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Acute Delta- and Kappa- Opioid Agonist Pretreatment Potentiates Opioid Antagonist-Induced Suppression of Water Consumption

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Abstract

The primary objective of this study was to determine whether pretreatment with kappa- and delta-opioid agonists potentiates naltrexone-induced suppression of water consumption following 24-hr of deprivation. This study also examined the temporal effects of agonist-induced antinociception using the tail-flick and hot plate tests. Adult male Sprague-Dawley rats were water deprived 20 hr and then given an injection (s.c. or i.c.) of an opioid agonist or saline. Drugs included the mu-opioid agonists morphine and DAMGO, the kappa-opioid agonists spiradoline, bremazocine, and U69,593, and the delta-opioid agonists BW 373U86 and DPDPE). Three hr and 45 min later, animals received a single dose of naltrexone (0.1 – 30 mg/kg, s.c.) or saline. Fifteen minutes later, animals were allowed free access to water for 30 min. For the tail-flick and hot plate tests, animals were given a single injection of agonist and tested in both procedures every 30 min for up to 2 hr, then hourly up to 6 hr post-injection. Naltrexone dose-dependently suppressed fluid consumption 24 hr after deprivation. The effects of naltrexone on drinking were potentiated following pretreatment with at least one dose of the agonists tested except BW 373U86. With the exception of BW 373U86, DAMGO, and DPDPE, all of the opioid agonists produced significant antinociception in the hot plate test. Only BW 373U86 failed to have an antinociceptive effect in the tail-flick test. By 4 hr after treatment, drug-induced antinociception had largely waned, suggesting the potentiation of naltrexone-induced drinking suppression was not a result of a direct interaction with the agonists. In conclusion, kappa-opioid and delta-opioid receptors appear to contribute to the manifestation of acute opioid dependence, albeit to a lesser degree than mu-opioid receptors.

Keywords

Rats; Drinking suppression; Antinociception; Hot plate; Tail-flick; Acute dependence

1. Introduction

Opioid antagonists (e.g., naloxone and naltrexone) have only minor physiological and behavioral effects at low doses in non-opioid dependent subjects [18]. However, subjects treated chronically with mu-opioid receptor agonists (e.g., morphine) are more sensitive to a number of opioid antagonist-induced effects, including suppression of schedule-controlled behavior [14,39]. Sensitization to antagonists following chronic morphine administration is

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thought to be due to withdrawal and, as a result, is used as a measure of physical dependence [2,18].

Giving a single dose of morphine-like agonists followed by the administration of an opioid antagonist elicits many effects similar to those seen with chronic morphine, possibly reflecting a state of acute physical dependence [for review, see 22]. This includes sensitization to the behavioral effects of opioid antagonists. For example, opioid agonist pretreatment potentiates the suppressant effects of naltrexone on operant behaviors, such as responding for food and intracranial self-stimulation [1,14]. The suppressing effects of opioid antagonists have been demonstrated for unconditioned behaviors as well [6,26,42]. For example, naloxone dose-dependently decreases drinking in rodents following water deprivation, intracellular dehydration [6], extracellular dehydration, and angiotensin-induced polydipsia [8]. Previous work in our laboratory has also shown that naltrexone also dose-dependently suppresses drinking in rats following 24 hr water deprivation [42]. This effect was potentiated in a dose-dependent manner by pretreatment with mu-selective agonists (e.g. fentanyl, meperidine). Such findings demonstrate that drinking suppression serves as a useful and convenient measurement of acute opioid dependence.

Like mu-opioid agonists, chronic kappa-opioid and delta-opioid agonist administration results in physical dependence, albeit to a lesser degree [10,29,46]. Additionally, both delta-opioid and kappa-opioid antagonists have been shown to precipitate withdrawal in morphine-dependent rats [30], indicating further a possible role of these receptor systems in mediating physical dependence upon opioids. Studies have also been conducted looking at the involvement of delta-opioid and kappa-opioid receptor subtypes in acute opioid dependence. For example, 4-hr pretreatment with the systemically administered kappa-opioid-selective agonists ethylketocyclazocine (0.3 and 1.0 mg/kg) and U-50,488 (3.0 and 10 mg/kg) as well as the central administration of the kappa-opioid receptor-selective peptide dynorphin A₍₁₋₁₇₎ (30 µg) potentiated the suppressing effects of naltrexone on food-reinforcement by as much as 19-fold [1,3]. Similarly, pretreatment with the delta-opioid receptor-selective peptide DPDPE (30 and 100 µg) produced significant leftward shifts of the naltrexone dose-response curve, resulting in 11- and 33-fold decreases of the naltrexone ED₅₀, respectively [3].

