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Research report

The CRF_1 receptor antagonist SSR125543 prevents stress-induced long-lasting sleep disturbances in a mouse model of PTSD: Comparison with paroxetine and D-cycloserine

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HIGHLIGHTS

- The CRF_1 antagonist SSR125543 attenuates the long-term effects of stress.
- Sleep disturbances are commonly reported symptoms in post-traumatic stress disorder.
- Here we tested SSR125543 on sleep impairment induced by traumatic stress using EEG.
- The stress-induced effects were prevented by repeated administration of SSR125543.
- These findings confirm that SSR125543 can attenuate the effects of traumatic stress.

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ABSTRACT

The selective CRF_1 (corticotropin releasing factor type 1) receptor antagonist SSR125543 has been previously shown to attenuate the long-term behavioral and electrophysiological effects produced by traumatic stress exposure in mice. Sleep disturbances are one of the most commonly reported symptoms by people with post-traumatic stress disorder (PTSD). The present study aims at investigating whether SSR125543 (10 mg/kg/day/i.p. for 2 weeks) is able to attenuate sleep/wakefulness impairment induced by traumatic stress exposure in a model of PTSD in mice using electroencephalographic (EEG) analysis. Effects of SSR125543 were compared to those of the 5-HT reuptake inhibitor, paroxetine (10 mg/kg/day/i.p.), and the partial N-methyl-D-aspartate (NMDA) receptor agonist, D-cycloserine (10 mg/kg/day/i.p.), two compounds which have demonstrated clinical efficacy against PTSD. Baseline EEG recording was performed in the home cage for 6 h prior to the application of two electric foot-shocks of 1.5 mA. Drugs were administered from day 1 post-stress to the day preceding the second EEG recording session, performed 14 days later. Results showed that at day 14 post-stress, shocked mice displayed sleep fragmentation as shown by an increase in the occurrence of both non-rapid eye movement (NREM) sleep and wakefulness bouts. The duration of wakefulness, NREM and REM sleep were not significantly affected. The stress-induced effects were prevented by repeated administration of SSR125543, paroxetine and D-cycloserine. These findings confirm further that the CRF_1 receptor antagonist SSR125543 is able to attenuate the deleterious effects of traumatic stress exposure.

1. Introduction

Stress can have a significant negative impact on sleep and traumatic life events may produce at least temporary sleep disturbances that may include insomnia or subjective sleep problems [1]. There is evidence which supports the idea that disrupted sleep represents a core component of post-traumatic stress disorder (PTSD), involved both in the development and the maintenance of this condition [2]. Sleep disturbances may exacerbate the difficulties associated with PTSD, especially those related to increase arousal such as concentration, hypervigilance or irritability. As an example, Meewise et al. [3] suggested that in victims of a major industrial fire, sleep disturbances may contribute to attentional dysfunction many years after the traumatic event. From these observations it was concluded that efficiently addressing post-traumatic sleep symptoms during PTSD

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may be beneficial in optimizing clinical interventions [4]. There have been relatively few studies to directly examine the impact of pharmacological intervention on sleep problems in PTSD [5]. Maher et al. [6] have reported an improvement of post-traumatic-induced sleep disturbances following selective serotonin reuptake inhibitors (SSRIs), which represent the mainstay of treatment of PTSD.

A potential new therapeutic approach to prevent post-traumatic sleep disturbances may be to reduce the activity of the corticotropin-releasing factor (CRF) system using CRF_1 receptor antagonists. Several studies have reported elevated levels of CRF in the cerebrospinal fluid [7,8] and in the plasma of PTSD patients [9]. CRF is well known to be the main physiological regulator of the stress response. Following exposure to emotional and/or physical stressors, it is synthesized in neurons of the paraventricular hypothalamic nucleus and triggers the secretion of adrenocorticotropin (ACTH), which subsequently stimulates the release of cortisol from the adrenal cortex into blood and exerts a negative feedback on the hypothalamic pituitary adrenal (HPA) axis [10,11]. Several studies indicate that CRF may play a role in the regulation of stress-induced changes in arousal and sleep. For example, Opp et al. [12] demonstrated that rat strains differing in the synthesis and secretion of CRF, and in basal plasma concentrations of corticosterone showed significant differences in the amounts of sleep. Specifically, Lewis rats, known to display a deficiency in the synthesis and secretion of hypothalamic CRF, exhibit less wakefulness and more NREM than the genetically-related inbred Fischer 344 and outbred Sprague–Dawley [12,13] and Wistar rats [13]. Other studies have reported that blockade of the CRF_1 receptor reduced stress-induced sleep disturbances. For instance, the infusion of the CRF_1 receptor antagonist, antalarmin, into the central nucleus of the amygdala resulted in an attenuation of electric shock-induced reduction in REM sleep in rats [14]. In another study using depressed subjects, four weeks of treatment with the CRF_1 receptor antagonist, R121919, decreased the number of awakenings [15]. Based on this observation, it can tentatively be suggested that a CRF_1 receptor antagonist would also be able to attenuate sleep disturbances in other stress-related conditions, such as PTSD.

