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ORIGINAL INVESTIGATION

Inhibition of orexin-1/hypocretin-1 receptors inhibits yohimbine-induced reinstatement of ethanol and sucrose seeking in Long–Evans rats

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Abstract
Rationale Previous studies have shown that orexin-1/hypocretin-1 receptors play a role in self-administration and cue-induced reinstatement of food, drug, and ethanol seeking. In the current study, we examined the role of orexin-1/hypocretin-1 receptors in operant self-administration of ethanol and sucrose and in yohimbine-induced reinstatement of ethanol and sucrose seeking.
Materials and methods Rats were trained to self-administer either 10% ethanol or 5% sucrose (30 min/day). The orexin-1 receptor antagonist SB334867 (0, 5, 10, 15, 20 mg/kg, i.p.) was administered 30 min before the operant self-administration sessions. After these experiments, the operant self-administration behaviors were extinguished in both the ethanol and sucrose-trained rats. Upon reaching extinction criteria, SB334867 (0, 5, 10 mg/kg, i.p.) was administered 30 min before yohimbine (0 or 2 mg/kg, i.p.). In a separate experiment, the effect of SB334867 (0, 15, or 20 mg/kg, i.p.) on general locomotor activity was determined using the open-field test.
Results The orexin-1 receptor antagonist, SB334867 (10, 15 and 20 mg/kg) decreased operant self-administration of 10% ethanol but not 5% sucrose self-administration. Furthermore, SB334867 (5 and 10 mg/kg) significantly decreased yohimbine-induced reinstatement of both ethanol and sucrose seeking. SB334867 did not significantly affect locomotor activity measured using the open-field test.
Conclusions The results suggest that inhibition of OX-1/Hcrt-1 receptors modulates operant ethanol self-administration and also plays a significant role in yohimbine-induced reinstatement of both ethanol and sucrose seeking in rats.

Keywords Orexin · Hypocretin · Operant self administration · Reinstatement · Yohimbine · SB334867 · Stress · Ethanol

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Introduction

The orexins (or hypocretins) are neuropeptides produced exclusively in the lateral hypothalamus (de Lecea et al. 1998; Peyron et al. 1998; Sakurai et al. 1998), an area activated by consummatory rewards (Bernardis and Bellinger 1996; Levitt and Teitelbaum 1975) and strongly linked to preferences for cues associated with food and drug reward (Harris et al. 2005). Orexin/hypocretin-containing neurons have widespread projections throughout the central nervous system (CNS; Nambu et al. 1999; Peyron et al. 1998). There are currently two identified orexin/hypocretin peptides, orexin-A/hypocretin-1 and orexin-B/hypocretin-2, and two orexin/hypocretin receptors, the orexin-1/hypocretin-1 (OX-1/Hcrt-1) receptor and the orexin-2/hypocretin-2 (OX-2/Hcrt-2) receptor (de Lecea et al. 1998; Sakurai et al. 1998). The OX-1/Hcrt-1 receptor is selective for orexin-A/hypocretin-1, while the OX-2/Hcrt-2 receptor is nonselective for both orexin-A/hypocretin-1 and orexin-B/hypocretin-2 (Sakurai et al. 1998). Although both receptors are

expressed throughout the CNS, the ventral tegmental area (VTA) and locus coeruleus (LC) exhibit the highest expression levels (Sakurai 2007). Furthermore, the locus coeruleus, a key modulator of attentional state, has been shown to receive the densest innervation of orexin/hypocretin neurons (Hagan et al. 1999; Horvath et al. 1999). The orexin/hypocretin system was initially implicated in the regulation of homeostatic functions such as feeding and arousal (Hagan et al. 1999; Mignot 2001; Sakurai et al. 1998; Sutcliffe and de Lecea 2002; Willie et al. 2001). However, results from more recent studies indicate that orexins/hypocretins play a significant role in reward-related behaviors (Borgland et al. 2006; Boutrel 2006; DiLeone et al. 2003; Harris and Aston-Jones 2006; Harris et al. 2005; Lawrence et al. 2006; Narita et al. 2006).

