Comparison of concentrations of γ-aminobutyric acid and glutamate in cerebrospinal fluid of dogs with idiopathic epilepsy with and without seizure-related magnetic resonance imaging hyperintense areas in the limbic system

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Objective—To investigate differences in CSF concentrations of excitatory and inhibitory neurotransmitters in dogs with and without T2-weighted (T2W) MRI hyperintense areas in the limbic system.

Sample—Archived CSF samples and stored brain MRI images of 5 healthy research dogs (group 1), 8 dogs with idiopathic epilepsy (IE) with no abnormal MRI findings (group 2), and 4 dogs with IE with hyperintense areas in the limbic system detected by means of T2W MRI (group 3).

Procedures—Archived CSF samples and stored MRI images obtained from all dogs were evaluated. Dogs in groups 2 and 3 were matched on the basis of age and breed. High-performance liquid chromatography was used to evaluate glutamate and γ-aminobutyric acid (GABA) concentrations in CSF samples.

Results—Glutamate concentrations were higher in CSF of both groups of dogs with IE than in healthy dogs. However, glutamate concentrations in CSF were not significantly higher in dogs with IE and with hyperintense areas than in dogs with IE but no abnormal MRI findings. Concentrations of GABA in CSF were higher in group 3 than in group 2 and in group 2 than in group 1.

Conclusions and Clinical Relevance—No significant difference was evident between glutamate concentrations in CSF of dogs with IE and with and without hyperintense areas detected by means of T2W MRI. However, glutamate concentrations typically were higher in CSF of dogs with IE and MRI hyperintense areas. Future studies with larger sample sizes should be conducted to confirm this finding and to determine the clinical importance of high glutamate concentrations in CSF of dogs with IE. (Am J Vet Res 2013;74:1118–1125)

Abbreviations

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<tr>
<th>FLAIR</th>
<th>Fluid-attenuated inversion recovery</th>
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<tr>
<td>GABA</td>
<td>γ-Aminobutyric acid</td>
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<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<td>IE</td>
<td>Idiopathic epilepsy</td>
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<td>T1W</td>
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<td>T2W</td>
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Idiopathic epilepsy is a common cause of seizures in dogs and shares characteristics with IE in humans. The index of suspicion for IE is highest in purebred dogs with no abnormal findings on neurologic examination; confirmation of the diagnosis of IE necessitates exclusion of other causes of seizures. With the increasing availability of cross-sectional imaging in veterinary neurology, the use of MRI to exclude intracranial causes of seizures is now also part of the standard for diagnostic testing. Although the precise pathophysiologic processes for IE in dogs remain unclear, an imbalance of excitatory and inhibitory neurotransmitters is likely involved. Among the various neurotransmitters, glutamate is the predominant excitatory neurotransmitter and GABA is the predominant inhibitory neurotransmitter. Seizures experimentally induced by intraparenchymal infusion of kainic acid in dogs and pilocarpine in rodents have been reported to lead to increased glutamate concentrations and decreased GABA concentrations in the CSF before and shortly after seizures. Conversely, increases in extracellular GABA concentrations have been reported after experimentally induced seizures in rats and in humans with spontaneous epilepsy. Evaluation of neurotransmitter concentrations in the CSF of dogs with IE revealed increased concent-
tations of excitatory glutamate and decreased concentrations of inhibitory GABA.\textsuperscript{13-16} These studies generally identify an increase in glutamate concentration in the CSF of subjects with experimentally induced and spontaneous IE, but the findings regarding GABA concentrations in the CSF are less consistent.

Glutamate causes activation of N-methyl-D-aspartate receptors, which opens calcium channels and increases intracellular free calcium concentration. With excessive increases in the glutamate concentration, the increase in intracellular free calcium concentration within neurons activates metabolic pathways that ultimately trigger cell death (termed excitotoxicosis).\textsuperscript{17} Several areas of the brain, including the hippocampus, amygdala, and thalamus, have a selectively greater susceptibility to excessive glutamate concentrations.\textsuperscript{16-18} In particular, the hippocampus is especially susceptible to excitotoxicosis resulting in neuronal loss in the hippocampal CA1 and CA3 pyramidal cells and dentate hilar neurons.\textsuperscript{19,20} In humans, selective neuronal susceptibility related to excitotoxicosis in the limbic system has been observed as bilateral hyperintense areas on images obtained with T2W MRI.\textsuperscript{18,21-23} Similar hyperintense areas involving the limbic system have been observed with T2W MRI in some dogs with IE.\textsuperscript{24}

The purpose of the study reported here was to examine the relationship between CSF concentrations of the neurotransmitters glutamate and GABA and the MRI appearance of abnormalities in the limbic system of dogs with IE. We hypothesized that hyperintense areas in the limbic system detected with T2W MRI would be associated with a higher glutamate concentration in postictal dogs with IE, compared with the glutamate concentration in postictal dogs with IE with no abnormal MRI findings.

