The Mechanism of Carbamazepine Aggravation of Absence Seizures

Lige Liu,1 Thomas Zheng,1 Margaret J. Morris, Charlott Wallengren, Alison L. Clarke, Christopher A. Reid, Steven Petrou, and Terence J. O’Brien

Departments of Medicine (L.L., T.Z., C.W., T.J.O.), Surgery (T.J.O.), and Neurology (T.J.O.), University of Melbourne, Royal Melbourne Hospital, Melbourne, Victoria, Australia; Beijing Friendship Hospital, Affiliate of Capital University of Medical Sciences, Beijing, China (L.L.); Department of Physiology and Pharmacology, University of New South Wales, Sydney, Australia (M.J.M.); and Howard Florey Institute of Physiology and Medicine, Melbourne, Victoria, Australia (A.L.C., C.A.R., S.P.)

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ABSTRACT

Carbamazepine (CBZ) aggravates many generalized seizures types, particularly absence seizures, but the mechanisms underlying this are poorly understood. GABA signaling within the reticular nucleus of the thalamus and the ventrobasal complex of the thalamus is critical to the neurophysiology of absence seizures. The hypothesis that CBZ aggravates absence seizures by acting at the VB thalamus via a GABAA receptor-mediated mechanism was investigated in a genetic rat model, generalized absence epilepsy rats from Strasbourg (GAERS). Seizure activity was quantified by a 90-min electroencephalogram recording postdrug injection. Intracerebroventricular injections of CBZ (15 μg in 4 μl) resulted in seizure aggravation versus vehicle treatment, with a mean increase in seizure time of 40%. This indicates that CBZ acts directly, rather than via a metabolite, on the brain to aggravate seizures. Seizure aggravation also occurred following bilateral microinjection of CBZ (0.75 μg in 0.2 μl) into the VB (53%) but not following injection into the Rt (−9%). However, seizure aggravation was blocked when the GABAA receptor antagonist, bicuculline (BIC, 0.04 μg in 0.2 μl), was coinjected with CBZ into the VB. Injection of BIC alone (versus vehicle) into the VB also blocked seizure aggravation following systemic administration of CBZ (15 mg/kg i.p.). In vitro studies in Xenopus oocytes expressing recombinant GABAA receptors demonstrated that CBZ produced a dose-dependent potentiation of the GABA current at a physiological relevant concentration range (1–100 μM). These data demonstrate that CBZ acts at the VB thalamus to aggravate absence seizures in GAERS and that activation of GABAA receptors is critical to this effect.

Aggravation of seizures by antiepileptic drugs (AEDs) is an important clinical problem that is often overlooked in practice (Lerman, 1986; Perucca et al., 1998). The neurobiological mechanisms underlying seizure aggravation are poorly understood. One of the drugs most implicated is carbamazepine (CBZ), a major first line AED for the treatment of focal seizures. In patients with generalized epilepsy syndromes, CBZ commonly causes an increase in a variety of seizure types, including typical and atypical absence seizures, myoclonic, atonic, and tonic seizures, and also on occasions generalized tonic-clonic seizures (Perucca et al., 1998). Of these, the aggravation of absence seizures is the most predictable and, with the availability of good animal models (Snead et al., 1999), the most amenable for mechanistic studies. In agreement with human studies, CBZ has also been demonstrated by our group and others to exacerbate spontaneous absence seizures in well validated rat models, i.e., low-dose pentylentetrazole (McLean et al., 2004) and the generalized absence epilepsy rats from Strasbourg (GAERS) (Marescaux et al., 1984; Micheletti et al., 1985; Wallengren et al., 2005). The primary neuropathological correlate of absence seizures is bursts of highly synchronized rhythmic oscillatory glutamatergic firing between neurons in the first order thalamic relay nuclei and neocortical pyramidal neurons, which are recorded on the scalp EEG as spike and wave discharges (SWDs). This activity is synchronized by GABAergic neurons.
in the reticular nucleus of the thalamus (Rt), with the critical involvement of rhythmically generated low-threshold calcium spikes (Steriade and Deschenes, 1984). Recent evidence in genetic rat models indicates that the site of initiation of the SWDs is the somatosensory cortex (Meeren et al., 2002; Pinault, 2003). However, the ventrobasal (VB) complex of the thalamus, consisting of the ventroposteromedial and ventro-posterolateral nuclei and representing the primary thalamic input into the somatosensory cortex, plays a critical role in the synchronization and maintenance of the seizure discharges. In animal models, SWDs can be obliterated by lesions placed in the VB or the somatosensory cortex, demonstrating the critical involvement of both structures to the generation and maintenance of the seizures (Danobe et al., 1998).

