Portosystemic shunts (PSS) are abnormal communications between the portal venous system and the systemic circulation. Single intra- or extrahepatic PSS are a common congenital abnormality in dogs.1-17 Multiple extrahepatic PSS can also occur in young dogs as congenital abnormalities or secondary to portal hypertension.18,19 Hepatic encephalopathy (HE) is a spectrum of neurologic abnormalities found in animals and humans associated with advanced liver disease.20 Neurologic signs compatible with HE are found in young dogs with PSS.19 Despite extensive investigation in experimental animal models and affected humans, the pathogenesis of HE has been incompletely elucidated.20-22 Multiple abnormalities in CNS metabolism, the monoamine and amino acid neurotransmitter systems, and endogenous benzodiazepines and their receptors have been implicated in this complex pathophysiologic state.20-22 Many of these abnormalities have been linked to the effects of ammonia on the CNS.21 Ammonia is associated with an increase in CSF tryptophan (TRP) concentrations, either through direct stimulation of neutral amino acid transport across the blood brain barrier23,24 or through an increase in production of glutamine (GLN) in the CNS.21,25 The brain has no urea cycle; consequently, ammonia in the CNS is removed by transamination of glutamate into GLN.21 Glutamine shares an antiport transport mechanism with TRP and other large, nonpolar amino acids across the blood-brain barrier (Fig 1).25 Few studies26-28 have used dogs with spontaneous PSS to investigate the neurobiologic characteristics of HE. We hypothesized that dogs with PSS have CNS biochemical changes similar to those described for experimental models of HE and that increases in CSF GLN concentrations might be associated with an increase in

Objective—To determine whether glutamine (GLN), tryptophan (TRP), and tryptophan metabolite concentrations are higher in cerebrospinal fluid (CSF) dogs with naturally occurring portosystemic shunts (PSS), compared with control dogs.

Animals—11 dogs with confirmed PSS and 12 control dogs fed low- and high-protein diets.

Procedure—Cerebrospinal fluid and blood samples were collected from all dogs. Serum and CSF concentrations of GLN, alanine, serine, TRP, 5-hydroxyindoleacetic acid (5-HIAA), and quinolinic acid (QUIN) were measured.

Results—Cerebrospinal fluid concentrations of GLN, TRP, and 5-HIAA were significantly higher in PSS dogs, compared with control dogs fed high- or low-protein diets. Cerebrospinal fluid QUIN concentration was significantly higher in PSS dogs, compared with control dogs fed the low-protein diet. Serum QUIN concentration was significantly lower in PSS dogs, compared with control dogs fed either high- or low-protein diets.

Conclusions and Clinical Relevance—An increase in CNS GLN concentration is associated with high CSF concentrations of TRP and TRP metabolites in dogs with PSS. High CSF 5-HIAA concentrations indicate an increased flux of TRP through the CNS serotonin metabolic pathway, whereas high CSF QUIN concentrations indicate an increased metabolism of TRP through the indolamine-2,3-dioxygenase pathway. The high CSF QUIN concentrations in the face of low serum QUIN concentrations in dogs with PSS indicates that QUIN production from TRP is occurring in the CNS. High concentrations of QUIN and other TRP metabolites in the CNS may contribute to neurologic abnormalities found in dogs with PSS and hepatic encephalopathy. (Am J Vet Res 2002;63:1167–1171)
Materials and Methods

