 167 ± 40.0 ; and midbrain, 132 ± 37.4 ng/g). This was followed by a concomitant depletion of dopamine and norepinephrine in these brain regions (postshunt: dopamine 104 ± 20.0 , $3,697 \pm 977$, and 105 ± 14.1 ; norepinephrine 521 ± 71.6 , 81.6 ± 13.7 , and 218 ± 31.7 ng/g; vs. sham group: dopamine 532 ± 83.1 , $8,210 \pm 1,126$, and 192 ± 35.0 ; norepinephrine $1,338 \pm 425$, 124 ± 21.3 , and 449 ± 89.7 ng/g) of encephalopathic dogs with portacaval shunt. Furthermore, tyramine, tyrosine, dopamine, and norepinephrine levels in plasma and CSF increased markedly as clinical features in the dogs' behavior characteristic of hepatic encephalopathy occurred, including hypersalivation, ataxia, flapping tremor, somnolence, and coma.

Cerebral hypertyraminemia and a defect in sympathetic neurotransmission may contribute to the development of hepatic encephalopathy of liver disease.

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INTRODUCTION

Tyramine concentration is abnormally elevated in the plasma of patients with different categories of liver disease including cirrhosis (1), hepatitis (2), Reye's syndrome (3), as well as in dogs with hepatic insufficiency or portacaval shunt (4). In all cases, hypertyraminemia was correlated in severity with hepatic encephalopathy.

We have recently demonstrated that tyrosine metabolism was impaired in dogs with end-to-side portacaval shunt (4). These animals also exhibited a decreased tyrosine clearance along with an appreciable increase in the conversion of tyrosine to tyramine after an oral dose of tyrosine. Furthermore, these elevations became more pronounced when they manifested symptoms of hepatic encephalopathy.

Tyramine possesses varied pharmacologic actions on the cardiovascular and central nervous systems (5, 6). If this substance does accumulate in blood of patients with liver disease, its action could play a part in the cardiovascular and neurologic complications of liver disease.

In this study, dogs with portacaval shunt were chosen as an experimental model in order to determine the influence of nutrient portal perfusion on tyramine levels in brain and cerebrospinal fluid (CSF).¹ How diversion of portal blood from liver by portasystemic shunt affects the relative concentration of dopamine, norepinephrine, and epinephrine in plasma, CSF, and brain was also studied. Tyrosine metabolism in central and peripheral nervous systems was correlated with clinical status of these shunted animals.

METHODS

Materials. [³H]Tyramine (9 Ci/mmol sp act) and S-[³H]adenosylmethionine (8 Ci/mmol sp act) were obtained

¹Abbreviations used in this paper: CSF, cerebrospinal fluid; SGOT, serum glutamic oxaloacetic transaminase.

from New England Nuclear (Boston, Mass.). Radiochemical purity of S-[³H]adenosylmethionine determined by descending paper chromatography at 5°C on Whatman No. 1 paper (Whatman Inc., Clifton, N. J.) using *n*-butanol:1.0 N HCL:ethanol (50:50:20; retardation factor [*R_f*], 0.00) was >98.7%. Radiochemical purity of [³H]tyramine checked by analytical thin layer chromatography (silica gel GF; Analabs Inc., Foxboro Co., North Haven, Conn.) with three different solvent systems (system 1, ammonia:methanol:chloroform:*n*-butanol, 15:16:15:40, *R_f* 0.78; system 2, *n*-butanol:acetic acid:water, 25:4:10, *R_f* 0.51; system 3, *n*-butanol:pyridine:acetic acid:water, 15:2:3:5, *R_f* 0.55) was >98%. Tyramine was obtained from Sigma Chemical Co. (St. Louis, Mo.). The antibody against tyramine was prepared according to the method of Faraj et al. (7). Radioactivity was measured in a liquid scintillation spectrometer (Beckman LS-330, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) using a scintillation fluid (Amersham Corp., Arlington Heights, Ill., counting efficiency, 40%).

