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Distinctive modulation of ethanol place preference by delta opioid receptor-selective agonists

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Abstract

Background—Naltrexone is one of the few drugs approved by the Federal Drug Administration for the treatment of alcoholism. However, naltrexone is only effective in a subpopulation of treatment-seeking alcohol abusers, and suffers from compliance issues. The non-selective nature of this opioid antagonist likely contributes to its side effects and poor therapeutic efficacy. Drugs selectively targeting delta opioid receptor subtypes offer a potential way to treat alcohol abuse disorders. We have recently shown that delta subtype-selective agonists TAN-67 and SNC80 can have opposing effects on alcohol consumption, while having similar effects on alcohol withdrawal-induced anxiety.

Methods—We studied the ability of TAN-67 and SNC80 to induce place preference in naïve and ethanol exposed C57BL/6 mice and determined the effect of these agonists on the expression of ethanol place preference.

Results—We show that TAN-67 and SNC80 have opposing actions on ethanol place preference. However, neither of the drugs induces place preference by themselves at doses that are therapeutically effective in mice. Interestingly, SNC80, like naltrexone reduces ethanol place preference, however we have previously shown that SNC80 increases ethanol consumption at the tested dose. Similar to naltrexone, TAN-67 reduces alcohol consumption, but we show here that it may be due to an increase in ethanol place preference. Importantly, we found that chronic ethanol exposure does not increase the rewarding properties of the DOR subtype selective agonists.

Conclusions—Our results provide a better understanding of how DOR subtype selective drugs could potentially be used for treatment of alcohol abuse disorders.

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Role of contributors

This study was designed by RMvR. Data collection and analysis was done by RMvR and DB. RMvR wrote the first draft of the manuscript. All authors contributed to the interpretation of the data and composition of the manuscript. All authors have approved the final manuscript

Author contribution: RMvR (designed research, performed research, analyzed data, wrote paper), DIB (performed research), JLW (designed research, analyzed data, wrote paper)

Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

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Keywords

delta opioid receptor; subtypes; alcoholism; ethanol; reward; place preference

1. Introduction

Alcohol use disorders pose a large burden on society (Rehm et al., 2009; Nutt et al., 2010). Currently only a small number of FDA-approved drugs are available for treatment seeking individuals. The non-selective opioid antagonist naltrexone is frequently prescribed to assist alcohol abusers in reducing their ethanol consumption. However, the efficacy of this drug is still under debate, and may only be effective in heavy drinkers (Pettinati et al., 2006; King et al., 2009) and those with a (genetic) predisposition/family history of alcohol use (Monterosso et al., 2001; Oslin et al., 2003; Krishnan-Sarin et al., 2007; Anton et al., 2008). Naltrexone has several side effects including limiting the utility of opioid analgesics in people actively taking the drug. Therefore, there is still a need to develop better therapeutics to treat alcohol use disorders. One potential mechanism is to selectively target delta opioid receptors (DORs). DORs are involved in ethanol consumption (Roberts et al., 2001) as well as anxiety (Filliol et al., 2000). Importantly, two DOR subtypes have been described *in vivo* (Zaki et al., 1996; Dietis et al., 2011). Moreover, DORs are upregulated after chronic exposure to stimuli including morphine (Cahill et al., 2001), stress (Commons, 2003) and inflammation (Cahill et al., 2003). Recently, we demonstrated that DOR subtypes have opposing effects on ethanol consumption (van Rijn and Whistler, 2009; van Rijn et al., 2010) and that ethanol exposure can reveal anxiolytic-like properties of the DOR-1 agonist TAN-67, that are absent in naïve mice (van Rijn et al., 2010). Also, we have shown that chronic ethanol exposure increases the potency of some DOR selective ligands in the spinal cord of mice (Van Rijn et al., 2011).

To further investigate the mechanism of action of DOR subtype-selective agonists on ethanol consumption we studied the effects of TAN-67 and SNC80 on ethanol place preference (PP). We find that these DOR agonists do not produce PP themselves but have a significant effect on ethanol place preference. Interestingly, we show for the first time that these two DOR agonists have opposite effects in the way that they modulate ethanol PP.

