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Research report

The role of endogenous dynorphin in ethanol-induced state-dependent CPP

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ABSTRACT

The aim of this study was to determine the role of the endogenous dynorphin/kappa opioid receptor (DYN/KOP) system in ethanol-induced state-dependent conditioned place preference (CPP). To this end, mice lacking the pro-DYN gene and their wild-type littermates/controls were tested for baseline place preference on day 1, received 15-min morning and afternoon conditionings with saline or ethanol (2 g/kg) each day for three consecutive days and were then tested for CPP under a drug-free state on day 5 and following a saline or ethanol (1 or 2 g/kg) challenge on day 8. Given that compensatory developmental changes may occur in knockout mice, the effect of nor-binaltorphimine (nor-BNI), a KOP antagonist, on state-dependent CPP induced by ethanol was also studied in wild-type mice. On day 1, mice were tested for baseline place preference and, 4 h later, treated with saline or nor-BNI (10 mg/kg). On days 2–4, mice received 15-min morning and afternoon conditionings and were tested for CPP under a drug-free state on day 5 and following an ethanol (1 g/kg) challenge on day 8. A comparable CPP was observed in mice lacking the pro-DYN gene and their wild-type littermates/controls as well as in wild-type mice treated with nor-BNI and their saline-treated controls. However, these mice compared to their respective controls exhibited a greater CPP response following an ethanol (1 g/kg) challenge, suggesting that the endogenous DYN/KOP system may negatively regulate ethanol-induced state-dependent CPP.

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1. Introduction

Alcoholism and alcohol-related disorders represent a major public health and socioeconomic issue. Alcohol is a powerful reinforcing agent and its chronic use leads to compulsive alcohol-taking and alcohol-seeking behaviors. However, only limited pharmacotherapeutic agents are available to treat this chronic relapsing brain disorder. This is, at least in part, due to the fact that the neurobiological mechanisms underlying the rewarding and reinforcing actions of alcohol are not fully understood. Notably, alcohol administration has been shown to affect numerous neurotransmitter/neuropeptide systems. Therefore, understanding factors that regulate the rewarding and addictive action of alcohol has paramount importance for the development and design of pharmacotherapy to curb alcohol addiction.

The opioid peptides (beta-endorphin, enkephalin and dynorphin) and their receptors (namely mu, delta and kappa opioid receptors) have been implicated in the rewarding and addictive actions of ethanol and other drugs of abuse [1–6]. For example, kappa opioid receptor (KOP) agonists are shown to reduce alcohol reward [7] and self-administration [8,9]. In addition, the dynorphin (DYN)/KOP system may be important in stress-mediated reinstatement of ethanol-seeking behaviors [10].

The endogenous DYN/KOP receptor system may also be involved in alcoholism and alcohol-related disorders [3,6,7,10,11]. Thus, mice lacking KOP compared to their wild-type littermates had a higher dopamine outflow in the nucleus accumbens (NAc) following an acute ethanol challenge [7]. Moreover, ethanol withdrawal was reported to be associated with changes in the level of DYN and its precursor in brain areas relevant to reward and reinforcement [8,12]. Surprisingly, however, mice lacking DYN exhibited a conditioned place preference (CPP) response that was comparable to their wild-type controls [13]. Nevertheless, given that changes in the level of DYN and pro-DYN mRNA have been reported shortly after withdrawal from ethanol [8,12], we assessed whether ethanol CPP would be altered if mice were tested at a later time point following ethanol conditioning.

