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Alterations of L-type voltage dependent calcium channel alpha 1 subunit in the hippocampal CA3 region during and after pilocarpine-induced epilepsy

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ABSTRACT

Voltage-dependent calcium channels (VDCC) have been shown to regulate neuronal excitability and their antagonists have been used clinically for the control of seizures. While functional studies of VDCC in epileptogenesis in the CA1 area of hippocampus or the dentate gyrus have been done, few studies were carried out in the CA3 area. Given the bursting characteristics of the CA3 neurons, we speculated that VDCC in the CA3 area might play an important role in the epileptogenesis. In the present study in the mouse pilocarpine model of temporal lobe epilepsy, we investigated the alterations of alpha 1 subunits of L-type VDCC in the CA3 area of the hippocampus at different stages of epileptogenesis, i.e., acute stage from 10 min to 1 day during and after pilocapine-induced status epilepticus (SE), latent period at 1 week, and chronic stage with spontaneous recurrent seizures at 2 months after SE. We found that an immediate redistribution of alpha 1 subunits in the CA3 area occurred during SE which might be involved in the seizure occurrence indicated by the Racine score record. Alterations of alpha 1 subunits were also demonstrated in the latent period and chronic stage, which might be related to the epileptogenesis and occurrence of epilepsy. $Ca_v1.3$, but not $Ca_v1.2$, was expressed in reactive astrocytes of the CA3 area, indicating the involvement of $Ca_v1.3$ in the modulation of astrocytic Ca^{2+} homeostasis during epileptogenesis.

1. Introduction

With significant morbidity and mortality, status epilepticus (SE) has been considered as a major medical and neurological emergency (DeLorenzo et al., 1996). SE induced injury may be epileptogenic leading to temporal lobe epilepsy (TLE) eventually. Ion channels, such as sodium, potassium and calcium channels, play important roles in neuronal excitability, and are involved the pathologenesis of SE. Of calcium channels, voltage-dependent calcium channels (VDCC) (Beck et al., 1997, 1998; Heinemann and Hamon, 1986; Kohr et al., 1991; Mody et al., 1990; Speckmann and Walden, 1986) may modulate neurotransmitters release (Dooley et al., 1988; Herdon and Nahorski, 1989; Horne and Kemp, 1991; Luebke et al., 1993; Turner et al., 1993; Wessler et al., 1990; Woodward et al., 1988), neural cell migration and differentiation, and gene expression (Leclerc et al., 2006; Platel et al., 2007; Weissman et al., 2004).

TLE is the most common type of intractable epilepsy, and pathological change of the hippocampus plays an important role in the epileptogenesis of TLE. The hippocampal CA3 area has 1.5–2.0 times neurons than CA1 area. A hallmark of CA3 hippocampal neurons is intrinsic burst firing (Xu and Clancy, 2008). In the hippocampus of primates, 95% CA3 neurons are bursting neurons (Buckmaster and Amaral, 2001), In rodents, distinct

Abbreviations: AE, acquired epilepsy; GFAP, glial fibrillary acidic protein; PISE, pilocarpine-induced status epilepticus; SE, status epilepticus; TLE, temporal lobe epilepsy; VDCC, Voltage-dependent calcium channels.

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populations of bursting and non-bursting CA3 neurons have been identified (Masukawa et al., 1982). The apical dendrites of these neurons make synapses with the mossy fibers which originate from the granule cell of the dentate gyrus. The Schaffer's collateral branches of the axon of the CA3 pyramidal neurons project to the stratum radiatum of CA1 to form synapses with the trunk of the apical dendrites. Both of the above mentioned synapses are excitatory (Storm-Mathisen and Fonnum, 1972), and together take part in the excitatory recurrent circuit in the hippocampal formation. Furthermore, CA3 neurons were demonstrated to directly synapse onto glial cells (Volterra and Steinhauser, 2004). The neuron-to-glia signaling may disturb astrocyte receptor functioning and be involved in epileptogenesis (Seifert et al., 2004). Previous studies in epileptic rats have demonstrated that CA3 neurons had long-lasting depolarization shifts and accompanying repetitive firings, suggesting this subgroup of neurons may play a vital role in the occurrence of epilepsy (Ishihara et al., 1993; Momiyama et al., 1995). The long-lasting depolarization shifts and accompanying repetitive firings of CA3 neurons in epileptic rats were subsequently demonstrated to be associated with enhanced Ca^{2+} influx consistent with increased L-type Ca^{2+} channel activity (Amano et al., 2001). In the rat hippocampus and resected hippocampus of patients with therapy-refractory temporal lobe epilepsy, L- and N-type Ca^{2+} channel currents are demonstrated to account for >95% of the high-threshold Ca^{2+} currents, and the high-threshold Ca^{2+} currents density was significantly larger in patients and in rat kainate epileptic model compared to the adult control rats. It indicates the important implication of L- and N-type Ca^{2+} channels in epilepsy (Beck et al., 1997, 1998). Previous studies have shown many morphological and electrophysiological changes of L- and N-type Ca^{2+} channels in the hippocampal neurons of patients with TLE or acquired epilepsy(AE) animals. However, the controversies still remain. For instance, increase (Amano et al., 2001; Beck et al., 1998; Djamshidian et al., 2002; Faas et al., 1996; Hendriksen et al., 1997; Kang et al., 2004; Kelly et al., 2003; Vreugdenhil and Wadman, 1992, 1994; Xu et al., 2007, 2010), decrease (Djamshidian et al., 2002; Hendriksen et al., 1997; Kang et al., 2004; Kelly et al., 2003; Xu et al., 2007, 2010), as well as unchanged Ca^{2+} channels (Su et al., 2002) have been reported from different research groups. Interestingly, simultaneous regional decrease and increase of L-type (Kelly et al., 2003) and N-type calcium channels (Xu et al., 2010) were also observed indicating their complex implication in the mechanism of epileptogenesis.