Relapse to drug seeking is a hallmark of addictive behaviors, yet there are currently few effective treatments for preventing relapse after a protracted period of abstinence. A number of methods have been developed in animals to study reinstatement of drug seeking and have adequate validity to model aspects of relapse in humans (Epstein et al. 2006, no. 16). For example, reinstatement of drug seeking in laboratory animals is induced by environmental cues and contexts associated with drug availability, reexposure to the drug itself, and stress. These conditions have been reported to induce relapse to drug taking in humans (Epstein et al. 2006; Katz and Higgins 2003; Spanagel 2003). The orexin/hypocretin system has been shown to play a key role in cue-induced reinstatement of morphine, nicotine, and ethanol seeking (DiLeone et al. 2003; Lawrence et al. 2006; Narita et al. 2006; Pasumarthi et al. 2006) and has been proposed to play a role in footshock stress-induced reinstatement of cocaine seeking (Boutrel et al. 2005); however, the role of the orexin/hypocretin system in stress- or yohimbine-induced reinstatement of ethanol seeking is not known.

It has been shown that yohimbine, an α-2 adrenoceptor antagonist, induces a stress-like state in both humans and laboratory animals (Bremner et al. 1996a, b; Vythilingam et al. 2000). In contrast to other stressors, yohimbine activates c-fos and CRF mRNA in the same brain regions as that elicited after footshock-induced stress (Funk et al. 2006). Furthermore, the brain areas activated by yohimbine and footshock were found to be regionally specific in brain regions associated with the rewarding effects of ethanol and other substances of abuse, notably, the shell of the nucleus accumbens, basolateral and central amygdalar nuclei, and the bed nucleus of the stria terminalis (Funk et al. 2006). Footshock-induced stress has been shown to be effective in inducing reinstatement of cocaine, heroin, ethanol, and nicotine seeking (Buczek et al. 1999; Erb et al. 1996; Funk et al. 2006; Le et al. 2000; Le et al. 1998; Liu and Weiss 2002; Lu et al. 2003; Shaham et al. 1997); however, its ability to reinstate seeking of natural rewards such as sucrose has been inconsistent (Buczek et al. 1999). Based on the neurochemical data and the increasing evidence showing that yohimbine reinstates reward-seeking behavior (Funk et al. 2006; Gass and Olive 2007; Ghitza et al. 2006; Le et al. 2005; Lee et al. 2004; Marinelli et al. 2007; Nair et al. 2006; Shepard et al. 2004), we used yohimbine in the current study to induce reinstatement to determine the role of the OX-1/Hcrt-1 receptors in the reinstatement of ethanol and sucrose seeking.

Materials and methods

Subjects

Male Long–Evans rats weighing 180–200 g upon arrival (Harlan Indianapolis, IN, USA), were individually housed in ventilated Plexiglas cages in a climate-controlled room on a 12-h light–dark cycle (lights on at 7 A.M.). Operant training occurred between 8 A.M. and 12 P.M., Monday through Friday. Food and water were available ad libitum, except for short periods during initial self-administration training, as outlined below. All procedures were preapproved by the Gallo Center Institutional Animal Care and Use Committee and were in accordance with NIH guidelines for the Humane Care and Use of Laboratory Animals.

Drugs

SB334867 (1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthydrin-4-yl urea HCl; Tocris, MI, USA) was made up in a vehicle solution containing saline with 25% cyclodextrin (*w*/*v*) and 5% dimethyl sulfoxide (*v*/*v*) and was administered at doses of 5, 10, 15, or 20 mg/kg (as outlined in the methods for each experiment). Yohimbine (Sigma Aldrich, MO, USA) was made up in distilled water and administered at a dose of 2 mg/kg, in accordance with previous reinstatement studies (Gass and Olive 2007; Ghitza et al. 2006; Le et al. 2005; Marinelli et al. 2007; Nair et al. 2006; Shepard et al. 2004). Vehicle injections were administered in the same volume as the injected drug as a control condition. Sucrose solutions [5 or 10% sucrose (*w*/*v*) Fisher Scientific, NJ, USA] were prepared using filtered water. The 10% ethanol (*v*/*v*) solution was prepared using 95% ethyl alcohol (Gold Shield Chemical, Hayward, CA DSP-CA-151, USA) and filtered water. In the sucrose fade experiments, 10%, 5%, 3%, and 1% sucrose, respectively, were dissolved in 10% ethanol (*w*/*v*).

