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Research Report

Ketamine reduces neuronal degeneration and anxiety levels when administered during early life-induced status epilepticus in rats

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ABSTRACT

Status epilepticus (SE) when occurred during brain development can cause short- and long-term consequences, which are frequently associated with NMDA-mediated glutamatergic excitotoxicity. In the present work, we investigated the putative neuroprotective role of ketamine, an NMDA receptor antagonist, on early life SE-induced acute neuronal death and long-term behavioral abnormalities. Male Wistar rats (16 postnatal days) were induced to SE by LiCl–pilocarpine i.p. administration (3 mEq/kg; 60 mg/kg, respectively). Fifteen or 60 min after pilocarpine injection, animals received a ketamine administration (22.5 mg/kg i.p.). Neuronal degeneration was assessed 24 h after SE induction. Another subset of animals was destined to behavioral tasks in adulthood (75–80 postnatal days). Fluoro-Jade C labeling revealed a marked neuronal death on CA1 hippocampal subfield, habenula, thalamus and amygdala in SE animals. Ketamine post-SE onset treatment prevented neuronal death in all regions assessed. In the elevated plus maze, SE induced an increase in anxiety-like behaviors whereas ketamine administration during seizures was able to prevent this alteration. Ketamine administration in non-SE animals resulted in high anxiety levels. There were no observed differences among groups in the open field task in all parameters analyzed. Our results suggest that ketamine post-SE onset treatment was effective in preventing acute and long-standing alterations caused by SE early in life, which indicates a putative role of glutamatergic system on SE-induced brain damage as well as long-lasting behavioral consequences.

1. Introduction

Status epilepticus (SE) is a life-threatening neurological disorder defined as a seizure or repeated seizures lasting more than 30 min (Chen and Wasterlain, 2006) and its incidence is higher during infancy and childhood (Gross-Tsur and Shinnar, 1993; Holmes, 1997). Previous studies using animals models have reported that prolonged epileptic activity, when occurred during

Abbreviations: CTRL, control; EPM, elevated plus maze; FJC, Fluoro-Jade C; i.p., intraperitoneal; KET, ketamine treated group; NMDAR, *N*-methyl-D-aspartate receptor; PND, postnatal day; POm, posteromedial thalamic nucleus; SE, status epilepticus; SE+KET15, status epilepticus plus ketamine at 15 min; SE+KET60, status epilepticus plus ketamine at 60 min; VPM, ventral posteromedial thalamic nucleus

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central nervous system development, can cause short- and long-term consequences (de Oliveira et al., 2008; Fujikawa, 1995; Kubova et al., 2004; Rice et al., 1998; Sankar et al., 1998).

One of the initial consequences of SE on the developing brain is a rapid neuronal cell death observed in specific areas. Rats submitted to LiCl–pilocarpine-induced SE during the first three weeks of life presented an intense neuronal loss in hippocampus, amygdala, thalamus and temporal cortical regions (such as perirhinal and entorhinal cortices) (de Oliveira et al., 2008; Kubova et al., 2004; Sankar et al., 1998). SE-induced nerve cell damage was considered to occur through both necrosis and apoptosis, whereas eosinophilic cells and nuclear fragmentation in TUNEL staining was observed in SE-submitted animals (Kubova et al., 2004; Sankar et al., 1998).

In addition to the acute neuronal death, early life-induced SE can cause long-standing structural and functional changes in the brain. Young rats (until 3 weeks old) submitted to SE presented a severe memory impairment in several tasks such as inhibitory avoidance and water maze at adulthood (de Oliveira et al., 2008; Hoffmann et al., 2004; Sayin et al., 2004). Moreover, animals also displayed alterations in their emotional behavior, which was characterized by higher levels of anxiety when exposed to the light–dark box and elevated plus maze (de Oliveira et al., 2008; Sayin et al., 2004).

