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SHORT COMMUNICATION

Pentylenetetrazol-induced seizures are associated with Na^+,K^+-ATPase activity decrease and alpha subunit phosphorylation state in the mice cerebral cortex

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Summary The present study aimed to investigate whether Na^+,K^+-ATPase activity and phosphorylation state of the catalytic α subunit are altered by pentylenetetrazol (PTZ)-induced seizures. PTZ (30, 45 or 60 g/kg, i.p.) was administered to adult male Swiss mice, and Na^+,K^+-ATPase activity and phosphorylation state were measured in the cerebral cortex 15 min after PTZ administration. Na^+,K^+-ATPase activity significantly decreased after PTZ-induced seizures (60 mg/kg). Immunoreactivity of phosphorylated Ser943 at α subunit was increased after PTZ-induced seizures. A significant positive correlation between Na^+,K^+-ATPase activity and latency to myoclonic jerks and generalized seizures was found. Conversely, a strong negative correlation between Ser943 phosphorylation and latency to generalized seizures was detected. Given the role of Na^+,K^+-ATPase as a major regulator of brain excitability, Ser943 at Na^+,K^+-ATPase α subunit may represent a potentially valuable new target for drug development for seizure disorders.

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Introduction

Na^+,K^+-ATPase (EC 3.6.3.9) is a plasma membrane protein which is expressed in virtually all living cells (Skou and Esmann, 1992). By driving sodium export and potassium import across the plasma membrane, Na^+,K^+-ATPase plays a key role in the maintenance and regulation of electrolyte homeostasis in both intracellular and extracellular environments (Skou and Esmann, 1992).

In the mammalian central nervous system, Na^+,K^+-ATPase activity significantly accounts for the maintenance of the electrochemical gradient across the plasma membrane underlying resting and action potentials as well as neurotransmitter release and uptake (Moseley et al., 2007). Accordingly, a decrease of Na^+,K^+-ATPase activity critically affects neurotransmitter signaling, neural activity, as well as animal behavior (Moseley et al., 2007). In addition, it has been suggested that Na^+,K^+-ATPase plays a role in several neurological disorders, including seizure activity and epilepsy (Aperia, 2007; Benarroch, 2011). In this context, ouabain elicits electrographically-recorded seizures in rats (Bignami and Palladini, 1966) and mutations in the Na^+,K^+-ATPase α subunit gene have been associated with epilepsy in mice (Clapcote et al., 2009) and humans (Deprez et al., 2008; Poulsen et al., 2010a). These studies are, to some degree, in agreement with those that have shown that prostaglandin E_2 decreases Na^+,K^+-ATPase activity (Oliveira et al., 2009), increases phosphorylated Na^+,K^+-ATPase α subunit Ser943 immunoreactivity (Oliveira et al., 2009) and facilitates PTZ-induced seizures (Oliveira et al., 2008). However, it is not clear whether alterations in the phosphorylation state of Ser943 are associated to seizures, in an individual basis. Therefore, considering that protein phosphorylation is an important mechanism of Na^+,K^+-ATPase activity control (Poulsen et al., 2010b, 2012), and the emerging role of Na^+,K^+-ATPase as a putative therapeutic target for selected neurological disorders (Aperia, 2007; Benarroch, 2011), the present study aimed to investigate whether the phosphorylation state of the catalytic α subunit is altered by pentylenetetrazol (PTZ), a classical convulsant agent that has been successfully used in the study of seizure mechanisms and screening of anticonvulsant drugs.

Methods

Adult male Swiss mice were used. All animal experimentation reported in this study has been conducted in accordance with national and international legislation and with the approval of the Institutional Committee on Animal Use and Care of the Federal University of Santa Maria (process #51/2010). Detail of the animals and reagents used, seizure evaluation, Na^+,K^+-ATPase activity measurements, immunodetection of Na^+,K^+-ATPase α subunit and statistical analyses are described in the Supplemental Methods section.

Seizure evaluation

Animals were injected intraperitoneally with PTZ (30, 45 or 60 mg/kg) or its vehicle (0.9% NaCl), and were monitored for 15 min for the occurrence of behavioral seizures. During the 15 min observation period, the latency to myoclonic jerks and generalized tonic-clonic seizures was recorded.

Neurochemical analyses

Immediately after seizure evaluation (15 min after PTZ administration) the animals were killed by decapitation and the cerebral cortex was rapidly dissected on an inverted Petri dish placed on ice, and gently homogenized in an appropriated solution for determination of Na^+,K^+-ATPase activity or immunodetection of Na^+,K^+-ATPase α subunit as described in detail by Oliveira et al. (2009).

Results

Administration of PTZ (45 and 60 mg/kg) caused the appearance of myoclonic jerks (Fig. 1A), but only 60 mg/kg PTZ elicited generalized tonic-clonic seizures (Fig. 1B). The administration of PTZ (60 mg/kg) decreased Na^+,K^+-ATPase activity by 37.65% in the cerebral cortex [$F(3,40) = 2.932$; $P < 0.05$; Fig. 1C]. Interestingly, Pearson correlation analyses revealed a strong positive correlation between Na^+,K^+-ATPase activity in the cerebral cortex and the latency to PTZ-induced myoclonic jerks ($r = 0.7425$; $P < 0.01$ — Fig. 1D) or generalized tonic-clonic ($r = 0.7799$; $P < 0.005$ — Fig. 1E) seizures.

In order to investigate whether the PTZ-induced decrease of Na^+,K^+-ATPase activity was due to a decrease in the levels of available enzyme molecules, we measured the total Na^+,K^+-ATPase α subunit immunoreactivity in the samples. Statistical analysis revealed that PTZ did not alter total α subunit immunoreactivity [$t(7) = 0.1715$; $P > 0.05$] (Fig. 2A), indicating that the content of the catalytic α subunit is similar between PTZ-treated and control animals.

Since Na^+,K^+-ATPase activity is regulated by phosphorylation of the α subunit at Ser943 (Poulsen et al., 2010b), we investigated whether PTZ-induced seizures are accompanied by changes in the phosphorylation state of this residue. PTZ administration significantly increased Ser943 phosphorylation by 82.5% [$t(10) = 2.616$; $P < 0.05$] (Fig. 2B). Pearson correlation analysis revealed a strong negative correlation between Ser943 phosphorylation and latency to generalized tonic-clonic seizures induced by PTZ (Pearson $r = -0.88$; $P < 0.005$ — Fig. 2C) in an individual basis.

Discussion

Na^+,K^+-ATPase is a major regulator of brain excitability. Accordingly, current evidence supports that decreased Na^+,K^+-ATPase enhances neuronal excitability and facilitate convulsions. In fact, ouabain increases the frequency of spontaneous postsynaptic potentials and decreases the amplitude and duration of CA3 pyramidal cell afterhyperpolarizations in hippocampal slices (Haglund and Schwartzkroin, 1990), and blocks an outward current which is critical to neuron hyperpolarization between bursts of action potentials (Johnson et al., 1992). Moreover, ouabain induces electrographically-recorded seizures in rats (Bignami and Palladini, 1966), and a point mutation in the Na^+,K^+-ATPase $\alpha 3$ isoform results in a phenotype which is