Neuroscience Letters 443 (2008) 108–112

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Expression of morphine-conditioned place preference is more vulnerable than naloxone-conditioned place aversion to disruption by nociceptin in mice

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ARTICLE INFO

Article history:
Received 17 June 2008
Received in revised form 15 July 2008
Accepted 17 July 2008

Keywords:
Nociceptin
Orphanin FQ
Reward
Addiction
Aversion
Opioid
Conditioned place preference
Conditioned place aversion
Locomotion

ABSTRACT

The opioid peptide nociceptin (orphanin FQ) suppresses the incentive and rewarding properties of drugs. Thus, targeting the nociceptin system may be beneficial in treating drug addiction. The effects of nociceptin (0–1.5 nmol intracerebroventricular) on the expression of morphine- (6 mg/kg subcutaneous) and naloxone-(6 mg/kg subcutaneous) induced place conditioning were examined in mice. Whereas doses of 0.5 nmol nociceptin and above disrupted expression of morphine-conditioned place preference (CPP), naloxone-conditioned place aversion (CPA) remained intact at all doses of nociceptin tested. Doses of 0.5 nmol nociceptin and above suppressed locomotion, though this appeared unrelated to the expression of place conditioning. These results suggest that nociceptin more potently blocks the ability of reward-associated cues than aversion-associated cues to influence behavioral biases.

Nociceptin (known also as orphanin FQ) is an endogenously occurring opioid peptide that may be a useful target for treating mental disorders such as depression, anxiety, anorexia and drug addiction (see Refs. [2,16]). In particular, the nociceptin receptor is highly expressed in brain regions related to reward and behavioral reinforcement [5] and nociceptin suppresses the acquisition of morphine, ethanol, cocaine, amphetamine and methamphetamine-induced conditioned place preference (CPP, see Ref. [18] for reference). These studies suggest that nociceptin blocks the acute rewarding effects of abused drugs, or possibly the creation of learned associations between rewarding and environmental stimuli. They are supported in part by studies showing that nociceptin receptor knockout mice display stronger CPP for cocaine [13], methamphetamine and alcohol [18]. However, the constitutive nature of the knockout mice used in the latter studies prevents differentiation between any roles of nociceptin in the acquisition of CPP, from roles in the expression of CPP.

Although the above studies suggest nociceptin blocks the initial rewarding effects of drugs, they do not address the question of whether targeting the nociceptin system could be beneficial for preventing drug seeking, craving or relapse once addiction has been established. In order to achieve this, it is necessary to examine the effect of nociceptin on behavioral measures more akin to those representing either active seeking of drugs, or the expression of a behavioral bias towards cues that predict availability of drugs [19]. A lesser number of studies have addressed this question and show that nociceptin itself, or agonists of the nociceptin receptor, variably suppress ethanol drinking and ethanol self-administration depending on conditions (see Refs. [3,4]). Although the expression of CPP is unlikely to represent all of the processes that underlie drug seeking, expression of CPP may address a subcomponent of these. That is, at the least, animals emit a behavioral bias towards drug-associated cues in the CPP paradigm that presumably depends on the rewarding property of drug. With respect to this, nociceptin has been shown to suppress the expression of ethanol [10] and cocaine- [9] induced CPP. However, the non-peptide nociceptin receptor agonists Ro 65-6570 and Ro 64-6198 appear to be less effective at blocking the expression of morphine-induced or cocaine-induced CPP [9,21]. Additionally, Ro 64-6198 does not suppress the expression of morphine-induced CPP, but suppresses reinstatement of extinguished morphine-induced CPP [21].

Here, we tested the effect of nociceptin on the expression of morphine-induced CPP and conditioned place aversion (CPA) induced by the general opiate antagonist naloxone. This was undertaken to study the selectivity of the effect of nociceptin on

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doi:10.1016/j.neulet.2008.07.043

behavioral biases towards stimuli conditioned to rewarding and aversive experiences. We chose morphine and naloxone as these drugs share a common site of action, though they induce opposite behaviors.

Experimental protocols were approved by the institutional review committee and were in accord with the National Institute of Health ethics guidelines. Male C57BL6J mice (Nihon Clea, Tokyo, Japan) were introduced into a temperature and humidity controlled room at least 4 days before surgery. Animals were housed as three animals per cage (12 h:12 h light/dark cycle, lights on 8.00 am) and received standard lab chow and water ad libitum. All mice were implanted with histologically verified indwelling cannula targeted at the lateral cerebroventricle under ketamine (100 mg/kg, Wako, Osaka, Japan) and xylazine (10 mg/kg, Sigma, Tokyo, Japan) anesthesia as previously described [17].

Place conditioning was conducted in a two compartment apparatus that recorded position and locomotion, as previously described [17]. Animals were aged 11 weeks at the initiation of the conditioning protocol. Conditioning was performed using an unbiased experimental design using a 20 min "pre-test" where animals were given free access to both compartments under drug-free conditions. The following day, each animal was given a subcutaneous (s.c.) injection of vehicle immediately before placement in a randomly assigned compartment for 40 min. The following day, each animal received an s.c. injection of vehicle, 6 mg/kg morphine or 6 mg/kg naloxone before being placed for 40 min in the opposite compartment to that of the day before. This process was repeated twice (i.e., a total of two vehicle, and two drug-conditioning sessions). The expression of place conditioning (referred to as "test") to the drug-paired compartment was assessed the following day under the same conditions as the pre-test (i.e., 20 min) with the exception that immediately prior to being placed in the apparatus, mice were administered either vehicle or 0.05, 0.1, 0.5 or 1.5 nmol nociceptin by i.c.v. injection as previously described [17]. Morphine hydrochloride (Sankyo Co., Tokyo, Japan), naloxone hydrochloride (Sigma, Tokyo, Japan) and nociceptin (Peptide Institute, Osaka, Japan) were all dissolved in sterile 0.9% NaCl vehicle at concentrations corrected for salt and water content.

