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IMPAIRED MONOAMINE OXIDASE ACTIVITY IN DOGS WITH PORTACAVAL SHUNT*†

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Abstract—In dogs with portacaval shunt, hypertyraminemia could result from either impaired degradation by monoamine oxidase (MAO) and/or from failure of tyramine to reach this enzyme. MAO activity was evaluated in liver obtained from dogs before and after the construction of an end-to-side portacaval shunt. Diversion of portal blood from the liver by a portacaval shunt resulted in a significant ($P < 0.05$) decrease in hepatic MAO activity [11.9 ± 4.1 nmoles of 4-hydroxyphenylacetic acid (PHA) formed·(mg protein)$^{-1}$·hr^{-1}] as compared to controls [28.7 ± 6.3 nmoles PHA formed·(mg protein)$^{-1}$·hr^{-1}]. Activity was maximally reduced in shunted dogs with stages II and III hepatic encephalopathy. In addition, more than a 50 per cent reduction in both the V_{max} and the K_m of hepatic MAO for tyramine was noted in shunted dogs as compared to controls. Similar kinetic abnormalities [post-shunt, K_m 52.63 ± 14.3, V_{max} 3.8 ± 1.2 vs sham group, K_m 120.1 ± 22.3 μM tyramine, V_{max} 14.3 ± 4.5 nmoles PHA·(mg protein)$^{-1}$·hr^{-1}], as well as decreased MAO activity [post-shunt, 1.85 ± 0.83 vs sham group, 6.7 ± 2.1 nmoles PHA formed·(mg protein)$^{-1}$·hr^{-1}], were found in cerebral cortex from encephalopathic dogs with portacaval shunt. In summary, defective MAO activity may contribute to many of the pathophysiologic events observed in dogs with portacaval anastomosis. Such abnormalities could explain the hypertyraminemia and encephalopathy that have been reported in patients and experimental animals with liver disease.

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

Tyramine is an aromatic amine found in a variety of body fluids and tissues. Endogenous tyramine arises by decarboxylation of its parent amino acid tyrosine, the responsible enzyme being the aromatic amino acid decarboxylase. Mammals also obtain tyramine from one or two other sources, either by ingesting it in food as the free amine or by bacteria metabolizing tyrosine within the large intestine.

Normally, aromatic amines (tyramine, phenethylamine, etc.) formed in the gastrointestinal tract by bacterial decarboxylases undergo extensive metabolism by monoamine oxidase (MAO) during their first passage through the intestinal wall and liver immediately after absorption. In liver disease, several factors that could favor an accumulation of tyramine in the general circulation are: lack of adequate MAO activity, natural or surgical portasystemic shunt, diminished mass of the liver, and curtailed perfusion of hepatocytes. The amines under consideration possess varied pharmacologic actions on the cardiovascular and central nervous systems. If these substances or their active metabolites do in fact accumulate in blood of patients with liver disease, their actions could play a part in the cardiovascular and neurologic complications of liver disease.

In this study, dogs with end-to-side portacaval shunts were used as experimental models to investigate the influence of nutrient portal perfusion on MAO activity. We have measured the activity and evaluated certain kinetic parameters of this enzyme in liver tissues obtained at laparotomy before and following the construction of the shunts in these animals. The enzymatic activity was correlated with the fasting plasma tyramine. How portacaval shunt affects the activity of MAO in nonhepatic tissues such as brain was also studied.

MATERIALS AND METHODS

Tyramine[^{3}H] (sp. act. 9 Ci/mmole) was obtained from the New England Nuclear Corp. (Boston, MA). Radiochemical purity checked by analytical thin-layer chromatography with three different solvent systems was above 98 per cent. 4-Hydroxyphenylacetic acid (PHA) was obtained from the Eastman Chemical Co. (Rochester, NY). Tyramine was obtained from the Sigma Chemical Co. (St. Louis, MO). The antibody against tyramine was prepared according to Faraj *et al.* [5]. Radioactivity was measured in a liquid scintillation spectrometer (Beckman

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LS 330, Beckman Instruments, Inc., Spinco Division, Palo Alto, CA) using a scintillation fluid (ACS, Amersham/Searle Corp., Arlington Heights, IL, counting efficiency, 40 per cent).