GABAergic mechanisms within the thalamus play a pivotal role in the regulation of rhythmic thalamocortical activity and of absence seizures. Drugs enhancing GABA function in the brain exacerbate both experimental and clinical absence seizures (Hosford and Wang, 1997; Sneed et al., 1999). GABA_A receptor agonists administered systemically consistently exacerbate experimental seizures (Sneed et al., 1999). In contrast, GABA_A agonists injected into the thalamus have variable effects on absences seizures depending on the site of administration. Microinjections of muscimol into the VB enhances the seizures, whereas paradoxically microinjections into the Rt inhibit them (Hosford et al., 1997). The pattern of activation of GABA_A receptors at different brain regions is, therefore, critically important in the regulation of the thalamocortical oscillatory activity and absence seizures. The antiepileptic action of CBZ is believed to primarily occur due to a use-dependent blockage of voltage-gated Na_+ channels (Willow et al., 1985). CBZ has also been shown to interact with several other channels and receptors including GABA receptors, K_+ channels, L-type Ca_2+ channels, and adenosine binding sites in the brain (Gasser et al., 1988; Olpe et al., 1991; Granger et al., 1995; Schirrmacher et al., 1995). Given the crucial role of thalamic GABA_A receptors in the control of the synchronization and desynchronization of thalamocortical circuitry, interaction with these receptors by CBZ is a prime hypothesis for the mechanism of absence seizure aggravation. Previous in vitro studies have demonstrated that CBZ acts as a positive allosteric modulator of GABA_A receptors in cultured cortical neurons (Granger et al., 1995), with the GABA-induced current being reversibly increased by CBZ in single-cell recordings. In this in vivo study, we use the well validated rat model of absence epilepsy, GAERS, to identify the neuroanatomical site within the GABAergic mechanisms within the thalamus play a pivotal role in the regulation of rhythmic thalamocortical activity and of absence seizures. Drugs enhancing GABA function in the brain exacerbate both experimental and clinical absence seizures (Hosford and Wang, 1997; Sneed et al., 1999). GABA_A receptor agonists administered systemically consistently exacerbate experimental seizures (Sneed et al., 1999). In contrast, GABA_A agonists injected into the thalamus have variable effects on absences seizures depending on the site of administration. Microinjections of muscimol into the VB enhance the seizures, whereas paradoxically microinjections into the Rt inhibit them (Hosford et al., 1997). The pattern of activation of GABA_A receptors at different brain regions is, therefore, critically important in the regulation of the thalamocortical oscillatory activity and absence seizures. The antiepileptic action of CBZ is believed to primarily occur due to a use-dependent blockage of voltage-gated Na_+ channels (Willow et al., 1985). CBZ has also been shown to interact with several other channels and receptors including GABA receptors, K_+ channels, L-type Ca_2+ channels, and adenosine binding sites in the brain (Gasser et al., 1988; Olpe et al., 1991; Granger et al., 1995; Schirrmacher et al., 1995). Given the crucial role of thalamic GABA_A receptors in the control of the synchronization and desynchronization of thalamocortical circuitry, interaction with these receptors by CBZ is a prime hypothesis for the mechanism of absence seizure aggravation. Previous in vitro studies have demonstrated that CBZ acts as a positive allosteric modulator of GABA_A receptors in cultured cortical neurons (Granger et al., 1995), with the GABA-induced current being reversibly increased by CBZ in single-cell recordings. In this in vivo study, we use the well validated rat model of absence epilepsy, GAERS, to identify the neuroanatomical site within the thalamocortical circuitry at which CBZ is acting to aggravate seizures and the neuropharmacological mechanisms underlying this effect.