Studies in dogs. 20 male mongrel dogs (20–25 kg) obtained from Emory University Animal Facility were conditioned for 3 wk. During this period, the dogs were checked periodically for the presence of parasites, kidney and liver disease, by both serological and histological methods. They were housed in individual fiberglass runs that meet all the present standards for humane care. They were fed Purina dog chow (Ralston Purina Co., St. Louis, Mo.) and water ad lib. Each dog received a full liver evaluation before and at determined intervals following the construction of the shunt as follows. Serum levels of glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, albumin, bilirubin, and blood urea nitrogen were measured according to standard laboratory techniques. Blood samples for tyramine, tyrosine, dopamine, norepinephrine, and epinephrine determinations were drawn from a peripheral vein into evacuated tubes (Vacutainer, Becton, Dickinson Co., Waltham, Mass.) containing glutathione and ethyleneglycol-*bis*-(β -aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA, Upjohn Co., Kalamazoo, Mich.), chilled to 0°C and centrifuged immediately at 500 g for 10 min. The plasma was then removed and quickly frozen at –80°C until analysis (within 1 mo).

Surgical procedure. 12 dogs were anesthetized with sodium pentobarbital (25–30 mg/kg). The surgical field was exposed through a midline incision, and the portal vein and inferior vena cava were exposed by reflecting the stomach and intestine to the left. The portal vein was isolated from the last major splanchnic branch to the division into lobar branches and any collateral branches between were divided. The proximal and distal veins were controlled with vascular tapes. The inferior vena cava was exposed from the renal veins to the point where it passes behind the liver and the anterior surface dissected for placement of a partial occlusion vascular clamp. The portal vein and vena cava were then approximated in a side-to-side fashion and longitudinal venotomies were made in each 2.5 cm in length. After the anastomosis was complete, the vascular clamps were removed and hemostasis maintained by gentle pressure. The hepatic side of the portal vein was then ligated with a Tevdek 2-0 suture (De Knatell, Inc., Queens Village, N. Y.) to convert the shunt with the end portal-to-side caval shunt. In situations where the portal vein and inferior vena cava did not approximate easily, the portal vein was divided just below the branches to the hepatic lobes and the end of the portal vein was anastomosed to the vena cava in a direct end-to-side fashion. Patency was determined by observation of clearance of the venous congestion of the intestine, which occurred while the portal vein was clamped.

After surgery, the dogs were maintained on 5% dextrose (500 ml) over a period of 4 h. The animals were then housed

in the appropriate cages. To prevent infection, penicillin G (Bicillin, Wyeth Laboratories, Philadelphia, Pa.) 2,500 U/d, was given intramuscularly daily for 2 d postoperatively. After a period of time, generally varying between 4 to 24 wk, animals manifested various signs of hepatic encephalopathy. Stage of coma was used as a factor in determining the end of the experiment. Dogs with stages 0 (*n* = 4) and I (*n* = 3), II (*n* = 2), and III (*n* = 3) hepatic encephalopathy were killed at 24, 8, and 4 wk postoperatively with an overdose of pentobarbital. The shunt was examined for patency. In the case of sham dogs, the 12 conditioned animals received anesthesia similar to the above group. Through a midline incision, the portal vein was exposed for a period of time, and then the incision was closed. Care and maintenance of these dogs were analogous to that described above. The postoperative time for the sham-operated dogs was the same as that of the shunted group.

Specimen preparation. The dogs were anesthetized with sodium pentobarbital (20–30 mg/kg). The superior aspect of the skull was removed to allow access to the cerebrum, cerebellum, and medulla oblongata. The animals were then killed with an overdose of sodium pentobarbital (75 mg/kg). Within the next 3–5 min, the brain was removed, the dura was separated, the optic chiasma was left intact as a landmark, and the cerebellum was gently manipulated to reveal the medulla oblongata. The brain was then gently placed on its dorsal cortex over dry ice and was allowed to reach a temperature suitable for dissection. All subsequent manipulations were carried out at 0–5°C. 2–3 g of temporal cerebral cortex and cerebellum were excised. The cerebellum was then removed to allow access to the midbrain. The posterior aspect of the midbrain transverse slice included the inferior colliculi and pineal body dorsally, and ventrally, the area immediately rostral to the pons. The hypothalamus was removed from an area between the optic chiasma, mammillary bodies, and piriform area to a depth of ~2 mm. After dissection of the hypothalamus, a transverse cut was made rostral to the optic chiasma. The bilateral corpus striatum was removed from the walls of the third ventricle, and the white matter was trimmed from its lateral aspects. A 25% wt/vol homogenate in 0.2 N perchloric acid was made of each specimen and then centrifuged at 10,000 g for 30 min. The supernatant fractions were removed and stored at –80°C until analysis. CSF samples were collected in ice-cold tubes over EGTA and glutathione and were stored at –80°C until analysis.