Studies in dogs

Twenty male mongrel dogs (20–25 kg) obtained from the Emory University Animal Facility were conditioned for 3 weeks. During this period, the dogs were checked periodically for the presence of parasites and 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*. Before and at various times after surgery, the following studies were performed. Fasting 8:00 a.m. blood samples were drawn from a peripheral vein for routine blood studies [total bilirubin, total protein, blood urea nitrogen (BUN), alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), and creatinine]. Blood samples (heparinized) for tyramine determinations were drawn, chilled (0°) and immediately centrifuged at 500 *g* for 10 min; the plasma was then removed and quickly frozen at −80° until analysis (within 1 month).

Surgical procedure

Ten 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 where it passes behind the liver, and the anterior surface was 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 an O-Tevdek to convert the shunt with the end portal to a 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) for a period of 4 hr. The animals were then housed in the appropriate cages. To prevent infection, penicillin G (Bicillin, Wyeth Laboratories, Philadelphia, PA), 2500 U/day, was given intramuscularly daily for 2 days postoperatively. After a period of time, generally from 4 to 16 weeks, each animal 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 and I (N = 6), stage II (N = 2), and stage III (N = 2) hepatic encephalopathy were killed at 16, 8 and 4 weeks postoperatively with an overdose of pentobarbital. The shunt was examined for patency. In the case of the sham dogs, ten conditioned animals received anesthesia just as the above group. Through a midline incision, the portal vein was exposed for a period time and, then, the incision was closed. Care and maintenance was analogous to that of the dogs described above. The postoperative time for the sham-operated dogs was the same as that of the shunted group.

Monoamine oxidase activity

Enzyme preparation. The preparation of enzymes containing MAO was done according to a modified procedure of Wurtman and Axelrod [6]. At laparotomy a 0.5 to 1 g wedge biopsy was taken from the free edge of the liver and was frozen immediately at −80° pending analysis (within 48 hr of collection). A 2.5% (w/v) homogenate (Polytron Brinkman Instruments, Westbury, NY) of liver was prepared in chilled isotonic KCl at 5°; the homogenate was centrifuged at 10,000 *g* for 10 min. Enzymes and reagents were kept on ice prior to use. In the case of brain MAO, a 1–2 g biopsy was taken from the cerebral cortex and a 10% (w/v) homogenate was made as described above.

Enzyme assay. Activity of MAO enzyme was determined in liver or brain supernatant fractions (10,000 *g*). The base for the reacting mixture was composed of sodium phosphate buffer (0.5 M, pH 7.4, 0.25 ml), tyramine[^{3}H] (58 μM, sp. act. 34.5 μCi/μmole, 0.025 ml), and the enzyme preparation [2.5% (w/v) for liver or 10% (w/v) for brain, 10,000 *g*, 0.20 ml]. The above mixture was vortex-mixed. Blank tubes were prepared by substituting distilled water for the enzyme preparation. The reaction mixture was incubated for 20 min at 37°. The reaction was stopped by the addition of 0.2 ml of 2 N HCl. The deaminated radioactive material was extracted into ethyl acetate by shaking the organic solvent for 30 min and then centrifuging (500 *g*) for 10 min. The transfer of the organic phase was then facilitated by rapid freezing of the aqueous phase in an isopropyl alcohol/dry ice bath. The organic layer was decanted into scintillation vials containing 6 ml of scintillation fluid. All samples were counted to a ± 2 per cent error. The 4-hydroxyphenylacetic[^{3}H] acid in the organic phase was characterized by radiochromatographic analysis according to a modified procedure of Faraj *et al.* [7], as follows. The extracted ethyl acetate fraction was evaporated to dryness under nitrogen at 40° (N-Evap, Organomation Associates, Inc., Northborough, MS). The residue was dissolved in 0.2 ml methanol and applied to 0.25 mm thin-layer chromatography plates (silica gel GF, AnaLabs, Inc., North Haven, CT). The plates were developed to 14 cm in a system made up of ammonia–methanol–chloroform–*n*-butanol (15:15:15:55). Under these conditions, the R_f value of tyramine and 4-hydroxyphenylacetic acid were 0.78 and 0.25 respectively. A freshly prepared solution of tyramine and 4-hydroxyphenylacetic acid (100 μg/10 μl) was applied as standard. After development, the spots were

visualized by ultraviolet light (250 nm). The plates were scraped with an Autozonal scraper (AnaLabs, Inc.) in 0.5-cm sections and placed in scintillation vials containing 1 ml methanol. The mixture was shaken vigorously and allowed to stand at least 3 hr before counting. The results indicated that, following enzymatic incubation, the counts extracted consisted primarily of PHA[^{3}H]. The final protein concentration of the enzyme solution was determined by Lowry *et al.* [8] using bovine serum albumin as the standard.

