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Effects of protease-activated receptor 1 inhibition on anxiety and fear following status epilepticus

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ABSTRACT

Protease-activated receptor 1 (PAR1) is an important contributor to the pathogenesis of a variety of brain disorders associated with a risk of epilepsy development. Using the lithium-pilocarpine model of temporal lobe epilepsy (TLE), we recently showed that inhibition of this receptor during the first ten days after pilocarpine-induced status epilepticus (SE) results in substantial anti-epileptogenic and neuroprotective effects. As PAR1 is expressed in the central nervous system regions of importance for processing emotional reactions, including amygdala and hippocampus, and TLE is frequently associated with a chronic alteration of the functions of these regions, we tested the hypothesis that PAR1 inhibition could modulate emotionally driven behavioral responses of rats experiencing SE. We showed that SE induces a chronic decrease in the animals' anxiety-related behavior and an increase of locomotor activity. PAR1 inhibition after SE abolished the alteration of the anxiety level but does not affect the increase of locomotor activity in the open field and elevated plus maze tests. Moreover, while PAR1 inhibition produces an impairment of memory recall in the context fear conditioning paradigm in the control group, it substantially improves contextual and cued fear learning in rats experiencing SE. These data suggest that PAR1-dependent signaling is involved in the mechanisms underlying emotional disorders in epilepsy.

1. Introduction

Temporal lobe epilepsy (TLE) is the most common type of focal epilepsy in adults and is frequently associated with psychiatric co-morbidities such as depression and anxiety [1–5]. It has been estimated that interictal psychiatric symptoms contribute more to lowering the health-related quality of life than the frequency, severity, and chronicity of seizures [5,6]. Unfortunately, little is known about the cause, prevention or treatment of psychiatric co-morbidities in epilepsy [5].

Using a classical model of TLE, we recently have shown that the inhibition of protease-activated receptor 1 (PAR1), a major thrombin receptor in the brain, after lithium-pilocarpine-induced status epilepticus (SE) results in a substantial decrease of post-SE animal mortality, SE-induced cell loss, and the likelihood of the subsequent occurrence of epilepsy [7]. As PAR1 is implicated in modulation of animal behavior, memory formation, and synaptic plasticity [8–10], here we examined the effect of PAR1 inhibition on the variety of behavioral measures in animals experiencing SE.

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2. Materials and methods

2.1. Animals and experimental design

This study was conducted under protocols approved by the National Institute of Health for the humane treatment of laboratory animals and the Animal Care Committee of Bogomoletz Institute of Physiology.

Status epilepticus (SE) was induced in adult Wistar rats at postnatal day (P) 50–60 by administration of lithium chloride and pilocarpine (Li-Pilo) as described previously [7,11]. Briefly, rats were injected intraperitoneally (i.p.) with lithium chloride (127 mg/kg, 1 ml/kg) 19–20 h before administration of pilocarpine, which was i.p. injected at a dose 10 mg/kg at 30-min intervals until Racine stage V seizures (SE) developed [27]. Only 3 of 29 (10.3%) rats failed to develop stage V seizures after five consecutive Pilo injections. These animals were excluded from further investigation. Pilo-induced seizures were terminated with sevoflurane at 90 min after SE onset.

The PAR1 specific blocker (SCH79797 [SCH], 25 μg/kg) or appropriate volume of vehicle was i.p. injected 20–30 min after SE termination in Li-Pilo-treated rats (SE + SCH group, n = 14; and SE + vehicle group, n = 12). Thereafter injections were repeated once a day for 10 consecutive days [7]. Two groups of Li-Pilo-untreated rats

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(Control + SCH group, n = 12; and Control + vehicle group, n = 10) received the same dose of SCH or vehicle injections.

All of the rats were monitored for spontaneous seizures using videotaping for 12 h per day for 6 days starting 60 days after SE. Videos were then examined by trained researchers blinded to treatment protocol. In our study, the presence of stage 3–5 seizures in rats was taken into account as they were most easily distinguishable in video recordings from normal animal behavior. Thereafter, all animals were subjected to a battery of behavioral tests. Rats were monitored for seizures for one hour before the behavioral studies. An a priori decision was made prior to testing to evaluate only rats that were seizure-free before the testing and to discard data from any trial in which the rat had a seizure.

2.2. Open field and elevated plus maze

The open field (OF) test was used to assay general locomotor activity levels and anxiety in rodents [12,13]. Each animal tested in OF was placed in the right top corner of the square arena (1.0 m × 1.0 m with 0.3 m - height walls that prevent the rat from escaping) divided into 25 (0.2 m × 0.2 m) squares by black lines. Total distance traveled and the amount of time spent in nine internal squares of the arena were evaluated during a 5-min period.

Another test measuring anxiety level, the elevated plus maze (EPM), is usually used for screening putative anxiolytic or anxiogenic compounds in rodents [14,15]. The test is based on the natural predisposition of rodents to avoid open spaces and tendency to be thigmotaxic. In the EPM, the level of anxiety is measured by comparing the amount of time spent on the open/closed arms (more time spent in the open arms – the lower the level of anxiety). The apparatus for EPM consists of two opposite open arms (0.5 m × 0.1 m) and two closed arms (0.5 m × 0.1 m × 0.4 m) elevated to a height of 0.5 m above the floor. The junction area of the four arms (central platform) measured 0.1 m × 0.1 m. For the EPM test, each animal is placed on the center platform of the maze facing an open arm. Total distance traveled, the number of open/closed arm entries, and the amount of time spent on the open/closed arms were evaluated during a 5-min period.

