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Research report

Clc-2 knockout attenuated experimental temporal lobe epilepsy in mice by tonic inhibition mediated by $GABA_A$ receptors

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ABSTRACT

Temporal lobe epilepsy (TLE), the most prevalent form of epilepsy, is often associated with drug-resistant seizures. In TLE, altered function of γ-aminobutyric acid (GABA)$_A$ receptors ($GABA_A$Rs) results in potentiation of excitatory and/or failure of inhibitory neurotransmission, which contributes to seizure induction and propagation. Our previous study suggested that chloride channel-2 (Clc-2) contributed to chronically elevated tonic inhibition mediated by $GABA_A$Rs in a rat model of TLE. In the present study, we used Clc-2 knockout mice to investigate further the role of Clc-2 and its interaction with tonic GABAergic inhibition in a model of TLE. The results revealed that knockout of Clc-2 decreased tonic seizure protection, latency of clonic seizure, seizure threshold and mortality protection in mice. Clc-2 knockout decreased the action potential $(AP)_{peak}$ and $AP_{threshold}$, Clc-2 currents and $GABA_A$R-mediated tonic inhibition in CA1 pyramidal neurons. Thus, the voltage-gated chloride channel Clc-2, which was functionally upregulated in CA1 pyramidal cells after TLE, may provide protection against TLE by its regulation of action potentials, Clc-2 currents and $GABA_A$Rs in the CA1 region of the hippocampus.

1. Introduction

Temporal lobe epilepsy (TLE), a form of adult focal onset epilepsy, is the most prevalent form of epilepsy and often associated with drug-resistant seizures (Blumcke et al., 2013). It is widely accepted that the abnormal neuronal discharge in patients with epilepsy is a result of potentiation of excitatory and/or failure of inhibitory neurotransmission. Altered function of γ-aminobutyric acid (GABA) receptors, which are the predominant inhibitory neurotransmitter in the mammalian nervous system, may contribute to seizure induction and propagation (Treiman, 2001). Of all of the GABA receptors, $GABA_A$ receptors ($GABA_A$Rs) are thought to be the most important in the induction of TLE (Gonzalez et al., 2015). Ivan and Walker suggested that persistent activation of $GABA_A$Rs could decrease the neuronal voltage response to incoming excitation due to its ability to decrease the membrane input resistance (Pavlov and Walker, 2013). As a result, the neuron is unable to generate an action potential, contributing to seizure induction and propagation (Pavlov and Walker, 2013).

Chloride channel-2 (Clc-2), a member of the Clc family of anion channels, is almost ubiquitously expressed in the human body. Clc-2 is activated upon hyperpolarization, acidic extracellular pH, and osmotic cell swelling (Grunder et al., 1992; Jordt and Jentsch, 1997; Thiemann et al., 1992). Clc-2 channels serve organ- and tissue-specific functional roles, including inhibitory GABA responses in neurons (Niemeyer et al., 2004). In hippocampal pyramidal neurons, the Clc-2 protein is localized to synaptic and perisynaptic regions within GABAergic neurons (Sik et al., 2000). Our previous studies revealed that Clc-2 was functionally upregulated in CA1 pyramidal cells in pilocarpine-treated rats and that an observed increase in Clc-2 currents in CA1 pyramidal cells was reversed by a specific antagonist of α5 subunit-containing $GABA_A$ receptors. These results suggest that Clc-2 contributed to chronically elevated tonic inhibition mediated by α5 subunit-containing $GABA_A$Rs in the CA1 region in experimental TLE rats. However, we could not predict whether the loss of chloride extrusion via Clc-2 would produce an increased susceptibility to seizures by impairing tonic GABAergic inhibition. Hence, in the present study, we used Clc-2 knockout mice to investigate further the role of Clc-2 in experimental TLE and its interaction with tonic GABAergic inhibition. The effects of Clc-2 knock-out on seizure parameters, AP properties in CA1 pyramidal neurons, Clc-2 currents and tonic currents (I_{tonic}) in CA1 pyramidal neurons were studied.

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2. Materials and methods

2.1. Animals

Wildtype (WT) C57BL/6 mice were purchased from the Shanghai animal center. The Clc-2$^{-/-}$ mice were generated as previously described by Nehrke et al. (2002). Animals were housed in the animal center of the Tongji University School of Medicine. The room temperature was kept at 24 ± 1 °C and humidity at 50–60% under a 12:12 light-dark cycle. The animals were allowed access to water and food ad libitum. All of the experiments were approved by the local Animal Care and Use Committee of Tongji University School of Medicine and performed under the guidelines in the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85-23, revised 1996).

2.2. Experimental TLE induction

The induction of experimental TLE was similar to that used in the study by Shafaroodi et al. (2015). Briefly, pentylenetetrazol (PTZ) was intraperitoneally injected (85 mg/kg) in mice, which were later moved to an open field (80 cm in diameter) and monitored for the latency of onset of clonic seizures and the incidence of tonic seizures and death (Loscher et al., 1991a; Moezi et al., 2012). The latency was defined as the time between PTZ injection and the onset of clonic seizures.