In spite of these studies, virtually no research exists examining the impact of acute kappa- and delta-opioid dependence on naltrexone-induced changes of unconditioned behaviors. Studying the impact of kappa- and delta-opioid receptors on acute opioid drug dependence could further elucidate the mechanisms involved in the transition from initial drug exposure, to dependence and addiction and possibly lead to better treatments for opioid dependence. Therefore the main goal of this study was to determine whether the acute administration of these agonists would influence opioid antagonist-induced suppression of water consumption following deprivation. Based on the studies outlined above, our hypothesis was that pretreatment with kappa- and delta-opioid agonists would produce acute opioid dependence as measured by the potentiation of naltrexone-induced drinking suppression.

From a previous study in our laboratory [42], sensitization to naltrexone was seen up to 6 hr after administration of mu-selective agonists. This effect occurred at a time when drug-induced antinociception had waned, suggesting that the changes associated with acute opioid dependence persist well after peak tissue levels of drug have dissipated. Therefore, the second objective of this study was to gain insight into the possible existence of a similar temporal relationship between agonist-induced sensitization to the effects of naltrexone and agonist-induced antinociception. This was accomplished by constructing a time course of antinociception following single injections of agonists in the tail-flick and hot plate procedures. Although both the tail-flick and hot-plate antinociceptive assays measure the response to thermal stimuli, the tail-flick response is mediated predominantly by spinal mechanisms,

whereas the hot-plate response is mediated predominantly by supraspinal mechanisms [21, 45]. Assessing the drugs in both assays afforded us an opportunity to assess their site of action in addition to their temporal effects.

2. Methods

2.1 Animals

The subjects were adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Raleigh, NC). Animals weighed between 250g–400g at the beginning of the experiment and were randomly assigned to treatment groups. Animals were singly housed in polycarbonate cages located in ventilated cabinets in the lab. The animals were maintained on a 12:12 light-dark cycle with food and water available ad libitum. During testing, animals were not provided free access to water, but food was available throughout testing. Animals were maintained according to the ‘Guide for the Care and Use of laboratory animals’ (National Academy of Sciences, 1996). All procedures were approved by Emory University’s Institutional Animal Care and Use committee.

2.2 Measurement of Water Intake

This procedure has been described previously [42]. In short, animals were deprived of water 24 hr before testing. On the test day, animals were weighed and injected either subcutaneously or intracisternally with saline (0.9% NaCl) or an opioid agonist [morphine (1.0 and 10 µg; 3.0 and 10 mg/kg), DPDPE (100 µg), DAMGO (0.1 µg), bremazocine (0.1 and 0.3 mg/kg), BW 373U86 (0.79–7.92 mg/kg), spiradoline (1.0 and 3.0 mg/kg), U69,593 (0.3 and 3.0 mg/kg)] 4 hr before measurement of water intake. Naltrexone (0.1–30 mg/kg) or saline was then given 15 min before testing (i.e. 3 hr 45 min after agonist administration). Rats were given free access to water for 30 min, using 100 ml calibrated water bottles (Wahmann Laboratories, Timonium, MD). Following testing, animals were given a minimum of 48 hr access to water prior to subsequent testing. All experiments occurred between 1200 and 1850 hr, two times per week. In generating dose-response curves, separate cohorts of subjects were used to test each pretreatment dose of agonist given 4 hr prior to testing. Subjects were exposed to the same agonist pretreatment throughout experimentation. However, for the second injection given 15 min prior to testing, subjects received saline and all doses of naltrexone (one dose per test session), which were randomly assigned. Additionally, each subject underwent two habituation sessions, wherein saline was given 4 hr prior to testing. Thus, each animal was tested with a single opioid-agonist dose, a total of six to seven times. A total of 122 animals were used for these experiments.

2.3 Antinociception Time Course

A time course of the effects of the various opioid agonists on nociception was constructed using the radiant heat tail-flick and hot plate tests [42]. Prior to drug administration, drug naïve animals went through the procedure (tail-flick test immediately followed by hot-plate test) two times, approximately 30 min apart, in order to establish a baseline for each subject. Subjects were then given a single injection of drug (s.c. or i.c., depending on the drug) and latencies were recorded at 30, 60, 90, 120, 240, and 360 min post-injection. Only drugs given s.c. were recorded up to the 360 min time point. Tail-flick response latencies were determined using a modified version [17] of the radiant heat tail-flick procedure [11] and a Model 33 Tail Flick Analgesia Meter (IITC, Inc., Life Science Instruments, Woodland Hills, CA). A 20-V bulb was focused on the lower third of the rat’s tail. An automatic timer, which began as soon as the light came on, was used to record the latency to tail movement. Tail movement activated a photocell, which subsequently turned off the light source and timer. A maximum cutoff time of 8.5 s was used to decrease the amount of tissue damage. For the hot plate (Model 39D, Hot Plate Analgesia Meter; IITC, Inc., Woodland Hills, CA), the surface measured 26.5×29×3 cm

and was surrounded by 28.5-cm-high Plexiglas walls and removable cover. The surface temperature of the plate was set at $52.0 \pm 0.2^\circ\text{C}$. In this procedure, animals were placed on the hot plate and the endpoint was defined by the animals licking their hind-paws or jumping off the surface. A maximum cutoff time of 30 s was used. A total of 70 animals were used for these experiments.