Analytical Procedure

Tyramine. Tyramine in plasma (usually 1 ml) was assayed according to the following procedure. To an aliquot of plasma, an equal volume of 6% wt/vol 5-sulfosalicylic acid was added, and the mixture was shaken vigorously for 1 min. Upon centrifugation (500 g for 20 min), an aliquot of the supernate (pH 1.5) was removed and placed in a 50-ml glass-stoppered centrifuge tube. Upon addition of 2 g NaCl, 0.3 g anhydrous Na₂CO₃, 4 ml of 0.5 M borate buffer (sodium borate, adjusted to pH 10.5 with 10 N NaOH), and 30 ml of ethyl acetate, the mixture was shaken (Eberbach Corp., Ann Arbor, Mich.) for 30 min and then centrifuged for 10 min. An aliquot (25 ml) of the organic phase was removed. To the two-phase mixture, 10 ml of ethyl acetate was added, and after shaking and centrifuging as above, 10 ml of solvent was withdrawn. The combined fractions of ethyl acetate (35 ml) were evaporated (N-Evap, Organomation Associates, Inc., Northborough, Mass.) to dryness under nitrogen at 40°C. The residue was reconstituted for radioimmunoassay in 0.2 M sodium phosphate buffer (pH 7.4). CSF samples were made basic, and

the tyramine in these samples was extracted with ethyl acetate as described above.

In 12 × 75-mm plastic tubes (Lab-Tek culture tube, Lab-Tek Products Div., Miles Laboratories, Inc., Naperville, Ill.) were placed 0.3 ml of 0.5% bovine serum albumin wt/vol in 0.2 M sodium phosphate buffer solution (pH 7.4). We then added 0.1 ml of antibody solution (1:50), 0.1 ml of [³H]-tyramine (0.15 ng, 8,000 cpm) in phosphate buffer, and one of the following: (a) for the development of a standard curve, 0.5–10.0 ng of tyramine in 0.1 ml of phosphate buffer; (b) 0.1-ml aliquot of the solution in phosphate buffer (pH 7.4) containing the extracted tyramine from plasma; (c) 0.1-ml aliquot of the solution in phosphate buffer (pH 7.4) containing the extracted tyramine from CSF; and, (d) 0.1 ml of brain supernatant fractions (10,000 g, pH 7.4). The tubes were capped and incubated at 4°C for 2 h. Antibody-bound [³H]-tyramine was separated from tyramine by the addition of 0.5 ml aqueous polyethylene glycol 6,000 (30% wt/vol) as described by Cheung and Slaunwhite (8). The tubes were vortex mixed vigorously and centrifuged (2,000 g, 4°C) for 40 min. The percentage free [³H]tyramine in the sample was determined by measuring the radioactivity in 0.2 ml of the supernate. The ³H was measured in a liquid scintillation spectrometer. Each sample was assayed in triplicate. All samples were counted to ±2% error. The radioimmunoassay of tyramine had a limit of sensitivity of 0.5 ng for tyramine. It showed a considerable degree of specificity since it did not cross-react with closely related analogs and metabolites of tyramine.

Catecholamines. Dopamine, norepinephrine, and epinephrine in supernatant fractions of selected brain regions (10,000 g, pH 5.1, 1:100 dilution), plasma, or CSF were assayed by radioenzymatic assay according to a procedure of Peuler and Johnson (9). The method was based upon the incubation (37°C, 40 min) of 50 μl of plasma, CSF, or brain supernatant fractions in the presence of catechol-O-methyltransferase, dopa decarboxylase inhibitor, and S-[³H]adenosylmethionine (5 μCi). The formed methylated metabolites [³H]3-O-methyldopamine, [³H]normetanephrine, and [³H]metanephrine were extracted and characterized by radiochromatographic analysis according to Peuler and Johnson (9). The assay had a sensitivity of 1 pg for norepinephrine and epinephrine, and 6 pg for dopamine. When known amounts of dopamine (580 pg), norepinephrine (550 pg), and epinephrine (540 pg) were added to plasma or tissue supernatant fractions, and the radioenzymatic assay was carried out, the recovery of dopamine, norepinephrine, and epinephrine from these biological materials was essentially quantitative (90–95%).