Enzyme kinetics. Kinetic analyses were carried out on liver and brain tissues. The apparent K_m of MAO for tyramine was determined by varying the tyramine[^{3}H] concentration between 7.3×10^{-3} and 1.38×10^{-1} mM (sp. act. 34.5 μCi/μmole). MAO activity was determined, and initial velocity for each substrate concentration was used in a Lineweaver–Burk plot from which K_m and V_{max} were obtained.

Analytical procedure

Tyramine in plasma (usually 1 ml) was extracted according to the following procedure. To an aliquot of plasma (1 part), methanol (2 parts) was added and the mixture was shaken vigorously for 1 min. Upon centrifugation (500 *g* for 20 min), the supernatant fraction was removed and placed in a 50-ml glass stoppered centrifuge tube (Kontes Co., Vineland, NJ) and then evaporated to dryness under nitrogen at 40°. The residue was reconstituted for radioimmunoassay in 0.01 M sodium phosphate buffer (pH 7.4).

The method used was a modification of that described by Faraj *et al.* [5]. In 12 × 75 mm plastic tubes (Lab-Tek culture tube, Lab-Tek Products, Division of Miles Laboratories, Inc., Naperville, IL) was placed 0.3 ml of 0.5% (w/v) bovine serum albumin in 0.01 M sodium phosphate buffer solution [pH 7.4, containing 0.03% (w/v) potassium phosphate, 0.8% (w/v) NaCl, and 0.05% (w/v) human gammaglobulin]. We then added 0.1 ml of antibody solution (1:50), 0.1 ml of tyramine[^{3}H] (0.16 ng, 7500 cpm) in phosphate buffer, and either unlabeled tyramine (0.5–10 ng for standard curve) or a 0.1-ml aliquot of the sodium phosphate buffer (pH 7.4) containing the extracted tyramine. The tubes were capped and incubated at 4° for 2 hr. Antibody-bound tyramine[^{3}H] was separated from tyramine by the addition of 0.5 ml aqueous polyethylene glycol 6,000 (30%, w/v) as described by Cheung and Slaunwhite [9]. The tubes were vortex-mixed vigorously and centrifuged (2000 *g*, 4°) for 40 min. The percentage of free tyramine[^{3}H] in the sample was determined by measuring the radioactivity in 0.2 ml of the supernatant fraction. The ^{3}H was measured in a liquid scintillation spectrometer. Each sample was assayed in triplicate. All samples were counted to ± 2 per cent error.

Statistical analysis

The results obtained in each series of experiments were expressed as the arithmetic mean ± standard deviation (S.D.). The sample means were then compared by Student's test for paired data when appropriate. Values of $P < 0.05$ were accepted as representing significant differences.

RESULTS

With the exception of two dogs, the shunted animals exhibited significant loss of weight starting at the end of the second week; after 6 weeks the average weight loss was 10–20 per cent. 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 and hypersalivation (stage I). Specific neurological symptoms, including ataxia, flapping tremor, narrow pupils and, sometimes, temporary paralysis appeared in stage II, whereas in stage III the dogs were asleep. In the ten dogs studied, the shunt was found patent upon autopsy (4–16 weeks following construction of the shunt) and no other vascular or other abnormalities were formed.

Biochemical data base

In each of 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, BUN and creatinine. The results indicated appreciable elevations of SGOT and alkaline phosphatase in shunted dogs (SGOT 180 ± 40 and alkaline phophatase 250 ± 57 mU/ml), compared to the serum levels of these enzymes determined preoperatively (SGOT 37.1 ± 7 and alkaline phosphatase 40.1 ± 10 mU/ml) or in a sham-operated group (SGOT 36 ± 9 and alkaline phosphatase 40 ± 10 mU/ml), respectively. This was followed by a concomitant decrease in serum protein, BUN, and creatinine in dogs with portacaval shunt. Although clinical jaundice was not detectable, serum bilirubin increased 2.2 times the pre-shunt values (Table 1).