2.4 Central Drug Administration

Several of the drugs (e.g., morphine, DAMGO, DPDPE) were given centrally. Rats were anesthetized with halothane and then shaved from the back of the head just behind the ears to the scapulae. The area was cleaned with 70% ethanol. Animals were then injected intracisternally (i.c.) at the base of the skull with one of the opioid agonists. Following injection, animals were placed back in their home cage and allowed to recover from anesthesia before being returned to their housing cabinets or tested for antinociception. The i.c. route of administration was used because it permits (relatively) quick injection of drugs into the central nervous system without the need to deeply anesthetize each rat and eliminates the need to perform lengthy stereotaxic surgery. Additionally, it has no apparent lingering effects upon motor activity [24]. Other studies from our laboratory have also shown that pretreatment with systemic and central saline produces very comparable results [3,15]. Therefore, groups receiving drug i.c. were compared to that receiving systemically administered saline.

2.5 Drugs

The drugs used and their source were: morphine sulfate (Penick, Co., Nutley, NJ); [(+)-4-[alpha (R)-alpha-[(2S,5R)-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl]-(3-hydroxyphenyl)methyl]-N,N-diethylbenzamide dihydrobromide (BW 373U86), [D-Ala², NMePhe⁴, Gly-ol]-enkephalin (DAMGO); [D-Pen², D-Pen⁵]-enkephalin (DPDPE) and naltrexone hydrochloride (Sigma Chemical Co., St. Louis, MO); bremazocine hydrochloride and [5a,7a,8b)-(+)-N-{7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl)]benzene-acetamide (U69,593; Research Biochemicals, Inc., Natick, MA); spiradoline methanesulfonate (Pharmacia and Upjohn Co., Kalamazoo, MI). With the exceptions of bremazocine and U69,593, drugs were dissolved in 0.9% saline. Bremazocine was prepared in de-ionized water, while U69,593 was dissolved in 3 parts 8.5% lactic acid and 2 parts 1.0 N sodium hydroxide. Agonist doses chosen for this study were based on previous work, demonstrating behavioral effects [3,14,34,42]. Drug doses are expressed as the free base. It should be noted that initial free base dose calculations for BW 373U86 were based on the hydrochloride salt form of the drug. However, the salt was changed to dihydrobromide by the source company without notification until after completion of experimentation. As such, the true free base doses were recalculated based on the new salt form.

2.6 Data Analysis

Water intake data were converted to mL of water consumed per kg of body weight. The converted data are expressed as percent saline control in order to facilitate comparisons among the different experimental groups. Results are expressed as mean $\pm$ S.E.M. The transformed data were analyzed using a mixed two-factor analysis of variance (ANOVA; repeated measures: agonist pretreatment $\times$ naltrexone dose) and Newman-Keuls test post hoc comparison to determine significant differences (GB-STATv7.0, Dynamic Microsystems, Inc., Silver Spring, MD). Naltrexone ED₅₀ values were calculated for each subject by linear regression. From this, means and 95% C.I. were derived for each pretreatment group. ED₅₀ data from the pretreatment groups were analyzed using a one factor ANOVA and Newman-Keuls test *post hoc* to determine significant differences.

Tail-flick and hot plate latencies are expressed as percentage maximum possible effect (% MPE; [12]):

$$\%MPE = \frac{(\text{test latency} - \text{baseline latency})}{(\text{cut-off time} - \text{baseline latency})} \times 100$$

The transformed latency data (%MPE) were analyzed using a one-factor, repeated measures ANOVA and Newman-Keuls test *post hoc*.

P-values less than 0.05 were accepted as statistically significant.

3. Results

3.1 Naltrexone-induced Drinking Suppression Following Saline or Agonist Pretreatment

Four hr after saline pretreatment subjects drank an average of 59 ± 2.4 ml/kg (Table 1). Naltrexone dose-dependently decreased drinking following saline pretreatment ($F(3,66) = 100.8, p < 0.001$), with 30 mg/kg naltrexone suppressing drinking by 81% (Fig. 1).

The volume of water consumed 4 hr after systemically ($F(2,36) = 4.58, p < 0.05$) or centrally ($F(2,26) = 4.36, p < 0.05$) administered morphine alone was dose-dependently decreased (Table 1). Pretreatment with 3.0 or 10 mg/kg morphine suppressed drinking by 14 and 32%, respectively, while centrally administered morphine (e.g., 1.0 and 10 μ g) suppressed drinking by 17 and 23% (Table 1). Conversely, pretreatment with another μ -opioid receptor agonist DAMGO did not significantly alter basal water consumption (Table 1). With the exception of 0.1 mg/kg bremazocine, which increased water consumption (Table 1; $F(2,29) = 7.03, p < 0.01$), drinking was not affected by pretreatment with kappa-opioid agonists (e.g. either dose of spiradoline, $F(2,27) = 0.73$; U-69,593, $F(2,29) = 1.34$). Finally, pretreating the animals with delta-opioid agonists significantly suppressed basal water consumption: BW 373U86 (0.79–7.92 mg/kg) reduced drinking by 17–24% ($F(3,34) = 7.83, p < 0.01$) and DPDPE reduced water consumption by nearly 20% ($t(1,19) = 3.11, p < 0.01$).

