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N-methyl-D-aspartate receptor NR2B subunit involved in depression-like behaviours in lithium chloride-pilocarpine chronic rat epilepsy model

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ABSTRACT

Depression is a common comorbidity in patients with epilepsy with unclear mechanisms. This study is to explore the role of glutamate N-methyl-D-aspartate (NMDA) receptor NR1, NR2A and NR2B subunits in epilepsy-associated depression. Lithium chloride (Licl)-pilocarpine chronic rat epilepsy model was established and rats were divided into epilepsy with depression (EWD) and epilepsy without depression (EWND) subgroups based on forced swim test. Expression of NMDA receptor NR1, NR2A and NR2B subunits was measured by western blot and immunofluorescence methods. The immobility time (IMT) was significantly greater in Licl-pilocarpine model group than in Control group, which was also greater in EWD group than in EWND group. No differences of spontaneous recurrent seizure (SRS) counts over two weeks and latency were found between EWD and EWND groups. The number of NeuN positive cells was significantly less in Licl-pilocarpine model group than in Control group, but had no difference between EWD and EWND groups. The ratios of phosphorylated NR1 (p-NR1)/NR1 and p-NR2B/NR2B were significantly greater in the hippocampus in EWD group than in EWND group. Moreover, the expression of p-NR1 and p-NR2B in the CA1 subfield of hippocampus were both greater in Licl-pilocarpine model group than Control group. Selective blockage of NR2B subunit with ifenprodil could alleviate depression-like behaviours of Licl-pilocarpine rat epilepsy model. In conclusion, glutamate NMDA receptor NR2B subunit was involved in promoting depression-like behaviours in the Licl-pilocarpine chronic rat epilepsy model and might be a target for treating epilepsy-associated depression.

1. Introduction

Depressive disorders are very common in patients with epilepsy. According to the data from a recent meta analysis, patients with epilepsy had an overall prevalence of active depression of 23.1% and lifetime depression of 13% (Fiest et al., 2013). Previous clinical data also indicated that the prevalence of depression in epilepsy was higher than most of other neurological and non-nervous diseases, and depression had significantly negative influence on quality of life in patients with epilepsy (Cramer et al., 2003; Ettinger et al., 2004).

Recent evidences indicated that there were common neurobiological mechanisms between the two conditions (Harden, 2002; Sankar and Mazarati, 2010). Kanner et al. proposed that there were bidirectional relations between epilepsy and depression, and several pathogenic mechanisms identified in animal models and patients with psychiatric disorders were found to facilitate the occurrence of seizures or process of epileptogenesis in animals (Kanner, 2012a). These mechanisms included: (1) disturbances of neurotransmitters such as serotonin (5-HT) and 5-HT_{1A} receptor in the central nervous system; (2) endocrine

Abbreviations: DG, dentate gyrus; EWD, epilepsy with depression; EWND, epilepsy without depression; FST, forced swim test; ^{1}H-MRS, proton magnetic resonance spectroscopy; IMT, immobility time; Licl, lithium chloride; NeuN, neuronal specific nuclear protein; NMDA, N-methyl-D-aspartate; SCT, sucrose consumption test; SE, status epilepticus; SRS, spontaneous recurrent seizures; SucroRate, sucrose consumption rate; TLE, temporal lobe epilepsy.

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disturbances such as hyperactivity of hypothalamic-pituitary-adrenal axis; and (3) inflammatory mechanisms (Kanner, 2012b). The common underlying mechanisms may explain the high comorbidity of epilepsy and depression. However, Mazarati et al. (2008) found that depression-like behaviours in Licl-pilocarpine rat chronic epilepsy model were resistant to fluoxetine, a selective serotonin reuptake inhibitor, suggesting epilepsy-associated depression might have other underlying mechanisms beyond alterations of serotonergic pathways.

Glutamate is the principal excitatory neurotransmitter in central nervous system. Excessive glutamate release has pathogenic roles in epilepsy which has been established for a long time (Nadler, 2012). In recent years, evidences demonstrated that glutamatergic system might also take part in the pathogenesis of depression (Mitchell and Baker, 2010; Szakacs et al., 2012). Glutamate N-methyl-D-aspartate (NMDA) receptor is an ionotropic glutamate receptor both involved in the normal neuronal function and pathological process of some neurological disorders (Waxman and Lynch, 2005). Antagonists of NMDA receptor, such as MK801 and ketamine, have been shown to have antidepressant properties in animal models and patients with depression (Pittenger et al., 2007).

