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Research report

Orexin receptor 1 signaling contributes to ethanol binge-like drinking: Pharmacological and molecular evidence

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HIGHLIGHTS

- Orexins (OX) contribute to voluntary ethanol intake and ethanol seeking.
- Icv OXR1 antagonist (SB) blunted ethanol binge consumption in a DID procedure.
- Icv SB increased ethanol sedation without altering ethanol metabolism.
- Ethanol (but not saccharin) binge-intake failed to reduce OXR1 mRNA in the LH.
- Targeting OXR1 could help to prevent the development of binge-consumption disorders.

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ABSTRACT

Orexins (OX) have been recently implicated in ethanol seeking and self-administration. A few recent studies have provided additional evidence that OX receptor antagonists effectively reduce voluntary ethanol consumption in subjects spontaneously showing high levels of ethanol intake. The present study further evaluates the contribution of OXR1 to excessive binge-like drinking of ethanol in ad libitum-fed C57BL/6J mice from a pharmacological and molecular approach. The main findings in the study are: (1) Icv administration of SB-334867 (3 μg/μl) blunted ethanol (20% v/v), but not saccharin (0.15% w/v) binge-like drinking in a drinking in the dark procedure, without any alteration of chow consumption or total calories ingested; (2) Icv administration of SB-334867 (3 μg/μl) increased the latency to recover the righting reflex after a sedative dose of ethanol without any significant alteration in ethanol peripheral metabolism; (3) four repetitive, 2-h daily episodes of saccharin, but not ethanol binge-like drinking blunted OXR1 mRNA expression in the lateral hypothalamus. Present findings extend the current knowledge pointing to a role for OX signaling in ethanol sedation, which might partially explain the inhibitory effect of OXR1 antagonists on ethanol consumption. Combined pharmacological and molecular data suggesting the contribution of OXR1 in ethanol binge-drinking leading us to propose the idea that targeting OXR1 could represent a novel pharmacological approach to control binge-consumption episodes of ethanol in vulnerable organisms failing to spontaneously reduce OX activity.

1. Introduction

Orexin (OX) A and B (also named as hypocretin-1 and 2, respectively) are hypothalamic neuropeptides cleaved from the protein precursor pre-proorexin (preprohypocretin) [1,2]. OX peptides are produced by a population of neurons in the perifornical (PFLH), lateral (LH) and dorsomedial hypothalamus (DMH) [3,4], which send widespread projections to brain regions [3–9]. The OX peptides have been involved in a variety of regulatory physiological functions including arousal [7,10] sleep-wakefulness [11,12], homeostatic aspects of feeding [13–15] and motivational activation [16]. The wide range of OX physiological actions are mediated by two individual membrane receptors located on cell bodies and presynaptic axons [17], orexin type 1 and type 2 receptors

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(OXR1 and OXR2 respectively). While orexin A (OX-A) acts on both OXR1 and OXR2, orexin B (OX-B) acts only on OXR2 [9].

During the last decade, cumulating experimental evidence has pointed to a key role for OX in drug addiction [18–24]. Moreover, consistent with OX regulation of motivated and reward-seeking behaviors [16,25,26], available pharmacological and molecular observations suggest the additional involvement of the OX system in ethanol seeking and ethanol self-administration [28]. Thus, intraperitoneal (ip) administration of the OXR1 antagonist SB-334867 reduces cue-induced reinstatement of alcohol seeking behavior in alcohol-preferring rats (iP) [29] and stress induced reinstatement of ethanol seeking in wistar rats [30] and Long–Evans rat [31]. Also, ip SB-334867 reduces progressive ratio responding for alcohol in inbred alcohol-preferring (iP) rats [32] and ethanol self-administration in outbred rats and alcohol-preferring P rats [33]. Peripheral administration of a novel OXR2 antagonist, JNJ-10397049, dose-dependently reduces ethanol self-administration in Wistar rats, ethanol-induced place preference and reinstatement of operant responding for ethanol in DBA/2 mice [34] while icv injection of the selective OXR2 antagonist TCS-OX2-29 reduced self-administration of ethanol, but not sucrose [35].

A few recent studies have provided additional evidence that OXR antagonists effectively reduce voluntary ethanol consumption in subjects spontaneously showing high levels of ethanol intake. Thus, in a 2-bottle free choice paradigm, peripheral administration of the OX antagonist SB-334867 reduced voluntary ethanol consumption in high, but not low, ethanol preferring Sprague-Dawley rats [36]. Also, a significant reduction in voluntary ethanol self-administration has been reported in response to OXR antagonism in rats selectively bred for high ethanol preference [29,32,33,37] and also in mice showing ethanol binge-drinking [37].