**Materials and Methods**

**Animals.** All experiments were approved by the Animal Ethics Committee, Ludwig Institute for Cancer Research/Department of Surgery, University of Melbourne (Melbourne, VIC, Australia) and were performed in accordance with the guidelines set by the Australian Code of Practice for the Prevention of Cruelty to Animals. Every precaution was taken to minimize stress and the number of animals used in each series of experiments. GAERS, a strain of Wistar rats, were obtained from our breeding colony and used for the studies. Absence-type seizures are observed in 100% of rats by 13 weeks of ages that are accompanied by generalized spike and wave discharges on an EEG recording (Marescaux et al., 1992). As with our previous studies (McLean et al., 2004; Lohman et al., 2005; Wallengren et al., 2005), female rats were used because seizure aggravation is believed to be more prominent in females. Ovariectomy was performed to minimize the effects of circulating sex hormones. Rats weighing between 180 and 210 g and 13 weeks of age at the start of the experiment were kept in standard rat boxes in the Ludwig Institute animal facility with food and water ad libitum.

**Surgeries.** Animals were anesthetized with xylazine (10 mg/kg) and ketamine (75 mg/kg) i.p. Bilateral ovariectomies were performed. Two holes were drilled bilaterally in the frontal bone and two in the parietal bone, approximately 2 mm anterior and 4 mm posterior to bregma, respectively. Four extradural electrodes for EEG recordings were then affixed on the holes. The head of each rat was fixed to a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Bregma was taken as a reference point for placement of the intracerebral catheter(s). For the first experiment, a single i.c.v. catheter was implanted into the right lateral ventricle via a hole drilled 1.5 mm to the right and 1.0 mm posterior to bregma, and a 22-gauge guide cannula and injecting needle (Plastics One Inc., Roanoke, VA) were lowered into the hole to a depth of 3 to 3.5 mm ventral to the dura, until it entered the right lateral ventricle (confirmed by drawback of CSF in the injecting line). For the subsequent experiments, intracerebral microcatheters (26 gauge, Plastics One) were inserted bilaterally into either the VB or Rt, with coordinates based on a previous study from our group that developed and validated a method for accurately and reliably administering small volumes (0.2–0.5 µl) of drugs to these structures in female GAERS of the same age (i.e., VB, 3.0 mm posterior and 2.6 mm lateral from bregma and 5.5 mm ventral from dura; Rt, 3.0 mm posterior and 3.6 mm lateral from bregma and 5.8 mm ventral from dura) (Lohman et al., 2005). Electrodes and cannulae were held in place with dental cement (Vertex-Dental B.V., Zeist, The Netherlands). Bilateral ovariectomies were performed via flank incisions during the same anesthetic. Animals were given the analgesic Rimadyl (4 mg/kg) (Pfizer, Inc., New York, NY) and individually housed postoperatively. A 7-day recovery period was given during which rats were handled every day to habituate the animal to experimental procedures.

To verify the cannulae locations after completion of the experimental procedures, the animals were injected with 0.2 µl of methylene blue while freely moving and then terminally anesthetized (20 mg/kg xylazine and 150 mg/kg ketamine i.p.). The animals were transcardially perfused with 0.1 M phosphate-buffered saline, pH 7.4, followed by 4% paraformaldehyde. The brain was then extracted, postfixed in 4% paraformaldehyde at 4°C for 4 h. After being submerged in a cryoprotecting 20% sucrose solution at 4°C, for 48 h the brain was then snap frozen. Coronal sections (50 µm) were cut on a cryostat. The site and extent of the methylene blue staining in the brain was identified under light microscope by a reviewer who was blinded to the results of the EEG recordings.

**Drugs.** CBZ and bicuculline (BIC) were obtained from Sigma-Aldrich (St. Louis, MO) and dissolved in vehicle containing 40% propylene glycol, 10% ethanol, and 50% saline. This solvent has been well documented in the literature for similar in vivo studies (e.g., Korolkiewicz et al., 1996; Graumlich et al., 1999) and has no adverse effects on seizure activity (Fig. 1).