2. Methods

2.1 Ethanol place preference

Ethanol place preference was established by conditioning C57BL/6 mice (male, 18–22g, Taconic) to 2 g/kg (15%) ethanol (Lim et al., 2011). A locomotor box (Med Associates, St Albans, VT) was divided in two chambers. One chamber had plastic rod flooring and horizontally striped “wallpaper,” the other chamber had plastic hole flooring and vertically striped “wallpaper.” Mice were paired with ethanol or saline in an unbiased approach. Mice were injected intra peritoneally (i.p.) once daily with saline or ethanol and confined to one of two chambers for five minutes directly after injection. Each mouse received four pairings with ethanol and saline (Day 2–8). On day one and day ten each mouse was placed in the saline-paired chamber and allowed to freely explore both chambers for 30 minutes. Time spent in both chambers was recorded on these days. Chambers were cleaned with 70% isopropanol between each measurement. Mice were injected with saline on day one and day ten to balance for any handling stress. To determine the effect of opioid selective ligands on the expression of ethanol PP mice were injected sub-cutaneously (s.c.) with drug 30 minutes prior to placement in the chamber.

2.2 Drug place preference

Drug place preference was performed as previously described (van Rijn and Whistler, 2009). On day one naïve or ethanol exposed (see below) C57BL/6 mice were placed randomly in one of two PP chambers and each mouse was allowed to freely explore both chambers for 30 minutes. An unbiased approach was used to determine in which chamber each mouse would receive drug or vehicle. Each mouse would be injected twice daily (morning and afternoon) with either vehicle or drug for three days (days 2–4) and confined to one of the chambers for 30 minutes directly after injection. On day five each mouse was placed in the saline-paired chamber and allowed to freely explore both chambers for 30 minutes. Time spent in both chambers was recorded on day one and day five. Chambers were vigorously cleaned with 70% isopropanol between each measurement and the isopropanol smell was allowed to dissipate before the start of the next experiment. Mice were injected with saline on day one and day five to balance for any handling stress.

2.3 Chronic voluntary ethanol consumption

Mice were individually housed in ventilated plexiglass cages at ambient temperature (21°C) in a room maintained on a reversed 12L:12D cycle (lights off at 10.00, lights on at 22.00). Food and water was provided ad libitum. The mice were given two weeks to acclimatize to the individual housing conditions and reverse light cycle before the start of the experiments. A 2-bottle limited access (4 hours/day) drinking paradigm was employed for three weeks to train mice to increase their intake of a 10% ethanol solution, as previously described (van Rijn and Whistler, 2009).

2.4 Drugs

Ethanol solutions were prepared in tap water using 95% (vol/vol) ethanol (Gold Shield Chemical Co., Hayward, CA, USA). SB205607 dihydrobromide (TAN-67) and SNC80 were purchased from Tocris (MO, USA). Naltrexone hydrochloride (NTX) and morphine sulphate, were purchased from Sigma-Aldrich (MO, USA). All compounds were dissolved in saline. All opioid drugs were prepared immediately prior to injection and were administered s.c. at a volume of 10 ml/kg. The drug concentrations used were chosen for their ability to affect ethanol consumption (van Rijn and Whistler, 2009; van Rijn et al., 2010). We have shown that the effects of 25 mg/kg TAN-67 can be blocked by the DOR-1 selective antagonist BNTX (van Rijn and Whistler, 2009).

3. Results

3.1 Delta opioid receptor subtype selective agonists uniquely modulate the expression of ethanol place preference

We have previously shown that DOR-1 and DOR-2 selective ligands have opposing effects on ethanol consumption (van Rijn and Whistler, 2009; van Rijn et al., 2010). Specifically, whereas the DOR-1 agonist TAN-67 (25 mg/kg) decreases ethanol consumption, the DOR-2 agonist SNC80 (20 mg/kg) increases ethanol consumption. To better understand the mechanism of these two agonists on ethanol consumption we trained mice to display PP to ethanol (see methods) and injected the DOR agonist prior to testing the PP to ethanol. Interestingly, we found that SNC80 completely abolishes the expression of ethanol-induced PP ($F_{2,42} = 8.60$, $p = 0.0007$). TAN-67, on the other hand, enhances the PP to ethanol, nearly doubling the time spent in the ethanol-paired chamber compared to saline.

