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Research report

The kappa-opioid receptor antagonist, nor-binaltorphimine (nor-BNI), decreases morphine withdrawal and the consequent conditioned place aversion in rats

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HIGHLIGHTS

- We examined the role of a selective kappa-receptor antagonist on opioid withdrawal.
- Nor-BNI 5 h prior to withdrawal reduced feces excreted in morphine-dependent rats.
- Nor-BNI 5 h prior to withdrawal reduced the conditioned place aversion 2 days later.
- Nor-BNI 2 h following withdrawal did not affect the conditioned place aversion.
- Dynorphin appears to enhance opioid withdrawal and its aversive consequences.

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ABSTRACT

Much data suggest that the binding of dynorphin-like peptides to kappa-opioid receptors (KORs) during the administration of and withdrawal from a variety of addictive drugs is aversive and serves to limit the reinforcing properties of those drugs and to enhance tolerance, withdrawal, and the probability of stress-induced relapse. In this study, we examined the role of KORs in mediating opioid withdrawal and its aversive consequences in rats. We found that selective blockade of KORs by i.p. administration of 20 mg/kg nor-binaltorphimine (nor-BNI) 5 h prior to naltrexone-precipitated withdrawal in morphine-dependent rats decreased feces excreted during a 30-min withdrawal session. More critically, this injection of nor-BNI decreased the subsequent conditioned place aversion (CPA) for the withdrawal chamber 2 days later. The subsequent finding that administration of nor-BNI 2 h following withdrawal did not affect the CPA 2 days later suggested that nor-BNI reduced the CPA in the prior experiment because it reduced the aversive effects of withdrawal, not because it reduced the aversive/anxiogenic effects of the withdrawal chamber at the time of CPA testing. These data indicate that the binding of dynorphin-like peptides to KORs during opioid withdrawal serves to enhance withdrawal and its aversive consequences and suggest that selective KOR antagonists may be useful in reducing these aversive effects and consequent relapse.

1. Introduction

While much evidence suggests that binding of the endogenous endorphin and enkephalin peptides to mu- and delta-opioid receptors, respectively, is generally rewarding (e.g. [1–3]), binding of the endogenous dynorphin-like peptides to kappa-opioid receptors (KORs) produces dysphoric-, aversive-, and stress-like states in humans [4] and laboratory animals [5–7]. For example, injections of selective KOR agonists produce conditioned place aversions [8–10], increase electrical self-stimulation thresholds [11], and reverse the rewarding effects of morphine [8,12] and other addictive drugs (e.g. [13–16]). The additional findings that dynorphin-like peptides and their precursors are increased in the striatum and elsewhere during administration of opioids and other addictive drugs (e.g. [17–19]) and during opioid [20] and nicotine [21] withdrawal have led to the hypothesis that these aversive peptides may play a neuroadaptive role in drug addiction serving to limit the rewarding effects of the drugs and to enhance tolerance, withdrawal, and the probability of subsequent relapse (e.g. [22–26]). Indicating that the binding of dynorphin-like peptides to KORs contributes to withdrawal, selective KOR antagonists attenuated the development of elevations in electrical self-stimulation thresholds during cocaine withdrawal

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[27], decreased measures of anxiety [28–30] and ultrasonic vocalizations [31] during ethanol withdrawal, and decreased somatic signs during nicotine withdrawal [32,33].

The data indicating the involvement of KORs in opioid withdrawal are, however, more mixed. Consistent with the view that activation of KORs contributes to opioid withdrawal, KOR-deficient morphine-dependent mice exhibited fewer naloxone-precipitated withdrawal symptoms than did wild-type mice [34]. Similarly, intraperitoneal (i.p.) injections of the selective KOR antagonist JDTic during the establishment of morphine dependence reduced spontaneous withdrawal signs in rats [35], and i.p. injections of 5 mg/kg of the selective KOR antagonist nor-binaltorphimine (nor-BNI) just prior to naltrexone-precipitated withdrawal in morphine-dependent mice reduced several withdrawal signs [36]. Finally, an i.p. injection of 30 mg/kg nor-BNI prior to heroin self-administration reduced the anxiety produced by spontaneous withdrawal in rats [37], and intra-cranial nor-BNI after 1 day of morphine abstinence reduced the enhanced aversive effects of electrical stimulation of the dorsal periaqueductal gray and inferior colliculus 24 h later in adolescent rats [38]. On the other hand, other investigators found that intra-cranial and systemic injections of nor-BNI precipitated or enhanced some opioid-withdrawal symptoms in rats and mice [39–44], and intra-ventricular nor-BNI prior to the development of morphine dependence increased the subsequent conditioned place aversion produced by naloxone-precipitated withdrawal in rats [42]. Although it is likely that some of these differences in the effects of KOR antagonists reflect differences in the dose, site, or timing of injections (see Section 4), there is as yet no consensus.