Apparatus

Self-administration testing was conducted in standard operant conditioning chambers (Coulbourn Instruments,

Allentown, PA, USA) enclosed in sound-attenuating cubicles with a fan for ventilation and background white noise. Each operant conditioning chamber housed two retractable levers on the right wall (4 cm above the grid floor, 12 cm apart). A liquid dipper system was placed centrally between the two levers. A house light was present on the wall opposite the levers and remained on at all times during the operant conditioning session. Stimulus lights were present 2 cm above each lever. An apparatus to emit a tone under specific conditions for operant responding was also present. Upon correct (active) lever press(es), the stimulus light above the active lever was illuminated and accompanied by a tone for 3 s to indicate availability of reward in the dipper receptacle. The dipper port was illuminated for 10 s, while the dipper cup was available. If the cup was not actively licked during the 10 s, the cup fell, and this event was recorded as a null response. For the cup to be available again, the rat had to perform another fixed ratio. The number of reinforcers obtained per operant self-administration sessions was determined as the total number of reinforcers offered minus the null responses. Stimulus, fluid delivery, and operant responses were all controlled and recorded by computer (Coulbourn Instruments, Allentown, PA, USA) using Graphic State 2.0 software. Inactive lever presses were also recorded as a measure of general activity but had no programmed consequences.

Operant self-administration procedure

Before beginning the operant self-administration training, rats were exposed to 10% ethanol or 5% sucrose solution (depending on which test group they were assigned) as the only liquid source in their home cages for 3 days. Rats were then fluid restricted for 22 h before being placed in the operant conditioning chambers for a 14-h overnight session on an FR1 schedule of reinforcement (0.1 ml after a single lever press) with either 10% or 5% sucrose as the reinforcer for the ethanol and sucrose assigned group, respectively. During this phase, only the active lever was available for the rat to press, to facilitate learning. Initial daily training consisted of 45 min FR1 operant conditioning sessions and 2-h daily water access, with water access immediately after the training sessions. Once conditions for operant responding were established (2–4 days), rats were given free access to water in the home cage and continued on a 45-min FR1 schedule for 3–4 days. Subsequently, operant conditioning training sessions were reduced to 30 min, and the work ratio increased to an FR3 schedule of reinforcement (three active lever presses required for 0.1 ml reward). A second, inactive lever was also introduced at this time. Upon pressing the inactive lever, no reinforcer, cue light, or auditory stimuli were presented, and the event was merely recorded as a measure of nonspecific behavioral activation. For rats assigned to the 10% ethanol group, a sucrose fading technique began at this point, and 10% ethanol was added to the sucrose solution (Samson 1986). Throughout the next eight to ten operant conditioning sessions, the sucrose concentration was gradually decreased (5%, 3%, and 1%) until the rats responded on an FR3 schedule for 10% ethanol alone. Stable baseline responding was achieved when the number of responses for each rat varied within 20% of the average for the group over three consecutive sessions. Both ethanol and sucrose groups had reached stable baseline responding at the FR3 level after 20 sessions.

Experiment 1—effect of SB334867 on operant self-administration of ethanol and sucrose

Rats were trained to self-administer either 10% ethanol or 5% sucrose in operant conditioning chambers as outlined above. Once the rats had achieved stable baseline responding levels, they were tested once per week with SB334867 (5, 10, 15, 20 mg/kg i.p.) or vehicle. Drug (or vehicle) was administered 30 min before the start of the operant conditioning session. All ethanol self-administering rats received all drug doses and vehicle conditions in a counterbalanced, Latin square design. In sucrose self-administering rats, initial indications showed no effect of SB334867 at 20 mg/kg, so rats were only tested with this dose or vehicle. Regular 30 min operant self-administration sessions were run on days between test sessions.

Experiment 2—effect of SB334867 on general locomotor activity

Testing was conducted using 40×40 cm open-field locomotor activity-monitoring chambers equipped with horizontal photobeams (Med Associates, St Albans, VT, USA). Horizontal locomotor activity was monitored at 100 ms, and stereotypic behaviors were also recorded throughout the session. Locomotor studies were run in daily 2 h sessions over four consecutive days. On day 1, rats were placed into the activity chamber for 2 h, no data were collected, and this session was used solely for habituation purposes. On days 2 and 3, animals were placed into the activity chamber, and the session was paused after 60 min, while animals were removed from the chamber and given a single i.p. injection of saline (3 ml/kg) to habituate animals to the injection procedure before the test day. Animals were returned to the activity chambers and the session continued for an additional 60 min. Data from the third day was used to assign animals to one of three treatment groups (0, 15, 20 mg/kg SB334867), six animals per group. On day 4, the animals were once again placed in the activity chambers, and the session was paused after 60 min, while the animals received a single i.p. injection of either vehicle or drug,

SB334867 (15 or 20 mg/kg). The animals were returned to the activity chamber immediately after the injections, and the session continued for an additional 60 min, drug challenge. At the end of each session, animals were returned to the home cage. Data were collected across the entire 2 h (120 min) session and recorded as distance traveled in centimeters and total number of stereotypic counts. Data were separated into habituation (0–60 min) and drug challenge (60–120 min) for statistical analysis. Data analysis was performed by one-way analysis of variance (ANOVA) using the between subjects factor of treatment (SB334867 dose).

