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RESEARCH****Research Report**

Ultrastructural GABA immunocytochemistry in the mossy fiber terminals of Wistar and genetic absence epileptic rats receiving amygdaloid kindling stimulations

Dilek Akakin^{a,*}, Serap Sirvanci^a, Ayten Gurbanova^b, Rezzan Aker^b,
Filiz Onat^b, Tangul San^a

^aDepartment of Histology and Embryology, Faculty of Medicine, Marmara University, Istanbul, Turkey

^bDepartment of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Marmara University, Istanbul, Turkey

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ABSTRACT

The existence of absence epilepsy and temporal lobe epilepsy in the same patient is not common in clinical practice. The reason why both types of seizures are rarely seen in the same patient is not well understood. Therefore, we aimed to investigate kindling in a well known model of human absence epilepsy, genetic absence epilepsy rats from Strasbourg (GAERS). In the present study, we analyzed whether the GABA content of GAERS that received kindling stimulations was altered in the hippocampal mossy fiber terminals compared to non-epileptic control (NEC) Wistar rats. For this purpose, we used an immunocytochemical technique at the ultrastructural level. Ultrathin sections were immunolabeled with anti-GABA antibody and transmission electron microscopy was used for the ultrastructural examination. The number of gold particles per nerve terminal was counted and the area of the nerve terminal was determined using NIH image analysis program. The GABA density was found to be higher in sham-operated GAERS than sham-operated Wistar rats. The density was increased in kindling Wistar group compared to sham-operated Wistar and kindling GAERS groups. No statistical difference was observed between sham-operated GAERS and kindling GAERS groups. The increase in GABA levels in stimulated Wistar rats may be a result of a protective mechanism. Furthermore, there may be strain differences between Wistar rats and GAERS and our findings addressing different epileptogenesis mechanisms in these strains might be a basis for future experimental studies.

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1. Introduction

Disturbances between GABA-mediated inhibitory and glutamate-mediated excitatory neurotransmissions in the central nervous system play an important role in the pathogenesis of epilepsy in experimental animal models and humans.

Typical absence epilepsy, a form of nonconvulsive idiopathic generalized childhood epilepsy, is characterized by a sudden interruption of behavioral activity and responsiveness associated with bilateral synchronous spike-and-wave discharges (SWDs) in the EEG. A well known animal model of absence epilepsy is an inbred strain derived from Wistar rats that show genetically

* Corresponding author. Marmara Universitesi Tıp Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, Haydarpaşa, 34668 Üsküdar, İstanbul, Turkey. Fax: +90 216 348 05 58.

E-mail address: dilekbangir@yahoo.com (D. Akakin).

determined spontaneous absence seizures, Genetic Absence Epilepsy Rats from Strasbourg (GAERS) (Danover et al., 1998; Marescaux et al., 1992). This strain was introduced by Vergnes et al. (1982) in the beginning of 1980s and is a well-established model of human absence epilepsy on the basis of neurophysiological, pharmacological, and genetic studies (Danover et al., 1998; Marescaux and Vergnes, 1995).

Cellular and molecular mechanisms underlying absence epilepsy may manifest themselves in brain regions other than thalamocortical circuitry. For example, metabolic activity and energy metabolism were shown to be increased in limbic structures including the hippocampus of both adult and immature GAERS (Nehlig et al., 1992, 1998). Moreover, basal levels of extracellular glutamate within the hippocampus, as measured by microdialysis, were shown to be significantly increased in GAERS compared to non-epileptic controls (NECs) (Richards et al., 2000). Therefore, there has been particular interest in the molecular and cellular alterations in hippocampus.

Amygdala kindling in rats is considered as one of the best-suited models of temporal lobe epilepsy (TLE) in humans (Löscher and Schmidt, 1988). Kindled rats are characterized by induction of partial-onset secondarily generalized convulsion (Goddard, 1967; Racine, 1972). Laboratory and clinical studies on focal epilepsy revealed increasing evidence that the hippocampus can provide a primary or secondary focus site. In this respect, kindling model of TLE has been used in many studies suggesting that the hippocampal–parahippocampal loop functions as a control point in limbic system epileptogenesis (Lothman et al., 1991; Ribak et al., 1992).

The granule cells of DG send strong glutamatergic projections called “mossy fibers” toward the hippocampal CA3 region, where they monosynaptically excite pyramidal neurons and neighboring inhibitory interneurons that inhibit CA3 pyramidal cells (Dichter and Spencer, 1969; Miles and Wong, 1987). Despite their well known excitatory nature, studies have shown evidence about the presence of vesicular GABA transporter (VGAT) mRNA, VGAT, glutamic acid decarboxylase (GAD) 65 and 67 as well as GABA in mossy terminals (Lamas et al., 2001; Lehmann et al., 1996; Sandler and Smith, 1991; Sloviter et al., 1996; Zander et al., 2010).

