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***N*-(2-Methyl-6-benzoxazolyl)-*N'*-1,5-naphthyridin-4-yl urea (SB334867), a hypocretin receptor-1 antagonist, preferentially prevents ethanol seeking: comparison with natural reward seeking**

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Abstract

Orexins/hypocretins (Orx/Hcrt) are hypothalamic peptides that regulate a wide range of physiological processes and have been shown to be recruited by drugs of abuse. This study was designed to test the effect of the specific Orx/Hcrt receptor-1 (Hcrt-r1) antagonist SB334867 on reinstatement elicited by ethanol (EtOH)-associated stimuli *vs.* stimuli associated with a conventional reinforcer (i.e., SuperSac: consisting to 3% glucose and 0.125% saccharin w/v). SB334867 (1–10 mg/kg, IP) dose-dependently reduced reinstatement induced by the EtOH- but not SuperSac-associated stimuli. These findings support a differential role of Hcrt-r1 in mediating EtOH seeking *vs.* natural reward seeking.

Keywords

Ethanol-seeking behavior; Orexin/Hypocretin; SB334867

Orexins/hypocretins (Orx/Hcrt) are peptide neurotransmitters that have been shown to regulate a range of physiological processes and to be recruited by drugs of abuse. Orx/Hcrt occurs in two forms, orexin A (Orx-A or hypocretin-1 [Hcrt-1]) and orexin B (Orx-B or hypocretin-2 [Hcrt-2]), and are expressed exclusively in hypothalamic nuclei (de Lecea et al., 1998; Sakurai et al., 1998). Orx-A and Orx-B regulate feeding, energy metabolism (Teske et al., 2010; Willie et al., 2001), and arousal (Sutcliffe and de Lecea, 2002) and bind to two Orx/Hcrt receptors (Hcrt-r1 and Hcrt-r2) with different affinity (Sakurai et al., 1998). A central role of Orx/Hcrt neurons in the lateral hypothalamus (LH) in drug addiction exists. Recent findings show that LH Orx/Hcrt neurons play a significant role in modulating reward function and particularly drug-directed behavior (Harris et al., 2005). Orx/Hcrt neurons in the LH become activated by stimuli associated with food, morphine, cocaine, and EtOH (e.g., Harris et al., 2005; for review, see Martin-Fardon et al., 2010). With regard to EtOH, blockade of Hcrt-r1 decreased EtOH self-administration (Lawrence et al., 2006) and cue- (Jupp et al., 2011b; Lawrence et al., 2006) and stress-induced reinstatement of EtOH seeking

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Author Contributions

R.M.F. and F.W. participated in the study concept and design. R.M.F. performed the experiments, undertook the statistical analysis, interpreted the findings, and drafted the manuscript. Both authors critically reviewed the content and approved the final version for publication.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

(Richards et al., 2008). Thus, behavioral and functional evidence shows a role for Orx/Hcrt signaling in the neurobehavioral and motivational effects of EtOH and other drugs of abuse. The aim of the present study was to test the effect of the specific Hcrt-r1 antagonist SB334867 on reinstatement elicited by EtOH-related stimuli *vs.* stimuli conditioned to a potent conventional reinforcer and whether this effect is specific to EtOH seeking.