Furthermore, most of the previous studies on the epileptic related alterations of calcium channels focused on the hippocampal CA1 and/or the dentate gyrus, with few on whole hippocampus (Kelly et al., 2003; Xu et al., 2007) and rare on CA3 area (Amano et al., 2001). Most of previous studies took samples at least 3 h (Kang et al., 2004) after the initial of induced SE and there is a short of data from study at early time points. This is important because it will be more meaningful to control SE and prevent subsequent epileptogenesis than treat epilepsy symptomatically. In our previous studies, we have reported detailed alterations of L- and P/Q-type Ca^{2+} channel alpha 1 subunits (including $Ca_v1.2$, $Ca_v1.3$ and $Ca_v2.1$) in CA1 and dentate gyrus (Xu et al., 2007) and N-type Ca^{2+} channel alpha 1 subunit ($Ca_v2.2$) in the whole hippocampus (Xu et al., 2010) of pilocarpine induced epileptic mice. In the present study using immunohistochemistry and immunofluorescence labeling, we aimed to show changes of L-type Ca^{2+} channel alpha 1 subunits (including $Ca_v1.2$ and $Ca_v1.3$) in CA3 area of the mouse hippocampus during and after pilocarpine induced epilepticus. We hoped this would broaden our knowledge of the role of VDCC in the mechanism of epileptogenesis in TLE.

2. Materials and methods

2.1. Pilocarpine treatment and behavioral record

Female C_{57}BL/6 mice weighing 25–30 g were used for the study according to our established procedures (Xu et al., 2007, 2009). Mice were given a single subcutaneous injection of methylscopolamine nitrate (1 mg/kg) 30 min before the injection of either saline in the control or pilocarpine in the experimental group. For the experimental group, mice were given a single injection of 300 mg/kg pilocarpine (i.p.) and experienced acute SE. Seizure behavior was scored using the following adapted Racine scale: score 0, no seizures observed; score 1, rhythmic mouth and facial movement; score 2, rhythmic head nodding (bobbing); score 3, forelimb clonus; score 4, rearing and bilateral forelimb clonus; and score 5, rearing and falling (stay fallen on rear side; tonic-clonic seizures).

An established SE was defined as continuous seizures (with a Racine score of 4–5, without returning to lower stages for at least 5 min) last for at least 2 h. In this study, only mice with established SE were used for immunohistochemistry and immunofluorescence labeling. SE lasting more than 3 h was terminated by an injection (5 mg/kg, i. p.) of diazepam to reduce mortality. To monitor animal behavior, the highest score reached in every 5 min during the three 30 min-sections (within an hour and a half of the onset of SE) was recorded and the percentages of Racine scores in each 30 min section were calculated.

All experiments were approved by Xi'an Jiaotong University Health Science Center Institutional Animal Care & Use Committee. In the handling and care of all animals, the guidelines for animal research of Xi'an Jiaotong University Health Science Center were strictly followed. Efforts were made throughout the study to minimize animal suffering and to use the minimum number of animals.

2.2. Immunohistochemistry study of the expression of c-fos, $Ca_v1.2$ and $Ca_v1.3$ in the hippocampus of control and experimental mice

A total of 49 mice (female) were used for immunohistochemical study. Six mice were killed at each of the survival intervals, i.e., at 10 min, 30 min, 1 h, 2 h during pilocarpine-induced status epilepticus (PISE), 1 day, 1 week and 2 months after PISE respectively. Seven mice (one at each time point) with saline instead of pilocarpine injection were sacrificed for the control group.

Following deep anesthesia with chloral hydrate (0.4 g/kg), mice were perfused transcardially with 10 ml of saline initially, followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB) plus 15% picric acid (final pH 7.4) for 15 min. After perfusion, the brain was removed, and kept overnight in 30% sucrose in 0.1 M PB. Coronal sections in brain at 40 μm thickness were cut in a cryostat (HM505E, Microm, Zeiss, Germany). A set of three from each of eight sections were prepared and transferred to different wells of a 24-well tissue culture dish for c-fos (Rabbit anti-c-fos polyclonal IgG, Calbiochem, PC05T) staining and calcium channel alpha1 subunits $Ca_v1.2$, $Ca_v1.3$ (Rabbit polyclonal IgG, Chemicon International, CA; Sigma, MO) immunocytochemical reaction.

Briefly, freely floating sections were treated in 4% normal goat serum for 2 h at room temperature. All sections were then washed in 0.1 M phosphate-buffered saline (PBS) containing 0.1% Triton X-100 and placed overnight in primary rabbit antibody for c-fos (1:10000), $Ca_v1.2$(1:400) and $Ca_v1.3$(1:200) in PBS/Triton X-100. After incubation, sections were washed in PBS and placed for 1 h in biotinylated goat anti-rabbit IgG (Vector Laboratories Burlingame, CA, USA) diluted 1:200 in PBS/Triton X-100. After three washes in PBS, they were placed in avidin–biotin complex (ABC) reagent