**Drug Administration and EEG Recording and Analysis.** The studies took place in a well-lit, quiet room with the rats in their home cages. Rats were connected via wires attached to the electrodes by gold crimp pins to a computer running Compumedics EEG acquisition software (Melbourne, Australia). After a 15-min habituation, a 30-min baseline recording was acquired. The drug/vehicle injections were administered over 5 min via a syringe pump. Ninety-minute postinjection EEG recordings were then acquired during which time the animals were able to move freely around their cage with access to food and water. Animals were constantly monitored by an investigator during the recording period to ensure that they did not fall
asleep. The second and subsequent treatment arms were performed at the same time of the day for each animal, with at least a 2-day interval between treatments. The order of the administered of the treatments was randomized for each experiment.

The seizure expression for the 90-min postinjection EEG recording was quantified by an investigator who was blinded to the nature of the drug administered by visual inspection of the EEG. Standard criteria described for adult GAERS were used to classify the seizures, i.e., an SWD burst of amplitude of more than three times baseline, a frequency of 7 to 12 Hz, and a duration of longer than 0.5 s (Marescaux et al., 1992). The start and end of each seizure was determined by manually marking the beginning and end of each SWD on the EEG (Figs. 1 and 5). From this, the total percent time spent in seizure over the 90-min postinjection EEG recording was determined, (percent time in seizure) – the primary outcome variable for comparison of the effect of the treatments on seizure expression. Two secondary outcome variables were also determined: the mean number of seizures occurring per minute and the mean duration of each seizure. These variables were compared between the different treatments arms for each of the experiments.

In Vitro Examination of CBZ Modulation of GABA_A Current. Oocytes from adult female *Xenopus laevis* were prepared as outlined previously (Petrou et al., 1997) and placed in 96-well plates. cRNA-encoding human α1, β3, and γ2L GABA_A receptor subunits was injected (~20–30 ng) into the cytoplasm of stage 5 or 6 oocytes using the Roboocyte Robot (Multi Channel Systems, Reutlingen, Germany) and stored at 18°C for 1 to 2 days prior to experimentation. GABA_A currents were measured with the two-electrode voltage clamp mode of the Roboocyte and were impaled with two glass electrodes containing 1.5 M potassium acetate and 0.5 M KCl and held at a membrane potential of ~80 mV. Oocytes were continually perfused with a ND96 solution (96 mM NaCl, 2 mM KCl, 0.1 mM CaCl₂, and 5 mM HEPES, pH 7.5) using a Gilson 222 XL Liquid Handler and Gilson Minipuls 3 Peristaltic Pump (Gilson Medical Electronics, Middleton, WI). Oocytes were perfused with a 20-s application of bath solution containing GABA (EC20: 6 × 10⁻⁶ M) followed by a 1-min application of bath solution alone in which the current levels returned to baseline levels. This GABA application was repeated three times to determine a baseline response to GABA. This was followed by a 20-s application of solution containing both GABA (EC20: 6 × 10⁻⁶ M) and various concentrations of CBZ (0.1, 1, 10, or 100 μM). The effect of CBZ on the GABA induced current was then expressed as the relative change in EC20 GABA current caused by the addition of CBZ according to the formula (I_GABA + CBZ - I_GABA)/I_GABA and termed the potentiation ratio.

Statistical Analysis. Statistical analysis was performed using the software package Statistica (StatSoft, Inc., Tulsa, OK). Where there were two treatments administered, the matched-pairs Student’s *t* test was used to assess for the level of statistical difference. Where more than two treatments had been administered to each animal, ANOVA for repeated measures was used, with a subsequent planned comparisons analysis to compare two specific treatments. For the comparison of the seizure aggravation following CBZ microinjections into the VB versus Rt, Student’s *t* test was performed. All data were expressed as mean ± S.E., and *p* < 0.05 was considered significant.

