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Effects of levetiracetam on spike and wave discharges in WAG/Rij rats

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Summary Effects of the novel anti-epileptic drug levetiracetam (50 and 100 mg/kg) on spike and wave discharges (SWDs) of WAG/Rij rats were studied. Levetiracetam decreased the incidence, average duration, total duration and peak frequency of the SWDs. There was no difference between the two doses. These results agree with results obtained in Genetic Absence Epilepsy Rat from Strasbourg (GAERS). Furthermore, the decrease of the SWD peak frequency might support the suggestions that levetiracetam might have a GABAergic mechanism of action.

Introduction

Levetiracetam has anti-epileptic properties in a wide range of animal models.[1—4] Gower et al.[2] investigated its effects on spike and wave discharges (SWDs) in the electro-encephalogram (EEG) of the Genetic Absence Epilepsy Rat from Strasbourg (GAERS), a model for absence seizures. In the GAERS, levetiracetam decreased the total duration of the SWDs, during the 2 h after injection, although in a non-dose-dependent manner (dose range: 5.4—170 mg/kg).[2]

A similar genetic absence epileptic rat model, the WAG/Rij strain is studied at our department.[5] However, the effects of levetiracetam on SWDs in the EEG of the WAG/Rij rat have not previously been investigated. Therefore, the effects of levetiracetam (50 and 100 mg/kg) on SWDs in the EEG of the WAG/Rij rat were investigated in order to replicate and extend the effects found in GAERS.

Method

This study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethical committee for animal studies was obtained.

Eight male WAG/Rij rats, 12 months old, with a mean body weight of 337 g (S.E.M. = 3 g), housed on a reversed day—night cycle, were used. A permanent cortical tripolar electrode (Plastics One MS-332/2-A) was implanted under isoflurane anaesthesia, coordinates according to Paxinos and Watson:[6] A 2.0, L 3.5 (frontal); A —6.0, L 4.0 (parietal) and the ground electrode over the cerebellum. All rats were used in a previous experiment, after which the animals were allowed to recover for at least 2 weeks.

Rats were connected to an EEG cable allowing free movements and habituated to the experimental conditions for 30 min. Experimental sessions were performed between 13:30 and 16:00 h in a room with red lights, on 3 separate days with 7 days between experimental days. Each rat received all three treatments. The rats were injected i.p. with saline (2 ml/kg) or 50 mg/kg of levetiracetam

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counterbalanced on the first 2 days and 100 mg/kg of levetiracetam on the 3rd day. Levetiracetam (UCB Pharma) was dissolved in saline (2 ml/kg). Directly after the injections the EEG was recorded during 2 h. The EEG in a bandwidth between 0.1 and 100 Hz, was digitalized with a sample frequency of 512 Hz, and stored for off-line analysis using the Windaq system (DATAQ Instruments, Akron, OH, USA). SWDs were marked after visual inspection based on the criteria for SWDs as proposed by Van Luijtelaar and Coenen.[5]

SWD incidence, average duration and total duration were determined. The SWD spectral content was analyzed using a Fast Fourier Transform (FFT) procedure with a window of 2 s, starting at the beginning of the SWD. The peak frequency was obtained from the results of the FFT.

Statistical analyses on each parameter were performed using a Repeated Measures Procedure in SPSS 11.5 (SPSS Inc., Chicago, IL, USA) (treatment as within subject factor, $\alpha = 0.05$), followed by post hoc paired *t*-tests ($\alpha = 0.05$).

Results

Fig. 1 shows the incidence of SWDs in the upper panel, the average duration of the SWDs in the first middle panel, the total duration of SWDs in the second middle panel and the mean peak frequency in the lower panel.

Statistical analyses showed:

(1) A decrease in incidence after levetiracetam ($F(2,6) = 8.29$; $P = 0.019$), with a significant decrease after 50 mg/kg ($P < 0.01$) and 100 mg/kg of levetiracetam ($P < 0.01$), as compared to saline.
(2) A decrease in average duration after levetiracetam ($F(2,6) = 25.52$; $P = 0.001$), with a significant decrease after 50 mg/kg ($P < 0.001$) and 100 mg/kg of levetiracetam ($P < 0.01$), as compared to saline.
(3) A decrease in total duration after levetiracetam ($F(2,6) = 10.17$; $P = 0.012$), with a significant decrease after 50 mg/kg ($P < 0.01$) and 100 mg/kg of levetiracetam ($P < 0.01$), as compared to saline.
(4) A decrease in peak frequency after levetiracetam ($F(2,6) = 7.86$; $P = 0.021$), with a significant decrease after 50 mg/kg ($P < 0.01$) and 100 mg/kg of levetiracetam ($P < 0.05$), as compared to saline.

There was no significant difference between the two doses of levetiracetam on any of the parameters.