**Materials and Methods**

**Sample**—The study was conducted with archived CSF samples and stored MRI images. Samples and images had been obtained from healthy research dogs as well as client-owned dogs that were brought to the University of Georgia Veterinary Teaching Hospital Neurology Service for evaluation of seizures.

**Healthy research dogs**—Samples of CSF obtained from the cerebellomedullary cistern and brain MRI images had been obtained from 5 healthy research dogs during a previous, unpublished study conducted by our research group. All protocols for procedures on these research dogs were approved by the University of Georgia Institutional Animal Care and Use Committee. Archived CSF and stored MRI images were retrieved, and these clinically normal dogs served as a control group (group 1).

**Client-owned dogs**—Client-owned dogs with IE were retrospectively identified through a search of medical records. Diagnosis of IE had been made on the basis of no abnormal findings on neurologic examination, with a normal interictal state (including normal mental state and behavior); results of a CBC, serum biochemical analysis, and urinalysis within respective reference ranges (except for dogs receiving phenobarbital treatment, which were allowed to have increases in serum alkaline phosphatase activity\textsuperscript{25}); results of CSF analysis within anticipated limits (nucleated cell count < 5 cells/µL, predominance of mononuclear cells, and protein content < 25 mg/dL\textsuperscript{26}); no history or clinicopathologic data that indicated exposure to a toxin; no abnormal results on any additional tests performed at the discretion of the attending clinician; and an MRI of the brain, with results excluding primary intracranial disease. An additional inclusion criterion for this study was the availability of stored MRI images and archived CSF collected at the time of diagnostic investigation. All diagnostic testing had been performed on client-owned dogs under the guidance of attending clinicians and with consent of the owners.

Dogs with IE were eligible for inclusion in 2 groups (groups 2 and 3). Dogs with no abnormal MRI findings were eligible for inclusion in group 2, and dogs with MRI abnormalities consisting of hyperintense areas involving structures of the limbic region (frontal lobes of the cerebral cortex, pyriform lobes, cingulate gyrus, parahippocampal gyrus, septal area, amygdala, and hippocampus)\textsuperscript{27} detected during T2W MRI were eligible for inclusion in group 3. To qualify, lesions must have remained hyperintense on T2W FLAIR and T2*-weighted gradient echo images, must have been isointense to hypointense on T1W FLAIR, and must have had no enhancement after IV administration of 0.1 mmol of gadopentetate dimeglumine/kg. Four dogs with hyperintense areas in the limbic region of the brain were identified and enrolled in group 3. For each dog in group 3, the age at the time of MRI and CSF collection was determined, and a dog within 6 months of that age was sought from the available dogs with IE with no abnormal MRI findings for inclusion in group 2. Three age-matched dogs were enrolled in group 2; a fourth randomly selected dog that was not matched on the basis of age was also enrolled in group 2. The breed of each dog in group 3 was determined, and a dog of the same breed or a genetically closely related breed\textsuperscript{28} was sought from the available dogs with IE with no abnormal MRI findings. Three breed-matched dogs were enrolled in group 2; a fourth randomly selected dog was also enrolled in group 2. Thus, there were 8 dogs in group 2.

**Data collection from medical records**—Information for dogs in groups 2 and 3 was collected from the medical records. Data collected for all dogs included age at first seizure, frequency of seizures, owner description of seizures (including typical duration of seizure and presence or absence of cluster seizures, status epilepticus, focal seizures, and postictal clinical signs), chronic (> 1 week) and acute (< 1 week) administration of anticonvulsant treatments, age at time of MRI and CSF collection, interval between last seizure and CSF collection, interval between last seizure and MRI, and results of CSF analysis. For this study, status epilepticus was defined as a single seizure lasting > 5 minutes or 2 or more seizures with an incomplete recovery between seizures. Cluster seizures were defined as > 2 seizures within 24 hours in which a dog had a complete recovery between seizures. Clinicopathologic results obtained during medical investigations (including results of a CBC, serum biochemical analysis, and urinalysis; determination of serum bile acid concentrations [when performed]; and diagnostic imaging findings)
were recorded. Interval for the storage of CSF between the time of the clinical investigation and neurotransmitter analysis was calculated for all dogs.