As shown in Figs. 1 and 2A, both systemically and centrally administered morphine dose-dependently potentiated the effects of naltrexone ($F(2,42) = 25.3, p < 0.001$ and $F(2,33) = 46.7, p < 0.001$, respectively) with 10 mg/kg (s.c.) significantly reducing the ED_{50} of naltrexone by more than 80-fold (Table 2; $F(2,37) = 22.5, p < 0.001$). An ED_{50} for naltrexone following 10 μ g morphine pretreatment could not be calculated as consumption following the lowest dose of naltrexone (i.e. 0.1 mg/kg) was suppressed by more than 60% (Fig. 2A). There was also a significant interaction between centrally administered morphine and naltrexone ($F(4,101) = 3.4, p < 0.01$). Compared with saline pretreatment, DAMGO also significantly potentiated the effects of naltrexone ($F(1,25) = 20.9, p < 0.001$), resulting in significantly less water consumption following 1.0 and 10 mg/kg naltrexone (Fig. 2B) and more than a threefold decrease of the ED_{50} for naltrexone (Table 2; $t(1,8) = 2.64, p < 0.05$).

Pretreatment with spiradoline 1.0 mg/kg and 3.0 mg/kg potentiated the effects of naltrexone, significantly reducing water consumption following 1.0 mg/kg naltrexone compared to saline pretreatment (Fig. 3A; $F(2,33) = 5.1, p < 0.05$). Spiradoline pretreatment also reduced the ED_{50} of naltrexone 1.7- and 3.4-fold, respectively, but these shifts were not significant (Table 2; $F(2,34) = 1.9$). Similarly, pretreatment with 0.1 and 0.3 mg/kg bremazocine ($F(2,36) = 3.0, p < 0.05$) and 3.0 mg/kg U-69,593 ($F(2,36) = 4.7, p < 0.05$) potentiated the effects of naltrexone at 1.0 and 10 mg/kg (Figs. 3B, 3C). However, both drugs failed to significantly reduce the ED_{50} of naltrexone (Table 2; bremazocine, $F(2,36) = 1.6$; U-69,593, $F(2,36) = 2.3$). There was no interaction between spiradoline and naltrexone ($F(4,101) = 1.5$). However, bremazocine and U-69,593 did interact significantly with naltrexone ($F(4,110) = 6.1, p < 0.01$ and $F(4,110) = 4.2, p < 0.01$ respectively).

Pretreatment with the delta-opioid agonist BW 373U86 up to a dose of 7.92 mg/kg had no effects on naltrexone-induced suppression of water consumption (Fig. 4; $F(3,42) = 0.4$). BW 373U86 pretreatment also failed to alter the ED_{50} of naltrexone (Table 2; $F(3,39) = 1.8$). In contrast, pretreating subjects with DPDPE significantly potentiated the effects of naltrexone at 0.1 and 10 mg/kg as compared to saline pretreatment (Fig. 4B; $F(1,26) = 13.4$, $p < 0.001$). Consequently, DPDPE pretreatment reduced the ED_{50} of naltrexone, albeit non-significantly, by threefold (Table 2; $t(1,6) = 1.5$).

3.2 Tail-flick and hot-plate antinociception time course

In the tail-flick test, except for BW 373U86 (2.38 and 7.92 mg/kg), all of the agonists significantly increased the % MPE over baseline values within 30 min of administration (Figs. 5A, 5B). There was also a significant effect of time on the antinociceptive effects of morphine (3.0 mg/kg, $F(7,71) = 13.9$, $p < 0.001$; 10 mg/kg, $F(7,71) = 31.1$, $p < 0.001$; 1.0 μ g, $F(6,62) = 7.2$, $p < 0.001$; and 10 μ g, $F(6,62) = 17.3$, $p < 0.001$), bremazocine ($F(7,71) = 9.2$, $p < 0.001$), spiradoline ($F(7,71) = 12.4$, $p < 0.001$), 2.38 mg/kg BW 373U86 ($F(7,71) = 3.6$, $p < 0.05$), DAMGO ($F(6,62) = 3.3$, $p < 0.05$), and DPDPE ($F(6,62) = 7.2$, $p < 0.01$) but not 7.92 mg/kg BW 373U86 ($F(7,71) = 1.4$). For systemically administered agonists (Fig. 5A), tail-flick latencies returned to basal levels by 6 hr (or sooner) post-administration- with the exception of 10 mg/kg morphine, which remained at 40% MPE. Similarly for the centrally administered agonists, latencies returned to basal values by 90 min or less post-injection (Fig. 5B). Only the 10 μ g morphine dose maintained an antinociceptive effect until the last time point, 4 hr.

