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INTERLEUKIN-1 RECEPTOR IS A TARGET FOR ADJUNCTIVE CONTROL OF DIAZEPAM-REFRACTORY STATUS EPILEPTICUS IN MICE

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Abstract—Proinflammatory cytokine interleukin-1 beta (IL-1β) may accumulate in the brain during status epilepticus, but whether it contributes to the progressive refractoriness of SE remains unclear. By using a kainic acid-induced SE mice model, we tested whether pharmacological blockade or knock-out of interleukin-1 receptor type 1 (IL-1R1) could influence the diazepam-refractory phenomenon of prolonged SE. We confirmed diazepam failed to terminate prolonged SE (allowed to continue for 40 min before diazepam administration). The expression level of IL-1β in the hippocampus during prolonged SE was significantly higher than that of baseline. Interestingly, prolonged SE was not diazepam-refractory in IL-1R1 knock-out mice. Moreover, administration of interleukin-1 receptor antagonist (IL-1RA) combined with diazepam terminated established prolonged SE, while IL-1RA alone is not capable to terminate prolonged SE. On the contrary, administration of recombinant human IL-1β weakens the efficacy of diazepam by prolonging its latency to terminate non-prolonged SE. Thus, the present study provides direct evidence that accumulated IL-1β contributed to the diazepam refractoriness of prolonged SE, and suggests that interleukin-1 receptor is a target for adjunctive control of diazepam-refractory SE.

Key words: status epilepticus, neuroinflammation, epilepsy, diazepam, interleukin-1 receptor, interleukin-1 beta.

INTRODUCTION

Status epilepticus (SE) is a life-threatening neurological emergency, characterized by continuous or intermittent seizures without full recovery between seizures (Betjemann et al., 2015). Refractory SE is defined by the failure of adequate amounts of two intravenous drugs to stop seizures, which occurs in about one-third of patients (Chen and Wasterlain, 2006). Those SEs without timely termination may result in later neuronal damage, cognitive alterations and epileptogenesis (Sadarangani et al., 2008; Ferlisi and Shorvon, 2012; Ferro et al., 2014). By convention, first-line antiepileptic drugs (ADEs) of convulsive SE are benzodiazepines, such as diazepam (DZP). DZP refractory/resistant SE refers to SE that cannot be terminated following the rational intravenous DZP, which was usually also resistant to other parenteral benzodiazepines and high possibly resistant to a second or third line of ADEs (Treiman et al., 1998; Chen and Wasterlain, 2006). DZP (or benzodiazepine) refractory SE seems dependent on the duration of SE, and it occurs especially when SE was prolonged (typically 0.5–1 h) in both patients and rodents (Loscher, 2009; Prasad et al., 2014). A recent prospective observational cohort study found that the time elapsed from SE onset to the first benzodiazepine administration is delayed with a median time of 30 min (Sanchez Fernandez et al., 2015). DZP refractory SE may be further controlled using other classes of AEDs and even general anesthesia (Shorvon, 2011). However, most of these drugs may lead to severe side-effects and complications (Bell, 1969; Sutter et al., 2014). Therefore, it is urgent to develop new therapeutic strategies for SE, especially for the benzodiazepine refractory prolonged SE.

Although several potential mechanisms for benzodiazepine refractory SE have been previously reported, such as internalization of GABAergic receptors (Naylor et al., 2005) and increase in membranous AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) as well as NMDA (N-methyl-D-aspartic acid) receptors during SE (Wasterlain and Chen, 2008), the exact mechanisms underlying the DZP refractoriness of SE remains obscure. Recently, increasing evidence supports that the neural inflammations may be a crucial factor promoting the refractoriness of SE. For example, Spatola et al. found that inflammatory SE was more often refractory to initial antiepileptic treatment (Spatola et al., 2015). Besides, Gaspard et al, reported autoimmune encephalitis is the most commonly identified cause of new-onset refractory SE (Gaspard et al., 2015). Moreover, purinergic P2X7 receptor

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Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ANOVA, analysis of variance; EEG, electroencephalogram; DZP, diazepam; GABA, gamma-aminobutyric acid; IL-1β, interleukin-1 beta; IL-1R1, interleukin-1 receptor type 1; IL-1RA, interleukin-1 receptor antagonist; i.p., intraperitoneally; KA, kainic acid; KO, knock-out; NMDA, N-methyl-D-aspartic acid; SE, status epilepticus; WT, wild-type.

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antagonists can enhance the inhibitory effect of DZP on SE (Jimenez-Pacheco et al., 2013), which may also act anti-inflammatory effects (Kim et al., 2010). Thus, these results lead us to consider the neuroinflammatory mechanism of refractory SE, which is still little studied.