## Results

**Carbamazepine Aggravates Seizures in GAERS by Acting Directly on the Brain.** CBZ is metabolized into several active substrates, including CBZ-10,11-epoxide (Eichelbaum et al., 1985), which contribute to its wide range of therapeutic and adverse effects. To determine whether CBZ-aggravated seizures in GAERS by acting directly on the brain (as opposed to via a metabolite after biotransformation in the liver), serial i.c.v. injections of either CBZ or vehicle were administered to female ovariectomized GAERS. Because, to our knowledge, there have not been any previous studies conducted where CBZ has been injected i.c.v. in rodent models of generalized seizures, a preliminary dose-response trial was performed to select a CBZ dose that would produce optimal seizure aggravation without excessive drowsiness in the rats. GAERS received one injection of 4 μl vehicle alone followed by two doses of CBZ (15 and 30 μg) in 4 μl of vehicle, each separated by at least 2 days. The doses were chosen based on a previous pharmacokinetic study where microdialysis was used to determine plasma and cerebrospinal fluid concentrations of CBZ following i.p. injections at anticonvulsant doses in rodent epilepsy models (Graumlich et al., 1999). Both the 15- and 30-μg CBZ i.c.v. doses resulted in increased percent time in seizure for the 90-min EEG recording compared with vehicle alone (mean, 18.5 and 21.3%, respectively, versus 11.5%); however, the
higher dose resulted in excessive sedation, whereas the lower dose resulted in little obvious behavioral effects. Therefore, the 15-μg dose was chosen for the subsequent randomized CBZ versus vehicle study.

Serial i.c.v. injections of either CBZ (15 μg in 4 μl of vehicle) or vehicle, in a randomized order, were administered to nine GAERS and seizure expression compared between the two treatments. The results of this experiment showed significant seizure aggravation following i.c.v. injection of CBZ compared with vehicle treatment (mean percent time in seizure = 16.9 versus 12.0%, p = 0.04, n = 9) (Figs. 1 and 2). There was a nonsignificant trend for the CBZ treatment to result in a greater number of seizures per minute (1.3 versus 1.0, p = 0.19) and a greater mean duration of seizures (8.3 versus 6.9 s, p = 0.09). These results show aggravation of seizures in GAERS following i.c.v. injection of CBZ, which was of similar magnitude to that previously reported with i.p. injections by our group and others (Micheletti et al., 1985; Wallengren et al., 2005) and demonstrate that CBZ acts directly on the brain to aggravate seizures, without requiring initial conversion into a metabolite.

**CBZ Aggravates Seizures in GAERS by Acting at the VB but Not the Rt Thalamic Subregions.** This experiment aimed to determine the anatomical region within the thalamocortical circuitry at which CBZ acts to aggravate absence seizures in GAERS. Here, CBZ or vehicle alone was microinjected into either the VB or Rt to determine whether CBZ acted directly at one or both of these regions to aggravate seizures in GAERS.

Bilateral microinjections of CBZ or vehicle were administered into either the VB (n = 7) or, in a separate cohort of rats into the Rt (n = 7). Each rat received two injections (CBZ and vehicle) in a randomized order separated by at least 2 days. The CBZ was administered (0.75 μg in 0.2 μl) at the same concentration as used in experiment 1, with the volume based on that used in our previous validation study of focal VB or Rt injections (Lohman et al., 2005). Significant seizure aggravation was seen after microinjection of CBZ into the VB (mean increase in percent time in seizure = 53%, p = 0.03, n = 7) compared with vehicle, but not after the Rt injections (mean increase in percent time in seizure = −8%, p = 0.61, n = 7) (Fig. 3). There were also a significantly greater number of seizures per minute following the VB injections of CBZ versus vehicle injections (2.7 versus 1.9, p = 0.03) but not for the Rt injections (1.8 versus 2.0, p = 0.31). There were no significant differences in the mean duration of individual seizures between the CBZ and vehicle arms for either the VB injections (5.2 versus 4.9 s, p = 0.61) or the Rt injections (4.7 versus 5.4 s, p = 0.61).