Tyrosine. The tyrosine in CSF, plasma, or brain supernatant fractions was measured on a Beckman amino acid analyzer (Beckman Instruments, Inc.) according to Benson and Patterson (10).

Statistical analysis

The results obtained in each series of experiments were expressed as the arithmetic mean and standard deviation (SD). The sample means were then compared by the Student's *t* test for paired data when appropriate. Values of *P* < 0.05 were accepted as representing significant differences.

RESULTS

Survival and clinical course. With the exception of two dogs, the shunted animals exhibited significant loss of weight starting at the end of the 2nd wk; after

6 wk the average weight loss was 10–20%. The physical weakness and general deterioration were the most obvious effects following portacaval shunt without the presence of infection. Hepatic encephalopathy in these dogs was characterized by hyperactive motor movement (stage I). Specific neurological symptoms, including ataxia, flapping tremor, narrow pupils, hypersalivation, and sometimes temporary paralysis appeared in stage II; while in stage III the dogs were asleep. In 12 of the dogs studied, the shunt was found patent upon autopsy (4–24 wk following the construction of the shunt) and no other vascular or other abnormalities were formed.

Biochemical data base. In all the dogs studied, hepatic function was assessed by routine laboratory tests that determined the serum level of alkaline phosphatase, SGOT, total protein, total bilirubin, albumin, blood urea nitrogen, and creatinine. The results indicated appreciable elevation of SGOT and alkaline phosphatase in shunted dogs (SGOT 204 ± 47.2, and alkaline phosphatase 269.4 ± 67.2 mU/ml) as compared to the serum levels of these enzymes determined preoperatively (SGOT 40.0 ± 7.8 and alkaline phosphatase 45.3 ± 12 mU/ml), respectively. This was followed by a concomitant decrease in serum protein (5.05 ± 2.03 vs. 6.60 ± 1.07 gm/dl), blood urea nitrogen (7.76 ± 2.12 vs. 15.7 ± 3.80 mg/dl), and creatinine (0.60 ± 0.20 vs. 1.00 ± 0.15 mg/dl) in dogs with portacaval shunt. Whereas clinical jaundice was not detectable, serum bilirubin increased 3.33 times the preshunt values in shunted dogs with stage II and III hepatic encephalopathy (0.40 ± 0.15 vs. 0.18 ± 0.03 mg/dl).

Plasma tyramine and tyrosine. Plasma tyramine was determined in a group of dogs (*n* = 12) before and following the construction of end-to-side portacaval shunt, as well as in a group of sham-operated dogs (*n* = 12). In sham-operated dogs, plasma tyramine did not differ significantly from the levels in control dogs. In postshunted dogs with stage 0 or I hepatic encephalopathy, plasma tyramine from fasted dogs (2.25 ± 0.70 ng/ml) did not differ significantly from that in control or sham-operated dogs. However, in encephalopathic dogs with stage II and III coma, plasma tyramine (8.50 ± 2.50 ng/ml) was significantly higher than the level in control, sham-operated, and non-encephalopathic dogs with portacaval shunt. Concentration of plasma tyrosine, 30–45 μmol/liter in control plasma, respectively, was significantly elevated in encephalopathic dogs (135 ± 30 μmol/liter) (Table I).

Plasma dopamine, norepinephrine, and epinephrine. Plasma catecholamines were determined in a group of dogs (*n* = 12) before and following the construction of end-to-side portacaval shunt, as well as in a group of sham-operated dogs (*n* = 12). In encephalopathic dogs with stage II and III coma, plasma dopamine (212 ± 51.3 pg/ml), norepinephrine (1,031 ± 300

TABLE I
Average (\pm SD) Levels of Dopamine (D), Norepinephrine (NE), Epinephrine (E), Tyramine (T), and Tyrosine (Tyr) in Plasma of Sham-operated and Dogs before (Preop) and after (Postop) the Construction of a Portacaval Shunt (PCS) with Different Stages of Hepatic Encephalopathy (HE)

