Acute Opioid Pretreatment Potentiates Naltrexone-Induced Drinking Suppression in Water-Deprived Rats

DAVID A. WHITE and STEPHEN G. HOLTZMAN

Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia

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ABSTRACT

Pretreatment with morphine-like agonists potentiates the behavioral effects of opioid antagonists, possibly reflecting a state of acute physical dependence. Several studies have used operant behavior to quantify these effects. However, little research has been done using unconditioned behavior. One objective of this study was to determine whether opioid agonist pretreatment (e.g., morphine, fentanyl, and meperidine) potentiated naltrexone-induced suppression of water consumption following deprivation. Another objective was to determine whether the agonist pretreatment interval was functionally related to efficacy for the manifestation of acute dependence. Finally, we compared temporally the effects of the three agonists. Adult male Sprague-Dawley rats were water deprived for 18, 20, or 22 h and given an injection (s.c.) of an agonist or saline. After 1.75, 3.75, or 5.75 h, animals received a single dose (s.c.) of naltrexone (0.01–30 mg/kg) or saline. Fifteen minutes later, subjects had access to water for 30 min. A time course of antinociception was constructed after agonist administration, using the tail-flick procedure. All three agonists dose dependently potentiated naltrexone-induced drinking suppression, decreasing the ED50 of naltrexone by as much as 150-fold. There was no clear relationship between agonist efficacy and pretreatment interval. Sensitization to naltrexone was seen up to 6 h after agonist administration, occurring in the apparent absence of an antinociceptive effect. These data extend the range of behavioral effects of opioid antagonists potentiated by opioid agonist pretreatment to suppression of drinking and show that such potentiation can occur in the absence of a prototypical agonist effect.

Low doses of opioid antagonists, such as naloxone and naltrexone, have relatively few physiological or behavioral effects in otherwise drug-free subjects (Martin, 1967; Blumberg and Dayton, 1973). However, in subjects physically dependent on morphine or other mu-opioid agonists following chronic treatment, the picture is quite different. Antagonist administration precipitates a number of prominent physiological and behavioral effects associated with withdrawal. In rats, these include loss of body weight, diarrhea, jumping, and “wet dog” shakes (Way et al., 1969; Cicero and Meyer, 1973; Wei, 1973). Additionally, chronic morphine treatment sensitizes subjects to pre-existing effects of antagonists. In pigeons trained to discriminate naltrexone versus saline, chronic daily morphine treatment increased the potencies of naltrexone and naloxone by greater than 100-fold for producing naltrexone-appropriate responding and other effects (e.g., suppression of the response rate) (Valentino et al., 1983). Consequently, sensitization to opioid antagonists is often used as a measure of physical dependence upon opioids.

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 (Kosersky et al., 1974; Eisenberg, 1982b; Krystal and Redmond, 1983; Ramabadran, 1983; Schnur, 1991). One such similarity is sensitization to the behavioral effects of opioid antagonists. Several studies have used operant behavior to quantify the effects of acute dependence. For example, in rats responding for food or brain stimulation, 4-h pretreatment with a single dose of morphine increased the potency of naloxone and naltrexone for suppressing responding by 2 orders of magnitude (Young, 1986; Adams and Holtzman, 1990a; Easterling and Holtzman, 1997).

