The Mechanism of Carbamazepine Aggravation of Absence Seizures

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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 (Rt) and the ventrobasal complex (VB) 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 GABA<sub>A</sub> 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 GABA<sub>A</sub> receptor antagonist, bicuculline (BIC, 0.04 μg in 0.2 μl), was co-injected 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 GABA<sub>A</sub> 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 GABA<sub>A</sub> 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 absence aggravation are poorly understood. One of the drugs most implicated is carbamazepine (CBZ), a major first line AED for the treatment of focal and generalized tonic-clonic 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 pentylenetetrazole (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 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 blockade 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 Ca2+ 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 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 and 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., Korolikiewicz et al., 1996; Graumlich et al., 1999) and has no adverse effects on seizure activity (Fig. 1).

**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 age 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 Ca2+ 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.
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 (Marescau 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 for arms of each for the experiments.

**In Vitro Examination of CBZ Modulation of GABA<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> 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 CaCl<sub>2</sub>, 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 (EC<sub>20</sub>, 6 × 10<sup>-6</sup> 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 (EC<sub>20</sub>, 6 × 10<sup>-6</sup> 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 EC<sub>20</sub> GABA current caused by the addition of CBZ according to the formula (I<sub>GABA + CBZ</sub> - I<sub>GABA</sub>)/I<sub>GABA</sub> 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 ovariotomized 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\textsubscript{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\textsubscript{A} receptor-
mediated mechanism and, therefore, would be blocked by coadministration of the GABA<sub>A</sub> receptor antagonist, BIC. It has previously shown that BIC may induce conformational changes in the GABA<sub>A</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 μg in 0.2 μl), 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>A</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>A</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>A</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>A</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>A</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>A</sub> Current Expressed in *Xenopus* Oocytes. To confirm earlier in vitro studies demonstrating CBZ potentiation of GABA<sub>A</sub> current,
we expressed GABA<sub>λ</sub> 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).

Fig. 6. Mean percent time in seizure (±S.E.) in GAERS over a 90-min EEG recording following bilateral microinjection into the VB of carbamazepine (CBZ) (0.75 μg), BIC (0.037 μg), a mixture of carbamazepine and bicuculline (CBZ-BIC), or vehicle. Two groups of seven animals were used (graphs A and B). 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 showed significant seizure aggravation following the CBZ injection (versus vehicle) (226.3%, n = 7, p < 0.01) (A) but not following BIC (−7.0%, n = 7, p = 0.77) (B) or CBZ-BIC (A and B combined) (21%, n = 14, p = 0.47).

Fig. 7. Mean percent time in seizure (±S.E.) in GAERS over a 90-min EEG recording following randomized drugs treatments of vehicle injections into the VB and vehicle injection i.p. (Veh VB, Veh IP); vehicle injections into the VB, CBZ injection i.p. (15 mg/kg) (Veh VB, CBZ IP); BIC (0.037 μg) microinjection into the VB followed by CBZ i.p. (BIC VB, CBZ IP); and BIC microinjection into the VB and injection of vehicle IP (BIC VB, Veh IP). Repeated measures ANOVA showed a significant difference between the treatment arms (n = 8, p < 0.001), with planned comparison analysis showing that Veh VB, CBZ IP injection was followed by significantly longer time in seizures than the other three treatments (*, p < 0.001), which did not significantly differ from each other (p > 0.05).

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<sub>λ</sub> receptor-mediated mechanism. GABA<sub>λ</sub> 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<sub>λ</sub> receptors in the generation of spontaneous spike and wave discharges (Staak and Pape, 2001).

Previous studies have demonstrated that the GABA<sub>λ</sub> 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<sub>λ</sub> 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<sub>λ</sub> 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<sub>λ</sub> 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<sub>λ</sub> 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>λ</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>λ</sub> receptors, that CBZ has a dose-dependent effect to enhance the response of GABA<sub>λ</sub> receptors to the application of GABA (Figs. 8 and 9). Our in vivo data in GAERS suggest that CBZ may also have a regionally specific effect on GABAA channels in the brain, acting on neurons in the VB but not those in the Rt. This would promote de-inactivation of calcium T-channels, thereby enhancing oscillatory thalamocortical activity and absence seizures (Danober et al., 1998).