Experiment 3—effect of SB334867 on yohimbine-induced reinstatement

For the reinstatement study, ethanol- or sucrose-seeking behavior was extinguished in rats trained to self-administer 10% ethanol or 5% sucrose, respectively, under FR3 conditions. During the extinction training, lever pressing on the active lever resulted in light and tone cue presentation but no reward delivery. Neither 10% ethanol nor 5% sucrose was available throughout extinction training. Extinction training continued until the rats responded with less than ten active lever presses per session or less than 10% of their previous baseline pressing on the active lever for two consecutive sessions (Fig. 2). The rats were assigned to one of six groups (three ethanol groups and three sucrose groups), matched for their previous operant self-administration responding. We used the between subjects factor of SB334867 dose (0, 5, 10 mg/kg i.p.) and the within subjects factor of yohimbine dose (0, 2 mg/kg, i.p.) to assess the effect of SB334867 on yohimbine-induced reinstatement. Animals received two injections per test day as outlined in Table 1. On test days, the rats were first injected with either SB334867 or its vehicle and 30 min later with either yohimbine or the yohimbine vehicle. Rats were placed into the operant self-administration chambers 30 min after the second injection for the 30-min-long reinstatement test session. Reinstatement sessions were run under the same conditions as the extinction sessions; successful FR3 responses at the previously active lever resulted in light and tone cue presentation with no reward delivery. Again, inactive lever responding had no programmed consequences and was used as a measure of nonspecific behavioral activation. Rats were tested with vehicle and yohimbine consecutively over two test sessions, 7 days apart. Regular 30-min extinction sessions were run on the between-test days.

Statistics

The data from the ethanol and sucrose groups in the operant self-administration and reinstatement experiments were analyzed separately. The data from the operant self-administration studies were analyzed by repeated measures two-way ANOVA using the within-subjects factors of SB334867 dose and lever. The data from the reinstatement studies were analyzed by two-way ANOVA using the between subjects factor of SB334867 dose and within subjects factors of yohimbine dose. Active and inactive lever data were analyzed separately, as there was no effect of either SB334867 or yohimbine on inactive lever pressing and therefore no need for determination of any drug × lever interactions. For the locomotor activity study, drug challenge (60–120 min) data were analyzed by one-way ANOVA using the between subjects factor of SB334867 dose. All statistical analyses were performed using SigmaStat software, and in all cases, post hoc analysis was determined by Student Newman–Keuls test where overall significance was $p<0.05$.

Results

Experiment 1: effect of SB334867 on operant self-administration of ethanol and sucrose

SB334867 significantly attenuated operant self-administration of 10% ethanol in a dose-dependent manner compared

Table 1 Injection procedure for reinstatement control and test sessions

	Week 1 (controls)		Week 2 (reinstatement test)	
	Injection 1	Injection 2	Injection 1	Injection 2
Group 1	Vehicle SB334867	Vehicle Yohimbine	Vehicle SB334867	2 mg/kg Yohimbine
Group 2	5 mg/kg SB334867	Vehicle Yohimbine	5 mg/kg SB334867	2 mg/kg Yohimbine
Group 3	10 mg/kg SB334867	Vehicle Yohimbine	10 mg/kg SB334867	2 mg/kg Yohimbine

Ethanol (three groups) and sucrose (three groups) trained rats were tested over 2 weeks. The control injections (week 1) determined any effect of SB334867 on extinction baseline responding. Reinstatement (week 2) determined any effect of SB334867 on yohimbine-induced reinstatement of ethanol or sucrose seeking. Injections were administered i.p. 30 min apart. The second injection (yohimbine vehicle or yohimbine) was given 30 min before the start of the operant session