Despite such studies, little research has been done on the effects of acute agonist pretreatment on antagonist-induced changes of unconditioned behaviors. One well documented effect of opioid antagonists on an unconditioned behavior is suppression of deprivation-induced drinking (Brown and Holtzman, 1979; Brown and Holtzman, 1981b). Therefore, the first objective of this study was to determine whether acute pretreatment with the mu-opioid agonists morphine, fentanyl, and meperidine would enhance naltrexone-induced drinking suppression.
In drug discrimination, a finite period of time is required for the development of maximally discriminable effects. For example, in rats trained to discriminate acutely administered morphine followed by naltrexone 3.75 h later versus saline followed by naltrexone, full morphine-naltrexone appropriate responding only occurred when morphine was given at least 3 h before naltrexone (Easterling and Holtzman, 1999). Results from the first part of our study confirmed that naltrexone dose dependently suppressed drinking and demonstrated that 4-h agonist pretreatment potentiated this effect dose dependently. Therefore, the second objective of this study was to determine whether there was a functional relationship between intrinsic efficacy and agonist pretreatment interval (e.g., 2, 4, or 6 h) on naltrexone-induced drinking suppression. We hypothesized that there would be an inverse relationship between the intrinsic efficacy and the pretreatment interval required to elicit maximal potentiation of naltrexone-induced drinking suppression. Morphine (intermediate efficacy), fentanyl (relatively high efficacy), and meperidine (relatively low efficacy) (Paronis and Holtzman, 1992) were administered either 2 or 6 h before naltrexone.

Results from the intrinsic efficacy studies showed that the peak effects of morphine, fentanyl, and meperidine occurred at a time when agonist activity should be declining based on the half-lives ($t_{1/2}$) of the three drugs (e.g., morphine $t_{1/2}$ in rat = 0.9 ± 0.2 h) (Dahlstrom et al., 1979; Hug and Murphy, 1981; Barjavel et al., 1995). No explicit comparisons of time courses for agonist activity and agonist-induced sensitization to naltrexone have been reported. Therefore, the third objective of this study was to compare the temporal relationship of agonist-induced sensitization to naltrexone with the prototypical agonist effect of antinociception in tail-flick procedure.

Materials and Methods

Animals. The subjects were adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Raleigh, NC), weighing between 200 and 400 g at the start of the study. For the drinking experiments, animals were individually housed in polycarbonate cages and maintained in ventilated cabinets located in the laboratory. For the analgesia time course experiment, animals were housed two to three per cage and maintained in the Emory School of Medicine Division of Animal Resources Care Facility. All animals were maintained on a 12-h light:dark cycle and were provided with food ad libitum. Subjects also had free access to water, except during testing. Animals were maintained according to the “Guide for the Care and Use of Laboratory Animals” (National Academy of Sciences, 1996), and all procedures were approved by the Institutional Animal Care and Use Committee.

Measurement of Water Intake. Subjects were deprived of water for 24 h before testing. On the day of testing, animals were weighed and administered saline (0.9% NaCl) or an opioid agonist (morphine (1.0–10 mg/kg), fentanyl (0.056 or 0.1 mg/kg) or meperidine (10–30 mg/kg)) 2, 4, or 6 h before measurement of water intake. Naltrexone (0.01–30 mg/kg) or saline was then given 15 min before testing. Water intake was measured for 30 min, using 100-ml calibrated water bottles (Wahmann Laboratories, Timonium, MD). Food was available during the testing period. At the end of the test, water intake was determined to the nearest milliliter. Rats were given free access to water for at least 48 h after testing. All experiments were conducted between 1200 and 1850 h, two to three times per week. In generating dose-response curves, each animal was tested with saline and all doses of naltrexone but was given only one pretreatment dose of an agonist or saline. Therefore, each animal was tested six to seven times. Prior to the start of drug testing, each subject underwent two habituation sessions in which it received injections of saline.

Analgesia Time Course Assay. A time course of the effects of a single injection of morphine (3.0 mg/kg), fentanyl (0.056 mg/kg), or meperidine (17.8 mg/kg) on antinociception in rats was constructed, using a modified version (Gellert and Holtzman, 1978) of the radiant heat tail-flick procedure (D’Amour and Smith, 1941) and a model 33 Tail Flick Analgesia Meter (ITC Inc., Life Science Instruments, Woodland Hills, CA). At the beginning of the test, radiant heat from a 20-V bulb was focused on the lower third of the rat’s tail. An automatic timer was also activated. Tail movement activated a photo-cell, which subsequently turned off the light source and the timer. The light intensity was adjusted so that the baseline latencies ranged from 2.0 to 3.0 s, and an 8.5-s maximum cutoff time was used to minimize tissue damage. Tail-flick was recorded to the nearest tenth of a second. Each rat underwent two predrug trials conducted approximately 30 min apart. The mean of these trials served as the baseline measure for that subject. Tail-flick latencies were recorded at 30, 60, 90, 120, 240, and 360 min after drug administration. Each animal was used once.