Status	n	D	NE	E	T	Tyr
			pg/ml		ng/ml	μ mol/liter
PCS						
Preop	12	58.2 \pm 21.2	177 \pm 41.3	221 \pm 61.7	1.85 \pm 0.45	45.5 \pm 15.7
Postop						
Stage 0 and I HE	7	71.4 \pm 33.7	179 \pm 45.3	140 \pm 42.7	2.25 \pm 0.70	68.7 \pm 21.2
Stage II and III HE	5	212 \pm 51.3	1,031 \pm 300	1,760 \pm 500	8.50 \pm 2.50	135 \pm 30.5
Sham						
Preop	12	75.1 \pm 30.3	168 \pm 50.8	147 \pm 50.7	1.70 \pm 0.40	55.2 \pm 17.5
Postop	12	45.7 \pm 15.8	151 \pm 49.8	170 \pm 53.7	2.03 \pm 0.16	37.4 \pm 12.7

pg/ml), and epinephrine (1,760 \pm 500 pg/ml) were significantly higher than the level in control, sham-operated and nonencephalopathic dogs with portacaval shunt (Table I).

Brain catecholamines

Dopamine. A decrease from 16 to 32% in dopamine concentration was observed in the cortex, hypothalamus, corpus striatum, midbrain, and cerebellum of postshunt dogs with stage 0 and I hepatic encephalopathy. This depletion in dopamine became more pronounced in shunted dogs with stage II and III hepatic encephalopathy (cortex, 12.54 \pm 4.5; hypothalamus, 104 \pm 20; corpus striatum, 3,597 \pm 977; midbrain, 105 \pm 14; cerebellum, 5.41 \pm 1.2; and hippocampus, 11.8 \pm 1.7 ng/g) as compared with the levels of dopamine found in these different brain regions of sham-operated dogs serving as controls (33.0 \pm 11.0; 532 \pm 83.1; 8,210 \pm 1,126; 192 \pm 35.0; 30.7 \pm 12.7; and 19.1 \pm 9.30 ng/g; Tables II-IV).

Norepinephrine. A decrease from 13 to 37% in norepinephrine concentration was found only in the cortex, hypothalamus, and midbrain of dogs with end-to-side portacaval shunt with stage 0 and I hepatic

encephalopathy (Table III). However, in postshunted dogs with stage II and III hepatic encephalopathy (Table IV), there was 61, 61, 52, 74, and 50% decrease in norepinephrine levels in cortex, hypothalamus, mid-brain, cerebellum, and hippocampus as compared with the norepinephrine concentration found in these regions of sham-operated dogs (cortex, 200 \pm 50.7; hypothalamus, 1,338 \pm 425; corpus striatum, 124 \pm 21.3; midbrain, 449 \pm 89.7; cerebellum 320 \pm 52.4; and hippocampus, 145 \pm 28.5 ng/g; Table II).

Epinephrine. A decrease from 21 to 30% in epinephrine concentration was observed in cortex, hypothalamus, midbrain, and cerebellum in postshunted dogs with stage II and III hepatic encephalopathy (Table IV).

Brain tyramine and tyrosine

Tyramine. In postshunted dogs with stage II and III hepatic encephalopathy, tyramine concentration in corpus striatum (1,312 \pm 371), hypothalamus (400 \pm 67.0), and midbrain (660 \pm 78.7) was significantly ($P < 0.05$) higher than the level in sham-operated dogs serving as controls (Tables II and IV). However, in dogs with stage 0 and I hepatic encephalopathy, near normal

TABLE II
Average (\pm SD) Levels of Dopamine (D), Norepinephrine (NE), Epinephrine (E), Tyramine (T), and Tyrosine (Tyr) in Various Brain Areas of 12 Sham-operated Dogs

Tissue	D	NE	E	T	Tyr
			ng/g		μ g/g
Cortex	33.0 \pm 11.0	200 \pm 50.7	2.40 \pm 1.60	67.5 \pm 7.71	12.5 \pm 4.1
Hypothalamus	532 \pm 83.1	1,338 \pm 425	122 \pm 55.0	167 \pm 40.0	10.0 \pm 3.23
Corpus striatum	8,210 \pm 1,126	124 \pm 21.3	238 \pm 59.0	964 \pm 175	—
Midbrain	192 \pm 35.0	499 \pm 89.7	24.8 \pm 8.01	132 \pm 37.4	8.57 \pm 2.72
Cerebellum	30.7 \pm 12.7	320 \pm 52.4	1.92 \pm 0.63	100 \pm 20.7	—
Hippocampus	19.1 \pm 9.30	145 \pm 28.5	1.83 \pm 0.50	84.0 \pm 14.2	—

