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Effects of a putative antidepressant with a rapid onset of action in defeated mice with different coping strategies

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ABSTRACT

There is evidence suggesting that stressful social events may result in depressive-like disorders, but the development of these disorders depend on the way in which people cope with stress. Although antidepressants are useful their drawback is a delay in the therapeutic effects, moreover not all the patients show an adequate response to this treatment. The aim of this study was to analyse the effect of RS 67333, which is a 5-HT_4 receptor partial agonist and a putative antidepressant which exhibits a rapid onset of action and to determine whether this drug reverses the behavioural and physiological effects that are generated by chronic defeat in subjects who manifest a more vulnerable profile in their response to stress. Male mice were exposed to defeat for 21 consecutive days using a sensorial contact model. After 18 days of defeat, 2 groups of subjects were established, active and passive, in accordance with the behaviour that was manifested during social confrontation, and drug treatment was initiated for 5 days. Finally, the animals were subjected to a forced swimming test (FST). The results revealed higher corticosterone levels in passive mice after the last defeat. Additionally, 3 days after the last defeat, they showed lower corticosterone levels and higher splenic IL-6 and TNF-α levels and hypothalamic GR mRNA levels when compared to their active and manipulated control counterparts. Passive mice had higher 5-HT_{1A} receptor mRNA levels than the manipulated controls and a lower MR/GR ratio than active mice. Similar to stress, the drug increased hypothalamic GR mRNA levels, but it did not affect other measured physiological variables or social behaviour, which suggested that the mechanism of this drug is not the most adequate for reversing stress-induced effects in this model. Nevertheless, the treatment increased swimming and decreased immobility in the FST, suggesting an antidepressant potential for this drug.

1. Introduction

Chronic social stress is considered a trigger factor for many affective disorders, including major depression. Research has demonstrated that stressful life events generate a series of behavioural and psychological effects that are similar in nature to the symptoms that are manifested by patients who are diagnosed with depression. As has been observed in depression, chronic social stress produces an increase in the release of proinflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) at a peripheral (Avitsur et al., 2003; Bailey et al., 2009; Stark et al., 2001) and central level (Wohleb et al., 2011), alterations in the hypothalamic pituitary adrenal (HPA) axis at a number of different levels (Blanchard et al., 1995; Buwalda et al., 1999, 2001) and changes in monoaminergic transmission (Van Praag, 2004). These alterations that are caused by stressful events can be considered biomarkers of depression (Dowlati et al., 2010; Hirschfeld, 2000; Howren et al., 2009; Lopez-Duran et al., 2009), and can be reversed by the administration of antidepressant treatments (Beitia et al., 2005; Reul et al., 1993; Wu et al., 2011).

Selective serotonin reuptake inhibitors (SSRIs), which are the most commonly prescribed antidepressants, may reestablish the activity of the HPA axis, probably by increasing the functionality of the glucocorticoid receptors (GRs) (Carvalho and Pariante, 2008; Pariante et al., 2004), and this neuroendocrine improvement is

Abbreviations: 5-HT_{1A}, 5-hydroxytryptamine 1A; 5-HT_4, 5-hydroxytryptamine 4; CEBA, Ethical Committee for animal welfare; cDNA, complementary deoxyribonucleic acid; DNA, deoxyribonucleic acid; Dnase, deoxyribonuclease; FST, forced swimming test; GAPDH, glyceraldehyde-6-phosphate dehydrogenase; GR, glucocorticoid receptor; HPA axis, hypothalamic pituitary adrenal axis; IL-6, interleukin-6; MR, mineralocorticoid receptor; mRNA, messenger ribonucleic acid; OF1, oncins France 1; RNA, ribonucleic acid; RS 67333, (1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propane); RT-PCR, reverse transcription polymerase chain reaction; SSRIs, selective serotonin reuptake inhibitors; TNF-α, tumour necrosis factor alpha; UV, ultraviolet.

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deemed necessary for clinical remission (Binder et al., 2009; Heuser et al., 1996; Ising et al., 2007; Ribeiro et al., 1993). SSRIs may also reestablish secretion patterns for proinflammatory cytokines such as IL-6 and TNF-α (Hannestad et al., 2011; Kubera et al., 2011).

However, a drawback of SSRIs is that their therapeutic effects are observed after a few weeks and, on occasions, after several months of treatment. This delay may correspond to the time that is required for these drugs to desensitise the 5-HT_{1A} receptors at a presynaptic and postsynaptic level, thus increasing the release of serotonin, which produces the antidepressant effect (Berton and Nestler, 2006; Blier and De Montigny, 1994; Duman et al., 1997). For this reason, the search for fast-acting antidepressants is currently one of the top priorities within the field of biomedicine. Recently, 5-HT_4 receptors have been the object of much attention with respect to their involvement in psychopathologies, and behavioural and neurochemical studies indicate that these receptors are involved in affective disorders and their treatments (Bijak et al., 1997, 2001; Duman, 2007; Vidal et al., 2009). Thus, it has been proposed that 5-HT_4 receptor agonism may constitute a new fast acting antidepressant mechanism because it has been observed that these receptors exert excitatory control over the activity and firing of serotonergic neurons that are located in the dorsal raphe nucleus (Lucas and Debonnel, 2002; Lucas et al., 2005). In support of this proposal, various studies using animal models of depression have found that the administration of 5-HT_4 agonists induces the same functional, morphological, molecular and behavioural changes as conventional antidepressants but within a shorter period of time (Licht et al., 2010; Lucas et al., 2007; Pascual-Brazo et al., 2011). Nevertheless, the effect of these agonists on the endocrine and immune alterations that are involved in depression has yet to be studied.