Plasma tyramine

Fasting plasma tyramine levels were determined in a group of dogs (N = 10) before (controls) and after the construction of end-to-side portacaval shunt, as well as in a group of sham-operated dogs (N = 10). In sham-operated dogs, fasting plasma tyramine levels did not differ significantly from the levels in control dogs. In post-shunted dogs with stage I hepatic encephalopathy, fasting plasma tyramine (2.15 ± 0.7 ng/ml) did not differ significantly from that in control or sham-operated dogs. In encephalopathic dogs with stages II and III coma, however, fasting plasma tyramine (7.5 ± 2.1 ng/ml) was significantly higher than the levels in control, sham-operated, and non-encephalopathic dogs with portacaval shunts (Table 2).

MAO activity

MAO activity was determined in liver supernatant fractions (10,000 *g*) prepared from dog liver tissue samples obtained before and after the construction of the shunt. A marked decrease in MAO activity (Table 2) was observed in canine liver from 4 to 16 weeks following end-to-side portacaval shunt [postoperative 11.9 ± 4.1, vs preoperative 28.7 ± 6.3, and sham-operated dogs 30.11 ± 6.8 nmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}]. Deterioration in hepatic MAO activity was more pronounced in dogs

Table 1. Biochemical data base of sham-operated and dogs before (preop, controls) and after (postop) construction of end-to-side portacaval shunt (PCS) with various stages of hepatic encephalopathy (HE)*

Status	N	SGOT (mU/ml)	Total bilirubin (mg/dl)	Albumin (g/dl)	Total protein (g/dl)	Alkaline phosphatase (mU/ml)	BUN (mg/dl)	Creatinine (mg/dl)
PCS								
Preop	10	37.1 ± 7.0	0.18 ± 0.03	3.0 ± 0.7	7.0 ± 1.0	40.1 ± 10	21.0 ± 5.0	1.00 ± 0.15
Postop	10	180.0 ± 40.0†	0.40 ± 0.15†	2.0 ± 0.2	5.1 ± 2.0‡	250.0 ± 57†	7.7 ± 2.1†	0.60 ± 0.2‡
Stages 0 and I HE	6	60.0 ± 14.0‡	0.20 ± 0.04‡	2.5 ± 0.6‡	6.0 ± 0.7‡	80.0 ± 17†	14.0 ± 4.0‡	0.90 ± 0.4‡
Stages II and III HE	4	300.0 ± 54.0†	0.60 ± 0.2†	1.5 ± 0.3†	4.0 ± 0.6‡	420.0 ± 72†	1.4 ± 0.5†	0.30 ± 0.06†
Sham								
Preop	10	36.0 ± 9.0‡	0.20 ± 0.07‡	3.1 ± 0.5‡	6.8 ± 0.9‡	40.0 ± 10‡	21.0 ± 7.0‡	0.87 ± 0.1‡
Postop	10	39.0 ± 9.7‡	0.18 ± 0.03‡	3.0 ± 0.4‡	7.2 ± 1.1‡	37.0 ± 8.1‡	18.0 ± 6.0‡	0.81 ± 0.13‡

* Values are mean ± S.D.
† $P < 0.05$, compared to controls (preoperative dogs).
‡ Not significantly different from controls.

Table 2. Hepatic MAO activity, enzyme kinetics, and fasting plasma tyramine in a group of sham-operated and in dogs before (preop, controls) and after (postop) construction of end-to-side portacaval shunt (PCS) with various stages of hepatic encephalopathy (HE)