TABLE III
Average (\pm SD) Levels of Dopamine (D), Norepinephrine (NE), Epinephrine (E), Tyramine (T), and Tyrosine (Tyr) in Various Brain Areas of Seven Shunted Dogs with Stage 0 and I Hepatic Encephalopathy

Tissue	D	NE	E	T	Tyr
	ng/g			μ g/g	
Cortex	25.0 \pm 9.7	174 \pm 37.1	1.90 \pm 0.40	80.0 \pm 10.2	25.5 \pm 4.31
Hypothalamus	360 \pm 42.3	846 \pm 71.3	100 \pm 28.7	153 \pm 30.7	28.7 \pm 31.8
Corpus striatum	7,981 \pm 1,071	129 \pm 22.7	299 \pm 51.7	831 \pm 140	—
Midbrain	163 \pm 30.1	316 \pm 51.4	17.5 \pm 3.20	100 \pm 29.3	21.5 \pm 2.77
Cerebellum	24.0 \pm 10.7	345 \pm 42.0	1.33 \pm 0.31	66.5 \pm 12.2	—
Hippocampus	24.5 \pm 8.5	121 \pm 17.6	2.19 \pm 1.10	58.5 \pm 9.31	—

values were observed in tyramine concentration in these regions (cortex 80.0 \pm 10.2; hypothalamus, 153 \pm 30.7; corpus striatum, 831 \pm 140; midbrain, 100 \pm 29.3; cerebellum, 66.5 \pm 12.2; and hippocampus, 58.5 \pm 9.31 ng/g, Table III).

Tyrosine. A characteristic increase in brain tyrosine levels was noted in postshunted dogs with stage II and III hepatic encephalopathy, from four- to fivefold the normal values.

Dopamine, norepinephrine, and epinephrine levels in CSF

The concentration of dopamine and norepinephrine increased significantly during the development of hepatic encephalopathy (postshunt, stage II and III hepatic encephalopathy: dopamine, 37.7 \pm 10.3 and norepinephrine, 454 \pm 67.5 pg/ml), in the CSF to about threefold their original values (preshunt: dopamine, 12.0 \pm 5.51 and norepinephrine, 137 \pm 37.5 pg/ml) and twofold the values of postshunt dogs with stage 0 and I hepatic encephalopathy (dopamine, 15.0 \pm 6.71 and norepinephrine, 216 \pm 45.1 pg/ml) (Table V). No apparent increase in epinephrine concentration was found in CSF of encephalopathic dogs.

Tyramine and tyrosine levels in CSF

Normal values of tyramine and tyrosine in the CSF were lower than in plasma: 0.71 \pm 0.14 ng/ml and 10.0 \pm 2.73 nM/ml, respectively. In postshunt dogs with stage II and III hepatic encephalopathy, there was a significant increase in tyramine and tyrosine concentrations in the CSF (tyramine 4.26 \pm 1.45; tyrosine, 80.0 \pm 12.2 nM/ml) as compared with their original values or the levels of these compounds in the CSF of shunted dogs with stage 0 and I hepatic encephalopathy (tyramine, 1.51 \pm 0.55 ng/ml; tyrosine, 30.0 \pm 5.17 nM/ml) (Table V).

DISCUSSION

Derangement in the central metabolism of aromatic amino acids is a possible mechanism responsible for the development of hepatic encephalopathy in patients with liver disease. To test this hypothesis, we constructed end-to-side portacaval shunt in dogs to produce progressive hepatic failure and studied its effect on CSF and brain levels of tyramine, tyrosine, dopamine, norepinephrine, and epinephrine. The present studies demonstrated appreciable elevation of tyramine in hypothalamus, corpus striatum, and mid-

TABLE IV
Average (\pm SD) Levels of Dopamine (D), Norepinephrine (NE), Epinephrine (E), Tyramine (T), and Tyrosine (Tyr) in Various Brain Areas of Five Shunted Dogs with Stage II and III Hepatic Encephalopathy