Rats were trained to self-administer 10% (w/v) EtOH or a palatable sweet solution, “SuperSac” (3% glucose and 0.125% saccharin w/v; see Supplementary Information), in the presence of distinct olfactory and auditory discriminative stimuli (S^D) that signaled the availability (S^+) *vs.* nonavailability (S^-) of reward. At the end of the conditioning phase, the rats showed stable EtOH and SuperSac self-administration and a significant reduction of responding during non-reward sessions (EtOH, $t_{30} = 11.9$, $p < 0.001$, Fig. 1A; SuperSac, $t_{27} = 23.2$, $p < 0.001$, Fig. 2A). Following the conditioning phase, responding was extinguished in sessions during which the reinforcers and stimuli were absent (Fig. 1B and 2B). Then, the effects of SB334867 on reinstatement elicited by an EtOH or SuperSac S^+ were tested. Both the EtOH S^+ and SuperSac S^+ produced identical recovery of responding ($t_{12} = -1.06$, $p = 0.3$; Fig. 1C and 2C for comparison). SB334867 (1–10 mg/kg, IP) attenuated reinstatement induced by the EtOH S^+ but not by the SuperSac S^+ . This was reflected by an overall significant effect of dose ($F_{3,51} = 4.9$, $p < 0.01$) and a significant group (EtOH or SuperSac) $\times$ dose interaction ($F_{3,51} = 2.9$, $p < 0.05$). Individual one-way ANOVA confirmed that SB334867 dose-dependently reduced reinstatement induced by the EtOH S^+ ($F_{3,27} = 4.9$, $p < 0.01$; Fig. 1C) but did not interfere with behavior induced by the SuperSac S^+ ($F_{3,30} = 0.5$, $p = 0.7$; Fig. 2C). Furthermore, SB334867 reversed EtOH S^+ -induced reinstatement to extinction (or S^-) levels at 3 mg/kg ($F_{2,12} = 6.5$, $p < 0.05$; pairwise comparisons, $p > 0.05$; Fig. 1C) and 10 mg/kg ($F_{2,12} = 0.8$, $p = 0.5$; Fig. 1C). Responses at the inactive lever remained low (< 4 responses) throughout testing and were not modified by SB334867 (data not shown).

The results show that blockade of Hcrt-r1 effectively reverses EtOH-seeking behavior without interfering with the motivational effects of a stimulus conditioned to a potent conventional reinforcer. These findings further support a differential role of the Orx/Hcrt system in drug-seeking behavior *vs.* normal motivated behavior and are consistent with the hypothesis that the hypothalamic Orx/Hcrt system may be a substrate for EtOH seeking and that Hcrt-r1 plays an important role in the modulation of EtOH-seeking behavior.

One hypothesis concerning the control of drug-seeking behavior is that the neural circuits that mediate these effects are common motivational circuits that are more robustly activated by drug-related stimuli and not specific to addiction-related events. This activation that normally governs responding for natural rewards may have created new motivational states or tilted processes that normally govern responding for natural rewards toward drug-directed behavior (Kelley and Berridge, 2002). It has been shown that pharmacological manipulation of the Orx/Hcrt system is particularly effective in modifying the conditioned effects of drug (including EtOH) cues in conditioned place preference and reinstatement studies (Harris et al., 2005; Lawrence et al., 2006; Smith et al., 2009). Additionally, evidence of EtOH-induced dysregulation of the Orx/Hcrt system exists. For example, earlier findings revealed that prepro-Orx mRNA is upregulated in the LH in inbred alcohol-preferring rats following chronic EtOH consumption (Lawrence et al., 2006). Therefore, one explanation for the preferential effect of SB334867 on conditioned reinstatement for EtOH *vs.* SuperSac could be that during conditioning, EtOH neuroadaptively altered the neural systems that regulate motivation normally directed toward natural rewards which is revealed by pharmacological (e.g., SB334867) manipulations.

Although the data reported here are consistent with recent findings that showed that Hcrt-r1 antagonism interferes with the motivation to self-administer EtOH but not sucrose, another palatable conventional reinforcer (Jupp et al., 2011a), the findings are somewhat different from earlier observations that showed that SB334867 similarly prevented stress-induced reinstatement of EtOH or sucrose seeking (Richards et al., 2008). Partial but not complete overlap exists between the circuitries that control conditioned and stress-induced reinstatement (e.g., Dayas et al., 2007; Zhao et al., 2006). As a result, the inconsistent SB33467 effects on these two reinstatement models may be attributable to different neural events that mediate these two forms of reinstatement. Nevertheless, these data suggest that targeting Hcrt-r1 to specifically block EtOH craving and seeking associated with alcohol cue exposure may offer a new approach to treat EtOH seeking without producing nonspecific side effects that interfere with normal motivated behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

SB334867	<i>N</i> -(2-Methyl-6-benzoxazolyl)- <i>N'</i> -1,5-naphthyridin-4-yl urea
Orx/Hcrt	orexin/hypocretin
Hcrt-r1	hypocretin receptor-1

