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Research Report

Blockade of the sodium calcium exchanger exhibits anticonvulsant activity in a pilocarpine model of acute seizures in rats

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ABSTRACT

Recent evidence suggests that the sodium calcium exchanger (NCX) may contribute to the etiology of pentylenetetrazol-induced seizures. Here we further investigated the role of NCX in the etiology of seizures by quantifying the effects of KB-R7943 and SN-6, potent inhibitors of the reverse mode of NCX subtypes 3 (NCX3) and 1 (NCX1), respectively, on the occurrence of acute seizures and status epilepticus induced by intraperitoneal administration of pilocarpine, a muscarinic acetylcholine receptor agonist. Pretreatment with KB-R7943 significantly reduced the incidence of pilocarpine-induced seizures and status epilepticus in 22–56% of treated animals. In the remaining animals that exhibited seizures, KB-R7943 pretreatment delayed the onset of seizures and status epilepticus, and reduced seizure severity. Delayed onset of seizures and reduced seizure severity also were seen following pretreatment with SN-6. These findings suggest that altered NCX activity may contribute to the pathophysiology of pilocarpine-induced seizures and status epilepticus.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by spontaneous recurrent seizures. Approximately 1–2% of the global population suffers from various epileptic syndromes, and about 30% of patients with epilepsy live with uncontrolled seizures (French 2007). Evidence indicates that temporal lobe epilepsy is the most common type of refractory epilepsy in humans (Semah et al., 1998), and therefore, novel antiepileptic treatments based on new underlying mechanisms for temporal lobe seizures are needed. Experimental evidence indicates that altered levels of intracellular Ca^{2+} in hippocampal CA1 neurons play an important role in the underlying mechanisms of neuronal hyperexcitability that leads to pilocarpine-induced seizures, a validated model of temporal lobe epilepsy (DeLorenzo et al. 2005; Turski et al., 1983). The levels of intracellular Ca^{2+} are highly regulated by Ca^{2+} binding proteins and Ca^{2+} extrusion through transporters/exchangers. One transporter/exchanger of interest is the sodium/calcium exchanger (NCX), a bidirectional membrane ion transporter that couples the influx/efflux of Ca^{2+} to the efflux/influx of Na^{+} to regulate levels of intracellular Ca^{2+} in neurons (Blaustein and Lederer, 1999; Annunziato et al., 2004). Under physiological conditions, NCX transports one Ca^{2+} out of the cell and three Na^{+} into the cell. The Ca^{2+} exit is known as the "forward" mode of NCX (Blaustein and Lederer, 1999; Annunziato et al., 2004). However, under certain conditions such as during

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Abbreviations: NMDA, *N*-methyl-D-aspartate; NCX, sodium calcium exchanger; SE, status epilepticus; TRPCs, transient receptor potential channels

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membrane depolarization, the exchanger can reverse function and transport Na^+ out of the cell and Ca^{2+} into the cell. The Ca^{2+} entry represents the "reverse" mode of NCX (Baker and McNaughton, 1976; Annunziato et al., 2004). Three different isoforms of NCX including NCX1, NCX2, and NCX3 have been characterized, cloned, and found in the brain, with multiple splice variants of NCX1 and NCX3 (Kofuji et al., 1994; Papa et al., 2003; Quednau et al., 1997). Despite the fact that reverse mode of NCX allows Ca^{2+} entry into the cell and possibly thereby altering Ca^{2+} homeostatic mechanisms, the role of NCX in the pathophysiology of limbic epilepsy remains poorly understood (Keele et al., 2000; Ketelaars et al. 2004). Nevertheless, a recent study reports that genetic deletion of NCX1 confers resistance to pentylenetrazole-induced tonic flexion, indicative of the important role this NCX isotope has in the etiology of these seizures (Saito et al., 2009). Because dysregulation of Ca^{2+} homeostatic mechanisms is an important feature of limbic epileptogenesis and the reverse mode of NCX contributes to Ca^{2+} influx, we sought to determine the extent to which inhibition of the reverse mode of NCX may affect acute pilocarpine-induced seizures and status epilepticus (SE) in rats.

2. Results

The incidence of pilocarpine-induced motor limbic seizures and SE were first evaluated using two doses of intraperitoneally (i.p.) administered pilocarpine (280 and 380 mg/kg). Although no difference was found in seizure severity between the two groups (280 mg/kg: 4.7±0.3, $n=11$; 380 mg/kg: 5±0, $n=9$; $H=0.8$, $P=0.4$), the onset of motor limbic seizures and SE was significantly delayed in the 280 mg/kg group (motor limbic seizures: 19±3 min, $n=10$, $F=7.4$, $P=0.01$; SE: 30±1 min, $n=10$, $F=10.4$, $P=0.005$) compared to the 380 mg/kg group (motor limbic seizures: 10±1 min, $n=9$; SE: 23±2 min, $n=9$). Furthermore, higher mortality rates were found in the 380 mg/kg group (9/9) compared to those the 280 mg/kg group (1/11; Chi-square: 163, $P=0.0001$). Therefore, we used the 280 mg/kg dose of pilocarpine for pharmacological studies.