In the hot plate test, all of the agonists produced significant antinociception within 30 min of administration with the exceptions of BW 373U86, DAMGO, and DPDPE (Figs. 5C, 5D). Again there was a significant effect of time on the antinociceptive effects of morphine (3.0 mg/kg, $F(7,71) = 8.4$, $p < 0.001$; 10 mg/kg, $F(7,71) = 31.9$, $p < 0.001$; 1.0 μ g, $F(6,62) = 15.5$, $p < 0.001$; and 10 μ g, $F(6,62) = 6.6$, $p < 0.001$), bremazocine ($F(7,71) = 28.3$, $p < 0.001$), spiradoline ($F(7,71) = 23.1$, $p < 0.001$), BW 373U86 (2.38 mg/kg, $F(7,71) = 5.8$, $p < 0.01$; 7.92 mg/kg, $F(7,71) = 4.6$, $p < 0.01$), and DAMGO ($F(6,62) = 7.7$, $p < 0.001$) but not DPDPE ($F(6,62) = 2.0$). For systemically administered agonists, response latencies returned to basal levels by no later than 4 hr post-administration (Fig. 5C), while antinociception produced by centrally administered agonists waned by 90 min post-administration (Fig. 5D). DAMGO and DPDPE failed to produce antinociception. However, DAMGO administration resulted in significant hyperalgesia beginning 90 min post-administration and lasting throughout the remainder of the experiment (Fig 5D). Similarly, 10 μ g morphine produced hyperalgesia 3 and 4 hr post-administration (Fig 5D).

4. Discussion

The findings from this study generally were consistent with our hypothesis that opioid dependence would be manifested following acutely administered kappa- and delta-opioid agonists and extend the range of compounds affecting naltrexone-induced alterations in unconditioned behavior. Systemic and centrally administered mu-opioid agonists dose-dependently potentiated the effects of naltrexone on drinking, consistent with other studies from our laboratory on acute opioid dependence using conditioned [1,14] and unconditioned behaviors [26,42]. A similar, but much smaller, effect occurred following acute pretreatment with the kappa-selective opioid agonists, as well as the delta-opioid agonist, DPDPE. These receptor subtype-selective effects are again consistent with other studies from our laboratory [3]. All systemically administered agonists, with the exception of BW 373U86, produced some degree of antinociception in either the tail-flick or hot plate assays. Save for the highest dose of morphine (10 μ g), centrally administered agonists had only modest antinociceptive effects.

Lastly, drug-induced antinociception had largely waned by 3 hr (centrally administered) and 4 hr (systemic administered) post-administration.

In the current study, spiradoline, bremazocine, and U69,543 all potentiated the effects of naltrexone on drinking behavior. These findings are in keeping with studies by Adams and Holtzman [1,3], where 4-hr pretreatment with kappa-selective agonists (e.g. ethylketocyclazine, U-50,488, (+)-NANM, and dynorphin A-(1–17)) potentiated the suppressant effects of naltrexone on operant responding for food reinforcement. In these studies agonist pretreatment produced significant leftward shifts of naltrexone dose-response curves and decreases in the ED₅₀s for naltrexone ranging from two- to 19-fold, depending on the agonist and dose. In our hands, agonist pretreatment only decreased the ED₅₀ for naltrexone two- to three-fold. This discrepancy is likely attributable to the potency of naltrexone itself. In the current study, the ED₅₀ of naltrexone following saline pretreatment was 1.27 mg/kg. However, in a previous study from our laboratory [42], the ED₅₀ of naltrexone alone was 8.85 mg/kg- nearly sevenfold higher. The apparent increased potency of naltrexone in the current study may partially stem from the observed increased basal consumption of water under control conditions. In the current study, control animals consumed 59 ± 2.4 mL of water 24 hours after water deprivation, which was greater than control animals from previous studies in our laboratory (i.e., 48 ± 3.8 and 53 ± 2.4 mL, respectively) (Brown and Holtzman, 1979; White and Holtzman, 2001). The cause for the greater consumption of water in the current study is not clear, but this fact should be taken into consideration when comparing the effects of agonists alone. If basal water consumption was more in line with those from our previous work (Brown and Holtzman, 1979; White and Holtzman, 2001), then virtually none of the agonists-induced decreases in basal consumption would have been observed- possibly with the exception of that following 10 mg/kg morphine (see Table 1). In fact, it would appear as though the kappa-selective opioid agonists bremazocine and spiradoline may actually stimulate water consumption. Comparisons of the changes in kappa-opioid-induced sensitivity to naltrexone from the present work to the saline-naltrexone ED₅₀ value from our previous study (White and Holtzman, 2001) would bring our results more in line with those of Adams and Holtzman (1990; 1991). Regardless of the discrepancies in basal water consumption and the potency of naltrexone between the current study and previous ones, the present findings indicate a role, albeit small, of the kappa-opioid system in the manifestation of acute opioid dependence.