References

- Dayas CV, Liu X, Simms JA, Weiss F. Distinct patterns of neural activation associated with ethanol seeking: effects of naltrexone. *Biol Psychiatry*. 2007; 61:979–989. [PubMed: 17098214]
- de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A*. 1998; 95:322–327. [PubMed: 9419374]
- Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature*. 2005; 437:556–559. [PubMed: 16100511]
- Jupp B, Krivdic B, Krstew E, Lawrence AJ. The orexin receptor antagonist SB-334867 dissociates the motivational properties of alcohol and sucrose in rats. *Brain Res*. 2011a; 1391:54–59. [PubMed: 21439948]
- Jupp B, Krstew E, Deysi G, Lawrence AJ. Discrete cue-conditioned alcohol-seeking after protracted abstinence: pattern of neural activation and involvement of orexin receptors. *Br J Pharmacol*. 2011b; 162:880–889. [PubMed: 20973776]
- Kelley AE, Berridge KC. The neuroscience of natural rewards: relevance to addictive drugs. *J Neurosci*. 2002; 22:3306–3311. [PubMed: 11978804]
- Lawrence AJ, Cowen MS, Yang HJ, Chen F, Oldfield B. The orexin system regulates alcohol-seeking in rats. *Br J Pharmacol*. 2006; 148:752–759. [PubMed: 16751790]
- Martin-Fardon R, Zorrilla EP, Ciccocioppo R, Weiss F. Role of innate and drug-induced dysregulation of brain stress and arousal systems in addiction: Focus on corticotropin-releasing factor, nociceptin/orphanin FQ, and orexin/hypocretin. *Brain Res*. 2010; 1314:145–161. [PubMed: 20026088]

- Richards JK, Simms JA, Steensland P, Taha SA, Borgland SL, Bonci A, Bartlett SE. Inhibition of orexin-1/hypocretin-1 receptors inhibits yohimbine-induced reinstatement of ethanol and sucrose seeking in Long-Evans rats. *Psychopharmacology (Berl)*. 2008; 199:109–117. [PubMed: 18470506]
- Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*. 1998; 92:573–585. [PubMed: 9491897]
- Smith RJ, Tahsili-Fahadan P, Aston-Jones G. Orexin/hypocretin is necessary for context-driven cocaine-seeking. *Neuropharmacology*. 2009
- Sutcliffe JG, de Lecea L. The hypocretins: setting the arousal threshold. *Nat Rev Neurosci*. 2002; 3:339–349. [PubMed: 11988773]
- Teske JA, Billington CJ, Kotz CM. Hypocretin/orexin and energy expenditure. *Acta Physiol (Oxf)*. 2010; 198:303–312. [PubMed: 20070282]
- Willie JT, Chemelli RM, Sinton CM, Yanagisawa M. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu Rev Neurosci*. 2001; 24:429–458. [PubMed: 11283317]
- Zhao Y, Dayas CV, Aujla H, Baptista MA, Martin-Fardon R, Weiss F. Activation of group II metabotropic glutamate receptors attenuates both stress and cue-induced ethanol-seeking and modulates c-fos expression in the hippocampus and amygdala. *J Neurosci*. 2006; 26:9967–9974. [PubMed: 17005860]

Figure 1.

(A) Active lever responses during conditioning sessions in the presence of stimuli paired with EtOH (EtOH/S⁺) availability vs. non-availability (Non-Reward/S⁻). *** $p < 0.001$, vs. EtOH/S⁺. (B) Extinction (EXT) responses and responses during an initial reinstatement test in the presence of the stimulus paired with reward non-availability (S⁻). (C) Reinstatement responses in the presence of stimuli previously associated with EtOH availability (S⁺) in vehicle-treated rats (0) and modification of conditioned reinstatement across doses of SB334867. * $p < 0.05$, *** $p < 0.001$, vs. 0 mg/kg; ++ $p < 0.01$, vs. EXT and S⁻.

Figure 2.

(A) Active lever responses during conditioning sessions in the presence of stimuli paired with SuperSac availability (SuperSac/S⁺) vs. non-availability (Non-Reward/S⁻). *** $p < 0.001$, vs. SuperSac/S⁺. (B) Extinction (EXT) responses and responses during an initial reinstatement test in the presence of the stimulus paired with reward non-availability (S⁻). (C) Reinstatement responses in the presence of stimuli previously associated with SuperSac availability (S⁺) in vehicle-treated rats (0) and tests of SB334867 effects on SuperSac. ++ $p < 0.01$, vs. EXT and S⁻.