Pharmacology, Biochemistry and Behavior 98 (2011) 425–431

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The corticotropin-releasing factor 1 receptor antagonist, SSR125543, and the vasopressin 1b receptor antagonist, SSR149415, prevent stress-induced cognitive impairment in mice

Alexandre Urani [a], Julie Philbert [a], Caroline Cohen [b], Guy Griebel [a,*]

[a] Sanofi-Aventis, Exploratory Unit, Chilly-Mazarin, France
[b] Sanofi-Aventis, Therapeutic Strategic Unit Aging, Chilly-Mazarin, France

ARTICLE INFO

Article history:
Received 10 January 2011
Received in revised form 16 February 2011
Accepted 17 February 2011
Available online 26 February 2011

Keywords:
Vasopressin
V1b receptor
CRF1 receptor
Anxiolytic
Memory
Learning
Acute stress
Anxiety

ABSTRACT

The vasopressin 1b receptor antagonist, SSR149415, and the corticotropin-releasing factor 1 receptor antagonist, SSR125543, are orally active non-peptidic compounds with anxiolytic- and antidepressant-like activities in animals. In the present study, their effects on stress-induced deficit in cognitive performances as assessed in a modified object recognition test were investigated in mice. The object recognition task measures the ability of a mouse to remember an object it has previously explored in a learning trial. During this acquisition session, the mouse was stressed by the presence of a pair of rats under the grid floor of the apparatus. One hour later, it was placed again in the environment with the known and a novel object, but in the absence of the rats. While non-exposed mice spent more time exploring the new object, mice that had been exposed to the rats during acquisition failed to discriminate between the known and the new object during retrieval. This cognitive impairment in stressed mice was prevented by the administration of SSR149415 (10 mg/kg, ip), SSR125543 (10 mg/kg, ip) and the selective serotonin reuptake inhibitor, fluoxetine (10 mg/kg, ip). Under similar conditions, the cognitive enhancer donepezil (1 mg/kg, ip) failed to reverse object recognition deficit. These results indicate that the effects of SSR149415 and SSR125543 in the modified object recognition test, in stressed mice, involve the ability of mice to cope with stress rather than an effect on cognition *per se*. Together, these data suggest that SSR149415 and SSR125543 may be of interest to reduce the cognitive deficits following exposure to stress-related events, such as acute stress disorder.

1. Introduction

Stress is a potent double-edged modulator of learning and memory processes (McEwen and Sapolsky, 1995; Sandi and Pinelo-Nava, 2007). Stress has been shown to facilitate (Andreano and Cahill, 2006; Lupien et al., 2007; Oitzl and de Kloet, 1992; Roozendaal et al., 2006) or to impair (Diamond et al., 1996; Eysenck et al., 2007; Lupien et al., 2007; Nadel and Payne, 2002) cognitive performances in animals and humans. The beneficial or deleterious effect of stress on learning depends among other aspects, on the intensity, the repetition and the controllability of stress and the memory phase (Sandi and Pinelo-Nava, 2007).

Stress is largely dependent on the activity of the hypothalamic–pituitary–adrenocortical (HPA) axis, which is activated by exposure to emotional and/or physical stressors (Strohle and Holsboer, 2003). The release of corticotropin-releasing factor (CRF) from neurons of the paraventricular hypothalamic nucleus (PVN) into the pituitary portal blood triggers the secretion of adrenocorticotropin (ACTH) from the anterior lobe. Subsequently, corticosterone is secreted from the adrenal cortex into blood and exerts a negative feedback on the HPA axis activity via pituitary, hypothalamic, limbic, and cortical regions (de Kloet, 2000; Sapolsky and McEwen, 1985). Two CRF receptor subtypes, CRF1 and CRF2, with distinct anatomical localization and pharmacology have been identified. In addition to a major projection from the paraventricular nucleus of the hypothalamus to the pituitary corticotropes, CRF-containing neurons and receptors are also found in brain areas involved in stress responses, including the amygdala, lateral septum, locus coeruleus and brainstem raphe. Similar to CRF, the nonapeptide vasopressin (AVP) is also released during the stress response. It acts as a direct ACTH secretagogue and also potentiates the stimulatory effect of CRF in animals and humans (Aguilera and Rabadan-Diehl, 2000). AVP exerts its effects via a dense localization of vasopressin receptors (V1a and V1b receptors) expressed mainly in limbic areas and in the hypothalamus.