The role of the kappa-opioid receptors in opioid dependence following protracted drug exposure is less clear. For example, the kappa-opioid receptor antagonist Nor-BNI has been shown to elicit a moderate withdrawal syndrome in morphine-dependent rats [30]. However, studies in rats employing chronic kappa agonist administration have found little or no dependence following spontaneous or antagonist-precipitated withdrawal [4,10,46]. Conversely, robust physical dependence upon kappa agonists has been shown in monkeys [19]. The ability of monkeys (versus rats) to develop dependence upon kappa-opioid agonists probably stems from a species-related difference in kappa-opioid receptor population [31,32, 32].

In rats, perhaps kappa-opioid receptors serve more as modulators of opioid dependence by acting on the other opioid receptor subtypes rather than as direct mediators of opioid dependence. For example, receptor heterodimerization occurs amongst opioid receptors [20, 25,41], specifically kappa-delta [25] and mu-kappa [41], which can impact the functionality of those receptors [20,25]. Thus, it is possible that the behavioral response to kappa-opioid agonist administration could be mediated indirectly via the mu-opioid receptor. This possibility would require further investigation as heterodimerization has been shown to alter the binding properties of selective agonists for the individual receptors [20,25,41]. It may also be that kappa agonists are directly acting on other receptor systems. For example bremazocine exhibits antagonistic properties for both mu- and delta-opioid receptors [47]. Furthermore, spiradoline

may also possess very minor antagonistic properties at the mu-opioid receptor [40], despite having a very high affinity for the kappa-receptor. Therefore, it can't be ruled out that selectivity was exclusive to the kappa-opioid receptor, as the effect seen may have resulted from the kappa-agonistic properties coupled with the additive effect of the mu-antagonism.

In the current study, DPDPE pretreatment potentiated the effects of naltrexone, reducing the ED₅₀ of naltrexone by threefold. These results were consistent with another study [3], where 4-hr pretreatment with 30 and 100 µg of DPDPE potentiated naltrexone-induced suppression of food-maintained operant responding, resulting in 11- and 33-fold leftward shifts of the naltrexone dose-response curves, respectively. The approximate ten-fold lower potency of DPDPE in the current study again is likely due to the greater potency of naltrexone alone (see above). However, this finding does lend support to the notion that delta-opioid receptors, like kappa-opioid receptors, play a role in the manifestation of acute dependence. Evidence also suggests a potential role for delta-opioid receptors in chronic opioid dependence. For example, the delta-opioid antagonist naltrindole has been shown to precipitate withdrawal in morphine-dependent rats [30]. Additionally, prolonged exposure or repeated exposure to delta-opioid agonists not only results in tolerance to their antinociceptive effects [27,38] but also in modest dependence as demonstrated by naloxone-precipitated withdrawal [10,27,29] in rodents.

Despite these findings, there are data that suggest that the effects of delta-opioid agonists (DPDPE in particular) may, in part, be mediated by other receptor systems. For instance, DPDPE-induced antinociception was not antagonized by a phosphodiester antisense oligonucleotide directed against delta-opioid receptors but was antagonized by the mu-opioid selective antagonist CTOP [16]. Further, in delta-opioid receptor knockout mice, both DPDPE and deltorphin II produced full antinociception in the tail-immersion and hot-plate tests; however, both drugs had little or no effect in mu-opioid receptor knockouts [35]. Such findings, coupled with the fact that tolerance with little or no dependence develops following both acute and chronic administration of the delta-opioid-selective agonist SNC80 in primates [5], bring the involvement of the delta-opioid receptor system in the mediation of opioid dependence into question.

BW 373U86 is a potent nonpeptide agonist with high selectivity for delta-opioid receptors [9]. In the current study, BW 373U86 had no effects on drinking suppression or nociception at doses shown to be behaviorally active [9,13,28]. Both of these findings are consistent with the literature. For example, one study reported that BW 373U86 up to a dose of 50 mg/kg (s.c.) failed to alter the latency for rats to withdraw their tails from 55° C water [9]. This dose was more than six-fold larger than the highest dose tested in the current study. It is possible that BW 373U86 has antinociceptive properties that are masked by low efficacy and/or nociceptive stimulus intensity. However, its marginal effects in other assays of antinociception would argue against it [44]. The inability of BW 373U86 to potentiate the effects of naltrexone is also in keeping with a study demonstrating that naloxone and naltrindole failed to precipitate a withdrawal syndrome in rats continuously infused (2 mg/kg/hr) for six days with the compound [28].