SE-induced neuronal degeneration has been frequently associated with an excessive activation of NMDA ionotropic glutamate receptors (NMDAR) (Holopainen, 2008) and previous studies have demonstrated that pretreatment with NMDAR antagonists is neuroprotective against SE-induced neuronal death (Clifford et al., 1990; Fujikawa, 1995; Holmes, 2004). However, despite the treatment of patients with SE started after onset of seizures, there are no studies investigating the effects of NMDAR blockage during SE. Thus, it becomes important to know the effectiveness of post-SE onset treatments with NMDAR antagonists in order to avoid the short- and long-lasting alterations induced by SE.

Therefore, the aim of this study was to investigate the putative protective action of a post-SE onset treatment with ketamine, a non-competitive NMDAR antagonist, on SE-induced neuronal death as well as on long-term behavioral alterations in animals submitted to SE early in life.

2. Results

2.1. *Effect of ketamine on LiCl–pilocarpine-induced SE*

The convulsive pattern presented by LiCl–pilocarpine-treated animals was similar to that described by de Oliveira et al. (2008). Systemic administration of LiCl–pilocarpine produced defecation, salivation, body tremor, and scratching within 2 to 8 min. This behavioral pattern progressed within 8 to 13 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 righting reflex and falling.

Animals treated with ketamine after SE onset presented a distinct behavioral pattern of seizures when compared with LiCl–pilocarpine rats. Five minutes after antagonist administration, both groups that received ketamine at 15 min (SE+KET15) or at 60 min (SE+KET60) showed a reduction in the intensity of sustained orofacial automatisms, forelimbs clonus and chewing, without recovery of the loss of righting reflex. The SE-induced motor activity was stopped only 70 min after SE onset for both ketamine-treated groups. Ketamine when administered at doses higher than 45 mg/kg, caused death in all SE-induced animals (data not shown). Animals from ketamine group (KET) showed symptoms of anesthesia, such as sedation, 2 min after ketamine administration and were recovered 45 min later.

2.2. *Effect of ketamine on SE-induced neurodegeneration*

By using Fluoro-Jade C (FJC) staining in brain sections, a large number of degenerative neuronal cells were observed in brains from SE group (Fig. 1). The FJC-positive staining cells showed a bright green color in the somas and fine processes with neuronal profiles (Fig. 1 inserts). LiCl–pilocarpine administration induced a massive neurodegeneration in several brain regions, including CA1 hippocampal subfield, habenula (lateral habenular nucleus), thalamus (ventral posteromedial thalamic nucleus) and amygdala (medial amygdaloid nucleus) 24 h after SE onset (Fig. 1). Both ketamine post-SE onset treated groups presented a significant reduction in the number of FJC-positive neurons (85–100%) in all brain regions analyzed (Table 1). FJC-positive neurons were not observed in brain regions from control (CTRL) and KET groups.

2.3. *Effect of SE and ketamine on locomotor and exploratory activities*

The pattern of distance traveled, and number of animals rearing and grooming across time were similar in all groups (Fig. 2A–C). All animals showed intra-session habituation to apparatus approximately 7 min after the starting of the session. There were no differences in other parameters of locomotor and exploratory activities, temporal organization and spatial distribution in all groups (Fig. 2D–F and supplementary Fig. S1 A–F). Moreover, all groups showed a similar pattern of inter-session habituation of the distance traveled, and number of animals rearing and grooming during the three days of testing (data not shown).

2.4. *Effect of SE and ketamine on anxiety-like behaviors*

Animals from SE and KET groups spent significantly low time in open arms (62.9±16.8 and 40.1±6.9, respectively; $F=6.626$; $p=0.0004$) when compared to the CTRL group (150.1±10.3) (Fig. 3A). Ketamine post-SE onset treatment in both times (SE+KET15 and SE+KET60) increased the time spent in open arms (115.9±15.3 and 101.5±19.2, respectively), however these values were not different from both CTRL and SE groups. SE+KET15 and SE+KET60 groups, when compared with only KET, spent more time in open arms. The number of risk assessment behaviors was significantly increased in the KET group (7.3±1.4) when compared to the CTRL and SE+KET60 groups (2.8±0.6 and 2.6±0.6, respectively) (Fig. 3B; $F=4.679$; $p=0.0038$). Animals from SE (7.0±1.4), SE+KET15 (4.1±0.9), SE+KET60 and CTRL groups presented similar levels of risk assessment behaviors. All groups presented similar number of