2.3. Assessment of seizure threshold

To evaluate the changes in seizure susceptibility caused by Clc-2 knockout, we assessed seizure threshold using the method previously described (Loscher et al., 1991b). Briefly, we inserted a 30-gauge dental needle into the lateral tail vein. The PTZ solution (0.5%) was slowly infused into the tail vein using an infusion pump (Harvard, USA) at a constant rate of 0.5 ml/min. Once forelimb clonus was followed by full clonus of the body, we stopped the pump and recorded the total dose of PTZ given to the mouse as an index of seizure threshold.

2.4. Assessment of AP properties in CA1 py… …rons

Brain slices were prepared after … induction … LE by PTZ (85 mg/kg, i.p.) according to th… meth… f Brown … al. (2011). Briefly, after seizure was obs…ed, mice … sa…ced by cervical dislocation. The brai… as ra…ly rem… thereafter and transferred to a sucrose … d sli… solution (… 4 °C), which was continuously bubble… wit… …gen (95% …, 5% CO_2). After the cerebellum, fronta… d dors… arts w… removed, the sample was mounted o… …tal plate. H… …l sections (300 μm thickness) were … pal… nd submer…d in artificial cerebrospinal fluid, which was also … libirated with carbogen. To measure the AP pro… in CA1 py…idal neurons, single cell patch clamp reco…g was used simila… to the study of Brown et al. (2011). In b…f, slices containing neurons were perfused with carbogen-

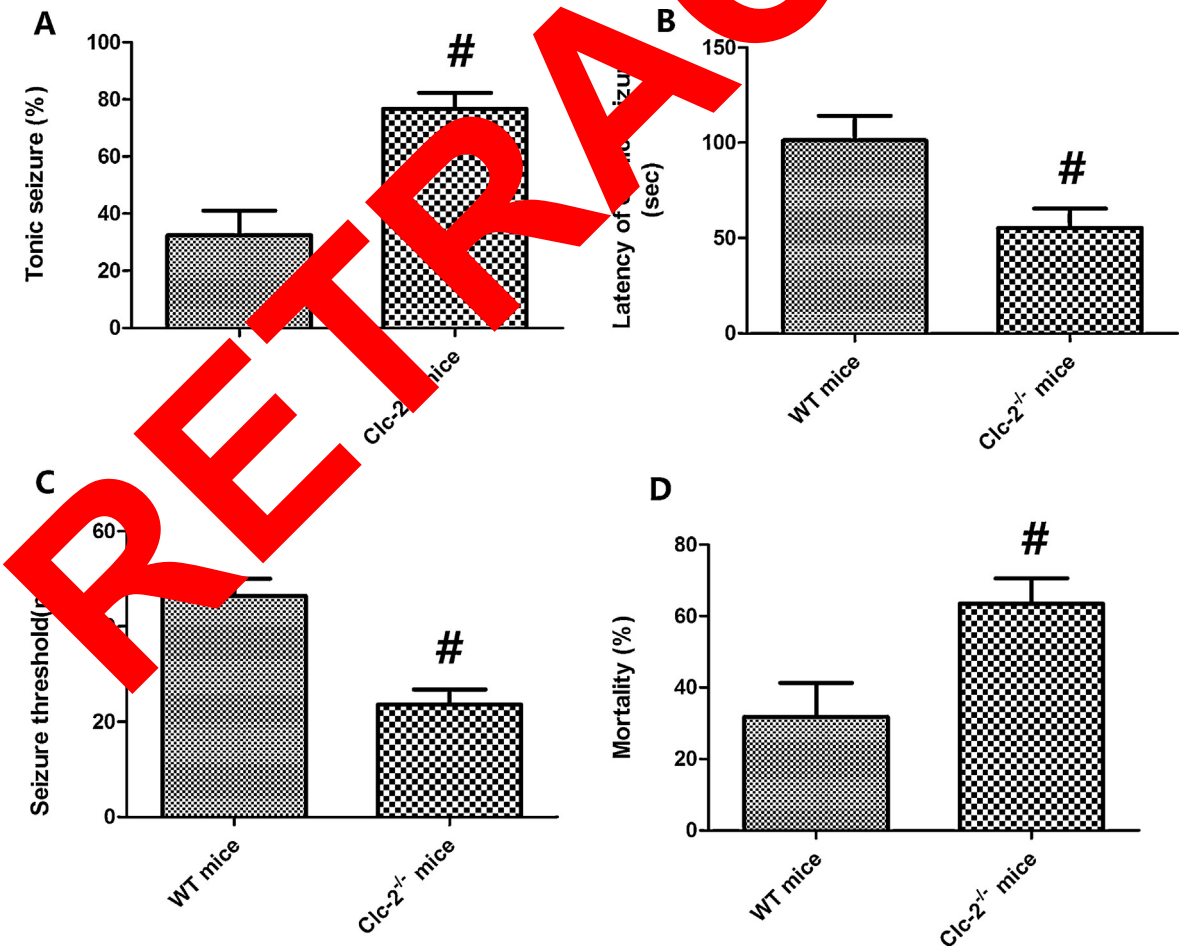

Fig. 1. Effects of Clc-2$^{-/-}$ on seizure parameters. WT: wild type; Clc-2$^{-/-}$: Clc-2 knock-out. Values are expressed as the mean ± SEM. *: $p < 0.05$ compared to WT mice. N = 12 per group.