Animals—Blood and CSF (cisternal puncture) samples were collected immediately prior to surgery from 11 dogs that were admitted to the Veterinary Hospital, University of Pennsylvania with signs compatible with PSS and HE (PSS dogs). Only a blood sample was collected from a twelfth PSS dog. Samples were taken immediately after the dog was anesthetized with either propofol (3 to 5 mg/kg, IV) or isoflurane in oxygen delivered via a cuffed endotracheal tube. Twelve Beagle dogs housed at an off-site facility not affiliated with the University of Pennsylvania were used as control dogs. Six dogs were fed a low-protein diet (14.8% protein; 430 Kcal/100 g metabolizable energy) ad libitum for 7 days then fed a high-protein diet (32.3% protein; 420 Kcal/100 g metabolizable energy) ad libitum for 7 days. The other 6 dogs were fed the high-protein diet initially, followed by the low-protein diet. At the end of the seventh day of each diet, food was withheld. The next morning, the dogs were anesthetized with thiopental sodium (5 to 10 mg/kg, given as a boluses to effect, IV), and CSF (cisternal puncture) and blood samples were collected. Serum and CSF samples were immediately frozen at -80°C and stored until assayed. The University Institutional Animal Care and Use Committee approved protocols for sample collection from the PSS and control dogs.

Serum and CSF tryptophan and amino acid measurements—Tryptophan concentrations were measured by use of an adaptation of a high-pressure liquid chromatography method. Fluorometric detection was obtained by use of a fluorometer set at 293 nm excitations with a 320 nm emission cutoff filter. Tryptophan was isocratically eluted from a C-18 reverse-phase column by use of a mobile phase that consisted of sodium acetate buffer (50 mM, pH 3.0) with di-sodium EDTA (5 μM), adjusted to between 10 and 15% methanol by use of 5-hydroxytryptophan as an internal standard and quinolinic acid (QUIN) as an internal standard and heptane sulfonate (2 mM) and 0.1% acetic acid.

Amino acid concentrations were measured by reverse-phase high-pressure liquid chromatography analysis with o-phthalaldehyde precolumn derivatization. Samples were precipitated with perchloric acid (0.4M final concentration) containing L-α-aminoadipate as an internal standard and centrifuged. The supernatant was adjusted to pH 8.3 to 9.0 with KHCO₃ and centrifuged again. Amino acids were derived from samples automatically by the use of o-phthalaldehyde and an automated sample processor was used to inject the derived amino acids into a C-18 column. Amino acids were eluted with a stepped linear gradient by use of sodium acetate buffer (50 mM, pH 6.0) with either 20 or 80% methanol and were detected with a fluorescence detector with a xenon mercury lamp set at 316 nm excitation and a 415 to 515 medium pass emission filter. Concentrations of QUIN were measured by electron-capture negative chemical ionization mass spectrometry and gas chromatography.

Statistical analyses—Data were analyzed by use of a commercial software package. Data were tested for normal distribution by use of skewness-kurtosis tests. Amino acid concentrations were then compared by use of the Kruskal-Wallis test for nonparametric data. Pairwise comparisons of amino acid concentrations were then performed by use of the Wilcoxon rank-sum test. The Pearson correlation method was used to test the association between serum and CSF QUIN concentrations. Differences were considered significant at a value of P < 0.05.

Results

Cerebrospinal fluid was obtained from 11 PSS dogs. Affected breeds included Yorkshire Terrier (n = 3), Jack Russell Terrier (2), Doberman Pinscher (1), Beagle (1), Miniature Schnauzer (1), Greyhound (1), Afghan Hound (1), and mixed-breed dogs (1). Ages of affected dogs ranged from 3 to 39 months (median, 8 months). There were 3 sexually intact females, 2 spayed females, 4 sexually intact males, and 2 castrated males. In addition, a blood sample, but not a CSF sample, was obtained from a 3-month-old sexually intact female Saint Bernard with an intrahepatic PSS.

Five PSS dogs were fed a low-protein diet for 1 week to 8 months (median, 2 weeks) prior to surgery. Three PSS dogs fed the low-protein diet and 1 PSS dog fed a regular diet received lactulose prior to surgery. Two PSS dogs fed the low-protein diet received antimicrobials (metronidazole, 1 dog; amoxicillin, 1 dog) for 2 weeks before surgery. Baseline plasma ammonia concentrations obtained 1 to 7 days before surgery ranged from 87 to 406 μmol/L (median, 291 μmol/L). Seven PSS dogs were anesthetized with propofol IV, and 4 dogs were anesthetized with isoflurane in oxygen by facemask. At surgery, 1 dog had a single, intrahepatic PSS, 7 dogs had a single extrahepatic PSS, and 3 dogs had multiple PSS.