Clinically, focal epilepsy and idiopathic generalized epilepsy coexistence is very rarely observed (Koutroumanidis et al., 1999; Nicolson et al., 2004; Jeha et al., 2006). The reason why both types of seizures are rarely seen in the same patient is not well understood. In recent kindling studies, it was reported that animals with absence epilepsy failed to show spontaneous convulsive seizures even after the maximum number of kindling stimulations (Eskazan et al., 2002; Onat et al., 2007). However in those studies, NEC animals reached stage 5 generalized convulsive seizure state with less number of kindling stimulations applied. It was suggested that GAERS are resistant to secondary generalization of limbic seizures during amygdala kindling (Eskazan et al., 2002). It was also revealed that hippocampal kindling resembles amygdaloid kindling in terms of delay of or resistance to secondary seizure generalization (Akman et al., 2008). TLE is the most common and surgery requiring seizure disorder in adults (Engel et al., 1997a; Engel et al., 1997b). Therefore, it is important to examine the underlying mechanisms of TLE for improving non-invasive treatment modalities in this type of epilepsy. The aim of the present study was to

investigate whether the resistance of GAERS to generalization of limbic seizures was related to the possible differences in GABA density in mossy terminals. For this purpose, we used immunocytochemical technique at the ultrastructural level in the CA3 region of the dorsal hippocampus of Wistar rats and GAERS.

2. Results

Our findings revealed that the mean number of stimulations to display the first stage 5 seizure was 14.75 ± 1.11 in NECs (expressed as mean $\pm$ SEM). In contrast, GAERS never showed seizures beyond stage 2 even after the maximal number of stimulation (30) was reached.

In all groups, the mossy fiber terminals (MFTs) in the CA3 region were localized around dendrites and characterized by the presence of spherical vesicles, dense core vesicles (DCVs), and their asymmetrical synaptic contact with multiple dendritic spines (Fig. 1). DCVs were much fewer in number than the spherical vesicles and well recognized with their electron dense center and larger diameter compared to the spherical vesicles.

We observed that MFTs were GABA immunoreactive (Fig. 1). Immunoreactivity was associated with the spherical and DCVs and the mitochondria within the MFTs in all groups.

In stimulated rats, synaptic vesicle distribution pattern of MFTs was altered and clusters of spherical vesicles were confined to restricted areas of the terminals (Figs. 1b, d). Non-stimulated rats appeared to have the entire MFTs full of synaptic vesicles (Figs. 1a, c). Quantitative work revealed that 66% of mossy terminals from the stimulated rats ($n=98$) and only 4% from the non-stimulated rats ($n=73$) were found to have depletion of vesicles.

There was no significant difference in the area of MFTs (sham-operated Wistar, $n=4$ animals, $n=39$ terminals: $4.48 \pm 0.44 \mu\text{m}^2$; sham-operated GAERS, $n=4$: $4.07 \pm 0.50 \mu\text{m}^2$; kindling Wistar, $n=4$ animals, $n=41$ terminals: $4.29 \pm 0.18 \mu\text{m}^2$; kindling GAERS, $n=4$ animals, $n=39$ terminals: $4.07 \pm 0.32 \mu\text{m}^2$) (Fig. 2). The density of GABA immunolabeling in the Wistar kindling group ($n=4$ animals, $n=41$ terminals) (38.95 ± 2.06 particles/ μm^2) was found to be significantly increased compared to the sham-operated Wistar group ($n=4$ animals, $n=39$ terminals) (13.45 ± 0.46 particles/ μm^2) (Fig. 3). GABA labeling in the sham-operated GAERS ($n=4$ animals, $n=39$ terminals) (25.44 ± 0.62 particles/ μm^2) was found to be significantly increased compared to the sham-operated Wistar group. GABA labeling in the kindling Wistar group was found to be significantly higher compared to the kindling GAERS ($n=4$ animals, $n=39$ terminals) (28.56 ± 1.03 particles/ μm^2) group. There was no significant difference between sham-operated GAERS and kindling GAERS groups.

3. Discussion

3.1. GABA labeling in mossy fibers of Wistar rats

The findings of the present study showed that MFTs of kindling Wistar group had increased GABA content compared to the sham-operated Wistar group. These data provide

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