The existence of multiple delta-opioid receptor subtypes has been suggested [23,36,37]. Perhaps BW 373U86 is acting on a particular subtype, as has been proposed [9]. It is also possible BW 373U86 is very short acting and the development of dependence may not occur. In support of this idea, the results of a study in monkeys [13] demonstrated that the effects of BW 373U86 on shock titration were short-lived, lasting no longer than 15 min. However, the study by Chang and colleagues [9] demonstrated BW 373U86-induced locomotion at one hour post-administration. Additionally, the lack of effects following chronic administration of BW 373U86 [28] would argue against it. At present these data would support our earlier postulation that the role of delta-opioid receptors in acute opioid dependence may only be minor.

A separate goal of this study was to gain insight into the temporal relationship between kappa- and delta-agonist-induced sensitization to the effects of naltrexone and antinociception. Previously we showed that sensitization to naltrexone lasted at least up to 6 hr after administration of mu-selective agonists [42]. This was well after drug-induced antinociception had waned and suggested that the changes associated with acute opioid dependence persist well after peak tissue drug levels have dissipated. In the current study, we found that drug-induced antinociception by systemically administered agonists was virtually absent in the hot-plate test but only largely reduced in the tail-flick test by 4 hr post-administration. Therefore the possibility of direct agonist-antagonist interactions can not be ruled out as an explanation for kappa-opioid agonist-induced changes in sensitivity to naltrexone. Conversely, antinociception produced by centrally administered DAMGO, DPDPE and morphine (1.0 µg) completely abated completely by 3 hr post-administration- a full hour prior to testing for drinking suppression by naltrexone. These results are in keeping with our previous report [42] and are particularly relevant given that acute opioid dependence is centrally mediated [3].

It is likely that activation of kappa- and delta-opioid receptor systems elicit phenomena that are pharmacologically related but qualitatively distinct from that produced following acutely administered mu-opioid agonists. There is precedence for such a postulation. For example, Martin and colleagues [33] demonstrated different “syndromes,” or constellations of physiological responses, produced by morphine (e.g. miosis, bradycardia, hypothermia, antinociception, etc.), the kappa-opioid receptor agonist ketocyclazocine (e.g. pupil constriction, flexor reflex suppression), and the delta-opioid receptor SKF-10,047 (e.g. mydriasis, tachypnea, tachycardia) in the spinal dog preparation. Further, from that study ketocyclazocine was unable to suppress abstinence-induced withdrawal in morphine-dependent dogs. In squirrel monkeys trained to discriminate 1.7 mg/kg morphine followed by 0.1 mg/kg naltrexone versus saline followed by naltrexone, the combination 0.3 mg/kg U69,593 followed by naltrexone failed to substitute for the morphine-naltrexone training regimen [43]. Moreover in rhesus monkeys, tolerance to response rate for food reinforcement seen with delta-opioid agonist SNC80 following acute and chronic administration did not confer cross-tolerance to morphine or U-50,488 [5].

In closing, these data suggest that single injections of kappa- and delta-opioid agonists influence the effects of opioid antagonists on unconditioned behaviors similar to mu-opioids agonists, only to a much lesser degree. The exact nature of the roles these receptor systems play in acute opioid dependence remains ambiguous and clearly requires further investigation.

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Fig. 1.

Morphine potentiates naltrexone-induced drinking suppression. Rats were deprived of water for 20 hr and administered morphine (MOR) or saline (SAL). After 3.75 hr, animals received a single dose of naltrexone or saline were tested 15 min later. Drinking was measured during a 30 min test period. Data are expressed as a percentage of within-subject saline control. Values are mean $\pm$ SEM (N=7–22). *Significantly different from SAL pretreatment group, mixed-two factor ANOVA, Newman-Keuls Test *post hoc*, $p < 0.05$.

Fig. 2. Centrally administered morphine and DAMGO potentiate naltrexone-induced drinking suppression. A, morphine (MOR) pretreatment and B, [D-Ala²,NMePhe⁴,Gly-ol⁵]enkephalin (DAMGO) pretreatment. Rats were deprived of water for 20 hr and administered an MOR or DAMGO (i.c.) or saline (s.c.). After 3.75 hr, animals received a single dose of naltrexone (s.c.) or saline were tested 15 min later. Drinking was measured during a 30 min test period. The SAL curve from Fig. 1 is reproduced in each panel. Values are mean $\pm$ SEM (SAL N=22; agonists N= 7–8). Data are expressed as a percentage of within-subject saline control. *Significantly different from SAL pretreatment group, mixed-two factor ANOVA, Newman-Keuls Test *post hoc*, $p < 0.05$.

Fig. 3.

Kappa-opioid agonists potentiate naltrexone-induced drinking suppression. A, spiradoline (SPIR) pretreatment; B, bremazocine (BRE) pretreatment; C, U-69,593 (U69) pretreatment. Rats were deprived of water for 20 hr and administered an opioid agonist or saline. After 3.75 hr, animals received a single dose of naltrexone or saline were tested 15 min later. Drinking was measured during a 30 min test period. The SAL curve from Fig. 1 is reproduced in each panel. Data are expressed as a percentage of within-subject saline control. Values are mean $\pm$ SEM (SAL N=22; agonists N= 7–9). *Significantly different from SAL pretreatment group, mixed-two factor ANOVA, Newman-Keuls Test *post hoc*, $p < 0.05$.