**MRI examination**—All dogs were anesthetized for imaging with a 3.0-T MRI unit with a single-channel extremity coil. All dogs received hydromorphone (0.04 to 0.05 mg/kg, IV) or butorphanol tartrate (0.2 mg/kg, IV) and midazolam (0.2 mg/kg, IV) or diazepam (0.2 mg/kg, IV) as premedications, except 1 dog in group 2 that did not receive a benzodiazepine. Anticholinergic medications (atropine or glycopyrrolate) were administered as needed, depending on heart rate and blood pressure. Anesthesia was induced with propofol (1 to 4 mg/kg, IV, to effect) and maintained with isoflurane.

Dogs were positioned in sternal recumbency. Images were obtained in the transverse and sagittal planes for T2W, T2W FLAIR, T1W FLAIR, and T2*-weighted gradient echo sequences. Additional T1W FLAIR sequences were obtained after IV administration of gadopentetate dimeglumine (0.1 mmol/kg). Archived images were reviewed by board-certified veterinary neurologists (MK and SRP) to establish a normal appearance of the anatomy (groups 1 and 2) or presence of T2W hyperintense areas in the limbic region (group 3); image review was performed with a commercially available Digital Imaging and Communications in Medicine (ie, DICOM) viewer.

**CSF analysis**—The CSF samples were collected from the cerebellomedullary cistern after completion of the MRI. One aliquot of CSF was analyzed within 30 minutes after collection to determine the nucleated cell count, differential cell count by use of a cytocentrifugation technique, and protein concentration. A second aliquot of CSF was placed into a freezer at –80°C within 15 minutes after collection and stored at that temperature until used for HPLC evaluation of neurotransmitter concentrations. The interval between collection and HPLC analysis was calculated for each sample.

Concentrations of neurotransmitters in the CSF were measured by means of HPLC electrochemical detection. Aliquots (20 µL) of CSF were used for the analysis of glutamate and GABA concentrations. Immediately prior to analysis, samples were thawed and derivatized with an autosampler in accordance with the manufacturer’s instructions. Each 20-µL sample was mixed 4 times with 15 µL of the derivatizing agent and incubated for 1 minute. The sample was then injected onto a 150 × 4.6-mm HPLC column with an in-line filter. A constant flow (0.70 mL/min) of mobile phase (0.1 mM Na2HPO4, 5.25% acetonitrile, and 29.75% methanol [pH 6.7]) was maintained by a high-pressure pump. Electrochemical detection potential was set at 150 and 550 mV. The positions and heights of the peaks of glutamate and GABA were compared with reference standard solutions diluted in the buffer used for the microdialysis procedure (artificial CSF). Peak areas were quantified by proprietary software. The coefficient of variation was ≤ 0.34 for glutamate concentrations and ≤ 0.10 for GABA concentrations. The limit of detection was 0.05 nmol/mL for glutamate and 0.15 nmol/mL for GABA.

**Statistical analysis**—Nonparametric results were reported as median and range. A Mann-Whitney-Wilcoxon test was used for pairwise comparison of neurotransmitter concentrations in CSF among groups of dogs. Unequal-variance t tests were used to compare neurotransmitter concentrations in dogs with and without particular characteristics of seizures. Pearson correlation analysis was used to assess relationships between typical duration of a seizure and neurotransmitter concentrations and between the time elapsed since the last seizure and neurotransmitter concentrations. Significance was set at values of P < 0.05. Statistical calculations were performed with publicly available software.

**Results**

**Dogs**—All dogs in group 1 were Beagles between 2 and 4 years of age. There were 3 males and 2 females, and none had abnormal findings on MRI examination of the brain. All 8 dogs of group 2 had no abnormal findings on MRI examination, and all 4 dogs of group...
3 had hyperintense areas in the limbic system observed with T2W MRI (Figure 1). The 8 dogs in group 2 (5 castrated males, 1 sexually intact male, and 2 spayed females) were 3 to 6 years old; there was 1 Cocker Spaniel, 1 English Bulldog, 1 Lhasa Apso, 1 Siberian Husky, 1 Silky Terrier, 1 West Highland White Terrier, 1 Labrador Retriever–crossbreed dog, and 1 mixed-breed dog. The 4 dogs in group 3 (1 castrated male, 1 sexually intact male, and 2 spayed females) were 2 to 11 years old; there was 1 Australian Shepherd, 1 Boxer, 1 Golden Retriever, and 1 Lhasa Apso.