Drugs. Morphine sulfate (Penick Co., Nutley, NJ), naltrexone hydrochloride (Sigma Chemical Co., St. Louis, MO), fentanyl citrate (McNeil Laboratories, Fort Washington, PA), and meperidine hydrochloride (National Institute on Drug Abuse, Bethesda, MD) were prepared in 0.9% saline. All drugs were injected s.c. in a volume of 1.0 or 2.0 (meperidine) ml/kg of body weight. Meperidine was also administered at multiple injection sites to minimize tissue irritation. All drug doses are expressed as the free base.

Data Analysis. Water intake data were converted to milliliters of water consumed per kilogram of body weight. The converted data were normalized to percentage of drug/saline or saline/saline control to facilitate comparisons among the different experimental groups. The amount of water consumed following drug/saline or saline/saline was defined as 100%. Results are expressed as mean ± S.E.M. The transformed data were analyzed by a two-factor analysis of variance (ANOVA; naltrexone dose × agonist pretreatment dose or naltrexone dose × agonist pretreatment time), with repeated measures for agonist pretreatment dose and agonist pretreatment time. Newman-Keuls post hoc comparison was used to determine significant differences among means (GB-STAT v6.0, Dynamic Microsystems, Inc., Silver Spring, MD). Naltrexone ED$_{50}$ values were calculated for each subject by linear regression. From this, means and 95% confidence intervals were derived for each pretreatment group. ED$_{50}$ data from pretreatment groups having two or more doses were analyzed using a one-factor ANOVA and Newman-Keuls test post hoc to determine significant differences. ED$_{50}$ data from pretreatment groups having only one dose were compared with the ED$_{50}$ from the saline pretreatment group using the Student’s $t$ test. $p$ values less than 0.05 were accepted as statistically significant.

TABLE 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/kg</th>
<th>2-h Pre-tx ml/kg</th>
<th>4-h Pre-tx ml/kg</th>
<th>6-h Pre-tx ml/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>N.A.</td>
<td>53.0 ± 2.4*</td>
<td>43.6 ± 3.5</td>
<td>36.4 ± 3.7*</td>
</tr>
<tr>
<td>Morphine</td>
<td>1.0</td>
<td>58.9 ± 4.5</td>
<td>47.3 ± 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>45.5 ± 3.5</td>
<td>49.7 ± 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>53.4 ± 6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.056</td>
<td>51.1 ± 4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>38.7 ± 3.4*</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>43.6 ± 3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meperidine</td>
<td>17.8</td>
<td>43.3 ± 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>47.3 ± 3.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.A., not applicable.

* Significantly different from combined saline pretreatment group, $p < 0.05$.

* Combination of 2- and 4-h saline pretreatment groups, which were not significantly different from each other ($t = 0.342, p = 0.7091$).

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Fig. 1. Potentiation of naltrexone-induced drinking suppression by pretreatment with morphine, fentanyl, or meperidine 4 h before testing. A, morphine (MOR) pretreatment. B, fentanyl (FEN) pretreatment. C, meperidine (MEP) pretreatment. Rats were deprived of water for 20 h and administered (s.c.) an opioid agonist or saline. After 3.75 h, animals received a single dose of naltrexone (s.c.) or saline and were tested 15 min later. Drinking was measured during a 30-min test period. Values are mean ± S.E.M. (n = 7–14). The curve for naltrexone after saline pretreatment is a combination of 2- and 4-h pretreatment groups, which were not significantly different from each other. The same saline-naltrexone curve appears in each panel. *Significantly different from within group saline control, p < 0.05. #Significantly different from the saline pretreatment group, p < 0.05.
Tail-flick latencies are expressed as the percentage maximum possible effect [% MPE; (Dewey and Harris, 1975)].