Tissue	D	NE	E	T	Tyr
	ng/g			μ g/g	
Cortex	12.5 \pm 4.50	78.7 \pm 13.2	2.00 \pm 1.2	146 \pm 45.7	50.3 \pm 9.51
Hypothalamus	104 \pm 20.0	521 \pm 71.6	98.0 \pm 14.5	400 \pm 67.0	53.4 \pm 11.0
Corpus striatum	3,697 \pm 977	81.6 \pm 13.7	250 \pm 27.6	1,312 \pm 371	—
Midbrain	105 \pm 14.1	218 \pm 31.7	16.0 \pm 5.60	660 \pm 78.7	44.5 \pm 7.51
Cerebellum	5.41 \pm 1.20	84.2 \pm 14.2	1.25 \pm 0.81	115 \pm 47.6	—
Hippocampus	11.8 \pm 1.70	73.5 \pm 10.7	2.00 \pm 0.97	70.3 \pm 1.71	—

TABLE V
Average (\pm SD) Levels of Dopamine (D), Norepinephrine (NE), Epinephrine (E), Tyramine (T), and Tyrosine (Tyr) in CSF of Sham-operated and Dogs before (Preop) and after (Postop) the Construction of a Portacaval Shunt (PCS) with Different Stages of Hepatic Encephalopathy (HE)

Status	n	D	NE	E	T	Tyr
		pg/ml			ng/ml	
PCS						
Preop	12	12.0 \pm 5.51	137 \pm 37.5	5.10 \pm 1.61	0.71 \pm 0.14	10.0 \pm 2.73
Postop						
Stage 0 and I HE	7	15.0 \pm 6.71	216 \pm 45.1	19.0 \pm 3.57	1.51 \pm 0.55	30.0 \pm 5.17
Stage II and III HE	5	37.7 \pm 10.3	454 \pm 67.5	9.12 \pm 2.17	4.26 \pm 1.45	80.0 \pm 12.2
Sham	12	10.0 \pm 5.11	130 \pm 35.2	3.80 \pm 1.41	0.65 \pm 0.27	8.57 \pm 3.44

brain, as well as in CSF, of dogs with portacaval shunt. Central hypertyraminemia correlated significantly with both plasma tyramine and stage of coma in these shunted animals. This was followed by a concomitant increase in brain and CSF tyrosine.

Two factors may favor the accumulation of tyramine in the central nervous system. These may include: (a) increased rate of transfer of tyramine across the blood-brain barrier; (b) decreased rate of tyramine degradation; and/or (c) increased rate of synthesis concomitant with increased brain tyrosine. As coma progresses, the blood-brain barrier probably becomes more permeable to tyramine that normally does not get into the brain from circulation (11). In support of this hypothesis, Livingstone et al. (12) assessed the functional integrity of the blood-brain barrier and found that in comatose animals, the barrier had become permeable to D-sucrose, inulin, and L-glucose, substances that usually do not cross the barrier. Furthermore, increased transport of tyramine and tyrosine into brain may also reflect upon changes in pH, ionic strength of the blood-brain barrier and decreases in concentrations of competing neutral amino acids during hepatic encephalopathy. Smith et al. (13) and Bloch et al. (14) recently demonstrated that tyrosine, phenylalanine, octopamine, and phenylethanolamine levels increased dramatically during the development of hepatic encephalopathy in CSF of dogs and pigs with portacaval shunt.

There is reason to believe that central and peripheral metabolism of tyramine may be impaired in liver disease. Under normal circumstances, the major metabolic pathway for tyramine proceeds via its oxidation to 4-hydroxyphenylacetic acid by monoamine oxidase (15). However, marked reduction in hepatic monoamine oxidase activity was noted in cirrhotics (16) and dogs with portacaval shunt (17). Decreased monoamine oxidase activity correlated significantly with stage of coma and plasma tyramine in dogs with hepatic insufficiency. Similar abnormalities in monoamine oxidase activity was also found in cerebral

cortex of these dogs (17). It is probable that depletion in brain monoamine oxidase activity observed in dogs with hepatic encephalopathy may have serious consequences. It may be partly responsible for the pronounced accumulation of tyramine in midbrain, hypothalamus, and corpus striatum seen in encephalopathic dogs with portacaval shunt. This in turn may have a disruptive effect upon the function of sympathetic neurons.

In liver failure, the increased brain tyrosine may be directed into the synthesis of tyramine. Consequently, this may represent another important mechanism for central hypertyraminemia in encephalopathic dogs with portacaval shunt. An example of such an increased flow of tyrosine into this pathway was recently demonstrated by us (18). We found that the hypertyraminemia of cirrhosis resulted primarily from overproduction of tyramine, as the production rate in these patients was significantly greater than in controls.