Characterization of seizures—For the dogs of group 2, typical seizure duration reported by the owners ranged from 45 seconds to 5 minutes for 6 dogs and was unknown for 2 dogs. Four owners reported cluster seizures, and 1 owner reported focal seizures (all others were generalized seizures); no owners reported episodes of status epilepticus. Five owners reported postictal clinical signs, and 1 owner was not asked about postictal clinical signs.

For the dogs of group 3, typical seizure duration reported by the owners ranged from 1 to 2 minutes for 2 dogs, was described as variable in 1 dog, and was not reported for 1 dog. Three owners reported cluster seizures, 2 owners reported episodes of status epilepticus, and 2 owners reported focal seizures; all others were generalized seizures. Three owners reported postictal clinical signs, and 1 owner was not asked about postictal clinical signs.

Prior to diagnostic evaluation, seizure disorders had been apparent for a median of 4.5 months (range, 1 to 48 months) for dogs of group 2 and 8 months (range, 1 to 36 months) for dogs of group 3. Since the onset of the seizure disorder, median number of recorded seizures was 9.5 (range, 2 to 35) for 6 dogs of group 2 and 18 (range, 10 to 19) for 3 dogs of group 3; the total number of seizures was not recorded for 2 dogs of group 2 and 1 dog of group 3. During the 48-hour interval before MRI and CSF collection, none of the dogs in group 2 had a seizure, whereas 2 of the dogs in group 3 had a seizure (1 dog at 12 hours before diagnostic testing and the second dog at 43 hours before diagnostic testing).

Anticonvulsant treatment—Overall, 7 dogs received anticonvulsant treatment chronically, and some dogs received >1 agent. Two dogs of group 2 and 1 dog of group 3 received a single medication chronically, 1 dog of each group received 2 medications chronically, 1 dog of group 2 received 3 medications chronically, and 1 dog of group 3 received 4 medications chronically. Three dogs of group 2 and 3 dogs of group 3 received phenobarbital chronically. Two dogs of group 2 and 1 dog of group 3 received potassium bromide chronically, and 1 dog of group 2 and 2 dogs of group 3 received levetiracetam chronically. One dog of group 2 received primidone chronically, and 1 dog of group 3 received gabapentin chronically.

Overall, 5 dogs received anticonvulsant treatment acutely. One dog of group 2 and 3 dogs of group 3 each received a single medication acutely, and 1 dog of group 2 received 3 medications acutely. One dog of group 2 and 3 dogs of group 3 received diazepam acutely, 1 dog of each group received potassium bromide acutely, 1 dog of group 2 received phenobarbital acutely, and 1 dog of group 2 received levetiracetam acutely.

CSF collection and storage—For 7 of 8 dogs of group 2, the median interval from the last seizure until CSF collection was 72 hours (range, 48 hours to 3 months); the time of the last seizure before CSF collection was not known for 1 dog in group 2. For the 4 dogs of group 3, the median interval from the last seizure until CSF collection was 48.5 hours (range, 12 to 141 hours). The last observed seizure was within 72 hours of CSF collection in 7 of 12 dogs with IE, including 4 of 8 dogs of group 2 and 3 of 4 dogs of group 3. For 2 dogs of group 2, the interval between the last observed seizure and CSF collection was >7 days (1 and 3 months, respectively).

For all dogs with IE, the interval from CSF collection until HPLC analysis (ie, amount of time the samples were stored at –80°C) ranged from 17 to 64 months. For group 2, the median interval from CSF collection until HPLC analysis was 43 months (range, 21 to 51 months), whereas the median interval for group 3 was 38 months (range, 17 to 64 months).