% MPE = \frac{(\text{test latency} - \text{baseline latency})}{(\text{cut-off time} (8.5 s) - \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. The % MPE data were used to calculate the cumulative area under the curve (AUC) for each drug at 2, 4, and 6 h. The AUC data were analyzed using a mixed two-factor ANOVA (drug dose \times time-repeated measure) followed by the Newman-Keuls test post hoc. The alpha level chosen for both analyses was 0.05.

Results

Saline Pretreatment. In separate experiments, subjects drank an average of 52.1 ± 3.0 ml/kg 2 h after saline pre-treatment and 53.8 ± 3.8 ml/kg 4 h after saline pretreatment. Because there was no difference between the means of the two groups, the groups were combined for subsequent analyses (Table 1). Naltrexone dose dependently decreased drinking following both 2- and 4-h saline pretreatment (F4,38 = 9.67 and F4,39 = 12.10, respectively). A comparison of the ED50 values of the two groups [2-h = 11.06 (0.2, 25.9) mg/kg; 4-h = 6.79 (2.3, 11.3) mg/kg] showed no differences in naltrexone-induced suppression of drinking at either interval after saline pretreatment; t = 0.246, p = 0.8274. Therefore, the two groups were combined for subsequent analyses. A one-way ANOVA of the combined data revealed a dose-dependent effect of naltrexone (F4,78 = 22.93), with 30 mg/kg naltrexone suppressing drinking by more than 50% (Fig. 1).

Four-Hour Agonist Pretreatment. There were no effects on the volume of water consumed following 4-h pretreatment with any dose of morphine alone (Table 1; F3,38 = 0.6121). Morphine dose dependently potentiated naltrexone-induced drinking suppression (Fig. 1A; F3,163 = 29.36), significantly reducing the ED50 of naltrexone by as much as 100-fold (Table 2; F2,35 = 3.16). In contrast, 4-h pretreatment with the highest dose of fentanyl (0.1 mg/kg) or meperidine (30 mg/kg) significantly reduced basal drinking in test subjects (Table 1; F2,29 = 4.44 and F3,39 = 4.56, respectively). Nevertheless, both fentanyl (F2,98 = 39.06) and meperidine (F3,122 = 15.98) dose dependently potentiated naltrexone-induced suppression of drinking, causing leftward shifts of the naltrexone dose-response curve (Fig. 1, B and C). Both 0.056 and 0.1 mg/kg fentanyl significantly decreased the ED50 of naltrexone by greater than 100-fold (Table 2; F2,29 = 7.59), while pretreatment with meperidine (17.8 and 30 mg/kg) significantly decreased it by up to 94-fold (Table 2; F3,37 = 4.61). Data analyses also revealed significant interactions between fentanyl and naltrexone dose (F4,98 = 6.39) and meperidine and naltrexone dose (F4,122 = 4.53).

From the 2-, 4-, and 6-h Agonist Pretreatment Studies. As shown in Fig. 2A, potentiation of naltrexone-induced drinking suppression following 3.0 mg/kg morphine was dependent on the pretreatment time (F3,191 = 28.05). Two-hour morphine pretreatment decreased the ED50 of naltrexone by 170-fold. This was more than 5 times greater than the effects of morphine pretreatment either at 4 or 6 h before testing (Tables 2 and 3). Analysis of the data also revealed a significant effect of naltrexone dose (F2,191 = 190.14) and a significant interaction between morphine pretreatment time and naltrexone dose (F6,191 = 9.17). Similar to the experiments with morphine, experiments with fentanyl also showed pretreatment time-dependent effects (Fig. 2B; F3,119 = 16.32), with the potency of naltrexone being highest at 2 h and lowest at 6 h (Tables 2 and 3). Again, there was a significant effect of naltrexone dose (F2,119 = 51.23) and a significant interaction between fentanyl pretreatment time and naltrexone dose (F6,119 = 3.02). Finally, meperidine potentiated naltrexone-induced drinking suppression at all three pretreatment intervals (Fig. 2C; F3,191 = 22.84). However, unlike morphine and fentanyl, meperidine was nearly equally effective at all three pretreatment intervals (Fig. 2C; Tables 2 and 3).