Earlier studies have shown a greater CPP response under a drugged compared to drug-free state, a phenomenon referred to as state-dependent CPP. This phenomenon has been reported with methamphetamine [14] and even with agents that induce aversion [15–17]. However, there appears to be a lack of support for state-dependent CPP following ethanol administration at least in some mouse strains [18]. Given that ethanol possesses both rewarding and aversive effects [19,20], that DYN levels increase following alcohol administration [8,12], and that DYN

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and related KOP agonists can induce aversion [3], we also determined whether state-dependent CPP induced by ethanol would be regulated by the endogenous DYN/KOP system. Thus, using an unbiased CPP paradigm and mice lacking DYN [21] and their wild-type littermates/controls, we assessed the role of endogenous DYN in ethanol-induced CPP both under a drug-free state as well as in the presence of an ethanol challenge, i.e., state-dependent CPP. Considering that compensatory changes may develop in knockout mice, we also examined whether ethanol-induced state-dependent CPP would be altered in wild-type mice treated with nor-binaltorphimine (nor-BNI), a selective and long-acting KOP antagonist [22], compared to saline-treated controls.

2. Materials and methods

2.1. Subjects

Male and female C57BL/6 mice as well as female mice lacking the pro-DYN gene [21] and/or wild-type littermates/controls (2–4-month-old) fully backcrossed (F12) on the C57BL/6 mouse strain were used for the experiments. Mice were housed 2–4 per cage with free access to laboratory chow and water under a 12-h light/12-h dark cycle. All experiments were conducted according to the National Institute of Health Guideline and approved by the Western University of Health Sciences Animal Care and Use Committee (Pomona, CA, USA).

2.2. Experimental procedures

2.2.1. Experiment 1: to assess the role of gender in ethanol-induced CPP

A three-chambered CPP apparatus (ENV-3013, Med Associates Inc., St. Albans, VT, USA) was used. The apparatus consisted of a smaller (7.2 cm × 12.7 cm × 12.7 cm) central grey chamber with smooth surface, used as the neutral chamber, and two conditioning chambers on either side identical in size (16.8 cm × 12.7 cm × 12.7 cm) but distinguishable from each other by visual (horizontal or vertical 2.54 cm black and white stripes) and tactile (rod surface or mesh floor) cues. The CPP procedure was conducted over a 5-day period. On day 1 (D1), male and female mice were tested for baseline place preference. Mice then received conditioning twice daily (once in the morning and once in the afternoon) on days 2–4, in which they were injected with saline or ethanol (2 g/kg, i.p.) and confined to the saline- or ethanol-paired chamber for 15 min. In the afternoon session, mice received the alternate treatment and were confined to the opposite chamber for 15 min. The assignment of mice to the treatments and conditioning chambers was carried out in a counterbalanced manner in which some animals received alcohol in the chamber with rod floor and some in the chamber with mesh floor. Also, we made sure that some animals received ethanol conditioning in the chamber with horizontal stripes and some in the chamber with vertical stripes. On day 5, mice were tested for postconditioning place preference under a drug-free state. On each test day (days 1 and 5), each mouse was placed in the central neutral chamber and allowed to freely explore all CPP chambers. The amount of time that mice spent in each chamber was recorded and used as a measure of place preference.

2.2.2. Experiment 2: to characterize the role of endogenous DYN in ethanol-induced CPP

Mice lacking DYN and their wild-type littermates were tested for baseline place preference on day 1, received their twice-daily (once in the morning and once in the afternoon) conditionings on days 2–4 and were tested for CPP on day 5, as described above. We used this dose of ethanol to enable us to compare our results to a previous report [13], which also studied the role of endogenous DYN in alcohol reward using mice on a mixed background mouse strain (C57BL/6J × 129/Sv-Tac). Given that changes in the level of DYN and pro-DYN mRNA have been reported shortly after withdrawal from ethanol [8,12], we determined whether ethanol CPP would be altered in mice lacking DYN if mice were tested for CPP at a later time point following the conditionings. Thus, mice were also tested for CPP on day 8.