to vehicle-treated animals (Fig. 1a). Repeated measures two-way ANOVA of lever pressing revealed a significant effect of SB334867 dose (F[4,69]=4.4, p<0.01, Fig. 1a), a significant effect of lever (F[1,69]=31.7, p<0.001), and a significant interaction of SB334867 dose × lever (F[4,69]=4.5, p<0.01). Post hoc analysis further revealed that the SB334867 dose had no effect on inactive lever pressing (Table 2). One-way ANOVA analysis of number of reinforcers obtained also revealed an overall main effect of the SB334867 dose (F[4,38]=3.0, p<0.05). Post hoc analyses revealed that 10, 15, and 20 mg/kg doses significantly reduced the number of reinforcers obtained compared to vehicle (Fig. 1b). In contrast, SB334867 (20 mg/kg) did not affect 5% sucrose operant self-administration when compared to vehicle-treated animals (data not shown). Repeated measures two-way ANOVA of lever pressing revealed a significant effect of lever (F[1,31]=169.5, p<0.001), but nonsignificant effect of SB334867 dose, and no interaction of SB334867 dose × lever (data not shown). Furthermore, one-way ANOVA of the number of sucrose reinforcers also showed a nonsignificant effect of SB334867 dose (data not shown).

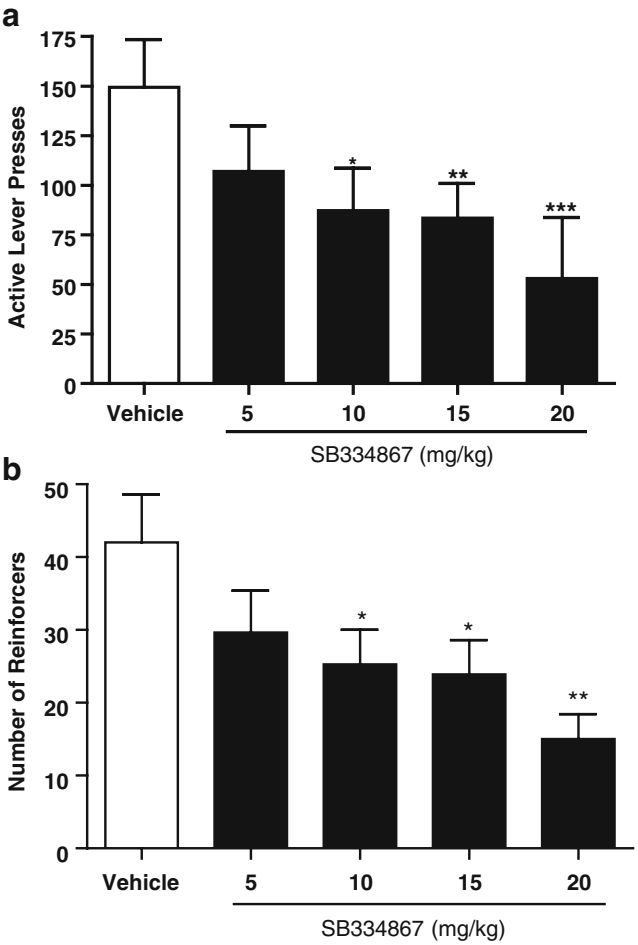

Fig. 1 Effect of SB334867 on operant self-administration of 10% ethanol. **a** Mean±SEM number of active lever presses. **b** Mean±SEM number of ethanol reinforcers obtained. Rats were pretreated with SB334867 or its vehicle 30 min before the 30-min self-administration sessions (n=8–9 per group). *p<0.05; **p<0.01; ***p<0.001 compared to vehicle condition

Table 2 Effect of SB334867 on inactive lever pressing in operant self-administration of ethanol

SB334867 (mg/kg)				
Vehicle	5	10	15	20
0±0	1±1	2±1	2±1	3±1

Mean±SEM inactive lever presses. Rats were pretreated with SB334867 or its vehicle 30 min before the 30-min ethanol self-administration sessions (n=8–9 per group)

Experiment 2: effect of SB334867 on general locomotor activity

There was no statistical difference in baseline general locomotor activity between treatment or vehicle groups during the 1-h habituation period. Furthermore, there was no overall main effect on the general locomotor activity during the 1-h drug challenge period that followed immediately after the SB334867 (15 or 20 mg/kg) or vehicle injection. One-way ANOVA of data during the drug challenge period revealed no effect of SB334867 on either total distance traveled or total number of stereotypic counts (data not shown).

Experiment 3: effect of SB334867 on yohimbine-induced reinstatement of ethanol and sucrose seeking

Ethanol and sucrose self-administering animals extinguished their lever pressing behavior at similar rates, requiring approximately 4 weeks to reach extinction criteria (Fig. 2). After extinction of the ethanol-seeking behavior, yohimbine (2 mg/kg, i.p.) induced reinstatement of lever responding on the active lever that had previously been associated with ethanol reinforcement. Two-way ANOVA analysis of active lever responding in ethanol-experienced rats revealed a significant interaction of yohimbine dose × SB334867 dose (F(2,57)=5.8, p<0.01). Post hoc analysis further revealed that yohimbine, in the absence of SB334867, significantly increased active lever responding (p<0.001, Fig. 3). Furthermore, the reinstatement was inhibited by pretreatment with SB334867 (5 and 10 mg/kg), as both doses tested significantly attenuated yohimbine-induced reinstatement of active lever responding (Fig. 3).

