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Research report

Selective orexin 2 receptor antagonism blocks cue-induced reinstatement, but not nicotine self-administration or nicotine-induced reinstatement

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HIGHLIGHTS

- Several orexin 1 receptor antagonists impact behaviors associated with drug abuse.
- The role of the orexin 2 receptor in these behaviors is unclear.
- We characterized 2-SORA 18, a selective orexin 2 receptor antagonist.
- Up to 60 mg/kg of 2-SORA 18 did not impact nicotine self-administration or nicotine-induced reinstatement.
- Doses as low as 15 mg/kg 2-SORA 18 completely blocked cue-induced reinstatement.

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ABSTRACT

The orexinergic system has been implicated in a number of behaviors, including reward and incentive motivation. Orexin 1 receptor antagonism has been reported to reduce drug self-administration, conditioned place preference, and reinstatement in rodents, but the role of the orexin 2 receptor is unclear. Here we evaluated the impact of the novel and selective orexin 2 receptor antagonist, 2-SORA 18, on motivation for nicotine as measured by responding on a progressive ratio schedule, as well as cue-induced reinstatement of a response previously associated with nicotine reward, and nicotine-induced reinstatement. 2-SORA 18 demonstrated selective effects on these behaviors. Specifically, doses up to 60 mg/kg 2-SORA 18 were without significant effect on nicotine self-administration or nicotine-induced reinstatement, but doses as low as 15 mg/kg 2-SORA 18 completely blocked cue-induced reinstatement. These findings indicate that orexin 2 receptor antagonism might have utility for attenuating relapse, particularly for patients sensitive to environmental stimuli associated with drug taking.

1. Introduction

Orexinergic neurons synthesizing the neuropeptides orexin A and B are located primarily in the lateral hypothalamus. The downstream targets of these neurons include brain regions implicated in incentive motivation, such as the dopamine synthesizing neurons in the ventral tegmental area (VTA) and their afferents in the nucleus accumbens [1,2]. Administration of either orexin A or B onto the VTA potentiates neuronal firing of VTA neurons [3–5] and increases dopamine efflux in the nucleus accumbens [6]. Knockout mice lacking the prepro-orexin gene demonstrate attenuated morphine-induced conditioned place preference, as well as morphine- and cocaine-induced dopamine release [6–8]. Based on these reports, it has been hypothesized that treatments that influence orexinergic signaling could represent a novel therapy for drug addiction.

There are two orexin receptors, OX1R and OX2R, both of which are G-protein coupled receptors that differ with regards to their localization and affinity for orexin A and B. Most research examining the role of orexin in mediating the behavioral effects of drugs

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of abuse has focused on OX1R, at least to some extent because of the availability of the partially selective OX1R antagonist SB-334867. For example, it has been shown that SB-334867 attenuates the self-administration of nicotine [9,10], alcohol [11–13], and cocaine [7,14]. Furthermore, this compound has been shown to reduce reinstatement produced by cues and contexts previously associated with the self-administration of alcohol [11] and cocaine [15–17]. Consistent with the involvement of OX1R on the incentive motivational effects of cues associated with drug administration, SB-334867 has also been shown to block conditioned place preference produced by ethanol [18] and morphine [19].

Due to the relative scarcity of available selective OX2R antagonists, much less is known regarding the involvement of OX2R in the incentive motivational effects of drugs and drug-paired cues. Smith et al. [16] reported that the partially selective OX2R antagonist 4PT failed to influence self-administration of cocaine or cue-induced reinstatement. In contrast, Shoblock et al. [20] recently reported that the OX2R antagonist JNJ-10397049 dose-dependently reduced alcohol self-administration and conditioned place preference. This discrepancy could result from a variety of factors, including the drug of abuse being examined (cocaine versus alcohol) or the OX2R antagonist being studied. Here we sought to further characterize the involvement of OX2R antagonism on the incentive motivational effects of drugs of abuse and drug-paired cues by examining the effect of the novel, selective, and brain penetrant OX2R antagonist 2-SORA 18 on nicotine self-administration under a progressive ratio schedule and the ability of nicotine or a cue previously paired with nicotine to promote reinstatement. 2-SORA 18 is a potent OX2R antagonist (IC50 = 12 nM) and is selective for OX2R relative to OX1R (over 1000-fold selective in terms of binding Ki and 24-fold selective in functional cell based assays [21]).