Interleukin-1 beta (IL-1β) is a seizure-related proinflammatory mediator, which may be accumulated in the brain during SE (Rijkers et al., 2009; Vezzani et al., 2011). Whether the IL-1β contributes to the pharmacological refractoriness of SE remains unclear. It has been reported that the mRNA of IL-1β highly increases at 7.5 h after the SE, suggesting that the induction of IL-1β by SE may not develop rapidly enough to explain time dependent resistance of SE to AEDs (Kuteykin-Teplyakov et al., 2009). However, recently, Librizzi et al. found that the IL-1β induced by the proceeding seizure is sufficient to exacerbate the seizures resurrected within a short time delay in the *in vitro* isolated guinea-pig brain (Librizzi et al., 2012). We also found interleukin-1 receptor antagonist (IL-1RA) enhances postictal suppression of kindled seizures in wild-type but not IL-1R1 knock-out (KO) mice (Tao et al., 2015). Athough these results were not in a SE condition, they at least suggest the increase of endogenic IL-1β or its receptor may be rapidly enough even during a self-limited seizure.

Therefore, we hypothesis that IL-1β may be accumulated during SE and contribute to the DZP-refractory phenomenon of prolonged SE. Using a DZP-refractory prolonged SE mice model induced by kainic acid (KA), we tested whether pharmacological blockade or KO of IL-1R1 could reverse the time-dependent DZP-refractory phenomenon of prolonged SE.

EXPERIMENTAL PROCEDURES

Animals

Age-matched wild-type (WT) C57BL/6 mice and IL-1R1-KO mice (generated on a C57BL/6 background, from Jackson Laboratory, stock number: 003245) were used in this study. All animals (aged 10–12 weeks) were maintained in individual cages with a 12-h light/dark cycle (lights on from 8:00 to 20:00). Water and food were given *ad libitum*. Experiments were conducted between 10:00 and 17:00. All experiments were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgery

The protocol of surgery is similar to our previous study (Xu et al., 2010; Tao et al., 2015). Briefly, under anesthesia by sodium pentobarbital (60 mg/kg i.p., sigma), mice were fixed in a stereotaxic apparatus (512600, Stoelting, USA). A bipolar electrode was adhered to a guide cannula (62003, RWD Life Science, China) with 0.5–1 mm beyond the tip of the guide cannula and implanted into the right ventral hippocampus (AP: −2.9, L: −3.0, V: −3.0) based on the atlas of mouse brain (Paxinos and Franklin, 2001), and another guide cannula was stereotaxically implanted into the lateral ventricle (AP: −1.0, L: −1.5, V: −2). The electrodes were made of twisted stainless steel teflon-coated wires (791500, A.M. Systems, USA) insulated except at the tip 0.5 mm and the maximal tip separation was about 0.5 mm. In addition, a ground screw was fixed above the prefrontal cortex (AP: +2, L: −1.5) and a referent screw above the cerebellar cortex (AP: −4.5, L: 0). Location of electrodes and cannulas was finally histologically verified.

SE model

After a period of 5–10 min of baseline electroencephalogram (EEG) recordings, KA (0.5 μg in 0.2-μl phosphate-buffered saline; Sigma) were injected into the ventral hippocampus by a 30-gauge needle inserted through the guide cannula. SE was allowed to continue for 10 min before DZP administration to induce a non-prolonged SE, and 40 min before DZP administration to induce a prolonged SE.

Drug administration

DZP (Kingyork, Tianjin, China) was administered intraperitoneally (i.p.) at 1, 10 and 20 mg/kg as previous study (Hanada et al., 2014). We found high dose of DZP (20 mg/kg) terminated prolonged SE in 4 out of 4 mice; DZP (10 mg/kg) can terminate prolonged SE in 3 out of 5 mice; and DZP (1 mg/kg) terminated prolonged SE in 0 out of 5 mice. In addition, high dose of diazapam may result in high risks of respiratory depression and other adverse reaction than low dose of diazapam, although it may terminate the prolonged SE. So, we finally used DZP at 1 mg/kg in our following experiments. Recombinant human IL-1β (10 ng in 1 μl, Prospec-Tany Techno Gene) or recombinant human IL-1RA (100 ng in 1 μl, Prospec-Tany Techno Gene.) was injected by a 30-gauge needle inserted through the guide cannula into the lateral ventricle as previous studies (Tao et al., 2015). The injection duration for IL-1β or IL-1RA was 5 min and the needle was remained in the position for another 5 min before retraction. For co-administration, IL-1β or IL-1RA was injected about 1 min after the injection of DZP.

EEG recording and analysis

Raw EEG signals were recorded with band-pass filters spanning 0.5–200 Hz and sampled at 1000 Hz with a digital amplifier (NuAmps, Neuroscan System, USA) similar to our previous studies (Xu et al., 2013). Recorded EEGs were offline analyzed by Scan 4.5. The entire EEG time series were direct-current shifted, digitally band-pass filtered from 0.5 to 100 Hz. Finally, the EEG data were imported into Labchart 7.0 software, where the power spectrum was calculated using fast Fourier transform with a Hanning window. Ictal discharge was defined as high amplitude (more than twofold of baseline discharges) discharges with frequency greater than 3 Hz. The SE onset was defined as appearance of continuous ictal discharge, and the termination of SE (or SE free) was defined as relatively flat EEG without ictal discharge.

Western blot

The right hippocampus were rapidly dissected out and homogenized in RIPA buffer (pH 7.5, in mmol/L; 20