Abnormal HPA activity has been implicated in a variety of conditions related to stress, including HPA overactivation in depression and some anxiety disorders. Infusion of CRF, CRF fragments or AVP into the rodent brain, or constitutive transgenic overexpression of CRF in mice, recapitulates some of the behavioural and neuroendocrine consequences of exposure to stress, such as increased anxiety-like behaviour

* Corresponding author at: Sanofi-Aventis, Exploratory Unit, 1 avenue Pierre Brossolette, 91385 Chilly Mazarin, France. Tel.: +33 1 60 49 54 30; fax: +33 1 60 49 78 45.
E-mail address: guy.griebel@sanofi-aventis.com (G. Griebel).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.02.019

and HPA dysfunction. In this context, it was postulated that CRF and AVP receptor antagonists may represent novel agents for the treatment of stress-related disorders. For example, the CRF1 receptor antagonist, SSR125543, has been reported to induce anxiolytic- and antidepressant-like effect in several animal models in rodents (Alonso et al., 2003; Griebel et al., 2002b; Gully et al., 2002; Louis et al., 2006). Likewise, the selective V1b receptor antagonist, SSR149415, was shown to block stress-induced elevation of plasma ACTH, and had anxiolytic and antidepressant-like effect in various animal models (Alonso et al., 2003; Claustre et al., 2006; Griebel et al., 2002a; Iijima and Chaki, 2007; Louis et al., 2006; Overstreet and Griebel, 2004; Overstreet and Griebel, 2005; Serradeil-Le Gal et al., 2002).

The aim of the present study was to evaluate the effects of SSR149415 and SSR125543 in a new animal model of acute stress disorder (ASD), which involves the assessment of cognitive performance following stress exposure. This idea is based on the observation that, among the symptoms of ASD, dissociative amnesia, i.e. the inability to recall an important aspect of the trauma, is predominant (DSM-IV-TR, 2000). In the present study, the deficit in recall performance was evaluated using a modified object recognition task (ORT) in mice, which is traditionally used to assess short-term visual episodic memory (Dodart et al., 1997; Ennaceur and Delacour, 1988) and serves as a screening model for compounds with potential promnestic activity. The ORT is based on the natural tendency of rodents to explore a novel object more than a known one and it has the advantage of not involving goal-oriented behaviours (e.g., reward, escape). In a first set of experiments we compared the potential deleterious effects of an exposure to different stimuli (i.e. mice or rats) during the acquisition phase on recall performance. Finally, to validate this procedure pharmacologically as a model of ASD, the effects of the selective 5-HT reuptake inhibitor (SSRI), fluoxetine, and the promnestic agent, donepezil, were evaluated.

2. Material & methods

2.1. Animals

Swiss male mice (Janvier, Le Genest St Isle, France) weighing 30 ± 2 g at the time of testing were used. For the predator stress procedure, male Sprague–Dawley rats (Charles River Laboratory, L'arbresle, France) weighing 300–350 g were used. Animals were fed ad libitum and kept in a controlled environment (12/12 h dark/light cycle, 21 °C, 50% humidity). The experiments made here fully comply with the European treaty on research involving living animals (n° 86/609/EEC) and the protocol was reviewed by Sanofi-Aventis ethical committee before the experiments started.

