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The orexin-1 receptor antagonist SB-334867 attenuates anxiety in rats exposed to cat odor but not the elevated plus maze: An investigation of Trial 1 and Trial 2 effects

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ABSTRACT

The orexins are hypothalamic neuropeptides most well known for their roles in regulating feeding and sleeping behaviors. Recent findings suggest that orexin-A may also modulate anxiety, although how and when the orexin system is involved remains unclear. To address this, we investigated the dose-dependent effects of the orexin-1 receptor antagonist SB-334867 in two rodent models of anxiety: the cat odor avoidance model and the elevated plus maze. In both models we tested the effects of SB-334867 when anxiety is novel (Trial 1) and familiar (Trial 2). In the first experiment, Wistar rats were treated with vehicle or SB-334867 (5, 10 or 20 mg/kg, i.p.) prior to their first or second exposure to cat odor. During Trial 1, rats treated with 10 mg/kg of SB-334867 approached the cat odor stimulus more than vehicle-treated rats. During Trial 2 the effects were more marked, with 10 mg/kg of SB-334867 increasing approach times, increasing the number of times rats exited the hide box to engage in exploratory behavior, and decreasing overall hide times. In addition, the 20 mg/kg dose decreased general activity during Trial 2. In the second experiment, the effects of SB-334867 (10 and 20 mg/kg) were tested in the elevated plus maze. There were no significant differences produced by drug treatment during either Trial 1 or Trial 2. Results suggest that SB-334867 decreases anxiety induced by some, but not all, stressors.

Introduction

Orexin-A and orexin-B (also known as hypocretin-1 and hypocretin-2) are neuropeptides produced exclusively within the lateral hypothalamus (LH), perifornical area (PFA) and dorsomedial hypothalamus (DMH), but affecting multiple neural substrates. The orexins act on two receptor subtypes distributed throughout the brain — orexin 1 (OX-1), which is selective for orexin-A, and orexin 2 (OX-2), which is a non-selective receptor for both orexin-A and orexin-B (de Lecea et al., 1998; Sakurai et al., 1998).

The neurotransmission of orexins influences a variety of neural processes. Most well-known for regulating feeding and sleeping behaviors (Smart and Jerman, 2002), the orexins were later implicated in addiction processes (Boutrel et al., 2010), and more recent findings suggest that they also regulate emotional processes, particularly those related to anxiety (Berridge et al., 2010; Mileykovskiy et al., 2005; Suzuki et al., 2005). For example, orexin-A mediates important components of anxiety such as arousal, vigilance and attention (Boschen et al., 2009; Boutrel et al., 2010). Physical stressors, such as immobilization and cold exposure, also involve activation of the orexin system (Ida et al., 2000), and microinjections of orexins can alter anxiety-related behaviors. For example, intracerebroventricular injections of orexin-A increases anxiety in rodents exposed to the elevated plus maze (EPM) and the light–dark exploration test, while injections of orexin-A directly into the bed nucleus of the stria terminalis increases anxiety in rats exposed to the EPM and social interaction test (Lungwitz et al., 2012; Suzuki et al., 2005). However these results contrast with the findings from Singareddy et al. (2006), that intracerebroventricular injections of either orexin-A or orexin-B decreases anxiety in the EPM and acoustic startle test.

The administration of orexin receptor antagonists have also shown mixed effects. Furlong et al. (2009) found that the dual orexin receptor antagonist almorexant did not reduce freezing induced by conditioned fear to foot shock or affect behavioral responses to restraint stress, but did affect some cardiovascular responses, while Steiner et al. (2012) found that almorexant did reduce fear-potentiated startle in a conditioned fear model but did not affect behaviors in the EPM. Similarly, the OX-1 receptor antagonist SB-334867 attenuated sodium lactate-induced panic in panic-prone rats, but had no effect on the control rats (Johnson et al., 2010). The implication of these findings is that the orexin system may mediate some, but not all forms of anxiety.

To further identify the role of the orexin system in anxiety related processes, we therefore tested whether the OX-1 receptor antagonist SB-334867 affects the anxiety-like behaviors produced by two animal models — the cat odor avoidance (COA) model and the elevated plus maze (EPM). The COA model exposes rats to cat odor in a custom built apparatus that allows avoidance, risk assessment and general activity

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to be measured (Staples, 2010). Experiments have repeatedly shown that cat odor exposure elicits a robust anxiety-like response in Wistar rats (McGregor and Dielenberg, 1999; Staples and McGregor, 2006; Staples et al., 2008). Cat odor exposure also activates a well-defined neural circuitry centered on the medial hypothalamus (Canteras, 2002; Dielenberg et al., 2001; Staples, 2010; Staples et al., 2009). Interestingly, moderate to high densities of orexin receptors (particularly OX-1 receptors) are located in many of the regions most commonly associated with cat odor-induced anxiety, including the olfactory bulbs, anterior olfactory nucleus, medial amygdala, bed nucleus of the stria terminalis, anterior hypothalamic nucleus, dorsal premammillary nucleus, dorsomedial part of the ventromedial hypothalamus, periaqueductal gray and locus ceruleus (Date et al., 1999; Hagan et al., 1999; Marcus et al., 2001; Peyron et al., 1998; Shibata et al., 2008). For this reason, we hypothesized that the anxiety produced by cat odor may depend, at least in part, on the orexin system.