3.2 Subtype selective delta opioid receptor agonists do not produce place preference in naïve or ethanol exposed mice

In order to determine if the changes to the ethanol PP could be attributed to an increase in the rewarding or aversive properties of the DOR-selective agonists, we tested place preference to the DOR agonists in naïve mice as well as in ethanol exposed mice, using a paradigm which we have previously shown upregulates functional DORs (van Rijn et al., 2010; Van Rijn et al., 2011). We found that, in naïve mice, DOR agonists do not produce significant PP. As controls, morphine (6 mg/kg) did produce PP, whereas the non-selective opioid antagonist naltrexone, at a dose (1.5 mg/kg) shown to decrease ethanol consumption (Le et al., 1993; Phillips et al., 1997; Middaugh and Bandy, 2000; van Rijn and Whistler, 2009) did not ($F_{3,42} = 3.54, p = 0.023$). Next, we exposed a new cohort of mice to ethanol in a voluntary 2-bottle choice limited access drink in the dark paradigm for 3 weeks. During the fourth week, we conditioned the mice to the DOR agonists and determined PP in the same manner as in naïve mice. In naïve and ethanol exposed mice TAN-67 shows a small, but non significant degree of place aversion, whereas SNC80 shows a small degree of PP. While there was a trend towards a difference between the effects of TAN-67 and SNC80 in naïve mice ($p = 0.07, n=10-14$), in ethanol exposed mice, the difference between the two agonists is significant ($p = 0.025, n=12$). A larger sample size might resolve if the DOR agonists are not just different from each other but also significantly different than saline.

4. Discussion

Based on the mechanism of action of naltrexone, the current understanding of how opioids reduce ethanol consumption is that an opioid antagonist makes alcohol less rewarding (Le et al., 1993; Phillips et al., 1997; Middaugh and Bandy, 2000; van Rijn and Whistler, 2009). However, here we show that the DOR-2 selective agonist SNC80 can prevent the expression of PP for ethanol, at a dose that actually leads to an increase in ethanol consumption (van Rijn et al., 2010). In contrast, the DOR-1 selective agonist TAN-67, which decreases ethanol consumption (van Rijn and Whistler, 2009) potentiates ethanol PP, which resembles its effects seen on morphine PP (Suzuki et al., 1996). Therefore, it may be that TAN-67 produces reduced ethanol consumption by increasing the rewarding properties of lower doses of ethanol. Conversely, SNC80 may reduce the rewarding effects of ethanol, which in turn promotes increased consumption. It is possible that DOR agonist induced changes in ethanol PP stem from effects on memory retrieval. Jutkiewicz and colleagues have previously shown that SNC80 has a small impact on learning and memory, but that it is not the reason for the antidepressant effects of SNC80 (Jutkiewicz et al., 2003). This may hold true for the effects on PP too. Whether the discrepancy between the effects on ethanol place preference of the non-selective *antagonist* naltrexone vs the DOR-selective *agonists* is due to a difference in target or circuitry is worth exploring.

Our results both in the ethanol PP and opioid PP provide additional evidence that DOR subtype-selective agonists can have opposing functions. Taken together with recent findings demonstrating requirement of not only DOR but also MOR for the actions of TAN-67 on ethanol consumption (van Rijn and Whistler, 2009) and antinociception, these data indicate that DOR subtypes are indeed distinct targets.

From the perspective of therapeutic utility, it is important that neither DOR agonist produces PP in naïve as well as ethanol exposed mice. However, the fact that they do alter the rewarding effects of ethanol suggests that their therapeutic utility may be different depending on whether the clinical endpoint is abstinence or reduced consumption. For example, increasing the rewarding properties of ethanol (with TAN-67 for example) could produce a decrease of ethanol consumption, but would not favor abstinence. In conclusion our data

provides new insight into the mechanisms with which DOR-selective ligands modulate ethanol driven behavior.

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Figure 1. The DOR agonists TAN-67 and SNC80 modulate ethanol place preference in opposite ways

C57BL/6 mice (n=12–16) were conditioned to i.p. ethanol (2 g/kg, E) or saline (S) paired with one side of a 2-chamber CPP box for five minutes per session for eight sessions (see drawing and methods section). On the days before (pre) and after (post) the conditioning sessions mice were allowed to freely explore both chambers of the CPP box for 30 minutes and time spent on each side was recorded. On the final test day mice were injected s.c. with either saline, 25 mg/kg TAN-67 or 20 mg/kg SNC80. Thirty minutes after injection, mice were injected i.p. with saline and placed in the CPP apparatus. (* $p < 0.05$; *** $p < 0.001$)

Figure 2. DOR selective agonists do not produce place preference in naïve or ethanol exposed mice

A, Naive C57BL/6 mice (n=10–14) were conditioned by twice daily injection with saline (s) or drug (d) [TAN-67 (25 mg/kg), SNC80 (20 mg/kg), naltrexone (NTX, 1.5 mg/kg) or morphine (6 mg/kg)] for three days. On the days before (pre) and after (post) the conditioning sessions mice were allowed to freely explore both chambers of the place preference set up for 30 minutes and time spent on each side was recorded. B, C57BL/6 mice (n=12) were trained to self-administer ethanol in a limited access 2-bottle choice paradigm for three weeks. During the fourth week animals were conditioned in a place preference paradigm to TAN-67 (25 mg/kg) or SNC80 (20 mg/kg) as described above. (* $p < 0.05$)