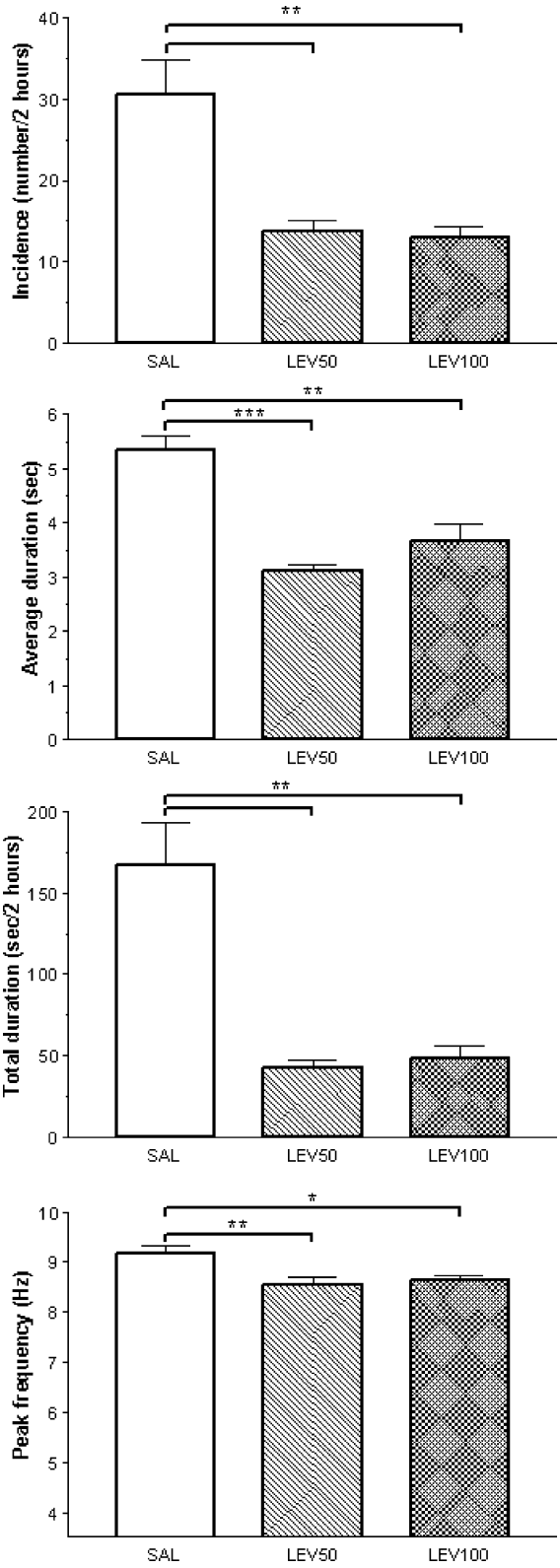

Figure 1 Means ($n = 8$) and standard errors of the incidence (upper panel), the average duration (first middle panel), the total duration (second middle panel) and the peak frequency (lower panel) of SWDs per treatment. Asterisks (*, ** or ***) indicating that $P < 0.05$, $P < 0.01$ or $P < 0.001$, respectively, in the post hoc paired *t*-tests.

Discussion and conclusions

The objective of this study was to investigate effects of levetiracetam on SWDs in WAG/Rij rats. The incidence, average duration, total duration and peak frequency of the SWDs were significantly decreased after levetiracetam as compared to the saline condition. However, no dose-dependent effects were observed.

The non-dose-dependent decrease in total duration of SWDs after levetiracetam agrees with findings of Gower et al.[2] This decrease in total duration of SWDs was shown to be the result of both a decrease in incidence and in average duration of SWDs. Furthermore, the peak frequency of SWDs was decreased, indicating that the morphology of the SWDs was affected by levetiracetam. The non-dose dependency of these effects suggest that the maximal effect of levetiracetam on SWDs might already be obtained by the lowest dose tested (50 mg/kg), suggesting that levetiracetam is not likely to completely abolish the SWDs. Indeed, a dose-dependent decrease in SWD activity after lower doses of levetiracetam (5.4 and 17.0 mg/kg) was observed on low dose pentylenetetrazol-induced SWDs in rats.[7]

Previously, we found that the gamma-aminobutyric acid (GABA) transaminase inhibitor vigabatrin decreased the peak frequency of SWDs, suggesting that a GABAergic component might be responsible for frequency modulation of SWDs.[8] Accordingly, Destexhe[9] suggests that the peak frequency of the SWDs can be determined by the balance between $GABA_A$ and $GABA_B$ conductances. Interestingly, levetiracetam decreases the peak frequency of SWDs as well. Indeed, Löscher et al.[10] reported some subtle significant effects on GABA turnover differing over time and brain region, and Rigo et al.[11] reported that levetiracetam reverses the negative allosteric modulation of $GABA_A$ receptors by Zn^{2+} and β-carbolines, both of these effects might affect the balance between $GABA_A$ and $GABA_B$ neurotransmission. However, despite suggestions that levetiracetam might have some GABAergic properties,[2,3,10–14] no clear effects of levetiracetam on other GABA-related parameters have been found so far: no direct effects at the $GABA_A$ or $GABA_B$ receptor,[15,16] no effects on brain GABA concentrations or release of GABA,[17,18] no effects on the activities of both GABA-synthesizing and GABA-degrading enzymes,[10,17] nor on neuronal voltage[14] and current responses to GABA,[11] have been found.

In conclusion, levetiracetam has some anti-epileptic properties in both the GAERS and the WAG/Rij rat models of absence epilepsy: levetiracetam, in contrast to the GABAergic drug vigabatrin, decreases the duration and the incidence of SWDs. Furthermore, levetiracetam decreased the peak frequency of the SWDs, similar to vigabatrin, suggesting some similar mechanism of action.

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