Following the observation that CBZ aggravates seizures following microinjection into the VB, a dose-response study was performed to further explore this effect. A separate cohort of three rats received serial microinjections of CBZ into the VB (in a random order) at 0.1, 0.4, and 0.75 μg in 0.2 μl of vehicle, as well as vehicle alone. Repeated measures ANOVA showed a significant effect of treatment on percent time in seizure. Vehicle alone did not affect seizure activity (18.3 before and 17.0% after injection). Injection of the two lower doses of CBZ (0.1 and 0.4 μg) increased the percent time in seizures, but this did not attain statistical significance compared with vehicle alone (percent time in seizure, 21.7 ± 3.28, 23.96 ± 1.66, respectively). However, the highest (0.75 μg) dose of CBZ resulted in a significant increase in percent time in seizures compared with vehicle (percent time in seizure, 36.59 ± 4.32, p = 0.005) (Fig. 4).

**Coadministration of a GABA\(_A\) Receptor Antagonist Blocks Seizure Aggravation by VB Injection of CBZ.** This experiment aimed to test the hypothesis that the aggravation of seizures in GAERS by focal injection of CBZ bilaterally into the VB was occurring via a GABA\(_A\) receptor-
mediated mechanism and, therefore, would be blocked by coadministration of the GABA<sub>α</sub> receptor antagonist, BIC. It has previously shown that BIC may induce conformational changes in the GABA<sub>α</sub> receptor (Luddens and Korpi, 1995) and is an allosteric inhibitor for steroids and barbiturates (Gee et al., 1987). Although systemically administered BIC aggravate seizures in GAERS, previous studies have found no significant effect of local administration of BIC into the VB (Danober et al., 1998). Two cohorts of rats were studied, both of which received three sequential injections into the VB bilaterally. Group A (n = 7) was treated with CBZ (0.75 g in 0.2 ml), a mixture of CBZ (0.75 µg) and BIC (0.037 µg), or vehicle alone. Group B received BIC (0.037 µg), a mixture of BIC (0.037 µg) and CBZ (0.75 µg), or vehicle. The dose of BIC was chosen on the basis of previous studies demonstrating effective blockade of GABA<sub>α</sub> receptors following central injections (Sanudo-Pena and Walker, 1997). As shown in Figs. 5 and 6, there was a significant difference between the treatments for group A (p = 0.004, repeated measures ANOVA) but not group B (p = 0.21). Planned comparison analysis again demonstrated a significant increase of the mean percent time in seizure following the CBZ injection but not following BIC or CBZ-BIC. These data demonstrate that BIC acts within the VB to antagonize the aggravation of the seizures by CBZ and, therefore, implicate a GABA<sub>α</sub> receptor-mediated mechanism. They do not exclude the possibility that CBZ was also acting at other sites within the brain to exert this effect.

**Administration of a GABA<sub>α</sub> Receptor Antagonist into the VB Blocks Seizure Aggravation following Systemic Administration of CBZ.** This experiment aimed to investigate whether injection of BIC bilaterally into the VB would block seizure aggravation in GAERS following i.p. injection of CBZ. This would provide strong evidence that GABA<sub>α</sub> receptor activation in the VB is critical to the seizure aggravating effects of systemically administered CBZ in this model. Rats that had been implanted with bilateral VB cannulae were injected centrally with either with BIC (0.037 µg in 0.2 µl of vehicle) or vehicle alone and i.p. either with CBZ (15 mg/kg in 2 ml of vehicle) or vehicle alone. Therefore, the rats received four sequential treatments, in a random order, separated by at least 2 days: vehicle VB, CBZ i.p.; BIC VB, CBZ i.p.; vehicle VB, vehicle i.p.; and BIC VB, CBZ i.p. The dose of CBZ administered i.p. was that which we and others had previously demonstrated to aggravate seizures in GAERS (Marescaux et al., 1984; Micheletti et al., 1985; Wallengren et al., 2005).

Significant seizure aggravation following i.p. injection of CBZ with vehicle injection into the VB was observed, which was completely blocked by the VB injection of BIC (Fig. 7). The VB injection of BIC alone had no significant effect on percent time in seizure. These results indicate that a GABA<sub>α</sub> receptor-mediated mechanism, localized to the VB, is responsible for the aggravation of seizures by CBZ in GAERS.