On the basis of these findings, we hypothesised that glutamatergic pathways might be involved in the pathogenesis of comorbidity of epilepsy and depression. There was a primary demonstration in our previous clinical study that glutamate and glutamine level in the right hippocampus significantly increased in patients with epilepsy and moderate depression and was the independent risk factor for depression (Peng et al., 2013). In this study, we employed Licl-pilocarpine chronic rat epilepsy model which were suggested by Mazarati et al. (Mazarati et al., 2009; Pineda et al., 2011) to be served as an animal model of the comorbidity of epilepsy and depression to further explore the role of glutamate NMDA receptor NR1, NR2A and NR2B subunits in epilepsy-associated depression. We found elevated phosphorylations of NR1 and NR2B (p-NR1 and p-NR2B) subunits level in the hippocampus in EWD group compared with EWND group, and in the CA1 subfield of hippocampus in Licl-pilocarpine model group compared with Control group. As NR1 subunit was the necessary subunit of NMDA receptor, blocking NR1 might produce some inevitable side effects. So selective blockage of NR2B subunit with ifenprodil was administered in Licl-pilocarpine chronic rat epilepsy model and changes of depression-like behaviours were observed to further investigate the role of NMDA receptor NR2B subunit in the pathogenesis of epilepsy-associated depression.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (SLRC laboratory Animal Corporation) weighing about 200–250 g were housed for 1 week before experiment. Housing temperature was maintained at 22 ± 1 °C, with 12 h light–12 h dark cycle and humidity of 35–40%. The experiments were done in accordance with the guidelines of the National Institutes of Health and the study was approved by Animal Care and Use of Committee of Zhongshan Hospital, Fudan University, China.

2.2. Establish Licl-pilocarpine chronic rat epilepsy model

The Licl-pilocarpine chronic rat epilepsy model was established according to previous studies (Mazarati et al., 2008). Animals received an intraperitoneal (i.p.) injection of lithium chloride (Licl, 127 mg/kg, dissolved in deionised water, Sigma, St. Louis, MO, USA). On the next day (22–24 h after the injection of Licl) animals were injected i.p. with scoporamine methyl nitrate (1 mg/kg, Sigma) to alleviate peripheral cholinergic effects of pilocarpine, and 30 min later they were injected i.p. with pilocarpine hydrochloride (40 mg/kg, Sigma). Continuous or repetitive limbic seizures started 15–30 min after pilocarpine injection. The stages of seizure degree were classified by the Racine scale (Racine, 1972). After 30 min from the first seizure with equal to or greater than Racine 4th stage, rats were injected i.p. with diazepam (10 mg/kg) to terminate further seizures. Control animals were injected i.p. with the same dose of Licl and scoporamine but used saline instead of pilocarpine.

One week after status epilepticus (SE), animals underwent two-week continuous video monitoring for detecting spontaneous seizures. All of the videos were analysed offline. To avoid immediate effects of spontaneous seizures on outcome of behavioural assay, further experiments were performed after verifying no seizures had developed for at least 6 h prior the forced swim test.

2.3. Sucrose consumption test (SCT)

This test for anhedonia is on the basis of the innate preference of rodents towards sweets (Pucilowski et al., 1993). All of the rats were habituated with 1% sucrose water for 2 days before beginning the test, and those drank too much or too little sucrose water were removed. On regular days the rats were housed in groups. Every 5 rats were kept in 1 cage. But on the day of SCT test, 1 rat was put in 1 cage to calculate the sucrose and common water consumption of every rat. Water deprivation was carried out for 24 h before the test. Then on the day of test, every cage was supplied with two identical bottles of water, the one was regular water, and the other was 1% sucrose. The test was performed starting from 9:00 to 10:00 AM. On half of the test time, the places of two bottles were exchanged. Regular and sucrose water intakes were calculated 1 h later. Sucrose preference rate (SucroRate) = sucrose consumption/(sucrose consumption + water consumption) × 100%. A low sucrose preference rate was interpreted as an equivalent of the state of anhedonia. The SCT was performed before experiment and every 1 week after injection of pilocarpine.

2.4. Forced swim test (FST)

The FST is carried out to test state of despair (Porsolt et al., 1979). A rat was put into a transparent tank filled with water (60 cm height and 30 cm diameter), and the water temperature was about 22–25 °C. Then 5 min of swimming behaviour was videotaped and analysed offline. There are 3 types of swimming behaviours in the modified FST: immobile behaviour, climbing behaviour, and swimming behaviour. The longer time of immobility is indicative of state of despair (Detke et al., 1995). The immobility time (IMT) was recorded for all of the tested rats. As the IMT was an important parameter to define depression-like behaviours in previous studies (Mazarati et al., 2008; Ghasemi et al., 2010), we set the average IMT of control rats add 1 standard deviation as the cutoff of depression-like behaviours in this study. The FST was performed at the end of SRS observation, that is, 21 days after SE.

2.5. Western blot analysis

After 21 days from SE and the FST was completed, half of the rats were transcardially perfused with 4 °C 0.1 M PBS 200 ml rapidly and the hippocampus and frontal lobe of both hemispheres were dissected out on ice, and then stored at −80 °C. The tissues of rat brains were homogenised in RIPA lyisis buffer (Beyotime Institute of Biotechnology, China) and centrifuged at 18,000 × *g* r.p.m. at 4 °C for 20 min. The supernatant was shifted out and total protein content was measured by BCA protein assay kit (Beyotime). About 50 μg proteins from each sample were used for measurement. SDS-PAGE