We have recently demonstrated that the CRF_1 receptor antagonist, SSR125543, was able to attenuate several behavioral (cognitive deficit), hormonal (increase in corticosterone level) and electrophysiological (hippocampal excitability impairment) effects following exposure to a traumatic event [16,17]. However, potential beneficial effects of SSR125543 on stress-induced sleep disturbances have not been studied. Therefore, the aim of the present study was to investigate the effects of SSR125543 on long-term sleep disturbances in stressed mice. We used a model of PTSD [16] based on exposure of mice to unavoidable electric foot-shocks. Sleep/wakefulness was investigated by using electroencephalographic (EEG) recording, which was performed prior to shock application and 14 days after stress. Our previous findings have shown that the current procedure produced at day 14 post stress a fragmentation of sleep [18]. SSR125543 was administered repeatedly and its effects were compared to those of paroxetine and D-cycloserine (DCS), two clinically active compounds in PTSD patients.

2. Material and methods

2.1. Animals

Swiss male mice (Janvier, Le Genest St-Isle, France) weighing 20–22 g at the start of the experiment were used. They were housed individually in plastic cages (30 × 18 × 18 cm) with free access to food and water *ad libitum*. They were maintained at a constant temperature of 21 ± 1 °C, humidity at 50 ± 10% and under a 12:12 light/dark cycle (light on at 7:00 a.m.). Experiments were conducted in accordance with the "Guide and Care and Use of Laboratory Animals" (National Institute of Health) and were approved by the in-house Animal Ethics Committee.

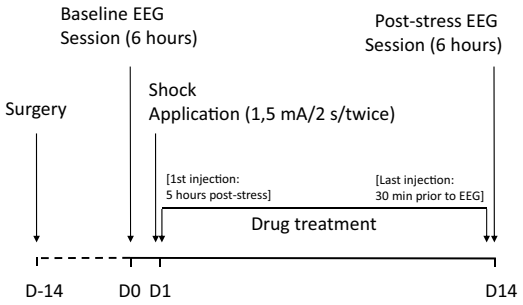

Fig. 1. Experimental design.

2.2. Shock application

Animals were placed into the shock chamber for a 190-s habituation period after which two electric foot-shocks (1.5 mA; for 2 s; 6 s apart) were delivered through the metal grid floor. Animals remained in the shock chamber for another 60-s period before they were returned to their home cage. Control animals were exposed to the same procedure, but without receiving any foot-shock.

2.3. Drug administration

Paroxetine (Sigma-Aldrich, CAS 110429-35-1), D-cycloserine (Sigma-Aldrich, CAS 68-41-7), and SSR125543, synthesized by Sanofi Medicinal Chemistry, were suspended in saline with methylcellulose (0.6%) and Tween 80 (0.1%) to obtain concentrations of 1.0 mg/ml. The treatments began five hours after stress. Mice received one intraperitoneal (i.p.) administration per day of 10 ml/kg. The last administration was performed 30 min before the start of EEG recordings. The doses were validated in a previous study using the same procedure and the same species. It showed that 10 mg/kg represented the optimal dose to seek efficacy in this model [17].

2.4. Sleep/wakefulness analysis

2.4.1. Surgery

Mice were anesthetized with Zoletil®50 (Tiletamine, Zolazepam, 60 mg/kg, i.p.), mounted in the stereotaxic apparatus and secured using blunt rodent ear bars. A scalp incision was made after local anesthesia with lidocaine 2% and the skin was retracted. The skull surface was cleaned to implant small stainless steel screw electrodes (0.9 mm in diameter). Three cortical electrodes were screwed into the bone over the sensorimotor cortex (1.5 mm lateral to the median and 1.5 mm behind the fronto-parietal sutures), the visual cortex (1.5 mm lateral to the median and 1.5 mm in front of the parieto-occipital sutures) and over the cerebellum. They were attached to a connector (Winchester®, 5-led) and fixed with dental cement (3 M® ESPE) to the cranium. Animals were allowed to recover from surgery in their individual cage for two weeks prior recordings.

2.4.2. Recording procedure

The experimental design is shown in Fig. 1. Mice were habituated in their home cage to the recording cable and room for one day prior to each EEG recording session. On the recording day, they were connected to the cable at 9:45 a.m. Recording sessions took