Despite the evident relationship among chronic social stress, the development of affective disorders and the validity of available treatments, it is important to highlight that individual differences exist in the way which people cope with stress and how they respond to these treatments. Many animal studies have found that individuals differ considerably in the manner in which they respond to stress and show a large degree of behavioural and physiological variability (Bartolomucci et al., 2005; Koolhaas et al., 1999; Veenema et al., 2003). For example, previous studies conducted in our laboratory have shown that mice that adopt a passive behavioural profile in response to chronic defeat-induced stress have higher levels of IL-6 and TNF-α in the spleen than those who adopt a more active profile. Additionally, these individuals respond to stress with higher glucocorticoid levels after defeat and lower levels of this hormone when the chronic social stress ceases (Gómez-Lázaro et al., 2011). Nevertheless, the physiological mechanism involved in the alteration of the HPA axis, which was only observed in passive subjects, has yet to be determined. These different biological patterns in response to stress may be related to the fact that not all individuals respond similarly to drug treatments.

The aim of this study was to analyse the effects of administering a 5-HT_4 receptor partial agonist, RS 67333, which has been described as a putative class of antidepressant with a rapid onset of action (Licht et al., 2010; Lucas et al., 2007; Pascual-Brazo et al., 2011), using a social stress model in mice. We hypothesised that a 3 and 5-day treatment with RS 67333 could reverse chronic defeat-induced HPA axis alterations in mice with a passive coping strategy and that this reversion may be mediated by hypothalamic GRs. Additionally, we expected the treatment to reduce the levels of the proinflammatory cytokines, IL-6 and TNF-α in the spleen and desensitise the 5-HT_{1A} and 5-HT_4 receptors in the hippocampus. Finally, we aimed to study the effectiveness of the treatment on the behavioural changes that are generated by chronic stress and on the behaviour that is manifested in the FST, which is the most widely used screening test for the antidepressant potential of novel compounds (Cryan and Holmes, 2005; Hunter et al., 2000; Nestler et al., 2002).

2. Methods

2.1. Animals

OF1 outbred mice, which are characterised by their aggressive behaviour, were used. One hundred and eighty, 6-week-old male mice (Charles River, Oncins, France) were individually housed for 7 days in transparent plastic cages that measured 24.5×24.5×15 cm. Food and water were available *ad libitum*, and the holding room was maintained at a constant temperature of 20 °C with a reversed 12-h light/dark cycle (white lights on from 20:00 to 08:00 h) to enable the nocturnal animals to be tested during their active phase, which was 1 h after the dark cycle began. All experimental procedures were conducted under dim red light conditions in a room that was adjacent to the holding facility. All procedures involving mice were carried out according to the European Directive (2010/63/EU) on the protection of animals used for scientific purposes (22 September 2010). The procedures were approved by the Ethical Committee for animal welfare of the Basque Country University (CEBA).

2.2. Experimental procedures

After a period of adaptation (7 days), a control group (i.e., manipulated control) and a group of socially stressed mice were established. Next, a social stress period was initiated, which lasted for 21 consecutive days. After the end of the social stress period, the FST (Porsolt et al., 1977) was performed over 2 days for all mice. The socially stressed group was divided into two subgroups (active and passive) according to the behavioural profiles that were manifested during defeat on day 18 (see below). The drug or vehicle treatment was initiated the next day and continued until the end of the experiment, which was 5 days later (days 19, 20 and 21 of chronic social stress and days 1 and 2 of the FST). The manipulated control group was also divided into drug and vehicle control groups. Therefore, a total of six groups were obtained. Three days before the experiment began and on day 21 of social stress, blood samples (50–100 μl) were collected from stressed mice (45 min after social defeat) and manipulated controls by submandibular puncture. This new method, which was developed by Golde et al. (2005), allows researchers to obtain a sufficient volume of blood from the submandibular vein in a short time while holding the mouse and without the use of anaesthesia. Mice were euthanised by cervical dislocation 24 h after the second swimming session, which corresponded to 72 h after the last defeat experience. Blood was immediately collected from each mouse by cardiac puncture, and only a few seconds elapsed between cervical dislocation and blood collection. The brain was then quickly removed and the hypothalamus and hippocampus were dissected. The spleen was also removed under sterile conditions and immediately processed to determine the cytokine secretion in response to mitogenic stimulation *in vitro* (Fig. 1).

2.2.1. Socially stressed mice

Mice were socially stressed using the sensorial contact model (Kudryavtseva et al., 1991) to obtain mice that experienced repeated social defeat. Eighty pairs of mice, which were matched by weight, were exposed to a 10-min confrontation, where half of the animals were placed in their opponent's cage (i.e., in the cage of a resident mouse), for 3 successive days to establish a dominance–submission relationship. After the third day, only those pairs that had clearly established a dominance–submission relationship during their agonistic confrontations continued the experiment, and this relationship was observed to remain unchanged throughout the subsequent stress period. From the fourth day until the last day of chronic stress (21 days), subordinate mice were exposed daily to 5 min of agonistic interaction with a different resident dominant mouse. As a result, the subordinate mice were repeatedly defeated by a different aggressive resident dominant mouse every day. After each daily confrontation,