In accordance with the ethanol data, two-way ANOVA analysis of active lever responding in sucrose-experienced rats revealed a significant effect of the yohimbine dose (F[1, 39]=5.0, p<0.05) and a significant interaction of the

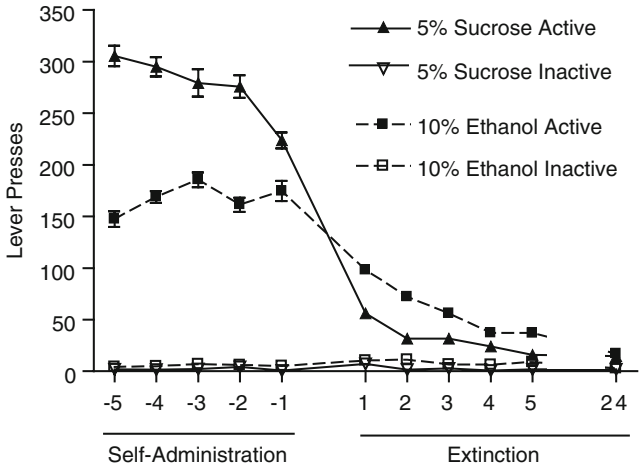

Fig. 2 Operant training and extinction of responding for 10% ethanol and 5% sucrose. Mean±SEM number of active and inactive lever presses during the last 5 days of operant training and first 5 days of extinction training. Last day of extinction training before reinstatement tests is also shown (day 24)

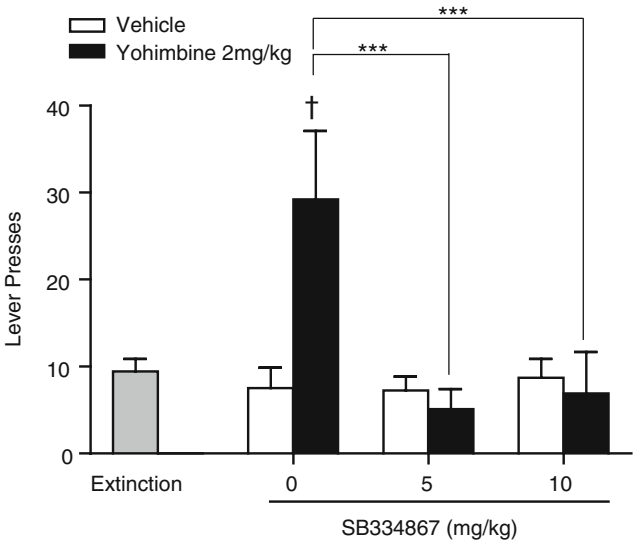

Fig. 3 Effect of SB334867 on yohimbine-induced reinstatement of ethanol seeking. Mean±SEM number of active lever presses during extinction and reinstatement tests. Extinction is shown as the average number of lever presses across the two sessions preceding the first reinstatement test for all rats. Rats were pretreated with SB334867 or its vehicle 30 min before yohimbine or its vehicle. Yohimbine (or vehicle) was administered 30 min before the start of the reinstatement session (n=8–11 per group). †p<0.001 compared to yohimbine-vehicle condition in rats injected with the SB334867 vehicle (0 dose); ***$p\leq$0.001 compared to SB334867 vehicle condition in rats injected with yohimbine

yohimbine dose × SB334867 dose (F[2, 39]=6.0, p<0.01). Post hoc analysis further revealed that yohimbine, in the absence of SB334867, significantly increased active lever responding (p<0.001, Fig. 4). Furthermore, SB334867 significantly attenuated yohimbine-induced reinstatement of active lever responding at both doses tested (Fig. 4).

After extinction of the ethanol- and sucrose-seeking behavior, SB334867 alone, in the absence of yohimbine, had no effect on active lever responding compared to vehicle in either ethanol- or sucrose-experienced rats (Figs. 3 and 4, respectively). Inactive lever responses were unaffected by any treatment in both ethanol- and sucrose-experienced rats (Table 3).