2.3. Contextual/cued fear conditioning test

After completion of OF and EPM tests, all rats were subjected to a contextual/cued fear conditioning test as reported previously with some modifications [16]. On Day 1, each rat was habituated to the experimental chamber during a 5-min period. On Day 2 (training day), after a 60-s delay, an auditory tone cue (70 dB, 10 kHz, 20 s) was presented followed by a mild foot shock (0.5 mA, 0.5 s). After the shock, the animal stayed in the experimental chamber for another 60 s. On Day 3 (24 h after the previous session; testing day), rats were subjected to a similar procedure except the foot shock was not given. Duration of freezing (the absence of movement for at least 1 s) was measured during the last 20 s before the onset of the auditory tone (contextual fear conditioning) and during the tone (cued fear conditioning) twice – on the training and testing days. Spontaneous freezing refers to freezing during the 20 s before the onset of the auditory tone during the training session.

2.4. Statistics

Statistical analysis was performed using the Origin 9 and GraphPad Prism 6 software programs. Data were analyzed using two-way repeated measures ANOVA with Bonferroni post hoc, one- way ANOVA with Tukey's post hoc or Students *t*-test and presented as mean ± S.D.

3. Results

3.1. Spontaneous recurrent seizures

Spontaneous seizures were observed in 9 out of 12 rats (75%) in the SE + vehicle group and 6 out of 14 rats (46%) in the SE + SCH group. No seizures were observed prior to or during the behavior testing.

3.2. OF and EPM

In the OF and EPM tests, both SE + vehicle and SE + SCH animals showed a significant increase in the distance traveled when compared to the Control + vehicle (OF: $t = 5.69$, $df = 19$, $p < 0.0001$; EPM: $t = 2.25$, $df = 20$, $p = 0.04$) and the Control + SCH groups (OF: $t = 2.15$, $df = 23$, $p = 0.04$, EPM: $t = 3.27$, $df = 16$, $p = 0.005$; Fig. 1A, C), respectively. The ratio of the time spent exploring internal squares to a total time in the OF was significantly increased in the SE + vehicle group compared to the Control + vehicle group ($t = 2.66$, $df = 20$ $p = 0.01$, Fig. 1B). We did not find any difference in this parameter between the SE + SCH, Control + vehicle, and Control + SCH groups ($p > 0.05$ in all cases, Fig. 1B). In the EPM, we observed a significant increase in the percent of entries into the open arms ($t = 2.90$, $df = 20$, $p = 0.009$, Fig. 1C) and time spent on the open arms ($t = 2.74$, $df = 20$ $p = 0.01$, Fig.1D) in the SE + vehicle compared to the Control + vehicle group. In the SE + SCH group, these parameters did not differ from controls ($p > 0.05$ in all cases, Fig. 1C, D).

3.3. Contextual/cued fear conditioning test

One-way ANOVA showed a statistically significant difference in spontaneous freezing between the treatment groups during training in the novel context ($F(3, 39) = 3.94$, $p = 0.02$). Tukey's post hoc analysis revealed that freezing in the SE + vehicle group was significantly increased compared to the Control + vehicle and the SE + SCH groups ($p < 0.05$ in both cases). We did not observe a significant difference in the level of spontaneous freezing between treatment groups during cue-dependent fear training ($F(3, 39) = 2.54$, $p = 0.07$).

Repeated measures two-way ANOVA of the contextual memory recall 24 h after the training showed a significant time × group interaction ($F(3,39) = 7.51$, $p = 0.0004$), a significant time effect ($F(1,39) = 82,85$, $p < 0.0001$), and a significant group effect ($F(3,39) = 3.36$, $p = 0.03$). Bonferroni's post hoc revealed a significant increase in freezing in the context testing session in Control + vehicle ($p < 0.0001$), Control + SCH ($p < 0.01$) and SE + SCH ($p < 0.01$), but not in the SE + vehicle ($p > 0.05$, Fig. 2A) group. Similar results were obtained after analysis of the cued-fear associative learning. Repeated measures two-way ANOVA revealed a significant time × group interaction ($F(3,39) = 6.99$, $p = 0.0007$) and a significant time effect ($F(1,39) = 101,40$, $p < 0.0001$). Bonferroni's post hoc revealed a significant increase in freezing in the cued testing session in Control + vehicle ($p < 0.0001$), Control + SCH ($p < 0.0001$), and SE + SCH ($p < 0.01$), but not in SE + vehicle ($p > 0.05$, Fig. 2B).

Interestingly, PAR1 inhibition reduced contextual fear freezing in the control group (testing day: Control + SCH vs Control + vehicle, $t = 2.83$, $df = 18$, $p = 0.01$), but did not impair the performance in conditioning to the tone paradigm (Control + vehicle vs Control + SCH, $t = 1.14$, $df = 18$, $p = 0.27$, Fig. 2).

4. Discussion

The major findings in the present study are that PAR1 inhibition restores the control level of anxiety-related behavior and improves contextual and cued fear learning following SE.

Experimental data concerning the effect of seizures on the level of animals' anxiety are ambiguous, and vary from an increase to decrease in anxiety depending on the experimental model, seizure severity,