2. Materials and methods

2.1. Animals

All studies were conducted in accordance with the Merck Institutional Animal Care and Use Committee and the National Research Council's Guide for the Care and Use of Laboratory Animals. Male Long Evans rats (250–275 g) purchased from Charles River Laboratories were housed singly in a humidity and temperature-regulated vivarium on a 12/12 h reverse light/dark cycle. The only exception was for the study characterizing the effects of 2-SORA 18 on locomotor activity, which was conducted in animals on a standard 12/12 h light/dark cycle. Water and chow were available *ad libitum* until training and subsequently fed 25 g chow per day following their daily operant session.

2.2. Apparatus

Nicotine self-administration occurred in operant chambers controlled by a computer interface system (Med Associates). Chambers were equipped with two levers 2.5 cm above a grid floor. Active lever presses activated a microliter syringe pump. Inactive lever presses were recorded but had no consequences. A cue light was positioned 7.5 cm above the active lever and a tone generator was located directly above the cue light. A house light was located on the opposite side of the chamber and signaled the onset of the session.

2.3. Food training and surgery

Before surgery, rats underwent operant training for 45-mg sucrose pellets on a fixed ratio (FR) 1 reinforcement schedule. Rats were then anesthetized and prepared with catheters implanted into the right jugular vein as previously described [22,23].

2.4. Effect of 2-SORA 18 on responding for nicotine under a progressive ratio (PR) schedules

Rats were trained to self-administer nicotine (0.03 mg/kg/infusion) on an FR1 schedule for 5 daily 1 h sessions. Each nicotine infusion was followed by a 40 s timeout in which the CS+ light was delivered but lever presses had no consequence. Rats were then placed under FR2 and FR5 schedules for 2 and 6 sessions, respectively. Animals were then trained for four days to respond for nicotine on a PR schedule until stable performance was achieved, as has been previously reported [24–26]. The sequence of PR schedule was determined using the exponential formula $5e^{(0.2 \times [\text{infusion number} + 3])} - 5$, with the first three values replaced by 3, 6 and 10. PR sessions concluded following 20 min of inactivity on the active lever.

Once animals reached stable PR performance, the effects of 2-SORA 18 on responding were examined using a between subjects group design ($n = 10$ per group). Animals were administered either vehicle or 2-SORA 18 (3, 15, or 60 mg/kg; p.o.) 1 h prior to testing.

2.5. Effect of 2-SORA 18 on cue- and nicotine priming-induced reinstatement for a response previously associated with nicotine reinforcement

Following PR testing, all rats were re-trained on an FR5 schedule (1 h session) for 3 days. Extinction of nicotine self-administration was then carried out, during which responding on the active lever did not result in a nicotine infusion. For half of the animals, the tone and cue light were still presented following an active lever press (priming group), whereas the tone and cue light were absent (cue group) for the other animals. Rats were given extinction sessions (10–24) until they achieved extinction criteria of less than 15 lever presses on the active lever on consecutive sessions. Eleven rats extinguished in the presence of tone and cue light successfully reached the extinction criteria by session 24 and were included in the nicotine-reinstatement study. Fourteen rats extinguished in the absence of tone and cue light successfully reached the extinction criteria by session 24 and were included in the cue-reinstatement study.

Cue-induced reinstatement of nicotine seeking was examined in rats that had undergone extinction in the absence of tone and light cues. The cue-induced reinstatement sessions were essentially identical to these rats' extinction sessions with the exception that active lever presses resulted in the delivery of the light and tone cue that were previously associated with nicotine delivery. The effects of 2-SORA 18 (3, 15, or 60 mg/kg; p.o.) on cue-induced reinstatement was evaluated in a Latin square design. Extinction sessions (minimum of one session) were conducted between test days. Allowing two days between treatment sessions was deemed to be enough time given that: (1) 2-SORA 18 has a half-life on 0.9 h in rat and so 48 h between test sessions represented >48 half-lives of the compound and (2) 15 mg/kg 2-SORA 18 produced EEG effects that lasted <4 h, consistent with the rat half-life of the compound [21].

Reinstatement of nicotine seeking induced by nicotine priming (0.15 mg/kg; s.c.) was examined in rats having undergone extinction in the presence of tone and light cues. Reinstatement sessions were essentially identical to the extinction sessions with the exception that nicotine was injected 15 min prior to the session. The effects of 2-SORA 18 (3, 15, or 60 mg/kg; p.o.) on reinstatement induced by priming were examined using a Latin square design.

2.6. Effect of 2-SORA 18 on locomotor activity

In order to examine the impact of 2-SORA 18 on locomotor activity, which potentially could confound the results observed during