A comparison of the ED50 values for naltrexone following 2-h pretreatment with morphine, fentanyl, and meperidine revealed no significant differences (F2,19 = 0.23), despite a trend toward a higher ED50 after meperidine pretreatment. Analysis of the ED50 values for naltrexone following 6-h pretreatment with all three agonists also revealed no significant differences (F2,19 = 0.16).

From the Tail-Flick Analgesia Time Course Assay. Morphine, fentanyl, and meperidine all significantly increased the % MPE over baseline values within 30 min of administration (Fig. 3). There was also a significant effect of time on the antinociceptive effects of morphine, fentanyl, and meperidine (F5,62 = 61.92; F2,119 = 51.23; and F2,119 = 51.23, respectively) as tail-flick latencies returned to baseline levels by 4 h after administration. Analysis of the AUC for morphine, fentanyl, and meperidine revealed a time-dependent effect (F2,74 = 42.45). Additionally, the overall antinociceptive effects of both fentanyl and meperidine were significantly less than that of morphine (Fig. 4).

Discussion

Naltrexone dose dependently suppressed drinking in water-deprived rats, consistent with previous studies (Brown et al., 1980; Brown and Holtzman, 1980, 1981a). Similar to the outcome of studies in which operant responding served as a baseline (see Introduction) (Young, 1986; Adams and Holtzman, 1990a; Easterling and Holtzman, 1997), acute pretreatment with morphine, fentanyl, and meperidine dose and time dependently potentiated the effects of naltrexone on drinking. The magnitude of the agonist-induced potentiation was comparable with that observed in studies of both food- and intracranial self-stimulation-maintained behavior (Young,
Fig. 2. The potentiation of naltrexone-induced suppression of drinking by opioid agonists following pretreatment intervals of 2, 4, and 6 h. A, 3.0-mg/kg MOR pretreatment. B, 0.056-mg/kg FEN pretreatment. C, 17.8-mg/kg MEP pretreatment. Rats were deprived of water for 18, 20, or 22 h and administered (s.c.) an opioid agonist or saline. After 1.75, 3.75, or 5.75 h, animals received a single dose of naltrexone (s.c.) or saline and were tested 15 min later. Drinking was measured during a 30-min test period. Values are mean ± S.E.M. (n = 7–14). The same curve for naltrexone after saline pretreatment appears in each panel. The 4-h pretreatment curves for morphine, fentanyl, and meperidine are reproduced from Fig. 1. *Significantly different from the saline pretreatment group, p < 0.05. The saline and 4-h data are taken from the experiment shown in Fig. 1.
There are no obvious differences in the pretreatment interval needed to potentiate naltrexone following pretreatment with morphine, fentanyl, or meperidine, three agonists with intrinsic efficacies that differ significantly from one another. The effects of naloxone-induced withdrawal from acute dependence have been reported as early as 30 min following morphine administration in rats (Eisenberg, 1982a) and 45 min in humans (Heishman et al., 1989). Perhaps there would have been a difference at pretreatment intervals shorter than 2 h. However, the direct behavior-suppressing effects of the agonists precluded examining shorter intervals. Intrinsic efficacy is a determinant of the magnitude of tolerance development to the antinociceptive effects of morphine-like drugs (Paronis and Holtzman, 1991). However, to our knowledge, no studies have explicitly compared the rate and magnitude of physical dependence development with equi-effective doses of agonists as a function of intrinsic efficacy. Our results suggest that efficacy is not a determinant of the rate at which a single dose of an agonist induces sensitization to an antagonist.

