C-Fos immunohistochemistry and central nervous system microinjections study of the mechanisms underlying the aggravation of absence seizures by carbamazepine in a genetic rat model

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Background and Objective: The mechanisms underlying carbamazepine (CBZ) aggravation of absence seizures are uncertain, but are thought to involve enhancement of neuronal activity within the thalamocortical circuitry. Critical to the oscillatory thalamocortical activity that underlies absence seizures is the reticular nucleus (Rt) and the ventrobasal nucleus of the thalamus (VB). Utilising c-Fos immunohistochemistry (cFos-ir) we examined patterns of neuronal activation and the relationship to seizure burden following administration of CBZ to female Generalised Epilepsy Rats of Strasbourg (GAERS). GAERS are a well-validated model of genetic generalized epilepsy. Additionally the effect on seizures expression of injections of CBZ intracerebroventricularly (icv), as well as locally into the VB and Rt was examined.

Methods: 13 week old female ovarectomised GAERS rats implanted with extradural EEG electrodes received, after one week, either 15 mg/kg of CBZ or vehicle i.p. Seizure burden post injection was quantitated by measuring the total duration and number of spike-wave discharges (SWD), and the individual burst length over a 90 min EEG recording. Results of the EEG analysis were correlated with cFos-ir in thalamocortical slices. For the second arm of the study female ovarectomised GAERS were implanted with either icv cannulae or bilateral microcatheters into the Rt or VB, using the following coordinates, Rt: AP 3, ML 3.6, DV 5.8; VB: AP 3, ML 2.6, DV 5.5 (relative to bregma, mm) and extradural EEG electrodes. The rats were studied following injection of CBZ (icv: 10ug in 4uL icv or Rt/VB: 3ug in 0.2uL) or vehicle (in random order). The treatment effect was quantified over a 90-minute EEG recording as for the cFos-ir.

Results: CBZ (ip) treated rats had significantly greater total duration of SWD vs. vehicle treated rats (17.9 vs. 8.8 %, n=5, p=0.04). The level of cFos staining did not differ between the treatment groups, however there was a positive correlation between staining intensity in the reticularis thalami (Rt) and the total seizure duration (r=0.66, p=0.04) and mean burst length (r=0.68, p=0.03). No significant correlation was found between seizure expression and cFos-ir for any other region examined. Seizure aggravation was seen in all rats (n=7) given CBZ icv (mean increase in seizure time = 49 ± 26% $p<0.05$). The cumulative time in seizures was significantly greater after administration of CBZ into the VB, compared to vehicle ($P = 0.01$, n=7). There was no difference in seizure duration between CBZ and vehicle. The number of seizures was significantly increased by CBZ ($P = 0.03$, n=7). No effects on seizures were observed following CBZ administration into the Rt.

Conclusions: The association between increased neuronal activation in the Rt and seizure burden in GAERS provides further support for the critical role of this structure in the generation and maintenance of absence seizures. Microinjection of CBZ into the VB aggravates absence seizures in GAERS with no effect in the Rt. This suggests that the VB is the neuroanatomical site at which CBZ aggravates absence seizures.

(EEG machine provided courtesy of Compumedics, Australia)