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Research Report

Orexin mediates morphine place preference, but not morphine-induced hyperactivity or sensitization

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ABSTRACT

Orexin (or hypocretin) has been implicated in mediating drug addiction and reward. Here, we investigated orexin's contribution to morphine-induced behavioral sensitization and place preference. Orexin–/– (OKO) mice and littermate wild-type (WT) controls ($n=56$) and C57BL/6J mice ($n=67$) were tested for chronic morphine-induced locomotor sensitization or for conditioned place preference (CPP) for a morphine- or a cocaine-paired environment. C57BL/6J mice received the orexin receptor 1 (Ox1r) antagonist, SB-334867, prior to test sessions. OKO mice did not significantly differ from WT controls in locomotor activity following acute- or chronic-morphine treatments. Similarly, mice treated with the Ox1r antagonist did not differ from vehicle controls in locomotor activity following acute- or chronic-morphine treatments. In contrast, while OKO mice did not differ from WT controls in preference for a morphine-paired environment, the Ox1r antagonist significantly attenuated place preference for a morphine-, but not a cocaine-paired, environment. These data suggest that orexin action is not required for locomotor responses to acute and chronic morphine, but Ox1r signaling can influence morphine-seeking in WT animals.

1. Introduction

Orexin- (or hypocretin-) containing neurons in the lateral hypothalamus (LH) project widely throughout the brain (Date et al., 1999; Nambu et al., 1999; Peyron et al., 1998). The orexin receptors, Ox1r and Ox2r, are located in multiple brain regions, including the ventral tegmental area (VTA) and nucleus accumbens (Acb) (Marcus et al., 2001), that are known to regulate drug addiction and reward processes. Behavioral analysis also supports a role for orexin in different components of drug addiction. We have shown that a mutation of the orexin gene (Georgescu et al., 2003) and blockade of Ox1r (Sharf et al., 2008) result in attenuated somatic withdrawal symptoms in morphine-dependent mice. Evidence suggests that orexin is also involved in drug reward and reinstatement. Preference for an environment previously paired with morphine, cocaine, or food is positively correlated with activation of orexin-producing neurons in the LH (Harris et al., 2005). Intra-VTA blockade of Ox1r dose-dependently reduced morphine-induced conditioned place preference (CPP) in rats and genetic deletion of orexin resulted in an attenuation of morphine-induced CPP (Narita et al., 2006). Orexin is also important for stress-mediated reinstatement of cocaine seeking (Boutrel et al., 2005). These results suggest a role for orexin in mediating dependence and behavioral plasticity that occurs in the absence of the actual drugs (i.e. during states of withdrawal or seeking).

Studies of locomotor activity also implicate orexin in response to both acute and chronic drug exposure. Orexin –/– (OKO) mice have been reported to display reduced

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locomotor activity in response to acute morphine (Narita et al., 2006) and intra-VTA Ox1r blockade attenuated the development, but not the expression, of cocaine sensitization (Borgland et al., 2006). It is, however, not known if orexin mediates sensitization to other drugs of abuse, such as morphine.

Here, we report that OKO animals display normal morphine sensitization, and in contrast to previous reports (Narita et al., 2006), normal locomotor response and CPP to morphine. The Ox1r antagonist also failed to block locomotor responses to morphine, but did attenuate morphine, but not cocaine, CPP. Together these data demonstrate that orexin may not be necessary for morphine-induced hyperlocomotion, but that altered orexin signaling can nonetheless modify drug–context associations.

2. Results

2.1. *Orexin −/− mice display normal acute locomotor and sensitization responses to morphine*

Locomotor activity was assessed during a 1-h habituation session, during which naïve OKO mice and WT controls were first exposed to the locomotor chambers. After habituation, animals received a saline injection and locomotor activity was measured for 1 h. Following the saline session, animals received a morphine injection (10 mg/kg, s.c.) and locomotor activity was again assessed for 1 h. Surprisingly, OKO mice did not differ from WT controls in locomotor activity during any of these phases (Fig. 1A). Repeated morphine treatments and locomotor tests were then conducted to determine if orexin was required for sensitization. Locomotor activity following the fifth morphine injection was significantly greater than that following the first morphine injection (Session effect, $F_{(2,28)}=33.57$, $P<0.0001$; day 1 vs. day 5, $P<0.002$ by Tukey's LSD), suggesting the development of locomotor sensitization (Fig. 1B). However, there was no significant effect of genotype nor a significant interaction between genotype and session indicating normal sensitization in orexin mutant mice.