**In Vitro CBZ Directly Potentiates GABA<sub>α</sub> Current Expressed in Xenopus Oocytes.** To confirm earlier in vitro studies demonstrating CBZ potentiation of GABA<sub>α</sub> current,
we expressed GABA_A receptors (α1β3γ2) in Xenopus oocytes. Typical traces demonstrating the potentiation of GABA currents by 10 and 100 μM CBZ are shown in Fig. 8. CBZ potentiation was clearly apparent and reproducible at 10 and 100 μM as shown. At 1 μM, the potentiation was smaller and could only be reliably determined by averaging multiple responses. Current records were averaged at a range of physiologically relevant CBZ concentrations and plotted (1–100 μM; Fig. 9).

**Discussion**

The results of this study support our hypothesis that CBZ acts to aggravate absence seizures in GAERS by acting at the VB thalamus via a GABA_A receptor-mediated mechanism. GABA_A receptors have been a major focus in absence epilepsy research due to their importance in synchronization and desynchronization of the thalamocortical circuitry. Recent in vivo neurophysiological studies in a phenotypically similar genetic model, the WAG/Rij rat strain, have demonstrated the critical role played by GABA_A receptors in the generation of spontaneous spike and wave discharges (Staak and Pape, 2001).

Previous studies have demonstrated that the GABA_A receptor antagonist, BIC, when injected systemically induces seizures in nonepileptic rats and enhance seizures in GAERS (Vergnes et al., 2000). In our study, microinjection of BIC into the VB thalamus did not affect the seizure activity in GAERS, which is consistent with previous studies (Danober et al., 1998). The contrasting effect seen with the systemic injection of GABA_A receptor antagonists is probably due to differential effects in different brain regions, with microinjections of BIC into the Rt shown to aggravate seizure in GAERS (Aker et al., 2002). Microinjection studies in animal models have also demonstrated contrasting effects of GABA receptor agonists in different subregions of the thalamus. Injection into the VB thalamus of the GABA transaminase inhibitor, γ-vinyl GABA, has been shown to aggravate absence-like seizures in GAERS (Liu et al., 1991). Conversely, injection into the Rt increases intra-Rt inhibition and therefore reduces the inhibitory input into the thalamic relay nuclei, thus resulting in inhibition of SWDs. Likewise, microinjection of the GABA_A receptor agonist muscimol into the VB region of the lh/lh genetic mouse model increased seizures, whereas injection into the Rt has the opposite effect (Hosford and Wang, 1997). Therefore, it is well established that drugs that enhance GABA_A activity within the VB aggravate experimental models of absence seizures. The results of this current study demonstrate that CBZ acts via a similar mechanism, enhancing GABA_A receptor activity in the VB regions of the thalamus to aggravate absence seizures in GAERS.
CBZ has been shown previously to be a positive allosteric modulator of GABA<sub>α</sub> receptors by single-cell recordings in cultured cortical neurons (Granger et al., 1995). In this we have reconfirmed, using neurophysiological recordings in vitro in Xenopus oocytes expressing recombinant GABA<sub>α</sub> receptors, that CBZ has a dose-dependent effect to enhance the response of GABA<sub>α</sub> receptors to the application of GABA (Figs. 8 and 9). Our in vivo data in GAERS suggest that CBZ may also enhance GABA action at physiologically relevant concentrations of CBZ (in values given in parentheses).

Subunit composition of the GABA<sub>α</sub> receptors strongly influences the physiological and pharmacological properties of the receptor. One prominent example is the lack of GABA potentiation effect of clonazepam in the VB compared with the Rt due to major differences in α and β subunit distributions between the two regions. The VB expresses high levels of benzodiazepine-sensitive subunits, particularly γ2 and α3 (Pirker et al., 2000; Browne et al., 2001; Wong and Snead, 2001). In contrast, progesterone, allopregnanolone, and pregnenolone, which are powerful enhancers of GABA<sub>α</sub> receptor-mediated responses (Hawkinson et al., 1996), exacerbate absence seizures in the genetic rodent models when injected into the VB, whereas injection into the Rt had no effect (Budziszewskia et al., 1999; Snejad et al., 1999). This suggests that the mechanism of absence seizure aggravation by these steroids may be via selective activation of a GABA<sub>α</sub> receptor subtype that is differentially expressed in the VB compared with the Rt.

