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Research report

Activation of Sigma-1 receptor ameliorates anxiety-like behavior and cognitive impairments in a rat model of post-traumatic stress disorder

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HIGHLIGHTS

- Rats subjected to RSPS showed anxiety-like behavior and cognitive impairments.
- The expression of BDNF, p-TrkB and p-ERK in the amygdala was significantly increased after RSPS exposure.
- Activation of Sigma-1 receptor effectively reduced PTSD-related behavioral abnormalities.

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ABSTRACT

Among learning and memory processes, fear memories are crucial in some psychiatric disorders like post-traumatic stress disorder (PTSD). Accumulating evidence shows that the sigma-1 receptor (Sig-1R) has comprehensive involvement in cognitive impairment and neuroprotective effects. It has also been reported that BDNF appears to enhance extinction of fear in anxiety disorders via the MAPK signaling cascade. However, it remains unclear whether BDNF-TrkB-MAPK pathway may be mechanistically involved in the therapeutic effect of sigma-1 receptor in the development of PTSD. To address this question, rats were subjected to a classical single-prolonged stress procedure (SPS) and kept undisturbed for 7 days. After that, rats were re-stressed by re-exposure to the forced swim component of SPS (RSPS). Behavior tests were subsequently performed to assess anxiety and cognitive impairments. Furthermore, we analyzed the expression of BDNF and the phosphorylation of TrkB and three MAPK pathways, namely, the ERK, JNK and p38. We found that the levels of BDNF and p-TrkB were increased following the RSPS procedure, which were reversed by the administration of PRE-084. Meanwhile, among the three MAPK signaling pathways, only the p-ERK expression was increased following the RSPS procedure. Collectively, our results indicate that BDNF-TrkB-ERK signaling pathway may be involved in the activation of sigma-1 receptor to yield therapeutic benefits for PTSD.

1. Introduction

Posttraumatic stress disorder (PTSD) is one of the most common psychiatric disease and is characterized by experience of a severe trauma or stress [1]. With the onset of PTSD, patients exhibit enduring re-experience of traumatic events and avoidance of the trauma-related stimuli, even though they actually know that the traumatic event no longer exists [2]. Although classified as an anxiety disorder and similar in some aspects to chronic stress, growing evidence suggests that PTSD is probably a deficit of learning and memory [3]. The three areas of the brain whose function may be altered in PTSD have been identified as the prefrontal cortex, the amygdala and the hippocampus [4]. It has been reported that the amygdala is crucial for encoding and retrieval of conditioned fearful memories [5], which is reciprocally connected with the hippocampus through the basolateral amygdala (BLA) [6].

Among the putative therapeutic targets being studied, the sigma-1 receptor (Sig-1R) has been gaining special attention for its comprehensive involvement in cognitive impairment and neuroprotective effects [7]. Hypofunction of the Sigma-1 receptor has been indicated to be involved in the pathophysiology of a variety of diseases [7–9]. The sigma-1 receptor is a small transmembrane protein located on the endoplasmic reticulum (ER) membrane [10]. The amino acid sequence of the Sig-1R does not resemble that of any other mammalian proteins [11]. Sig-1Rs reside at the specialized ER sub-region adjacent to mitochondria, known as the mitochondrial-associated membrane (MAM) [8].

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Not only is Sig-1R agonist regarded as one of the most efficient neuroprotective drugs, a body of evidence also exists of its beneficial effects in many disorders caused by disturbances of intercellular signaling pathways, particularly those associated with neurotrophic factors [11–13]. It is suggested that there is a direct relationship between Sig-1R agonists, Sig-1Rs and MAPKs and their possible roles in Sig-1R-mediated neuroprotection. Overexpression of Sig-1R enhances phosphorylation of the epidermal growth factor receptor, and leads to prolonged activation of downstream signaling pathways to promote neuritogenesis [14]. The Sig-1R agonist PRE-084 evokes phosphorylation of ERK1/2, but not p38-MAPK and JNK, while Sig-1R antagonist inhibits ERK1/2 phosphorylation in a concentration-dependent manner [15]. Sig-1R also plays a role in the regulation of both synaptic efficacy and spatial learning performance [16]. Administration of Sig-1R agonist has shown anti-amnesic effects in animal models of cognitive impairment, which could reverse the memory deficits caused by different lesion procedures [17]. However, the precise mechanism of this aspect of Sig-1R activity has not been fully elucidated [11]. Nevertheless, little is known, up to date, about the potential impact of Sig-1R agonists on the consolidation or extinction process of fear memory [17].