Status	N	Enzyme activity*, [nmoles PHA formed · (mg protein)$^{-1}$ · hr^{-1}]	Kinetic parameters* V_{max} [nmoles PHA · (mg protein)$^{-1}$ · hr^{-1}]	K_m (μM tyramine)	Plasma tyramine (ng · ml^{-1})
PCS					
Preop	10	28.70 ± 6.3	75.0 ± 11.6	112.00 ± 16.7	1.87 ± 0.5
Postop	10	11.90 ± 4.1‡	29.0 ± 5.0‡	73.80 ± 9.3§	4.82 ± 1.6‡
Stages 0 and I HE	6	18.00 ± 6.1§	50.1 ± 4.8§	125.10 ± 20.2§	2.15 ± 0.7§
Stages II and III HE	4	5.88 ± 2.1‡	7.7 ± 1.7‡	21.73 ± 5.0‡	7.50 ± 2.1‡
Sham					
Preop	10	30.11 ± 6.8§	70.3 ± 6.1§	95.40 ± 10.0§	2.03 ± 0.6§
Postop	10	29.07 ± 7.3§	68.7 ± 7.3§	100.30 ± 12.3§	1.70 ± 0.2§

* Values are means ± S.D.
† The concentration of tyramine[^{3}H] used to measure enzyme activity was 58 μM (sp. act. 34.5 μCi μmole).
‡ $P < 0.05$, compared to controls.
§ Not significantly different from controls (preoperative).

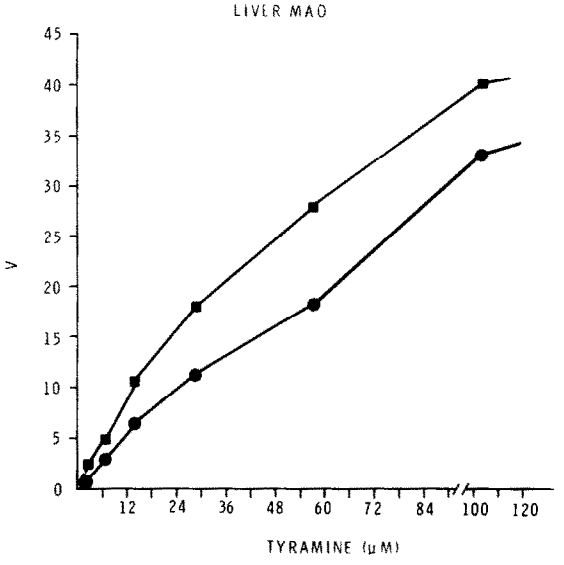

Fig. 1. Liver MAO activity, measured as velocity of PHA formation versus tyramine concentration, from preoperative (■——■) and shunted dogs with stages 0 and I hepatic encephalopathy (●——●). Tyramine[^{3}H] concentration varied from 7.3 to 146 μM (sp. act. 34.5 μCi/μmole). The velocity, V, is expressed as nmoles of 4-hydroxyphenylacetic acid (PHA) formed·(mg protein)$^{-1}$·hr^{-1}. Each point is the average for six dogs.

with stages II and III hepatic encephalopathy [5.88 ± 2.1 nmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}] than in shunted dogs with stages 0 and I coma [18.0±6.1 nmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}; Table 2, Figs. 1 and 2]. Furthermore, the activity of this enzyme was also determined in cerebral cortex supernatant fractions obtained from groups of sham-operated dogs and dogs with

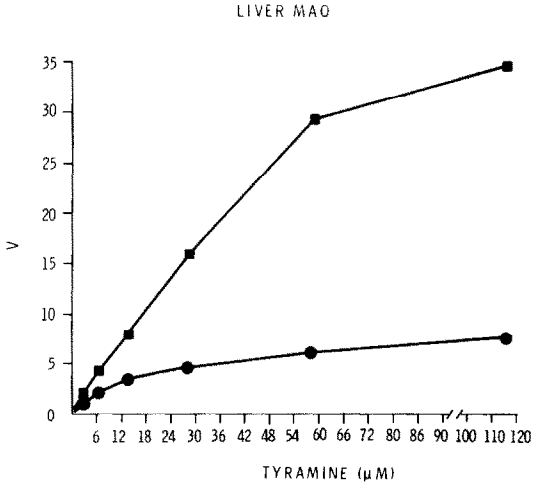

Fig. 2. Liver MAO activity, measured as velocity of PHA formation versus tyramine concentration, from preoperative (■——■) and shunted dogs with stages II and III hepatic encephalopathy (●——●). Tyramine[^{3}H] concentration varied from 7.3 to 146 μM (sp. act. 34.5 μCi/μmole). Velocity, V, is expressed as nmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}. Each point is the average for four dogs.