Allosteric modulators act on binding sites on receptors different from the (orthosteric) sites activated by the primary agonists for the receptor. They offer an increased opportunity for subtype selectivity in their effects because of the greater divergence in the domains that compose allosteric sites than those that interact with the primary endogenous agonist. The differential effects of CBZ on thalamic subregions may be explained by the molecular heterogeneity of GABA<sub>λ</sub> receptors in the thalamus. The GABA<sub>λ</sub> receptor is a heteromeric pentamer consisting of a combination of eight classes of subunits capable of forming a wide array of GABA<sub>λ</sub> receptor subtypes with varying pharmacological properties (DeLorey and Olsen, 1992). The subunit composition differs significantly in different regions of the thalamus. The VB contains high levels of α<sub>1</sub>, α<sub>3</sub>–5, β<sub>2</sub>, and δ subunits and is virtually devoid of α<sub>3</sub> and β<sub>3</sub> subunits. In contrast, the Rt is rich in α<sub>3</sub>, β<sub>1</sub>, and β<sub>3</sub> subunits and expresses little α<sub>1</sub>, α<sub>4</sub>, β<sub>2</sub>, and δ subunits (Pirker et al., 2000). Different subunit combinations in these two regions present functionally distinct receptor types. Although not directly tested in this study, the results of the current study would implicate α<sub>1</sub>, α<sub>4</sub>, β<sub>2</sub>, or δ as being important in CBZ action on GABA<sub>λ</sub> receptors in the VB.

Subunit composition of the GABA<sub>λ</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-insensitive GABA<sub>λ</sub> channels containing α<sub>4</sub> and δ subunits, whereas the Rt expresses higher levels of benzodiazepine-sensitive subunits, particularly γ<sub>2</sub> and α<sub>3</sub> (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>λ</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; Snead et al., 1999). This suggests that the mechanism of absence seizure aggravation by these steroids may be via selective activation of a GABA<sub>λ</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>λ</sub> receptor subunits differentially expressed within the VB compared with the Rt. CBZ has also been shown in HEK 293 cells transfected with α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub> subunits to potentiate GABA-induced current but not the other subunit combinations tested (α<sub>3</sub>β<sub>2</sub>γ<sub>2</sub> or α<sub>5</sub>β<sub>2</sub>γ<sub>2</sub>) (Granger et al., 1995). This direct effect of CBZ on GABA<sub>λ</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 α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub> 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

Fig. 8. Typical current traces showing potentiation of the EC<sub>20</sub> GABA response by 10 μM (A) and 100 μM CBZ (B) obtained in two electrode voltage-clamped Xenopus oocytes held at −80 mV. In each case, the four traces represent a series from the same oocyte showing first the response to GABA, then a reference 1 μM chlordiazepoxide (CDP) potentiation, followed by a recovery GABA response and finally the modulation by CBZ. CDP, a strong benzodiazepine site agonist, was used as a reference to ensure GABA<sub>λ</sub> γ<sub>2</sub> expression. The upper dashed line represents the leak corrected zero current level, and the lower dashed line is a guide to gauge the CBZ potentiation. Time and current scale bars apply to both figures.

Fig. 9. Dose-dependent GABA<sub>λ</sub> current potentiation by CBZ in Xenopus oocytes. Mean potentiation ratio (with S.E.M.) of GABA induced current in human α<sub>1</sub>, β<sub>3</sub>, γ<sub>2</sub>λ GABA<sub>λ</sub> subunit containing receptors by four concentrations of CBZ (in values given in parentheses).
α4 and δ subunits, may be even more important in mediating the differential effect of CBZ on GABA\(_\alpha\) 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 (Graumlich et al., 1999). The same concentration of CBZ was used for the VB microinjections 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\(_\alpha\) receptors indicate that CBZ enhances GABA\(_\alpha\) receptor responses at physiologically relevant concentrations (1–100 µM), within the range measured in CSF following the injection of 12

### References


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