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Orexin-1 receptor antagonist in central nucleus of the amygdala attenuates the acquisition of flavor-taste preference in rats

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ABSTRACT

Previous studies demonstrated that the intracerebroventricular administration of SB-334867-A, a selective antagonist of orexin OX_1R receptors, blocks the acquisition of saccharin-induced conditioned flavor preference (CFP) but not LiCl-induced taste aversion learning (TAL). Orexinergic fibers from the lateral hypothalamus end in the central nucleus of the amygdala (CeA), which expresses orexin OX_1R receptors. Taste and sensory inputs also are present in CeA, which may contribute to the development of taste learning. This study analyzed the effect of two doses (1.5 and 6 μg/0.5 μl) of SB-334867-A administered into the CeA on flavor–taste preference induced by saccharin and on TAL induced by a single administration of LiCl (0.15 M, 20 ml/kg, i.p.). Outcomes indicate that inactivation of orexinergic receptors in the CeA attenuates flavor–taste preference in a two-bottle test (saccharin vs. water). Intra-amygdalar SB-334867-A does not affect gustatory processing or the preference for the sweet taste of saccharin given that SB-334867-A- and DMSO-treated groups (control animals) increased the intake of the saccharin-associated flavor across training acquisition sessions. Furthermore, SB-334867-A in the CeA does not block TAL acquisition ruling out the possibility that functional inactivation of OX_1R receptors interferes with taste processing. Orexin receptors in the CeA appear to intervene in the association of a flavor with orosensory stimuli, e.g., a sweet and pleasant taste, but could be unnecessary when the association is established with visceral stimuli, e.g., lithium chloride. These data suggest that orexinergic projections to the CeA may contribute to the reinforcing signals facilitating the acquisition of taste learning and the change in hedonic evaluation of the taste, which would have important implications for the OX_1R-targeted pharmacological treatment of eating disorders.

1. Introduction

Innate preferences and aversions for certain flavors promote the survival of an organism and the selection of a diet nutritionally adapted to its requirements. However, the selection of most of the food in the human diet is largely determined by taste aversions and preferences that we have developed throughout our experience with different foods. In developed societies, these learned taste aversions and preferences can lead to the hedonic signals of food prevailing over its nutritional characteristics. In these cases, the diet is not limited to the satisfaction of metabolic requirements and can contribute to the development of obesity and other diseases (Berthoud, 2011; Cooper, 2007; Prescott, 2012). It is therefore important to understand the neurobiological mechanisms underlying the changes required for a previously neutral taste to become preferred or avoided as a function of experience. Questions of particular interest concern the site and manner of changes in the hedonic assessment of a gustatory stimuli, i.e., how the gustatory system is related to the reward system (Yamamoto and Ueji, 2011).

The amygdala is one of the sites where information on the reward value of food is encoded (Kenny, 2011). Because gustatory and visceral signals converge in the amygdala (Bernard et al., 1993; Price, 2003), it is one of the most widely studied structures in relation to taste learning (appetitive and aversive). The amygdala has also been associated with hedonic changes responsible for this learning (Yamamoto and Shimura, 2008). With regard to conditioned flavor preference (CFP), it has been confirmed that large electrolytic or excitotoxic lesions of the amygdala attenuate or block the acquisition of preference for a flavor associated with sucrose (Gilbert et al., 2003) or with an intragastric nutrient infusion (Touzani and Sclafani, 2005), although they do not appear to affect taste–nutrient preference learning (Touzani and Sclafani, 2005). Lesions centered in the basolateral amygdala (BLA) but not the central nucleus of the amygdala (CeA) attenuate appetitive (fructose) flavor–taste and flavor–nutrient learning (Dwyer, 2011; Touzani and Sclafani, 2005). Disruption of the dopamine function of the amygdala has produced similar results. Administration of the D1-like receptor

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antagonist SCH23390 in the CeA or BLA reduces the acquisition but not the expression of flavor preferences conditioned by intragastric glucose (Touzani et al., 2009). Whereas DA D1 or D2 receptor antagonists administered into the amygdala reduce the expression of flavor preferences conditioned by fructose (Bernal et al., 2009), only D2 receptor antagonism in the amygdala blocks the acquisition of fructose-CFP (Malkusz et al., 2012).These reports suggest that flavor–nutrient learning and flavor–taste learning may have distinct neuroanatomical and neurochemical bases (Bernal et al., 2009; Touzani et al., 2009).

In relation to taste aversion learning (TAL), it has been reported that i.p. LiCl induces c-Fos expression (Yamamoto et al., 1992, 1997) and mitogen-activated protein kinase (MAPK) activation in the CeA (Kwon and Houpt, 2012). Administration of SL327, a MAPK/ERK kinase inhibitor, reduced both LiCl-induced TAL and LiCl-induced pMAPK-positive cells in the CeA (Kwon and Houpt, 2012), indicating that this nucleus may also contribute to TAL acquisition.

In short, inputs of taste and visceral information converge in the amygdala, and this convergence is necessary for the development of both aversive and appetitive taste learning. It has also been suggested that flavor preference learning may be sustained by interactions between the amygdala and the lateral hypothalamus (LH) (Dwyer and Iordanova, 2010). Our group was able to verify the role in taste learning of orexin, a neuropeptide selectively expressed in the LH, given that the intracerebroventricular (i.c.v.) administration of SB-334867-A, selective antagonist of orexin OX_1R receptors, blocked the acquisition of saccharin-induced flavor preference but did not prevent LiCl-induced TAL (Mediavilla et al., 2011).