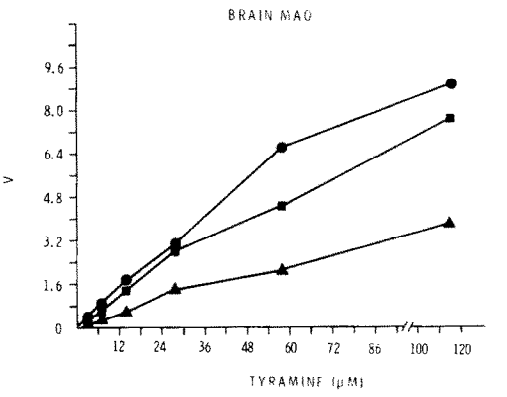

Fig. 3. Brain (cerebral cortex) MAO activity, measured as velocity of PHA formation versus tyramine concentration, from sham-operated (●——●) and shunted dogs with stages 0 and I (■——■) and stages II and III (▲——▲) hepatic encephalopathy. Tyramine[^{3}H] concentration varied from 7.3 to 146 μM (sp. act. 34.5 μCi/μmole). Velocity, V, is expressed as nmoles of PHA formed · (mg protein)$^{-1}$·hr^{-1}. Each point is the average for ten, six, and four dogs for sham and shunted dogs with stages 0 and I and stages II and III hepatic encephalopathy.

portacaval shunt. The results indicated that there had been a significant decrease of MAO activity in cerebral cortex from shunted dogs with stages II and III coma [1.85 ± 0.83 nmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}], compared to shunted dogs with stages 0 and I coma [4.46 ± 1.7 nmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}] and sham-operated dogs [6.7 ± 2.1 nmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}; Table 3, Fig. 3].

Kinetic studies of MAO

MAO from both liver and cerebral cortex was used for kinetic studies. Graph determinations of the apparent K_m values, when tyramine was the sub-

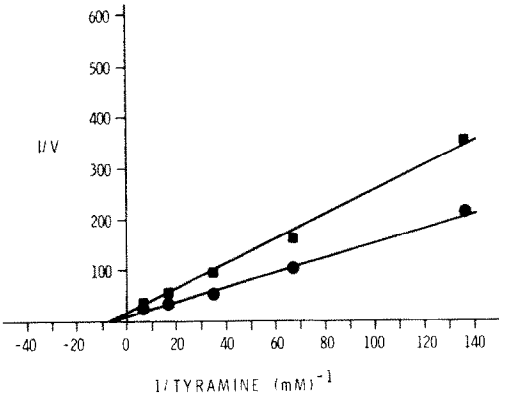

Fig. 4. Lineweaver–Burk plot for MAO in liver homogenates. Two hundred microliters of the supernatant fluid (10,000 g) from isotonic KCl homogenates of liver biopsy from preoperative (●——●) and shunted dogs with stages 0 and I hepatic encephalopathy (■——■) was incubated under standard conditions for 20 min with tyramine[^{3}H] varying in concentration from 7.3 × 10^{-3} to 1.38 × 10^{-1} mM (sp. act. 34.5 μCi/μmole). V is in μmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}. Each point is the average of six dogs.

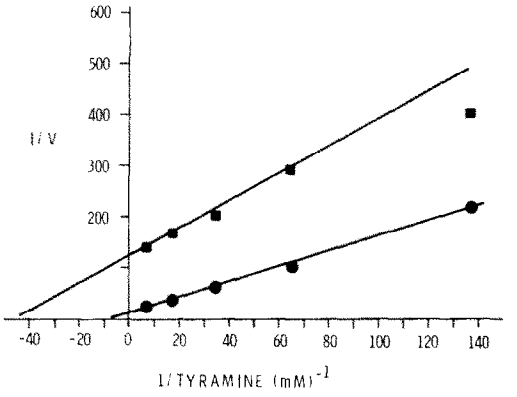

Fig. 5. Lineweaver–Burk plot for MAO in liver homogenates. Two hundred microliters of the supernatant fluid (10,000 *g*) from isotonic KCl homogenates of liver biopsy from preoperative (●——●) and shunted dogs with stages II and III hepatic encephalopathy (■——■) was incubated under standard conditions for 20 min with tyramine[^{3}H] varying in concentration from 7.3×10^{-3} to 1.38×10^{-1} mM (sp. act. 34.5 μCi/μmole). *V* is in μmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}). Each point is the average of four dogs.