2.2.3. Experiment 3: to characterize the role of endogenous DYN in ethanol-induced state-dependent CPP

Mice lacking DYN and their wild-type littermates/controls were tested for baseline place preference on day 1, received saline/ethanol or ethanol/saline conditioning on days 2–4 and were tested for CPP on day 5 and state-dependent CPP on day 8. To test for state-dependent CPP, mice were injected with ethanol (1 or 2 g/kg) and immediately thereafter were placed in the central neutral chamber and allowed to explore the CPP chambers. The amount of time that mice spent in each chamber was recorded. Mice used for the state-dependent CPP with the higher dose of ethanol (2 g/kg) were also tested for locomotor activity a week later. In order to reduce the number of mice used, we gave the same animal two doses of ethanol or one dose of ethanol and an injection of saline, so that they received two treatments separated from each other by 48 h. On the test day, mice were habituated to the locomotor activity chambers for 1 h, as described earlier [23], then injected with saline or ethanol (1 or 2 g/kg, s.c.), and locomotor activity was recorded for 1 h. To assure that our results were not skewed by the use of the same mice used for the CPP experiment, we also assessed locomotor activity in naïve DYN null mice and their wild-type littermates following single or repeated (once daily for 4 days) administration of the higher dose of ethanol (2 g/kg, i.p.).

2.2.4. Experiment 4: to examine the effect of nor-BNI, a selective and long-acting KOP antagonist, on ethanol-induced CPP

Wild-type mice were tested for baseline place preference on day 1 and treated with saline or nor-BNI (10 mg/kg) 4 h later. On the following day, mice received morning/afternoon saline/ethanol or ethanol/saline conditionings for three consecutive days and were then tested for postconditioning place preference on day 5, as described above. Mice were also tested for ethanol-induced state-dependent CPP on day 8, in which mice were injected with ethanol (1 g/kg) immediately prior to a CPP test.

3. Data analysis

Data are expressed as mean (±S.E.M.) of the amount of time that mice spent in the CPP chambers on preconditioning (D1), postconditioning (D5) and state-dependent (D8) test days or distance traveled (cm) following ethanol administration. Data were analyzed using two-factor or repeated measures analysis of variance (ANOVA) with the factors being test day and the amount of time that mice spent in the CPP chambers, genotype, or treatment with repeated measures over the test days. The Bonferroni post-hoc test was used to reveal significant differences in the amount of time that mice spent in the ethanol-paired vs. saline-paired chamber. A value of $p < 0.05$ was considered statistically significant.

4. Results

4.1. Ethanol induced a significant CPP in female but not male mice under the current unbiased CPP paradigm

The amount of time that male and female mice spent in the CPP chambers on the preconditioning (D1) and postconditioning (D5) test days is shown in Fig. 1. Repeated measures ANOVA of data in male mice revealed no significant effect of time that mice spent in the CPP chamber ($F(1,56) = 1.25$; p = NS), no significant effect of test day ($F(1,56) = 0.79$; p = NS) and no significant interaction between the two factors ($F(1,56) = 3.43$; $p = 0.07$), suggesting that male mice did not express CPP (Fig. 1, upper panel). In contrast, female mice exhibited a robust CPP response (Fig. 1, lower panel), as evidenced by a significant interaction between the two factors ($F(1,44) = 32.95$; $p < 0.0001$). This result illustrates that female but not male mice exhibited a significant CPP under our current unbiased CPP paradigm. Accordingly, we used female mice for the rest of the experiments.

4.2. Ethanol-induced CPP under a drug-free state was not altered in mice lacking DYN compared to their wild-type littermates

Fig. 2 depicts the amount of time that mice lacking DYN and their wild-type littermates/controls spent in the conditioning chambers on preconditioning (D1) and postconditioning (D5) test days as well as following a saline challenge on day 8 (D8). Repeated measures ANOVA revealed a significant interaction between the amount of time that wild-type mice spent in the CPP chambers and test day ($F(4,30) = 10.63$; $p < 0.0001$). The post-hoc analysis showed that mice spent a significantly greater amount of time in the ethanol-paired compared to saline-paired chamber on the postconditioning (D5) and state-dependent CPP (D8) test days (Fig. 2, upper panel). A similar result was observed in mice lacking DYN (Fig. 2, lower panel), as evidenced by a significant interaction between the two factors ($F(4,30) = 4.98$; $p < 0.01$). These results suggest that the rewarding action of ethanol was not altered in mice lacking DYN compared to their wild-type littermates/controls under a drug-free state at either time point following conditioning.