2.2. *Pharmacological blockade of Ox1r fails to affect acute locomotor and sensitization responses to morphine*

To complement the mutant studies, the Ox1r antagonist SB-334867 was administered to naïve C57BL/6J mice prior to each 10 mg/kg morphine treatment. Overall, total activity counts for OKO mice and their littermate WT controls were lower when compared to the C57BL/6J mice used in the pharmacological experiment. This reduced general locomotion may be the result of age differences among the cohorts as it has previously been reported that age can contribute to locomotor activity differences (Ingram et al., 1981). SB-334867 did not affect locomotor activity compared to saline and had no affect on acute hyperlocomotion to morphine (Fig. 2A), as confirmed by statistical analysis between SB-334867 and saline treated animals ($F_{(1,11)}=0.34$, $P=0.57$). While sensitization was clear (Session effect, $F_{(2,22)}=47.78$, $P<0.0001$; day 1 vs. day 5, $P<0.0001$ by Tukey's LSD), there were no significant SB-334867 or interaction effects (Fig. 2B). This suggests that, in normal animals, Ox1r blockade during morphine exposure does not influence motor response or the development of sensitization.

To further ascertain that orexin does not contribute to morphine-induced locomotor responses, a separate group of C57BL/6J mice underwent locomotor analysis with 5 mg/kg morphine injections and morphine-induced locomotor responses were recorded for 2 h. As seen with the higher morphine dose, SB-334867 failed to effect acute morphine induced hyperlocomotion (Fig. 3A). Although morphine significantly enhanced locomotion on day 1 ($F_{(2,20)}=23.44$, $P<0.000$), locomotor activity on day 5 was not significantly different than on day 1 ($P<.09$), suggesting that animals did not sensitize to the lower morphine dose (Fig. 3B). However, no significant interactions were found, thus further supporting that orexin is not required for morphine-induced locomotion at this lower dose.

2.3. *Pharmacological blockade of Ox1r, but not genetic mutation of the orexin gene, attenuates morphine CPP*

Based on previous reports on the role of orexin in morphine responsiveness (Narita et al., 2006), the minimal effects of genetic and pharmacological treatments on locomotor responses were unexpected. To explore further orexin-morphine interactions, Pavlovian reward responses to morphine were assessed using the conditioned place preference (CPP) paradigm. Here, animals were placed in an enclosed environment divided into two chambers, each containing distinctive environmental cues. During the habituation session, animals were allowed to freely explore both chambers. On days 1, 3 and 5 animals received an injection of saline or morphine (5 or 10 mg/kg, s.c.) and were confined to one of the chambers. On days 2, 4, and 6, mice received the alternate drug treatment and were confined to the other chamber. On the test day, animals were once again allowed to freely roam among the chambers and the time spent in each was recorded. Male OKO and WT mice showed statistically comparable preferences for an environment previously paired with either a 5 mg/kg or 10 mg/kg dose of morphine (Fig. 4A, Table 1). We also tested female OKO mice and their WT controls for the expression of morphine CPP to the higher morphine dose and again found no behavioral differences based on genotype (Fig. 4B). Furthermore, no significant gender differences were observed (Figs. 4A, B).

In contrast, C57BL/6J mice treated with SB-334867 demonstrated significantly reduced preference for a chamber previously paired with morphine ($t_{(24)}=2.30$, $P<0.03$; Fig. 5A). However, SB-334867 failed to alter CPP to cocaine (Fig. 5B), suggesting that SB-334867's effects are restricted to Pavlovian responses to opiates, and may not play a role in conditioned responses to psychostimulants.

3. Discussion

The present study investigates orexin's contribution to morphine locomotor sensitization and CPP. Surprisingly, we found that in response to acute morphine, OKO mice do not differ from WT controls in locomotor activity. Similarly, both genotypes displayed normal sensitization in response to