strate, of the normal enzyme from liver (preoperative group serving as controls) and the brain tissue (sham-operated group serving as controls) are shown in Figs. 4–6. The double reciprocal plots of velocity tyramine concentration yielded straight lines, indicating adherence to simple Michaelis–Menten kinetics. Upon extrapolation, the apparent K_m for tyramine was determined to be 112.0 ± 16.7 μM for liver and 120.1 ± 22.3 μM for brain, respectively. Michaelis constants for MAO isolated from liver (21.73 ± 5.0 μM) and brain (52.63 ± 14.2 μM) of dogs with stages II and III post-shunt hepatic encephalopathy were significantly lower than were those of the enzyme from normal liver and brain. Appreciable

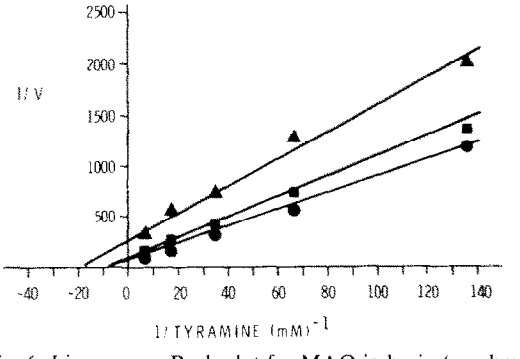

Fig. 6. Lineweaver–Burk plot for MAO in brain (cerebral cortex) homogenates. Two hundred microliters of the supernatant fluid (10,000 *g*) from isotonic KCl homogenates of cerebral cortex from sham-operated (●——●) and shunted dogs with stages 0 and I (■——■) and stages II and III hepatic encephalopathy (▲——▲) was incubated under standard conditions for 20 min with tyramine[^{3}H] varying in concentration from 7.3×10^{-3} to 1.38×10^{-1} mM (sp. act. 34.5 μCi/μmole). *V* is in μmoles of PHA formed·(mg protein)$^{-1}$·hr^{-1}. Each point is the average of ten, six, and four dogs for sham and shunted with stages 0 and I, and stages II and III hepatic encephalopathy.

Table 3. Brain MAO activity and enzyme kinetics in a group of sham-operated (controls) and in dogs with end-to-side portacaval shunt (PCS) with different stages of hepatic encephalopathy (HE)

Status	N	Enzyme activity* [nmoles PHA formed·(mg protein)$^{-1}$·hr^{-1}]	Kinetic parameters* V_{max} [nmoles PHA·(mg protein)$^{-1}$·hr^{-1}]	K_m (μM tyramine)
PCS	10	3.15 ± 1.0‡	7.45 ± 1.7‡	88.50 ± 17.1§
Stages 0 and I HE	6	4.46 ± 1.7§	11.11 ± 3.07§	124.50 ± 20.7§
Stages II and III HE	4	1.85 ± 0.83‡	3.80 ± 1.2‡	52.63 ± 14.3‡
Sham	10	6.7 ± 2.1	14.30 ± 4.5	120.10 ± 22.3

* Values are means ± S.D.
† The concentration of tyramine[^{3}H] used to measure enzyme activity was 58 μM (sp. act. 34.5 μCi/μmole).
‡ $P < 0.05$, compared to sham dogs serving as a control group.
§ Not significantly different from sham group.

reduction in V_{max} for both liver and brain MAO was also noted in post-shunt encephalopathic dogs, compared to controls (Tables 2 and 3).

DISCUSSION

We have demonstrated recently that plasma tyramine elevation was achieved by surgical construction of a portacaval anastomosis in dogs [4]. The hypertyraminemia seen in these dogs was of the same order of magnitude as that observed in cirrhotic patients. In both categories, elevated levels of tyramine seemed to correlate best with portal systemic shunting and hepatic encephalopathy.

The objective of this present study was to determine the cause for tyramine accumulation in plasma of dogs with portacaval shunt. Two factors that may influence the clearance of plasma tyramine in these shunted dogs are: impaired hepatic perfusion and/or reduced hepatic content of MAO enzyme. Under normal circumstances, the major metabolic pathway for tyramine is believed to proceed via its oxidation to 4-hydroxyphenylacetic acid by MAO enzyme primarily by that located in the liver.