To determine the effect of conditioning on locomotion during the test, locomotion was analyzed by a Student's *t*-test between the s.c. vehicle/i.c.v. vehicle-treated group and either the s.c. morphine/i.c.v. vehicle-treated group or s.c. naloxone/i.c.v. vehicle-treated group. To determine the effect of i.c.v. nociceptin administration on locomotion during the test, locomotion within the two conditioning conditions (i.e., morphine or naloxone) was analyzed by separate one-way analysis of variance (ANOVA) tests followed by Dunnett's multiple post hoc comparison. The establishment of place conditioning was defined as a statistically significant change in time spent in the compartment in which mice were conditioned to drug between the test and the pre-test by a paired Student's *t*-test. The effects of nociceptin on the magnitude of place conditioning in the morphine- or naloxone-conditioned animals were analyzed by separate one-way repeated measures ANOVA (where pre-test time and test time were the repeated measure) tests. Correlations between locomotion and the magnitude of place conditioning were tested using Pearson's correlation. Statistical significance was taken at *p*-values less than 0.05.

Mice conditioned with both morphine or naloxone had significantly higher locomotion (morphine conditioned: $t_{24} = -2.601$, $p = 0.0157$ and naloxone conditioned: $t_{26} = -2.649$, $p = 0.0135$) during the test than mice conditioned with vehicle and treated with vehicle during the test session (Fig. 1A). One-way ANOVA showed significant effects of nociceptin on locomotion (morphine conditioned: $F_{4,61} = 78.014$, $p < 0.0001$ and naloxone conditioned: $F_{4,66} = 42.905$, $p < 0.0001$). Generally, 0.05 nmol nociceptin stimulated locomotion, and 0.5 and 1.5 nmol nociceptin suppressed locomotion compared to the vehicle conditioned–vehicle treated group, and the magnitude of the locomotor differences did not depend on the nature of the previous conditioning (Fig. 1A).

As expected, vehicle-conditioned/vehicle-treated mice showed no change in bias towards any compartment (Fig. 1B). Amongst morphine-conditioned mice, vehicle, 0.05 and 0.15 nmol nociceptin-treated mice spent significantly more time in the morphine-paired compartment during the test (vehicle: $t_{11} = -3.155$, $p = 0.0092$; 0.05 nmol, $t_{13} = -2.597$, $p = 0.0221$ and 0.15 nmol, $t_{12} = -3.174$, $p = 0.0080$), i.e., they showed a CPP. Mice

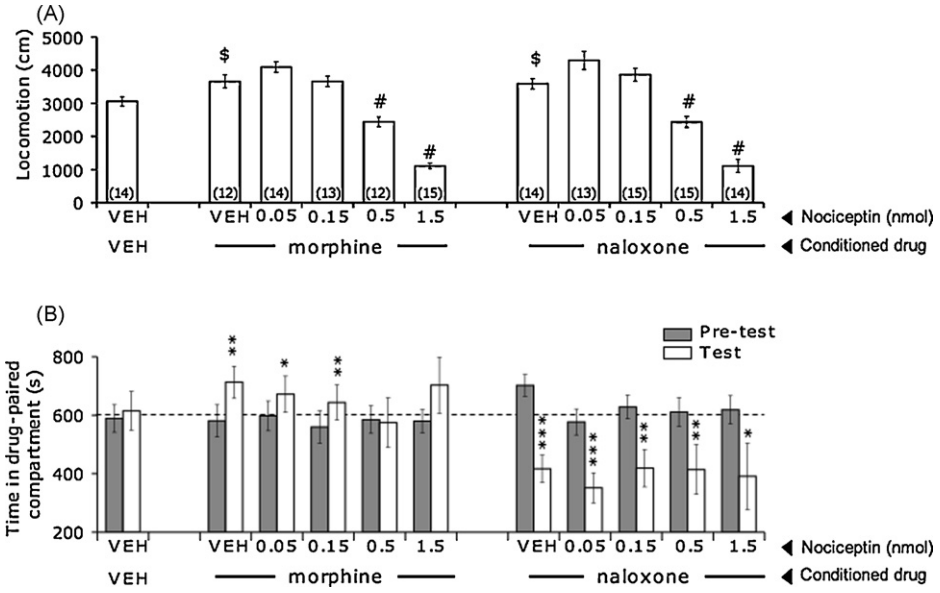

Fig. 1. (A) Locomotion during the test for expression of place conditioning. Numbers in parentheses indicate the number of mice in each group. (B) Time spent in morphine- or naloxone-conditioned compartments during the pre-test and test sessions. The dashed line represents an equal amount of time spent in each of the compartments. ($) $p < 0.05$ Compared to vehicle-conditioned and vehicle pre-treated group, (#) $p < 0.05$ compared to vehicle pre-treated group in the same drug-conditioned set, (*) $p < 0.05$ (**) $p < 0.01$ and (***) $p < 0.001$ compared to the corresponding pre-test time within groups. VEH = vehicle. Data are expressed as mean ± S.E.M.