The orexin system is activated by stimuli such as food and drug and may play an important role in reward-related learning and memory (Cason et al., 2010; Harris et al., 2005, 2007; Scammell and Winrow, 2011). Orexinergic fibers from the LH terminate in the amygdala (Nambu et al., 1999; Peyron et al., 1998; Schmitt et al., 2012), in which orexin OX_1R receptors are present (Lu et al., 2000; Scammell and Winrow, 2011). CeA receives a dense orexinergic projection (Schmitt et al., 2012) as well as gustatory and sensory inputs that may contribute to taste learning (Yamamoto and Shimura, 2008; Baxter and Murray, 2002; Price, 2003). With this background, we investigated whether the inactivation of orexinergic receptors in the CeA specifically prevents the acquisition of a gustatory preference in a flavor–taste learning procedure in which the positive hedonic value of the flavor is increased (Prescott, 2012) or whether it also affects TAL induced by a sucrose–LiCl pairing.

2. Methods

2.1. Animals

Male Wistar rats weighing 250–270 g on arrival at the laboratory were provided by Harlan Laboratories (Barcelona, Spain). They were individually housed in methacrylate cages (21.5 cm × 46.5 cm × 14.5 cm) that served as training chambers during the experiment. Animals were randomly distributed between experimental and control groups. Room temperature was maintained at 21°–24 °C under a 12:12 light-dark cycle, lights came on at 8:00. Food and water were available ad libitum except when otherwise reported. All procedures were carried out in accordance with guidelines established by the European Union (86/609/EEC) and Spanish Royal Law 1201/2005 and were approved by the Ethical Committee for Animal Research at the University of Granada. All efforts were made to minimize animal suffering and the number of animals used.

2.2. Drugs

Two doses (1.5 and 6 μg/0.5 μl) of the orexinergic antagonist SB-334867-A (1-(2-methylbenzoxazol-6-yl)-3-[1,5] naphthyridin-4-yl-urea hydrochloride, Tocris, Madrid, Spain) were bilaterally administered in the CeA. Dimethyl sulfoxide (DMSO-, Sigma, Madrid, Spain) was used as vehicle, because previous studies found no effects of this substance on learning or memory (Akbari et al., 2007; Mediavilla et al., 2011). LiCl (0.15 M, 20 ml/kg, Sigma, Madrid, Spain, i.p.) served as noxious visceral stimulus.

2.3. Surgery

Rats were deeply anesthetized with sodium pentothal (Lab. Abbot, Spain, 50 mg/kg i.p.) and placed in a stereotaxic apparatus (Digital Lab Standard Stoelting. Wooddale, IL). Bilateral cannulae (Plastic One, 26-gauge stainless-steel guide) were implanted in the CeA, with the tip of the guide cannula positioned 2.16 mm posterior to the bregma, 4.2 mm lateral to the midline and 7.5 mm below the skull surface. Stereotaxic coordinates were determined from the rat brain atlas of Paxinos and Watson (2005). The incisor bar was placed 3.3 mm below the interaural line. The guide cannula was secured to the skull with screws and dental cement and closed with a dummy cannula. After the intervention, all animals received an intramuscular injection of 0.1 cm^3 penicillin (Penilevel, Level, S.A., Barcelona, Spain) and were given at least 5 days for recovery with food and water ad libitum. During this recovery period, the rats were handled daily, and the dummy cannula was carefully removed and replaced.

2.4. Microinjection procedure

SB-334867-A and vehicle were administered through a guide cannula using an injection needle (33 gauge) connected by polyethylene tubing to a 5.0 μl Hamilton micro-syringe driven by an infusion pump (KD Scientific Inc., MA, USA). The injection needle was inserted 1 mm beyond the tip of the guide cannula. Infusions were delivered in an injection volume of 0.5 μl/side over a period of 60 s. After each infusion, the injector remained in place for 60 s to allow diffusion of the solution into the tissue and to minimize reflux along the injection track.

2.5. Experimental procedures

2.5.1. Flavor–taste preference procedure

Animals were water-restricted and habituated to the ingestion of water for 15 min a day from an inverted graduated cylinder (20 ml, 1-ml gradation) with a sipper spout that extended into the cage. The graduated cylinders were located centrally on the front side of the cage, and their position was counterbalanced following a double alternation sequence (LRRL) to prevent a side preference. Food pellets were removed during the drinking sessions. One hour after ending experimental sessions, all animals received 30 g of food. Four hours after the end of the experimental phase, there was a daily 30-min period of rehydration. Water and food intake and animal weight were recorded daily.

After 2 days of training sessions, all animals underwent three acquisition sessions (six days) to develop flavor preferences. The CS + was an 0.15% saccharin solution flavored with 0.05% (w/w) non-sweet cherry or grape flavor (Kool-Aid, General Foods, White Plains, NY), while the CS− was the same flavor diluted with tap water. Rats were offered cherry or grape-flavor on alternate days for 15 min on each day, and the flavor–saccharin pairs were counterbalanced across subjects. The administration of SB-334867-A (1.5 and 6 μg/0.5 μl per side) was also counterbalanced in the experimental groups: SB-334867-A was associated with the grape flavor on odd days and the DMSO with the cherry flavor on even days in half of the rats, while SB-334867-A was associated with cherry flavor on even days and the vehicle with grape flavor on odd days in the other half. SB-334867-A or DMSO was administered immediately after the 15-min intake period and after recording the amount of liquid ingested. In the control group, all animals received intra-amygdala DMSO immediately after the 15-min intake. Two control animals were removed because their guide cannula became detached before the completion of testing. The final number of animals