Glutamate concentration—The glutamate concentration was measurable in all CSF samples, and median values differed between dogs with IE and clinically normal dogs. Median glutamate concentration in the CSF of dogs of group 1 was 1.808 µg/mL (range, 0.165 to 0.296 µg/mL). This was significantly less than the median glutamate concentration for dogs of group 2 (1.014 µg/mL; range, 0.446 to 1.397 µg/mL [P = 0.001]) and group 3 (1.252 µg/mL; range, 0.724 to 1.835 µg/mL [P = 0.016]). There was not a significant (P = 0.368) difference in glutamate concentrations between groups 2 and 3 (Figure 2). Concentrations of glutamate in CSF were not correlated with cluster seizures (P = 0.98), status epilepticus (P = 0.88), focal seizures (P = 0.19), postictal clinical signs (P = 0.63), typical seizure duration reported by the owners (P = 0.77), or duration of CSF storage prior to HPLC analysis (P = 0.59).
Glutamate and GABA are the main excitatory and inhibitory neurotransmitters in the brain, respectively. Therefore, it has been postulated that abnormal glutamate and GABA concentrations play a major role in IE. An MRI of the brain is routinely performed as part of the diagnostic investigation of dogs suspected of having IE. There typically are no abnormal MRI findings in the brain of most dogs with IE, but hyperintense areas in the limbic region of the brain have been identified on T2W images in some dogs with IE, which are similar to the hyperintense areas in the limbic region seen on T2W images obtained from humans with glutamate excitotoxicosis. In dogs with IE with hyperintense areas on T2W images, it is the authors’ experience that there is often a recent history of seizures shortly before the MRI. Similarly, necropsy and microscopic evaluation of the brain of dogs with IE has revealed neuronal necrosis in areas of the limbic system. Experimen-

tally, injection of glutaminergic chemicals (eg, kainic acid) into the amygdala results in hyperintense areas on T2W images. Consequently, we hypothesized that glutamate concentrations are increased in the CSF of dogs with IE in which hyperintense areas are seen on T2W images of the brain. Although the concentrations did not differ significantly, the glutamate concentration in the CSF of dogs with IE and hyperintense areas on T2W images (group 3) typically were higher than that for dogs with IE and no abnormal MRI findings (group 2). Indeed, 2 of the 3 dogs with the highest glutamate concentration in the CSF were dogs with IE and hyperintense areas on T2W images.

An unanticipated result of the present study was the increase in GABA concentrations in the CSF of dogs with IE. In previous studies of dogs with IE, GABA concentrations in the CSF were below those of control dogs. Antithetically, in the present study, the GABA concentrations were significantly higher in the CSF of all dogs with IE, compared with the concentrations in the control dogs, and the GABA concentrations were highest in the CSF of dogs with IE and hyperintense areas on T2W images. Although we did not investigate the existence of a causal relationship between neurotransmitter concentrations and IE or neurotransmitter concentrations and MRI changes in the dogs of the present study, the results may have been related to the release of GABA in response to increased extracellular concentrations of glutamate. Steady-state control of extracellular concentrations of glutamate and GABA is achieved through glutamate and GABA transporters that remove these neurotransmitters from the extracellular space. Extracellular glutamate is removed primarily by glutamate transporters located on astrocytes near the synaptic cleft. Similarly, extracellular GABA concentrations are regulated through several GABA transporters (eg, GAT-1, GAT-2, and GAT-3). GAT-1 transporters are located on neurons, whereas GAT-2 and GAT-3 transporters are located on glial cells. Similar to the mechanism for glutamate transporters, physiologic activation of GABA transporters serves to remove extracellular GABA. However, an increased uptake of glutamate by glial cells causes a reversal of GAT-2 and GAT-3 transporters, which leads to a release of GABA from glial cells into the extracellular space.

It is theorized that this glutamate-GABA exchange mechanism may help limit the pathophysiologic effects of excessive glutamate concentrations. In humans with temporal lobe epilepsy, there is an upregulated expression of GAT-3 in hippocampal astrocytes, which may serve to augment the magnitude of GABA release. Importantly, these small changes in extracellular neurotransmitter concentrations occur at a cellular level and last for only a few minutes. Despite this, it has not been determined whether activation of glutamate-GABA exchange by glial
Another investigation\(^6\) found a decrease in GABA concentration and a return to baseline concentrations at 2 months. The kinetics of glutamate and GABA concentrations in the CSF are complex. One study\(^6\) revealed an increase in glutamate concentration and a decrease in GABA concentration for up to 48 hours during the postictal period, a decrease in concentrations of both neurotransmitters at 72 hours, and a return to baseline concentrations at 2 months. Another investigation\(^6\) found a decrease in GABA concentrations in Labrador Retrievers with IE at 72 hours following a seizure, but there was no difference in GABA concentrations in the CSF at 72 hours after a seizure between dogs with IE of other breeds and clinically normal dogs. In the study reported here, the last observed seizure was within 72 hours before the collection of CSF in most dogs and was ≤ 54 hours before CSF collection in 3 of 4 dogs of group 3. Similar to other investigations and given the wide variations in the interval between seizures and CSF collection coupled with a small sample size, definitive conclusions cannot be reached regarding the influence of the interval from the last known seizure and glutamate and GABA concentrations in the CSF. The ideal postictal window for collection of CSF for measurement of glutamate and GABA concentrations is not known, but it is possible that the shorter interval from the last observed seizure until CSF collection in most dogs in the present study may explain the difference between GABA concentrations for the present study and results of other studies.