Fig. 4. DPDPE, but not BW 373U86, potentiates naltrexone-induced drinking suppression. A, BW 373U86 pretreatment and B, [D-Pen²,D-Pen⁵]enkephalin (DPDPE) pretreatment. Rats were deprived of water for 20 hr and administered BW 373U86 (BW; s.c.), DPDPE (i.c.), or saline (SAL; s.c.). After 3.75 hr, animals received a single dose of naltrexone or saline were tested 15 min later. Drinking was measured during a 30 min test period. The SAL curve is reproduced from Fig. 1. Data are expressed as a percentage of within-subject saline control. Values are mean $\pm$ SEM (SAL N=22; N=7-9). *Significantly different from SAL pretreatment group, mixed-two factor ANOVA, Newman-Keuls Test *post hoc*, $p < 0.05$.

Fig. 5.

The effects of systemically and centrally administered opioid agonists on tail-flick and hot plate latency. Rats were given (s.c. or i.c.) single doses of morphine (MOR), spiradoline (SPIR), bremazocine (BRE), BW 373U86 (BW), [D-Ala²,NMePhe⁴,Gly-ol⁵]enkephalin (DAMGO), or [D-Pen²,D-Pen⁵]enkephalin (DPDPE). Tail-flick (A,B) and hot plate (C, D) latencies following systemically administered drugs were measured up to 360 min following drug administration (A, C), while latencies following centrally administered drugs were measured up to 240 min (B, D). Values are expressed as % MPE. Percent MPE values are mean $\pm$ SEM (N= 8–9). *indicates a significant difference from the within group baseline latency, $p < 0.05$.

Table 1

Basal water consumption 4 hr after saline or agonist administration

Drug	Dose (mg/kg)	Water consumed (mL/mg)
SAL	NA	59 ± 2.4
BRE	0.1 mg/kg	69 ± 3.7 [*]
	0.3 mg/kg	52 ± 3.1
BW	0.79 mg/kg	49 ± 1.9 [*]
	2.38 mg/kg	45 ± 1.6 [*]
	7.92 mg/kg	47 ± 1.8 [*]
DAMGO	0.1 µg	51 ± 4.1
DPDPE	100 µg	48 ± 2.3 [#]
MOR	1.0 µg	49 ± 3.3 [*]
	10 µg	46 ± 3.8 [*]
	3.0 mg/kg	51 ± 3.5
	10 mg/kg	40 ± 2.6 [*]
SPIR	1.0 mg/kg	60 ± 2.5
	3.0 mg/kg	63 ± 3.6
U69	0.3 mg/kg	54 ± 1.8
	3.0 mg/kg	57 ± 1.0

^{*} Significantly different from saline pretreatment group; ANOVA, Newman-Keuls test, $p < 0.05$.

[#] Significantly different from saline pretreatment group; Student's t-Test, $p < 0.05$. Values are mean ± SEM (SAL N=13; 3.0 MOR N=17; Others N=7–9).

Table 2

Agonist pretreatment decreases the ED₅₀ of NTX.

Drug	Dose	Route	N	ED ₅₀ (95% C.I.)	Fold Change
SAL	NA	s.c.	21	1.27 (0.83, 1.95)	-
BRE	0.1 mg/kg 0.3 mg/kg	s.c.	7 9	0.73 (0.48, 1.20) 0.63 (0.18, 2.21)	1.7 2.0
BW	0.79 mg/kg 2.38 mg/kg 7.92 mg/kg	s.c.	6 7 6	1.06 (0.49, 2.26) 2.50 (0.90, 6.99) 4.07 (0.29, 56)	1.2 0.5 0.3
DAMGO	0.1 µg	i.c.	6	0.37 (0.13, 1.093) *	3.4
DPDPE	100 µg	i.c.	6	0.42 (0.07, 2.31)	3.0
MOR	1.0 µg 10 µg 3.0 mg/kg 10 mg/kg	i.c. s.c.	6 3 11 6	0.17 (0.026, 1.11) * - 0.39 (0.23, 0.64) * 0.02 (0.001, 0.40) *	7.4 - 3.2 85
SPIR	1.0 mg/kg 3.0 mg/kg	s.c.	7 7	0.73 (0.073, 7.32) 0.37 (0.088, 1.58)	1.7 3.4
U69	0.3 mg/kg 3.0 mg/kg	s.c.	7 9	1.093 (0.80, 1.49) 0.59 (0.252, 1.35)	1.1 2.1

* Significantly different from ED₅₀ of saline pretreatment group; ANOVA, Newman Keuls test, $p < 0.05$.