Our current study suggests that CBZ may also be selective for GABA<sub>α</sub> receptor subunits differentially expressed within the VB compared with the Rt. CBZ has also been shown in HEK 293 cells transfected with α1β2γ2 subunits to potentiate GABA-induced current but not the other subunit combinations tested (α3β2γ2 or α5β2γ2) (Granger et al., 1995). This direct effect of CBZ on GABA<sub>α</sub> receptors to enhance GABA action at physiologically relevant concentrations has been confirmed by the results of our studies in vitro in Xenopus oocytes (Figs. 8 and 9). The α1β2γ2 subunit combination is expressed in the VB thalamus but not at all in the Rt (Pirker et al., 2000; Browne et al., 2001; Wong and Snead, 2001). It is also possible that other subunits that show increased expression in the VB compared with the Rt, such as
α4 and δ subunits, may be even more important in mediating the differential effect of CBZ on GABA<sub>A</sub> receptors; this will be investigated in future studies.

Prior to extrapolating the results of a pharmacological study in an animal model to the human situation, it is important to have an understanding of the relevance of the doses examined relative to those used in clinical practice. The dose of CBZ administered i.p. (15 mg/kg) has been shown to be anticonvulsant in many models of convulsive and limbic seizures (Graumlich et al., 1999) but also aggravate absence-like seizures in rats (Marescaux et al., 1984; Micheletti et al., 1985; Wallengren et al., 2005) without causing significant sedation or other adverse behavioral effects. In addition, the serum levels obtained by following the administration of this dose to rats (Graumlich et al., 1999) are within the ranges seen in human epilepsy patients treated with CBZ (Rambeck et al., 2006). The i.c.v. doses chosen (15 and 30 µg in 4 µl of vehicle) were based on a previous pharmacokinetic study where microdialysis was used to determine plasma and cerebrospinal fluid concentrations of CBZ following i.p. injections at anticonvulsant doses in rodent epilepsy models (Graumich et al., 1999). The same concentration of CBZ was used for the VB microinjection but at a lower volume (0.2 µl). The results of the study of Granger et al. (1995) and our new data from *Xenopus* oocytes expressing GABA<sub>A</sub> receptors indicate that CBZ enhances GABA<sub>A</sub> receptor responses at physiologically relevant concentrations (1–100 µM), within the range measured in CSF following the injection of 12 mg/kg CBZ to rats (i.e., 5 µM) (Graumlich et al., 1999). Likewise, human studies have demonstrated CSF concentrations of CBZ in patients with chronic epilepsy of 7 to 14 µM and brain concentrations of 38 to 69 µM (Rambeck et al., 2006). The amplitude of potentiation of GABA<sub>A</sub>-mediated responses by CBZ was >30%, which is comparable with those obtained by partial benzodiazepine agonists and therefore likely to have an impact on function (Granger et al., 1995).

In summary, the results of this study have localized the VB as the specific region of the thalamus at which CBZ acts to aggravate absence seizures in a genetic rat model. Bilateral microinjections of BIC demonstrated that activation of GABA<sub>A</sub> receptors is critical to this action. We cannot exclude the possibility that CBZ is acting on a distinct target and that the BIC blockade of the GABA<sub>A</sub> receptors inhibits seizure aggravation because these receptors are involved downstream in the expression of the phenomenon. However, the weight of converging evidence from the in vivo and in vitro studies would make this explanation seem less likely. A better understanding of the cellular mechanisms of underlying seizure aggravation will aid an improved clinical selection of AEDs for individual patients and guide more targeted drug design to incorporate the beneficial antiepileptic action without the seizure aggravating effects. The demonstration that CBZ is acting to aggravate seizures by a mechanism other than that which is believed to be its primary antiepileptic action (i.e., a use-dependent blockade of voltage-gated sodium channels) provides support for this proposition.

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Address correspondence to: Dr. Terence J. O’Brien, Department of Medicine, University of Melbourne, Royal Melbourne Hospital, Royal Parade, Parkville 3050, Victoria, Australia. E-mail: obrientj@unimelb.edu.au