2.2. Object recognition test

The object recognition test took place in a square open field (side: 52 cm) made of PVC as described before (Pichat et al., 2007); in this experiment, the PVC floor was pierced with small holes in order to let the smell go through. Light intensity was 50 lux and the walls were grey. The objects to be discriminated were a metal triangle and a plastic piece of construction game. The test consisted in 3 sessions. Mice were firstly habituated to the context for 3 min (session 1), 24 h prior to the acquisition. For the acquisition (session 2), mice were placed in the arena, in the presence of 2 identical objects, located 5 cm from the two opposite corners of the back wall. Animals were allowed to investigate the objects until they reached 15 s of exploration (cut-off: 5 min: mice not reaching 10 s after 5 min were removed from the experiment). Exploration of an object was defined as pointing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. The exploration time included only the time when the mouse was really investigating the object and no casually touching it or even "looking" at it. After a forgetting delay, mice were placed again in the enclosure containing one of the previous objects and a new one placed in a counterbalanced manner for 4 min (session 3). With a short (1 h) forgetting delay, mice usually remember the known object and spend more time exploring the new one. This behaviour reflects a significant recall of the previously presented object. With a longer (48 h) forgetting delay, mice usually do not remember the known object and spend the same amount of time exploring both objects. Exposure to predators was done during the acquisition (session 2) only (Fig. 1). The exposure paradigm was inspired by previous work using live rat/mouse exposure (Yang et al., 2004). A pair of male Sprague–Dawley rats was placed under floor, at a distance of 19 cm from it. At this distance, rats were able to touch the floor, but not to lift it up. For the control experiment, the effect of the presence of mice during the session 2 was evaluated using cage-mates of the mouse performing the test. Scoring was done manually online by an experimenter unaware of the treatment conditions.

2.3. Drug administration

Fluoxetine (Spectrum Chemical Mgf Corp, Gardena, CA) was dissolved in saline; SSR149415, SSR125543 and donepezil synthesized by the CNS Medicinal Chemistry Department of Sanofi-Aventis, were suspended in saline with methylcellulose (0.6%) and Tween80 (0.1%). Drugs were administered intraperitoneally (10 ml/kg of body weight), once, 30 min before session 2 (acquisition) in the short forgetting delay procedure and twice in the long forgetting delay procedure: 30 min before session 2 and 30 min before session 3 (retrieval). The acetylcholinesterase inhibitor, donepezil, was used as a negative control because of its cognitive-enhancing properties and since it represents the mainstay of treatment for the cognitive symptoms of diseases such as mild to moderate Alzheimer. The doses (i.e. 10 mg/kg for fluoxetine, SSR125543 and SSR149415, and 1 mg/kg for donepezil) were selected carefully on the basis on preliminary findings using the current procedure or on previously published findings showing that they are optimal to produce behavioural effects.

2.4. Statistical analysis

The data analysed were: the time to reach 15 s of exploration of the 2 identical objects in the acquisition session, the time of exploration of each object during retrieval session, the total time of exploration of the objects (sum of both objects exploration times), the ratio of the time exploration of the new object over the total time.

For exploration time, data were analysed using a two-way ANOVA with repeated measures with "object" as a fixed factor to analyse the ability of animals to discriminate between known and new object. The effect of "object" factor was then analysed by Winer analysis for each level of "group" factor. For ratios and total exploration time, a one-way ANOVA was performed to analyse the differences between groups, followed by a Dunnett's post-hoc analysis.

3. Results

3.1. Effect of an exposure to mice (no stress condition) or rats (stressful stimulus condition) on short-term memory performance in the object recognition test

The aim of this experiment was to verify whether the deleterious effect induced by the presence of two rats was specific to this species or if the presence of any animal disturbed learning. Performance in the object recognition test was evaluated in 3 conditions: mice were exposed either to mice (cage-mates, no-stress) or to a pair of rats (stress) or were left undisturbed (control). No physical contact was possible with the animals (mice or rats) located below the grid and the mouse performing the test.

During the acquisition session (session 2), the time needed to reach 15 s of exploration of the objects was not different between