Differences in EEG power between pediatric and adult rats exposed to nerve agent and treated with anticonvulsants

Peter Sheu
Mentored by Dr. McDonough and Dr. Miller

Introduction

Organophosphorus (OP) nerve agents such as VX and GB inhibit acetylcholinesterase (AChE) which is an enzyme that terminates neurotransmission signals from the neurotransmitter acetylcholine (ACh). With AChE inhibited, there are high amounts of ACh sending signals to the post-synaptic receptors leading to glandular secretions, muscular contraction, seizures, neuronal damage, and death in severe cases (McDonough et al., 1998).

Anticonvulsants like diazepam, which is the drug currently used to treat nerve agent-induced seizures, enhance gamma-Aminobutyric acid (GABA) actions and increase inhibitory neural activity in the brain. This prevents high reception of the signals being sent and therefore less neural activity. However, there are other clinical drugs like midazolam, that could be more effective and efficient as an anticonvulsant.

The purpose of this study was to evaluate the effectiveness in treating seizures induced by the nerve agents GB and VX in both pediatric and adult Sprague-Dawley rats with the anticonvulsants diazepam and midazolam.

Materials and Methods

The brain electroencephalographic (EEG) activity of Sprague-Dawley rats was recorded and the EEG power (energy) was used to evaluate the level of seizure activity. Ten animals of each gender, male and female, and each age, post-natal days (PND) 21, 28, 70, received one of the two nerve agents to induce seizures and were treated with one of the two anticonvulsants. Exposures started with establishing a baseline seen in Figure 1 (A), representing the brain activity at normal levels. Animals were then treated with supporting drugs that help keep them alive but did not interfere with inducing seizures. These include 2-PAM and atropine. GB or VX was then administered and the animals were observed until seizure onset was observed on the EEG. Seizure onset was identified by repeated graphical spikes in the EEG record such as seen in Figure 1 (B). Five minutes after seizure onset, treatment with diazepam or midazolam was given in attempt to stop the seizures. The rats were then recorded for the rest of the day to determine if the seizures were completely stopped. This was decided when the EEG graphs showed waves similar to baseline.

The data from the EEG recording apparatus was then exported to excel files via the NeuroScore™ data analysis program (Data Sciences International™, Minneapolis, MN). The channels were partitioned into non-overlapping 30-second blocks. Within each block the power spectra was determined and divided into 6 frequency bands: delta (0.1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz), gamma (30-180 Hz), and total power. Total power is the summation of the all frequencies (Howbert et al., 2014). Those animals that stopped seizing within four hours of anticonvulsant treatment were considered treatment successes and were used to populate the experimental groups. The graphs are of average EEG data. After organizing the data by age and treatment, the subsequent data was analyzed using three-way analysis of variance (ANOVA) statistical tests.

Results

Three-way ANOVA tests gave significant differences between not only time points but also treatments used. PND21’s’ total power p-value for drug was 0.014; post hoc tests showed midazolam treated animals overall had significantly lower total power values than animals treated with diazepam. The times factor had a p-value less than 0.001 indicating the seizure- and drug-induced changes over time. However, PND 28’s’ total power p-value for drug was 0.644 and thus not significant, indicating that both diazepam and midazolam affected seizure activity in a similar fashion.

Conclusions

This study was the first to compare the effectiveness of the anticonvulsant midazolam relative to diazepam across gender, agent and age. Agent and gender turned out to be factors that did not impact the results – seizure activity and response to anticonvulsant treatment were equivalent regardless of whether the seizures were elicited by VX or GB or whether males or females were involved. For the total power variable midazolam reduced power faster than diazepam in all three age groups. For the delta, theta, alpha, and beta bands similar trends as seen with total power were observed. Although the differences for PND28 may not be significant; more studies would have to be made before further conclusions could be made. At 160 minutes, there was almost no difference in levels of the brain activity while at seizures onset and up to 60 minutes after, there was a disparity between the brain levels in those that received midazolam compared to those that received diazepam. As supported by Graph 1 and 2, the three way ANOVA found that there was a significant effect of the drug used in animals of PND 21s. The mean brain activity of those that received midazolam was significantly lower than that of diazepam. This trend was found to be similar in adults, PND 70s. From this study, it was found that midazolam was more efficient at treating seizures than diazepam in the rats in the PND 21 and 70 age groups. It was not more effective than diazepam for those of PND 28, but was equivalent in efficacy. These results support the further development of midazolam as an improved anticonvulsant treatment for nerve agent-induced seizures across all age groups.

References