Concentrations of GLN, TRP, and HIAA were all significantly higher in CSF of PSS dogs, compared with control dogs fed either low- or high-protein diets (P < 0.001; P < 0.001; P = 0.004; P = 0.002, and P < 0.001, P = 0.005 for GLN, TRP, and HIAA, respectively, Table 1). Concentrations of QUIN were significantly (P = 0.03) higher in CSF of PSS dogs, compared with control dogs fed the low-protein diet; concentrations of QUIN in CSF of PSS dogs was not significantly (P = 0.056) different, compared with control dogs fed the high-protein diet. Cerebrospinal fluid concentrations of alanine and serine were not significantly different between PSS and control dogs. Serum GLN, TRP, and HIAA concentrations were not significantly different between PSS and control dogs. Serum QUIN concentrations were significantly lower in PSS dogs, compared with controls dogs fed either high- (P < 0.001) or low- (P < 0.001) protein diets. There was no correlation between serum and CSF QUIN concentrations (correlation coefficient = -0.1517; P = 0.676).
the uptake of nonpolar amino acids, including TRP, by
described for rats with portacaval anastomosis42,43 and
Increases in brain HIAA concentrations have also been
timations in PSS dogs of our study indicate an increase
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reported in another study of dogs with spontaneous
chronous PSS. Increases in CSF GLN concentrations in
humans with cirrhosis,40 and dogs with experimentally
found in the CNS of portocaval-shunted rats,38,39

Discussion

Hepatic encephalopathy is common in dogs with PSS. More than 90% of affected dogs have neurologic signs of HE.1 The pathogenesis of HE is complex and incompletely understood but is linked in part to accumulation of excess ammonia in the CNS.10,20,34 In the rat, ammonia in the CNS is converted to GLN in astro-
cyes by the astroglia-specific enzyme GLN syn-
thetase.35 A high CSF concentration of GLN results in
an increase uptake of TRP and other large nonpolar
amino acids across the blood-brain barrier via an
antiport transport mechanism (Fig 1).23-25 Methionine
sulfoximine, an inhibitor of GLN synthetase, prevents
the uptake of nonpolar amino acids, including TRP, by
the CNS36,37 and ameliorates the signs of HE in porto-
caval-shunted rats.37

In our study, dogs with PSS had significantly high-
er CSF concentrations of GLN and TRP than control
dogs fed either a high- or low-protein diet. These find-
ings indicate that biochemical changes, similar to those
found in the CNS of portocaval-shunted rats,35,36
humans with cirrhosis,36 and dogs with experimentally
created end-to-side PSS,1 occur in dogs with sponta-
neous PSS. Increases in CSF GLN concentrations in
our study were somewhat less striking than those
reported in another study of dogs with spontaneous
PSS, although in the latter study, units for amino acid
measurements were not clearly stated, making direct
comparisons difficult.29

To our knowledge, the consequences of high CSF
TRP concentrations have not been previously investi-
gated in dogs with spontaneous PSS. Tryptophan is the
precursor of serotonin, and TRP hydroxylation, the
rate-limiting step in CNS serotonin synthesis, is not
saturated at usual CSF TRP concentrations26; so in theory,
an increase in CSF TRP concentration could increase
serotonin synthesis. High CSF HIAA concen-
trations in PSS dogs of our study indicate an increase
of TRP through the CNS serotonin pathway.
Increases in brain HIAA concentrations have also been
described for rats with portacaval anastomosis44,45 and
in CSF of humans with cirrhosis and hepatic coma.44

In our study, the finding of high CSF TRP and 5-
HIAA concentrations in PSS dogs must be interpreted
cautiously. One might expect an increase in CSF TRP