With this respect, neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), have emerged as important downstream mediators of the Sigma receptor signaling pathway [13,18]. BDNF/TrkB pathway plays a key role in activity-dependent synaptic plasticity and the formation of fear memories [19,20]. BDNF has been recently identified as a molecular target for facilitating extinction learning and a potential treatment for fear associated disorders [21]. Another line of research indicated that blocking the activity of BDNF in the amygdala disrupted the retention of fear memory extinction [22]. Accumulating evidence, in fact, indicates reduced BDNF levels in the serum and hippocampus of PTSD patients [23]. Although these findings are not always consistent, evidence exists that the modulation of BDNF may represent an important functional mechanism associated with the exposure to stress [24]. Furthermore, studies indicated that extinction of memory could be facilitated by infusion of recombinant BDNF or by agonist of TrkB receptor [25,26].

A variety of animal paradigms have been adopted to mimic the behavioral and cognitive impairments in patients with PTSD, including predator-scent stress (PSS), trauma witness model (TWM) and single-prolonged stress (SPS) [27–29]. Here, we used a restressed single-prolonged stress model (RSPS), initially proposed by Liberzon et al. as it replicated the specific neuroendocrinological abnormalities observed in PTSD patients, such as enhanced glucocorticoid negative feedback [5,30].

In the present study, we used the RSPS paradigm to investigate the possible protective effect of Sig-1R and to characterize the correlated mechanisms, especially those involving BDNF/TrkB pathway. Furthermore, we analyzed the phosphorylation of three MAPK pathways, namely, the ERK, JNK and p38, one of which increased following neuronal activation, thus to clarify its downstream signaling cascade and better characterize its neuroprotective mechanism.

2. Materials and methods

2.1. Subjects

Adult male Sprague-Dawley rats (200–250 g) were used in this study. Rats were housed singly and maintained on a 12:12-h light/dark cycle, with free access to food and water. After arrival at the research facility, all rats were allowed to acclimate for at least 7 days before experiments. All experiments were conducted in accordance with the NIH guidelines using approved protocols from the Animal Care and Use Committee (China Medical University).

2.2. Single-prolonged stress and restress procedure

The rats in the RSPS and RSPS + PRE-084 groups were first subjected to a one-time combined stress paradigm (i.e. single-prolonged stress) applied consecutively in a day [30,31]. Briefly, Rats were restrained for 2 h inside a disposable restraint holder (58 mm diameter, 150 mm length). After that, they were placed in a clear cylindrical tank (600 mm × 400 mm × H500 mm) filled two thirds with water (24 °C), and were forced to swim for 20 min and then allowed to rest for 15 min, and finally exposed to ether anesthesia (with diethyl ether until loss of consciousness, 2–3 min). After 7-day's quiescent period (essential for the development of PTSD), rats were re-stressed by re-exposure to the forced swim component of SPS.

2.3. Drug treatment

PRE-084 (Sigma-Aldrich, St. Louis, MO USA) was used as sigma-1R agonist and was dissolved in 0.9% sterile saline and administered daily by intraperitoneal injections at 0.6 mg/kg for 7 consecutive days right after the SPS procedure. The concentration was based on previous reports. Control rats received only the vehicle.

2.4. Behavior tests

Open-field (*OF*) test was used to assess general motor ability and quantify anxiety-like behavior. The apparatus was made with black Plexiglas measuring 50 × 50 × 50 cm with a red fluorescent light illumination over the center of the arena. After 30 min of acclimation in the room, rats were put on a central start position in the open arena and allowed to explore for 5 min, during which their behavior was recorded and analyzed. The arena was cleaned with 70% ethanol after each session and individual rat was tested only once.

The *elevated plus maze* (*EPM*) test was performed as previously described [32]. Briefly, the EPM apparatus consisted of two opposing open and two opposing closed arms (50-cm arms, 50 cm off the ground). Rats were placed on the central platform facing an open arm and allowed to explore for 5 min. Each test was videotaped and subsequently scored by an independent observer. Arm entry was defined as entering an arm with all four paws. The percentage of time spent in the open arms and percentage of entries into the open arms relative to total (open + closed) arm were quantified as assessments of anxiety.

Morris water maze (*MWM*) was performed to evaluate the rat spatial learning and memory. Briefly, the task was conducted in a circular pool (120 cm in diameter) filled with water (23 ± 1 °C). The animals were subjected to 4 acquisition trials per day for 6 successive days. Before the first trial, animals were brought in the testing suite and allowed to find visible escape platform (12 cm diameter, 2 cm above the water surface), to determine if treatment affected the ability to swim or learn the water maze task. Rats were randomly placed into the middle of a quadrant (excluding the one containing the platform) with their noses facing the wall. If they could not find the hidden platform (2 cm below the water surface) in 60 s, the experimenters would gently assist the rats onto the platform and stay there for 10 s. Escape latency was recorded by a video-tracking system. Probe test was performed 24 h after the last acquisition trial to assess spatial memory, in which the platform was removed and the rats were allowed to swim freely. The time spent in the target quadrant was recorded.