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Research Report

Early life LiCl-pilocarpine-induced *status epilepticus* reduces acutely hippocampal glutamate uptake and Na^+/K^+ ATPase activity

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ABSTRACT

Status epilepticus-induced hippocampal neuronal loss is mainly associated with excitotoxicity induced by increased levels of extracellular glutamate which is normally neutralized by high-affinity uptake mechanism. The energy source for the glutamate uptake is the electrochemical Na^+ gradient maintained by Na^+/K^+ ATPase pump. In this study, we investigated the effect of early-life-induced *status epilepticus* on hippocampal Na^+/K^+ ATPase activity and glutamate uptake. Rat pups 15 days old were injected i.p. with LiCl (3 mEq/kg) 12–18 h prior to s.c. pilocarpine administration (60 mg/kg). Hippocampal Na^+/K^+ ATPase activity and glutamate uptake were evaluated 1.5, 12 and 24 h after SE induction. LiCl-pilocarpine-induced SE decreased Na^+/K^+ ATPase activity and glutamate uptake by 42 and 38%, respectively, 1.5 h after SE induction. However, 12 and 24 h after SE induction the pump activity and glutamate uptake returned to control levels. SE early in life increased hippocampal number of degenerating neurons in the CA1 subfield and dentate gyrus 24 h after SE induction. In conclusion, SE induced early in life causes short-term disruption in hippocampal Na^+/K^+ ATPase activity and glutamate uptake, which may be related to neuronal death found in CA1 subfield.

1. Introduction

Epilepsy is a common neurological disorder that occurs more frequently in children than adults. Prolonged seizure activity, i.e., *status epilepticus* (SE), or repeated, brief seizures affect neuronal structure and function in the developing nervous system which may lead to short-term brain damage and long-term behavioral alterations (Holopainen, 2008). One of the earliest brain consequences of SE early in life is the selective neuronal loss observed in specific brain areas. Rats with LiCl-pilocarpine-induced SE during the second week of life presented damage in the hippocampus, amygdala, thalamus, and septum as well as an elevation in the serum levels of neuron specific enolase (NSE) 24 h after SE induction (Sankar et al., 1997). Further analysis of hippocampal CA1 pyramidal cells showed DNA fragmentation (TUNEL analysis) and apoptotic bodies in electron micrographs of damaged neurons (Sankar et al., 1998).

The SE-induced neuronal loss is normally associated with overstimulation of the glutamatergic system due to elevated levels of glutamate in the synaptic cleft and excessive activation of ionotropic glutamate receptors (Clifford et al.,

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1990; Fujikawa, 1995). The excess of extracellular glutamate is normally neutralized by high-affinity uptake mechanism executed by a family of glutamate transporter proteins: GLAST/EAAT1, GLT1/EAAT2, EAAC1/EAAT3, EAAT4 and EAAT5 (Gether et al., 2006). Studies in humans and transgenic mice indicate that alterations in glutamate transporters can lead to epileptic phenotypes. "Knock-out" mice lacking astroglial transporter GLT-1 exhibit lethal spontaneous seizures (Tanaka et al., 1997). Intracerebroventricular administration of antisense oligonucleotide to EAAC1 produced epilepsy, characterized initially by facial twitches and freezing behavior that began 3–5 days of treatment. By 7 days, the animals showed tonic forepaws extension and clonic seizures (Rothstein et al., 1996).

The energy source for the glutamate uptake is the electrochemical Na^+ gradient maintained by Na^+/K^+ ATPase (adenosinetriphosphatase – EC 3.6.1.3) (Danbolt, 2001). Na^+/K^+ ATPase is a plasma membrane-embedded enzyme responsible for the active transport of sodium and potassium ions in all animal cells. It is present in higher concentrations in the brain and other nervous tissue, where it plays several roles in the complex and finely tuned control of the ionic environment which underlies nerve activity (Sweadner, 1992). Malfunction of Na^+/K^+ ATPase has been associated a several CNS disorders, including epilepsy (Rapport et al., 1975). Ouabain, a reversible inhibitor of the Na^+/K^+ ATPase pump, induces focal spikes discharges as well as contralateral focal seizures when applied locally on the cortical surface of rats (Lewin, 1971). Adult animals submitted to pilocapine-induced SE showed a reduction of hippocampal pump activity 1 h after SE induction and during silent period. However, during chronic phase, the enzyme activity increased up to 30% of control levels (Fernandes et al., 1996).