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control dogs</th>
<th>Dogs with PSS</th>
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<tbody>
<tr>
<td></td>
<td>High-protein diet (n = 12)</td>
<td>Low-protein diet (n = 12)</td>
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<tr>
<td>TRP (µmol/L)</td>
<td>3.85 (1.25-5.31)</td>
<td>3.93 (2.25-6.6)</td>
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<tr>
<td>GLN (µmol/L)</td>
<td>431 (253-1110)</td>
<td>500 (116-753)</td>
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<tr>
<td>ALA (µmol/L)</td>
<td>39.15 (25.9-63)</td>
<td>44.2 (27-166)</td>
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<tr>
<td>SER (µmol/L)</td>
<td>88.7 (71.2-103)</td>
<td>89.6 (1-148)</td>
</tr>
<tr>
<td>HIAA (nM/L)</td>
<td>128.5 (91.7-187)</td>
<td>119.5 (40.7-168)</td>
</tr>
<tr>
<td>QUIN (nM/L)</td>
<td>49.6 (28.5-81.5)</td>
<td>46.3 (16-88)</td>
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*Significantly (P < 0.01) different from control dogs fed either the high- or low-protein diet.‡Significantly (P < 0.05) different from control dogs fed the
low-protein diet.

PSS = Portosystemic shunts. TRP = Tryptophan. GLN = Glutamine. ALA = Alanine. SER = Serine. HIAA = 5-hydroxyindoleacetic acid. QUIN = Quinolinic acid.

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tion between serum and CSF QUIN concentrations in PSS dogs.

Quinolinate is an agonist of the N-methyl-D-aspartate subclass of excitatory amino acid receptors and has been proposed as a neurotoxin in hyperammonemic states. Activation of the N-methyl-D-aspartate receptors by QUIN can cause irreversible neuronal damage. The developing brain is more sensitive to N-methyl-D-aspartate receptor activation. Quinolinic acid can also cause neuronal necrosis through free radical formation that occurs independently of N-methyl-D-aspartate receptor activation. These findings have interesting implications for young dogs with congenital PSS. Although many dogs with PSS have clinical signs of CNS depression, some have seizure-like activity at the time of hospital admission. In addition, histologic lesions are sometimes found in the CNS of PSS dogs. Findings in a histologic study revealed polymicrocavitation of the brainstem, cerebellar nuclei, and sometimes polymicrocavitation at the border of the white and gray matter in the cerebrum cortex, in addition to an increase in numbers of protoplasmic astrocytes (Alzheimer type II astrocytes). In our study, we did not attempt to provide a link between QUIN and seizures or CNS pathologic changes; CSF QUIN concentrations in PSS dogs were generally below those sufficient to induce neuronal damage in vitro (250 to 990 nM/L). However, our findings of high CSF QUIN concentrations and the previous finding of high CSF glutamate concentrations in dogs with PSS raise the intriguing possibility that overstimulation of the N-methyl-D-aspartate class of receptors by QUIN or glutamate or free radical-mediated damage by QUIN are mechanisms for CNS pathologic changes found in dogs with PSS and HE.

Neurologic dysfunction and seizure-like activity also occur in a small percentage of dogs after surgery to correct PSS, regardless of the method of shunt attenuation used. Dogs euthanized because of uncontrollable seizures generally have more severe CNS lesions than nonsurviving dogs with PSS at postmortem examination. It is not clear whether the CNS lesions represent the cause or the pathologic consequence of the seizures. The cause of postoperative neurologic dysfunction, seizures, and CNS pathologic changes in dogs with PSS is not clear. Our pet owners did not consent to CSF sample collection from PSS dogs after surgery, so we could not investigate the effect of partial or complete shunt attenuation on CSF concentrations of TRP and its metabolites. If future studies implicates QUIN as a cause of seizures or neuronal necrosis in PSS dogs, inhibitors of QUIN synthesis or N-methyl-D-aspartate receptor blocking agents may enhance treatment for these dogs.

References