Like the COA model, the EPM allows avoidance, risk assessment and activity to be assessed (Carobrez and Bertoglio, 2005). Both the COA and EPM are ethologically relevant models in that they produce innate anxiety in rats (based on fear of predators and fear of open spaces, respectively). Both are also capable of producing one trial tolerance to anxiolytic drugs — a phenomenon whereby drugs such as benzodiazepines have a powerful anxiolytic effect in rats exposed to the stressor for the first time (Trial 1) but more limited effects on the second exposure (Trial 2) (File, 1993; McGregor and Dielenberg, 1999).

The main aims of the present experiments were to determine the dose-dependent effects of SB-334867 on anxiety-like behaviors and to distinguish these effects by the type of stressor (COA versus EPM) and number of exposures (Trial 1 versus Trial 2). In Experiment 1 we tested the dose-dependent effects of SB-334867 on anxiety-related behaviors in rats exposed to either a novel (Trial 1) or familiar (Trial 2) cat odor. We hypothesized that SB-334867 may have a significant effect on Trial 2 anxiety, given that the orexin system has been implicated in the regulation of anxiety elicited by a familiar stressor (Johnson et al., 2010; Li et al., 2010). In the second experiment we tested the dose-dependent effects of SB-334867 in rats given one (Trial 1) or two (Trial 2) exposures to the EPM.

Materials and methods

Subjects

Subjects were experimentally naïve male albino Wistar rats, obtained from the Animal Resource Centre (Perth, Australia) and weighing 253 ± 4 g (mean ± SEM) at time of testing. A total of 96 rats were used: 56 rats were tested in the COA apparatus, and 40 rats were tested in the EPM. Group allocation is summarized in Table 1. All rats were housed in cages of four in a temperature controlled holding room (22 ± 2 °C) on a reverse light–dark cycle (lights on from 1800 to 0600 h). Food and water were available ad libitum, and environmental enrichment was provided (consisting of hide cylinders, shredded paper and seeds scattered throughout the holding cages). Subjects were handled for seven days prior to the start of testing. Behavioral testing took place during the dark cycle under a red light, between 0900 and 1500 h. Experimentation adhered to the Australian NHMRC Code of Practice and was approved by the Macquarie University Animal Ethics Committee.

Drug treatment

Rats received 0, 5, 10 or 20 mg/kg of the OX-1 receptor antagonist SB-334867 (Tocris Biosciences), suspended in a vehicle solution composed of 10% beta cyclodextrin in sterile water. Drug and vehicle solutions were administered 10 min prior to testing via intraperitoneal (i.p.) injection, at a volume of 4 ml/kg.

Cat odor avoidance model

The COA apparatus consisted of a rectangular chamber (60 cm length × 26 cm width × 36 cm height), with black Perspex walls, a removable metal grid floor and a lockable wire mesh lid. A red Perspex "hide box" (21 cm length × 24 cm width × 22 cm height) with a small opening (6 cm × 6 cm) was positioned at one end of the chamber. The red Perspex hide box allowed rats to retreat from the stimulus while still being visible to the infrared cameras. At the opposite end of the chamber there was an alligator clip located 4 cm above the floor. The clip was used to secure the cat odor or control stimuli. The cat odor stimulus consisted of one piece of cat collar 50 mm in length (Kra-mar stretch collar, code 08010), worn by a domestic cat for six weeks. Collar pieces were kept in an airtight container and stored at. −20 °C when not in use. Prior to testing, the container holding the collar pieces was thawed by immersion in a hot water bath (55 °C) for 15 min. For tests requiring a control stimulus, one piece of odorless cat collar was attached to the alligator clip within the apparatus. All collar pieces were handled using disposable latex gloves. Testing using the COA model followed established procedures (Staples and McGregor, 2006; Staples et al., 2005) although with two notable differences: sessions were reduced to 5 min per trial, and the number of exits from the hide box was introduced as a new measure of anxiety-like behavior.

An infrared camera was positioned above the chamber and the output was fed to a computer using automated tracking software (Motmen Tracker 3, Motion Mensura, Sydney Australia). The software allowed the following behaviors to be recorded: Approach — the time that each subject spent within 7 cm of the collar stimulus; Hide — the time that

Table 1
Group allocation and treatment procedure for experiments.

Group	Familiarization	Trial 1	Trial 2
Experiment 1: Cat odor avoidance			
0 mg/kg	no odor + saline	cat odor + vehicle	cat odor + vehicle
5 mg/kg (Trial 1)	no odor + saline	cat odor + SB-334867	
10 mg/kg (Trial 1)	no odor + saline	cat odor + SB-334867	
20 mg/kg (Trial 1)	no odor + saline	cat odor + SB-334867	
5 mg/kg (Trial 2)	no odor + saline	cat odor + vehicle	cat odor + SB-334867
10 mg/kg (Trial 2)	no odor + saline	cat odor + vehicle	cat odor + SB-334867
20 mg/kg (Trial 2)	no odor + saline	cat odor + vehicle	cat odor + SB-334867
Experiment 2: Elevated plus maze			
0 mg/kg		EPM + vehicle	EPM + vehicle
10 mg/kg (Trial 1)		EPM + SB334867	
20 mg/kg (Trial 1)		EPM + SB334867	
10 mg/kg (Trial 2)		EPM + vehicle	EPM + SB-334867
20 mg/kg (Trial 2)		EPM + vehicle	EPM + SB-334867

Sessions lasted for 5 min, and each session was separated by 24 h. For all groups, n = 8. SB: SB-334867. Drugs were administered 10 min prior to each session.