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Research Report

Levetiracetam suppresses development of spontaneous EEG seizures and aberrant neurogenesis following kainate-induced status epilepticus

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ABSTRACT

Electroencephalographic (EEG) seizures and behavioral convulsions begin to appear spontaneously a few weeks after chemoconvulsant-induced status epilepticus (SE) and thereafter become more intense. This indicates the progressive development of a long-lasting epileptic focus. In addition, chemoconvulsant-induced SE increases neuronal proliferation in the dentate subgranular zone (SGZ) and ectopic migration of newborn neurons into the dentate hilus of adult animals. These seizure-induced newborn neurons, especially ectopic granule cells in the dentate hilus, are believed to facilitate the development of epileptic foci in animal models of temporal lobe epilepsy. In the present study, we examined the effects of a novel antiepileptic drug, levetiracetam, on the appearance of spontaneous EEG seizures and on the generation of newborn neurons, especially of ectopic granule cells in the dentate hilus, following kainate-induced SE. Levetiracetam treatment for 25 days, initiated 24 hours after induction of kainate-induced SE, significantly decreased the mean duration of spontaneous EEG seizures 58 days later. Levetiracetam treatment also prevented an SE-induced increase in the number of ectopic granule cells observed 58 days after kainate administration by suppressing neuronal proliferation in the dentate SGZ and abnormal migration of newborn neurons from the dentate SGZ to the hilus. These results are in accord with a previous report that an antimitotic agent that reduced the number of newborn neurons significantly decreased the frequency of spontaneous convulsions 1 month after pilocarpine-induced SE. This evidence from the kainate model of temporal lobe epilepsy suggests that levetiracetam may exert antiepileptogenic effects through the suppression of seizure-induced neurogenesis.

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1. Introduction

Levetiracetam has been reported not only to have a suppressive effect on the generation of acute seizures but also to inhibit the development of epileptic foci in various animal models of epilepsy. Continuous administration of levetiracetam using an osmotic mini-pump was found to prevent the development of spontaneous convulsions in a genetic model of epilepsy (Ji-qun et al., 2005). In the pilocarpine model of epilepsy, administration of levetiracetam for 20 days, starting from 1 day after status epilepticus (SE), also strongly inhibited the development of epileptic electrical activity in the dentate gyrus for at least 24 days following SE (Margineanu et al., 2008). Furthermore, the development of 'kindling', a progressive increase in seizure severity induced by repeated brain stimulation at certain intervals, as well as kindling-related gene expression, was significantly depressed by the daily application of levetiracetam (Husum et al., 2004; Loscher et al., 1998). These data suggest that levetiracetam may suppress the accumulation of functional and morphological plastic changes in neuronal circuits induced by seizures during the development of a long-lasting epileptic focus. Among numerous postseizure plastic changes, the marked increase in the number of newborn neurons in the adult hippocampal dentate gyrus and their disrupted migration have both received increasing attention because of their close relationship with the development of an epileptic focus in kindling and chemoconvulsant-induced SE models (Fournier et al., 2009; Jung et al., 2004; McCloskey et al., 2006).

Neurons are continuously born in the subgranular zone (SGZ) of the normal adult dentate gyrus (Doetsch and Hen, 2005). The generation of newborn neurons is strongly influenced by several environmental factors including environmental enrichment, physical exercise, and antidepressant treatment (Kempermann et al., 1997; Malberg et al., 2000; van Praag et al., 1999). Furthermore, an epileptic seizure significantly increases the number of newborn neurons for several weeks (Parent et al., 1997). Seizure-induced newborn neurons become incorporated into preexisting hippocampal circuits and have been proposed to contribute to the development of epileptic foci (Jung et al., 2004). This prediction is supported by the finding that an antimitotic agent applied for 14 days after the induction of pilocarpine-induced SE significantly decreased both the seizure-induced increase in newborn granule cells and the number of spontaneous seizures 1 month later (Jung et al., 2004). Moreover, 15% to 30% of the newborn neurons proliferating in the dentate SGZ after seizures were found to migrate ectopically and develop in the dentate hilus where newborn neurons are rarely observed in nonepileptic animals (Fournier et al., 2009; Jessberger et al., 2007b; Parent et al., 2006). This ectopic migration of newborn neurons was found to last for more than 5 months after SE (Hattiangady et al., 2004). Neurons that have ectopically migrated are commonly termed ectopic granule cells, because their histological properties are similar to granule cells in the dentate granule cell layer (GCL; Scharfman et al., 2000). The number of ectopic granule cells closely correlates with the number of spontaneous seizures after pilocarpine-induced SE (McCloskey et al., 2006). Furthermore, ectopic granule cells display spontaneous epileptiform burst discharges that are synchronized with firing in CA3 neurons (Scharfman et al., 2000). These results suggest that ectopic granule cells may play an important role in the development of long-lasting epileptic foci.

Based on these previous findings, we hypothesize that levetiracetam may prevent the development of epileptic foci following chemoconvulsant-induced SE through the suppression of the aberrant proliferation and migration of newborn granule cells. To elucidate the effects of levetiracetam on the development of an epileptic focus, we determined whether post-SE treatment with levetiracetam suppresses spontaneous electroencephalographic (EEG) seizures recorded 2 months after kainate-induced SE. We then conducted an immunohistochemical investigation to determine whether levetiracetam suppressed the generation of newborn neurons and the accumulation of ectopic granule cells over a period of 2 months after kainate-induced SE. We focused our attention on whether post-SE treatment with levetiracetam for 25 days could prevent long-lasting aberrant migration of newborn neurons 1 month after the cessation of treatment.

2. Results

2.1. *Effects of levetiracetam treatment on spontaneous EEG seizures 2 months after SE*

We first examined the effects of post-SE levetiracetam treatment (0.36 mmol/ml via osmotic mini-pumps, icv) on the development of spontaneous EEG seizures following SE induced by kainate (0.05 μg/μl, icv). To exclude the direct anticonvulsive effects of levetiracetam on spontaneous EEG seizures after kainate-induced SE, we ensured that continuous treatment with levetiracetam was finished before the EEG recording took place. It was previously reported that levetiracetam treatment for 28 days significantly suppressed the development of epileptic foci over a period of 35 days after the cessation of treatment in a genetic model of epilepsy (Yan et al., 2005). Following the study by Yan et al. (2005), we treated rats with levetiracetam (SE–LEV group) or saline (SE–vehicle group) for 25 days from 1 day after SE induction with kainate. The day of SE induction was defined as day 0. On day 58, i.e. 32 days after the cessation of levetiracetam treatment, spontaneous EEG seizures were recorded in freely moving rats.

Behavioral seizures in a period of SE induction with kainate lasted for less than 1.5 hours after kainate injection in all animals. There was no significant difference in the total duration of behavioral SE seizures between SE–vehicle and SE–LEV animals. During a period of 1.5 hours after kainate injection, there was also no significant difference in the total duration of epileptiform EEG activity between the SE–vehicle (2599.0±694.6 s) and SE–LEV (2869.2±274.0 s) animals. Two of eight animals in the SE–LEV group and two of eight animals in the SE–vehicle group in the EEG experiment died during kainate-induced SE. As such, spontaneous EEG seizures were recorded in the remaining six animals in each group.

On day 58, spontaneous EEG seizures were frequently observed in animals in the SE–vehicle group, demonstrating