Orally administered (p.o.) pretreatment with KB-R7943 at the doses of 10, 30, and 100 mg/kg significantly suppressed the occurrence of motor limbic seizures in 22% (2/9, Chi-square=5.5; $P=0.02$), 22% (2/9, Chi-square=5.5; $P=0.02$), and 33% (3/9, Chi-square=17; $P=0.0001$) of tested animals, respectively, compared to 91% (10/11) in the control group (Fig. 1A). In the remaining animals that exhibited seizures, KB-R7943 pretreatment significantly ($F=4$, $P=0.02$) delayed the onset of motor limbic seizures. This effect was observed at all doses tested (10 mg/kg: 29±3 min, $n=7$; 30 mg/kg: 31±3 min, $n=7$; 100 mg/kg: 33±4 min, $n=6$; $P<0.05$, Fig. 1B) as compared to the control group (19±2 min, $n=10$; Fig. 1B). Pretreatment with KB-R7943 also significantly reduced the severity of pilocarpine-induced seizures ($H=16$, $P=0.001$); this effect was observed with both 30 mg/kg (3.3±0.3, $n=7$, $P<0.05$; Fig. 1C) and 100 mg/kg (2.8±0.3, $n=5$, $P<0.05$; Fig. 1C) compared to the control group (4.7±0.3, $n=11$). Similarly, pretreatment with SN-6 (10 mg/kg; p.o.) also reduced the severity of pilocarpine-induced seizures (control group: 4.7±0.3, $n=11$; SN-6: 2.8±0.6, $n=5$; $H=7$, $P=0.01$) and delayed the onset of motor limbic seizures (control group: 19±2 min, $n=11$; SN-6: 34±3 min, $n=5$; $F=10$, $P=0.01$). However, SN-6 (10 mg/kg) pretreatment did not affect the incidence of motor limbic seizures (5/6 compared to the control group 11/12).

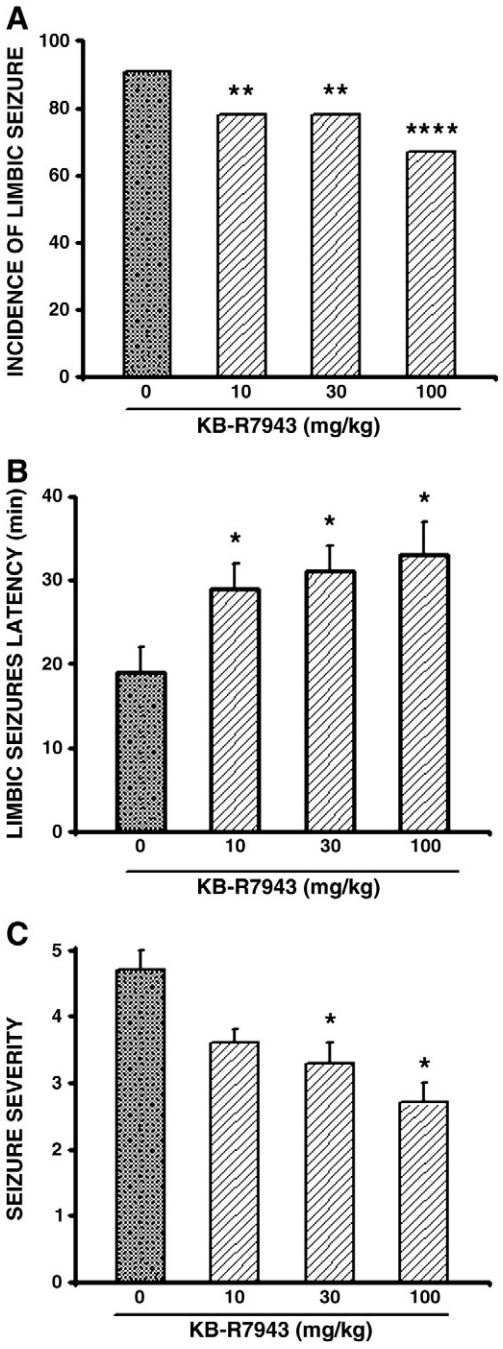

Fig. 1 – KB-R7943 pretreatment alters the expression of pilocarpine-induced seizures. A. KB-R7943 pretreatment (10, 30, or 100 mg/kg; p.o.) reduced the incidence of motor limbic seizures. B. KB-R7943 pretreatment (10, 30, or 100 mg/kg; p.o.) delayed the onset of motor limbic seizures. C. KB-R7943 pretreatment (10 or 30 mg/kg; p.o.) reduced the severity of pilocarpine-induced seizures. Data represent mean±S.E.M. *$P<0.05$, **$P<0.01$, ****$P<0.0001$.

In addition to limbic seizures, pilocarpine can also trigger myoclonic seizures reminiscent of brainstem bouncing seizures. Such seizures were seen in 9 of 11 (82%) of tested animals