This study also demonstrated a significant depletion in dopamine and norepinephrine levels in brain of dogs with portacaval shunt.² The levels of the catecholamines decreased markedly as clinical features of the dogs' behavior characteristic of hepatic encephalopathy occurred, including flapping tremor, somnolence, and coma. Two factors may influence the level of dopamine and norepinephrine in brain of shunted dogs: impaired synthesis and/or decreased neuronal uptake. Normally, catecholamines of brain sympathetic neurons are synthesized from the amino acid tyrosine by a series of enzymatic steps, as follows: tyrosine \rightarrow dihydroxyphenylalanine (dopa) \rightarrow dopamine \rightarrow norepinephrine \rightarrow epinephrine. The synthesis of these cate-

² No apparent change in the concentration of epinephrine was noted in the brain of encephalopathic dogs with portacaval shunt. Most of the literature indicates that brain epinephrine content in the caudate and corpus striatum of most mammals is very low (Gugten et al. 1976. *Brain. Res.* 107: 171-175). As reported here, however, its concentration in dog caudate is twofold greater than in any other brain regions sampled.

cholamines takes place mainly in the varicosities of the nerve terminals. The formation of dopa by the enzyme tyrosine hydroxylase and of dopamine by the enzyme L-aromatic amino acid decarboxylase takes place in the cytoplasm. Dopamine then enters the storage vesicle, where it is converted to norepinephrine by the enzyme dopamine- β -hydroxylase. The critical step in this process involves the conversion of tyrosine to dopa by the enzyme tyrosine hydroxylase (19).

Decreased tyrosine hydroxylase activity in the central nervous system of encephalopathic dogs with portacaval shunt is likely to affect the level of dopamine and norepinephrine in different brain regions. Chronic inhibition of brain protein synthesis after portacaval shunt could influence tyrosine hydroxylase activity in this organ. Wasterlain et al. (20) investigated the effects of chronic portacaval shunting, with or without additional ammonia loading, on brain protein synthesis in unanesthetized rats by continuous infusion of [^3H]lysine. The results indicated a 50% drop in the incorporation of [^3H]lysine into forebrain protein of shunted rats as compared with sham or control rats. Furthermore, increased content of tyramine in the brain of encephalopathic dogs with portacaval shunt may also affect the synthesis of norepinephrine and dopamine. Indirectly acting sympathomimetic amines and monoamine oxidase inhibitors, which exhibit an ability to release catecholamines from storage sites, profoundly reduced the activity of tyrosine hydroxylase in intact tissues (21–24). Tyramine also interferes with binding and neuronal uptake into adrenergic vesicles (25), and should, by this mechanism, lead to an increase in plasma and free cytosolic catecholamines. This may account for the significant accumulation of dopamine and norepinephrine seen in plasma and CSF of encephalopathic dogs with portacaval shunt.

The marked deficiency of dopamine in the hypothalamus and its subsequent elevation in CSF of encephalopathic dogs with portacaval shunt closely resembles our recent finding of decreased cerebral dopamine in Reye's syndrome (26), a disease associated with acute encephalopathy with evidence of hepatic dysfunction in children (27). Serum prolactin was significantly higher in the more severely encephalopathic patients examined than in those children with less severe Reye's syndrome who had normal prolactin concentrations. Secretions of prolactin by the anterior pituitary in man and in other mammals is under the control of hypothalamic neurohormones, including dopamine (28). We believe that hyperprolactinaemia in Reye's syndrome is caused primarily by dopamine depletion in the hypothalamus. The markedly increased concentration of dopamine in ventricular fluid from these patients (26) supports the idea that in hepatic coma there may be an increased release of dopamine

from effector neurons of the hypothalamus into the ventricular fluid. This may occur as a result of the displacement of this catecholamine by tyramine and its metabolite octopamine. Several workers (3, 29) have demonstrated a substantial increase in tyramine and octopamine levels in plasma, urine, and brain of patients with Reye's syndrome. Furthermore, an increase in hypothalamic and striatal octopamine content was associated with reduced brain dopamine and norepinephrine in these patients (29).

In summary, cerebral hypertyraminemia and a defect in dopaminergic neurotransmission may contribute to the development of hepatic encephalopathy in liver disease.

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