The present study had several limitations, including those inherent to a retrospective study with a small sample size. Perhaps with a greater sample size, a significant difference in glutamate concentrations between dogs with IE with and without hyperintense areas on T2W images would be detectable. Furthermore, the study reported here did not identify robust relationships between neurotransmitter concentrations in the CSF and specific seizure characteristics (eg, frequency, severity, and type) and was underpowered to identify more moderate or multifactorial relationships. The impact of such seizure characteristics on neurotransmitter concentrations in the CSF would require further investigation with a larger population.

With regard to inclusion criteria, a diagnosis of IE typically includes dogs with no abnormal findings during neurologic examination combined with the exclusion of other causes of seizures. Signalment, specifically age, also contributes to the diagnosis. In general, the age criterion for onset of seizures in IE is between 1 and 5 years. In the present study, 1 dog was 11 years old at the time of MRI. This is unusual for a dog with IE, but an alternate explanation for seizures was not identified, despite diagnostic testing. Moreover, the dog had no abnormal findings during neurologic examination. Arguably, cryptogenic epilepsy may have been a more appropriate classification for this dog. Ultimately, given our inability to identify an underlying disease process that might have altered neurotransmitter concentrations, this dog was included in the study despite not matching the typical age for onset of seizures.

Given the retrospective nature of the present study, CSF samples were collected in the course of clinical management of patients, with aliquots stored for various periods prior to measurement of neurotransmitter concentrations. Features of initial processing and storage both may have altered the concentration of neurotransmitters. Ideally, CSF should be frozen immediately to ensure there are no in vitro alterations in neurotransmitter concentrations. The interval from collection until freezing and the duration of storage play a role in the measurement of neurotransmitter concentrations. All CSF samples were collected and stored in small aliquots at ~80°C. Although care was taken to expediently freeze small aliquots, the interval from collection until freezing of samples was not standardized, although it is likely all samples were frozen within 15 minutes after collection. As a result of hydrolysis of CSF peptides (eg, homocarnosine) as well as nonenzymatic processes, the GABA concentration in CSF maintained at room temperature (approximately 20°C) increases in as little as 10 minutes and doubles in 2 hours.\(^{39,40}\) At room temperature, the GABA concentration in CSF increases linearly, and there may be additional increases during the process of analysis.\(^{39,41}\) Glutamate concentrations will also increase in samples stored at room temperature.\(^{42,43}\) Because the aliquots of CSF in the present study were probably frozen at ~80°C within 15 minutes after collection, the amount of time that CSF remained at room temperature likely had little influence on neurotransmitter concentrations. However, once CSF is frozen at ~80°C, GABA concentrations remain stable for as long as 25 months and glutamate concentrations remain stable for up to 9 months.\(^{39,42,43}\) In 1 study\(^4\) of dogs with IE, storage of CSF for 3 months prior to analysis had a minimal effect on neurotransmitter concentrations. Consequently, it is possible that there may have been artifactual in vitro increases in GABA or glutamate concentrations in samples that were not frozen quickly, were stored for prolonged periods, or were thawed and not analyzed immediately. Although the range of glutamate concentrations in CSF in previous reports differed widely and may even have varied greatly within a single study, the glutamate concentrations in the CSF of the control dogs of the present study were within the range reported in previous studies.\(^{44,46}\) In the present study, glutamate and GABA concentrations in the CSF of all dogs with IE were greater than those of dogs with IE in previous reports.\(^{5,16}\) However, the glutamate concentrations in the CSF were within the ranges reported for other disease processes.\(^{40,47}\)

Results of the present study should be interpreted cautiously. We were unable to detect a significant relationship between glutamate concentrations and the presence of hyperintense areas on T2W images, although the highest glutamate concentrations were in the CSF of dogs with IE with lesions on MRI. Future studies are needed to clarify the impact of glutamate concentrations in the CSF on hyperintense areas observed on T2W MRI. If glutamate plays a role in the development of these hyperintense areas, therapeutic interventions aimed at reducing release or enhancing extracellular removal of glutamate may help mitigate excitotoxic effects. Moreover, excessive extracellular...
glutamate may provide an explanation for the observed increase in GABA concentrations in the CSF.

References