Because of the importance of Na^+/K^+ ATPase in the control of neuronal excitability as well as in the maintenance of Na^+ gradient to clearance of extracellular glutamate, the aim of this study was to investigate hippocampal Na^+/K^+ ATPase activity and glutamate uptake in animals submitted to LiCl-pilocarpine-induced SE early in life.

2. Results

2.1. *Status epilepticus*

The behavioral pattern of SE correlated well with our previous study (de Oliveira et al., 2008) and was similar to the original description from Hirsch et al. (1992). Systemic administration of LiCl-pilocarpine produced defecation, salivation, body tremor, and scratching within 5–20 min. This behavioral pattern progressed within 30–45 min to increased levels of motor activity and culminated in SE in all animals. SE was characterized by sustained orofacial automatisms, salivation, chewing, forelimb clonus, loss of the righting reflex and falling. The mortality rate 24 h after the SE induction was 20%.

2.2. *Na^+/K^+ ATPase activity*

LiCl-pilocarpine-induced SE decreased Na^+/K^+ ATPase activity by 42% in hippocampal plasma membranes 1.5 h after SE induction when compared with control group (Fig. 1). However, 12 and 24 h after SE induction the activity of Na^+/K^+ ATPase returned to control levels. Application of 100 μM of pilocarpine *in vitro* did not alter the activity of Na^+/K^+ ATPase from hippocampal membranes (control: 1331±314.2 nmol Pi/min.mg protein; pilocarpine: 1339±264.8 nmol Pi/min.mg protein; data not shown).

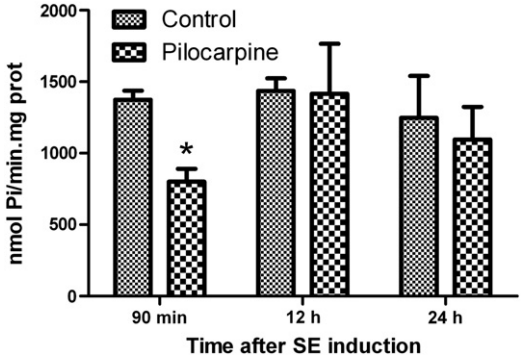

Fig. 1 – Hippocampal Na^+/K^+ ATPase activity in control and LiCl-pilocarpine-induced SE rats. Animals were sacrificed by decapitation 1.5 ($n=6$ control and 7 treated), 12 ($n=7$ control and 6 treated) and 24 h ($n=6$ control and 5 treated) after the SE induction. The data were expressed as mean±SD. * indicates $p<0.01$ as compared with the control group (one-way ANOVA followed by the Tukey´s post hoc test).

2.3. *Glutamate uptake*

When *ex vivo* hippocampal slices were incubated in lower concentration of glutamate (1 μM), LiCl-pilocarpine treated animals showed decreased levels of glutamate uptake 1.5 h after SE induction (Fig. 2). However, LiCl-pilocarpine-induced SE was not able to alter the glutamate uptake 12 and 24 h after SE induction. At a higher concentration of extracellular glutamate (100 μM), no alterations were observed in glutamate uptake levels between control and LiCl-pilocarpine-induced SE groups in all times tested.

2.4. *Degeneration of hippocampal neurons*

In the control group, a small number of Fluoro-Jade B-positive neurons were found in hippocampal CA1 subfield. However, in the LiCl-pilocarpine group, an increased number of labeled neurons were found in the stratum pyramidale (S. PYR) from CA1 subfield (Fig. 3).

3. Discussion

Pediatric neurologists have repeatedly described neuronal damage in several brain areas with subsequent cognitive impairment and development of epilepsy after SE in children. However cellular and molecular mechanisms involved in this damage are still under investigation. In order to contribute to this question, in the present study we investigated the effect of early-life LiCl-pilocarpine-induced SE on hippocampal glutamate uptake and Na^+/K^+ ATPase activity.