The present studies demonstrated significant impairment in hepatic MAO activity in these experimental animals; this enzyme was reduced to more than 50 per cent of control values. Furthermore, there was a negative correlation between plasma tyramine and hepatic MAO activity. Kinetic analysis revealed the existence of a prominent defect in the activity of MAO as shown by an appreciable reduction in the K_m for the substrate tyramine and lowered V_{max} for the enzyme. This suggests that smaller quantities of active enzyme were present in the liver from dogs with end-to-side portacaval shunt. Furthermore, activity was maximally reduced in shunted dogs with stages II and III hepatic encephalopathy. A similar kinetic abnormality, as well as decreased MAO activity, was also found in brain tissues of these shunted dogs.

Diversion of portal blood from the liver by a portasystemic shunt is often associated with postoperative deterioration of hepatic function and liver failure. The metabolic effect of the shunt is often a complex phenomenon that may include: (a) alterations in amino acid and hormonal metabolism; (b) dietary and nutritional imbalance; and (c) chronic inhibition of protein synthesis.

Any one or all of the above may have a disruptive effect upon the activity of MAO. There is reason to believe that portal flow diversion may produce abnormalities in estrogen or progesterone metabolism [10]. This was demonstrated by the decreased rate of hepatic conjugation of estrogen. Some evidence concerning the relative importance of some hormones (progesterone, estradiol and testosterone) in regulating MAO activity has been reported. Adrenocortical steroids can suppress MAO activity. This was first suggested by Avakian and Callingham [11], who found an increase in cardiac MAO activity after adrenolectomy which could then be antagonized by corticosteroids.

In the shunted group, weight loss, anemia and general malaise were apparent. The deterioration in their nutritional status became more persistent and distinct especially when these animals developed hepatic encephalopathy. These are the ones that had abnormally low liver and brain MAO activity, pronounced hypertyraminemia, and an abnormal biochemical liver profile.

We deduce from these observations that shunting of the portal blood directly into the vena cava will deprive the liver of essential nutrients (e.g. cofactors and minerals) that are needed for the maintenance of hepatocellular function. Consequently, this could have a devastating effect upon the activity of a series of hepatic oxidative enzymes including MAO.

Mitochondrial MAO contains covalently bound flavin-adenine dinucleotide as a cofactor [12]. Although its role is not known, evidence that nutritional iron is necessary for tissue MAO activity has accumulated from *in vitro* and *in vivo* studies [13–19]. As a result of lowered enzyme V_{max}, the possibility has been suggested that iron plays a role in the synthesis of MAO apoenzyme or active MAO. Consequently, 'iron deficiency' may be an important metabolic abnormality associated with decreased MAO activity seen in dogs with portacaval shunt.

This study also documents a decrease in brain MAO activity in a group of shunted dogs, especially in those that had several episodes of hepatic encephalopathy, as compared to non-encephalopathic or sham-operated dogs. Chronic inhibition of brain protein synthesis after portacaval shunt could result in low MAO activity in this organ. In support of this hypothesis, 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 intravenous infusion of [^{3}H]lysine. The results indicated a 50 per cent drop in the incorporation of [^{3}H]lysine into forebrain protein of shunted rats, compared to sham or control rats.

It is probable that the depletion in brain MAO activity observed in dogs with hepatic encephalopathy may have serious consequences. It could result in increased accumulation of false neurochemical transmitters (e.g. octopamine) in various brain regions, which in turn may influence the function of adrenergic and noradrenergic neurons. Recently, Dodsworth *et al.* [21], Bloch *et al.* [22] and James *et al.* [23] have shown octopamine to be increased in the brain of rats and pigs with experimental hepatic coma. Additionally, a concomitant deficiency of brain norepinephrine was demonstrated. Comparable results were obtained in post-mortem brains of patients with Reye's syndrome [24].

In summary, defective oxidative deamination of amine function is an attractive way to explain many of the pathophysiologic events observed in portacaval anastomosis in dogs. Such defects could explain the hypertyraminemia and encephalopathy that have been reported in both patients and experimental animals with liver disease.

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