Thus, one intent of our study was to further examine the role of KORs in mediating opioid-withdrawal symptoms in rats. More critically, a second intent was to examine the role of KORs in mediating the aversive consequences of withdrawal, as measured by a conditioned place aversion (CPA), as those aversive consequences are likely to mediate much of relapse via negative reinforcement (e.g. [45–47]). In Experiment 1, we show that 20 mg/kg nor-BNI given i.p. 5 h prior to naloxone-precipitated withdrawal in morphine-dependent rats reduced some symptoms of opioid withdrawal and reduced the consequent CPA 2 days later. We used 20 mg/kg, because that is the dose most commonly used in the studies cited above, and we gave nor-BNI 5 h prior to withdrawal because nor-BNI can block mu-opiate receptors (MORs) for 2–4 h following its injection [48], but becomes a very selective KOR antagonist thereafter [48–50], with its peak blockade of KORs occurring at 4 h [48]. In Experiment 2, we show that 20 mg/kg nor-BNI given 2 h following withdrawal, but still 2 days prior to CPA testing, did not significantly affect the CPA. This finding suggests that the nor-BNI-induced reduction of the CPA in Experiment 1 was likely due to a prior reduction of the aversive effects of opioid withdrawal rather than to a reduction of anxiety at the time of CPA testing.

2. Materials and methods

2.1. Experiment 1: 20 mg/kg nor-BNI 5 h prior to withdrawal

2.1.1. Subjects

The subjects were 48 male Long Evans rats (Charles River Laboratory, Wilmington, MA) that weighed between 250 and 400 g at the time of testing. They were single housed in 21.6 × 20.3 × 30.5-cm stainless-steel hanging cages in an animal colony that was lit from 8 AM to 8 PM. Food and water were available ad libitum except during testing. All procedures in this and the subsequent experiments were carried out in accordance with the principles of laboratory animal care established by the National Institutes of Health (NIH Publication No. 86–23, revised 1985) and were approved by the Bates College Institutional Animal Care and Use Committee.

2.1.2. Apparatus

Withdrawal and CPA testing occurred in two identical 20 × 49 × 31-cm galvanized-steel shuttleboxes that were divided into two 20 × 24.5-cm interconnecting metal chambers by a metal partition that had a 7 × 8-cm open arch flush with the floor. One of the chambers was silver gray and had a steel mesh screen with 1 × 1-cm openings covering the grid floor, and was illuminated by a 6 W bulb located inside a black Plexiglas box that was mounted on the transparent top and connected to a 60 V power source. The other chamber was painted black with a floor of stainless-steel grids 0.6 cm in diameter separated by 2 cm center to center running across the width of the chamber and was not illuminated. The grid floor pivoted in the middle below the partition such that when a rat moved into one chamber it would trigger a microswitch above the grid floor at the end of the other chamber. The room in which the shuttleboxes were contained was lit by three 25 W red bulbs and supplied with 75 dB (SPL) white noise.

2.1.3. Procedure

2.1.3.1. Initial place preference. Two rats at a time were taken to the experimental room in their cages and allowed to habituate for 5 min. During this time, weights on each end of the grid floors were adjusted such that the microswitch on each end was just tripped when the center of the rat was between the fourth and fifth grids of the opposite chamber. Each rat was then placed into the black chamber of one of the boxes facing the partition and was allowed free access to the apparatus for 15 min, after which they were returned to the colony. The time spent in both chambers of the shuttlebox was recorded with a microprocessor. The rats were then divided into four groups, with an approximately equal number of rats in each group preferring the white side of the shuttlebox and expressing an equivalent preference (measured in s) for the side designated as the withdrawal side.

2.1.3.2. Morphine dependence. Beginning on the following day, 30 rats received i.p. injections of morphine hydrochloride twice daily (at 8 AM and 4 PM) on the following schedule from a stock bottle of 15 mg/ml (Baxter, Deerfield, IL): Day 1, 10 and 10 mg/kg; Day 2, 10 and 15 mg/kg; Day 3, 20 and 20 mg/kg; Day 4, 30 and 40 mg/kg; Day 5, 40 and 50 mg/kg; and Day 6, 50 mg/kg in the morning in order to produce dependence. The remaining rats ($n = 18$) were injected i.p. on a similar schedule with similar volumes of 0.9% saline.

2.1.3.3. Precipitated morphine withdrawal. Immediately after the AM injection on day 6, eight non-dependent saline-injected and 15 morphine-dependent rats were injected i.p. with 2 ml/kg 0.9% saline in the colony, and the remaining 10 non-dependent and 15 dependent rats were injected i.p. with 20 mg/kg nor-BNI (Sigma, St. Louis, MO; 10 mg/ml in 0.9% saline). Five hours later, all rats were taken to the experimental room in pairs and injected i.p. with 4 mg/kg naltrexone hydrochloride (Sigma, St. Louis, MO; 4 mg/ml in 0.9% saline) in order to precipitate withdrawal. Each rat was then placed immediately into its designated withdrawal chamber of the shuttlebox in which its initial place preference had been attained, and confined there for 30 min. During this time period, the frequency of the following classic symptoms of opioid withdrawal was counted: jumps (all four legs off the ground), wet-dog shakes (a sudden, violent shake of the head, shoulders and trunk), and genital licks (presumably reflecting ejaculation), and the amount of feces excreted (g) and weight loss (g) were also measured. The rats were then returned to the colony.