Discussion

The present study shows that the OX-1/Hcrt-1 receptor antagonist, SB334867, dose dependently attenuates operant self-administration of ethanol, but not sucrose. After extinction of the ethanol- and sucrose-seeking behavior, we found that yohimbine induced a robust reinstatement of both ethanol and sucrose seeking that was significantly attenuated by SB334867 in both the ethanol- and sucrose-experienced rats.

Role of OX-1/Hcrt-1 receptors in operant self-administration of ethanol and sucrose

The OX-1/Hcrt-1 receptor antagonist SB334867 dose dependently attenuates self-administration of ethanol, but not sucrose, supporting previous studies demonstrating a role for OX-1/Hcrt-1 receptors in ethanol self-administration (Lawrence et al. 2006). The fact that SB334867 only

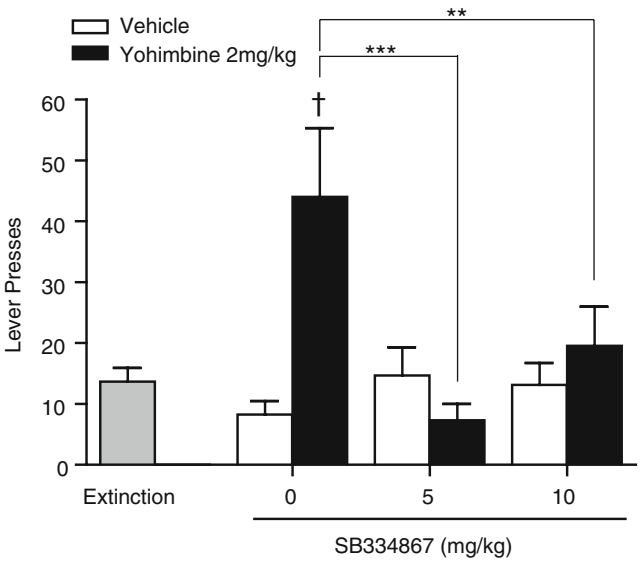

Fig. 4 Effect of SB334867 on yohimbine-induced reinstatement of sucrose seeking. Mean±SEM number of active lever presses during extinction and reinstatement tests. Extinction is shown as the average number of lever presses across the two sessions preceding the first reinstatement test for all rats. Rats were pretreated with SB334867 or its vehicle 30 min before yohimbine, or its vehicle. Yohimbine (or vehicle) was administered 30 min before the start of the reinstatement session (n=6–7, per group). †p<0.001 compared to yohimbine-vehicle condition in rats injected with the SB334867 vehicle (0 dose); **p<0.01; ***p<0.001 compared to SB334867 vehicle condition in rats injected with yohimbine

Table 3 Effect of SB334867 on yohimbine-induced reinstatement (inactive lever)

SB334867 (mg/kg i.p.)	10% Ethanol		5% Sucrose	
	Vehicle	Yohimbine	Vehicle	Yohimbine
0	1±0	1±0	2±1	3±2
5	1±1	0±0	1±1	0±0
10	0±0	1±1	1±1	2±2

Mean±SEM number of inactive lever presses during extinction and reinstatement tests. Extinction is shown as the average number of lever presses across the two sessions preceding the first reinstatement test for all rats. Rats were pretreated with SB334867 or its vehicle 30 min before yohimbine or its vehicle. Yohimbine (or vehicle) was administered 30 min before the start of the reinstatement session (n= 8–11 per group for 10% ethanol; n=6–7, per group for 5% sucrose)

attenuated self-administration of ethanol suggests that daily exposure to ethanol in combination with the presentation of the cues that signal ethanol availability leads to an increased activation of OX-1/Hcrt-1 receptors. This suggestion is further supported by the studies showing that orexin/hypocretin neurons are activated by ethanol-associated stimuli (Dayas et al. 2008). However, the finding that SB334867 had no effect on sucrose self-administration suggests that OX-1/Hcrt-1 receptors are not activated by sucrose or sucrose-associated cues. It remains to be determined if SB334867 reduced ethanol self-administration as a result of interference with ethanol-induced activation of OX-1/Hcrt-1 receptors and or the ethanol-associated cues.