Ethanol has both caloric and reward properties. Because OX receptor signaling contributes to drug and ethanol seeking [6,20,22,29] and homeostatic [13–15] and non-homeostatic aspects of food intake [38,39], those physiological mechanisms underlying the modulatory role of OXR1 on ethanol consumption remains unclear; nonetheless, some recent reports have suggested that OXR1 might modulate ethanol motivational properties independently of appetitive drive [32]. Thus, SB-334867 treatment significantly reduced self-administration for both alcohol and sucrose; however, under a progressive ratio it reduced responding for ethanol, but not sucrose, in ethanol preferring rats [32]. Nonetheless, additional research employing site-directed studies is needed to further dissociate the anatomical sites where independent aspects of ethanol intake are modulated by OXR1.

Adding complexity to the understanding of the role of OX in ethanol intake, it has been reported that central infusion of Orexin A reduces total sleeping time in ethanol induced coma [40] and recent pharmacological evidence indicates that the Orexin system modulates the sedative properties of several anesthetics such as sevoflurane and isoflurane [41], propofol [42], barbiturate [43] and ketamine [44]. Taking into account that enhanced sensitivity to ethanol sedative properties inversely correlates with voluntary ethanol consumption [45], there is the interesting hypothesis that reduced ethanol consumption in animals treated with an OXR antagonist might be the result, at least in part, of enhancement in ethanol-induced sedation.

In the present study we further explore physiological and psychological processes contributing to OXR1 modulation of binge-like voluntary ethanol consumption. To that aim, we test in a dose-response study the impact of the centrally administered OXR1 receptor antagonist SB-334867 (which is >1000-fold selective for OXR1 over OXR2 [46]), on ethanol and saccharin (a palatable, non-caloric substance) overconsumption in a "drinking in the dark" (DID) procedure [47,48], in adult mice under non caloric restriction. Secondly, we directly evaluate whether OXR1 antagonism enhance ethanol sedative properties, as measured by the ability of SB-334867 to modulate ethanol-induced loss of the righting reflex (LORR).

It has been shown altered OX mRNA expression within the LH after morphine [49], cocaine [50], saccharin, sucrose binge-like intake [39] and ethanol [51] administration as well. Given the impact of ethanol administration on OX synthesis and because ethanol and saccharin overconsumption engages OX peptides [37,39,51,52] the ability of OX-producing cells to develop compensatory changes in presynaptic OX receptors would be critical to successfully adjust altered neuropeptide release when engaged in ethanol and/or saccharin binge-drinking. Our third objective is aimed to explore OXR1 mRNA expression in the lateral hypothalamus (LH) following repetitive episodes of binge-like ethanol or saccharin consumption.

2. Methods

2.1. Animals

Male C57BL/6J mice (Charles River Laboratories, Spain) weighing 22–24 g at the beginning of the experiments were housed individually in an environmentally controlled room (22 °C temperature on a 12:12 h light–dark cycle). Standard rodent chow and water were provided ad libitum throughout the experiments and all the pharmacological manipulations were conducted at the onset of the dark phase. Behavioral procedures and pharmacological techniques were in compliance with the animal care guidelines established by the Spanish Royal Decrees 1025/2005 for reducing animal pain and discomfort and the protocols were approved by the University of Almería Bioethical Animal Care and Use Committee.

2.2. Surgery

Mice were anesthetized with a cocktail of ketamine (117 mg/kg) and xylazine (7.92 mg/kg) and surgically implanted with a 26-gauge guide cannula (Plastic One Inc., Germany) aimed at the left lateral ventricle, with the following stereotaxic coordinates: 0.2 mm posterior to bregma, 1.0 mm lateral to the midline, and 2.3 mm ventral to the surface. Mice were allowed to recover for approximately 2 weeks before experimental procedures were initiated. After experimental procedures, cannula placement was verified histologically. Intracerebroventricular (icv) infusions were given in a 1.0 μl volume over a 1-min period using a 33-gauge injector needle that extended 0.5 mm beyond that guide cannula. Compounds were administered manually with a 1-μl Hamilton syringe. The injectors were left in place for an additional 1 min to allow for drug diffusion and to minimize vertical capillary action along the injector tract when it was removed.

2.3. Experiment 1: Effects of icv SB-334867 on binge-like ethanol and saccharin drinking in a DID procedure

This study was aimed to test whether the OX receptors antagonist SB-334867 modulates binge-like ethanol and/or saccharin drinking as measured by a DID procedure.

2.3.1. Effects of icv SB-334867 on ethanol binge-like drinking

Male mice ($n = 30$) were trained and tested in a standard 4-day DID procedure [47,48,53,54]. On days 1–3, beginning 3 h into the dark cycle, all homecage water bottles were replaced with a single bottle of 20% (v/v) ethanol which were weighed and