Role of OX-1/Hcrt-1 receptors in yohimbine-induced reinstatement of ethanol and sucrose seeking

In the present study, we show that the OX-1/Hcrt-1 receptor antagonist, SB334867, attenuates yohimbine-induced reinstatement of ethanol and sucrose seeking. The protocol developed for studying reinstatement of drug seeking in animals has been shown to have validity for studying relapse to drug addiction in humans (Epstein et al. 2006; Katz and Higgins 2003; Spanagel 2003). Stress and reexposure to cues or to the context previously associated with drug availability are common reasons for relapse to drug seeking in humans and induce reinstatement of drug seeking in rodents (Liu and Weiss 2003; Shaham et al. 2000; Zironi et al. 2006). A "stress response" is generally believed to involve the CRF system and activation of the HPA-axis (for review see Koob 1999). However, emerging evidence suggests that the orexin/hypocretin system may also be involved in the response to stress (Paneda et al. 2005; Winsky-Sommerer et al. 2005). For example, the orexin/hypocretin peptides have been shown to induce dose-dependent activation of the HPA system; an effect that can be completely prevented using a CRF-1 receptor antagonist (Jaszberenyi et al. 2000), and recent data suggest that the CRF system directly innervates orexin/hypocretin expressing neurons (Winsky-Sommerer et al. 2005). Furthermore, SB334867 has been shown to attenuate footshock-induced reinstatement of cocaine seeking (Boutrel et al. 2005). Orexin-A/hypocretin-1 has been shown to induce noradrenaline release (Hirota et al. 2001) and orexin-A/hypocretin-1 induced reinstatement of cocaine seeking has been blocked using CRF-1 and noradrenergic receptor antagonists (Boutrel et al. 2005). It is also hypothesized that orexin/hypocretin neurons and CRF-expressing neurons constitute a feedback system which regulates arousal in response to stressful stimuli (Paneda et al. 2005; Winsky-Sommerer et al. 2003; 2005; Winsky-Sommerer et al. 2004).

The exact mechanism underlying yohimbine-induced reinstatement is still unknown; however, it is plausible to suggest that yohimbine may induce reinstatement through a mechanism similar to that of orexin-A/hypocretin-1. Evidence to directly support this hypothesis comes from the observation that orexin-A-/hypocretin-1-induced reinstatement and yohimbine-induced reinstatement can both be blocked by a CRF-1 receptor antagonist (Boutrel et al. 2005; Marinelli et al. 2007). In addition, yohimbine-induced increases in ethanol operant self-administration are inhibited by antalarmin, a CRF-1 receptor antagonist (Marinelli et al. 2007). Furthermore, yohimbine-induced reinstatement of palatable food seeking is reduced by inhibiting CRF-1 receptors (Ghitza et al. 2006). Both yohimbine and CRF induce release of noradrenaline in the locus coeruleus (Chen et al. 1992). Yohimbine has also been shown to up-regulate CRF expression in the central nucleus of the amygdala (Funk et al. 2006), and there is evidence to suggest that the orexin/hypocretin system can act through the amygdala to augment arousal and evoke behavioral responses associated with fear, stress, or emotion (Bisetti et al. 2006). We hypothesize that yohimbine induces a stress-like response that leads to activation of OX-1/Hcrt-1 receptors. Therefore, inhibiting OX-1/Hcrt-1 receptors attenuates the stress-like response leading to a reduction in yohimbine-induced reinstatement of reward seeking as seen in the present study.

Methodological considerations

There are two main methodological considerations regarding the present study. Firstly, it is possible that the observed decrease in operant self-administration of ethanol and the blockade of yohimbine-induced reinstatement were due to motor deficits. Secondly, the attenuated yohimbine-induced reinstatement of lever pressing could be due to nonspecific decreases in general locomotor behavior. However, there was no decrease in operant self-administration of sucrose after administration of the highest does of SB334867. In addition,

SB334867 had no effect on general locomotor activity in the open field locomotor activity test. Therefore, the inhibitory effect of SB334867 on ethanol self-administration and yohimbine-induced reinstatement does not appear to be based on reduced locomotor activity. Furthermore, in accordance with previous studies (Ghitza et al. 2006; Le et al. 2005; Shepard et al. 2004), there was no increase in responding at the inactive lever after the yohimbine injection in the reinstatement experiment, suggesting that the effect of the yohimbine treatment was specific to the active lever previously associated with the reward of ethanol or sucrose, respectively.

Conclusions

The results presented in the current study demonstrate that OX-1/Hcrt-1 receptors are involved in mediating operant self-administration of ethanol. These results support previous studies demonstrating a role for OX-1/Hcrt-1 receptors in ethanol self-administration and olfactory-cue-induced reinstatement (Lawrence et al. 2006). We further show that inhibiting OX-1/Hcrt-1 receptors attenuates yohimbine-induced reinstatement of both ethanol and sucrose seeking. Our findings suggest that OX-1/Hcrt-1 receptors may represent a possible target for the treatment of alcohol use disorders.

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