As shown in Fig. 2, the greatest potentiation of naltrexone-induced drinking suppression occurred at the shortest pretreatment interval, and the magnitude of potentiation declined thereafter. These results differ from a drug discrimination study, wherein rats were trained to discriminate between the combinations of acutely administered morphine followed by naltrexone (3.75 h later) and saline followed by naltrexone (Easterling and Holtzman, 1999). Maximal discriminative effects occurred at 3 to 4 h. Stimulus control was lower at 2 h and was almost nonexistent at 0.5 h. In this study, naltrexone-induced drinking suppression was equally potentiated by pretreatment with all three agonists (Table 3). This raises the question of whether the same phenomenon is being addressed in both studies. Potentiation of the effects of naltrexone with agonist pretreatment may reflect sensitization, which implies a pre-existing effect to which animals become more sensitive. Such a hypothesis is supported by the work of Adams and Holtzman (1990a, 1991), wherein naltrexone dose dependently decreased response rates for food reinforcement in rats. This effect was potentiated from severalfold to several orders of magnitude by a 4-h pretreatment with several systemically and centrally administered mu-selective agonists, including morphine, fentanyl, and \([\text{D-Ala}^2,\text{NMePhe}^4,\text{Gly-ol}^5]\) enkephalin.

It is also possible that agonist-induced potentiation of the

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**Table 3**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Pre-tx Time</th>
<th>Dose mg/kg</th>
<th>n</th>
<th>ED50 mg/kg</th>
<th>Fold Change</th>
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<tbody>
<tr>
<td>Saline</td>
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<td>14</td>
<td>8.85 (4.7, 16.8)*</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.0</td>
<td>7</td>
<td>0.052 (0.01, 0.2)*</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.0</td>
<td>6</td>
<td>0.29 (0.1, 0.8)*</td>
<td>30.5</td>
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<tr>
<td></td>
<td>2</td>
<td>0.056</td>
<td>6</td>
<td>0.072 (0.02, 0.2)*</td>
<td>123</td>
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<tr>
<td>Fentanyl</td>
<td>4</td>
<td>0.056</td>
<td>8</td>
<td>0.086 (0.05, 0.2)*</td>
<td>103</td>
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<tr>
<td></td>
<td>6</td>
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<td>7</td>
<td>0.34 (0.1, 1.1)*</td>
<td>26.0</td>
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<tr>
<td></td>
<td>2</td>
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<td>7</td>
<td>0.11 (0.07, 0.2)*</td>
<td>80.5</td>
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<tr>
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<td>4</td>
<td>17.8</td>
<td>8</td>
<td>0.16 (0.06, 0.4)*</td>
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<td>6</td>
<td>17.8</td>
<td>6</td>
<td>0.23 (0.03, 1.6)*</td>
<td>38.5</td>
</tr>
</tbody>
</table>

* N.A., not applicable.
* Significantly different from ED50 of combined saline pretreatment group, p < 0.05.
* Combination of 2- and 4-h saline pretreatment groups, which were not significantly different (t(6,8) = 0.246, p = 0.8274).

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**Fig. 3.** The effects of morphine, fentanyl, and meperidine on tail-flick latency. Rats were given a single dose of 3.0 mg/kg MOR, 0.056 mg/kg FEN, and 17.8 mg/kg MEP. Tail-flick latencies were measured 30, 60, 90, 120, 240, and 360 min following drug administration. Values are expressed as % MPE. Percent MPE values are mean ± S.E.M. (n = 8–9). *Significant difference from the within-group baseline latency, p < 0.05.
Fig. 4. The analgesic effects of fentanyl, morphine, and meperidine expressed as the area under the curve. Rats were given a single dose of 3.0 mg/kg MOR, 0.056 mg/kg FEN, and 17.8 mg/kg MEP. Tail-flick latencies were measured 30, 60, 90, 120, 240, and 360 min following drug administration and are expressed as % MPE. Transformed data were then used to calculate the cumulative AUC (% MPE \times \text{min}) at 2, 4, and 6 h. AUC values are mean \pm S.E.M. (n = 8–9). *Significant difference from morphine group, p < 0.05.
effects of naltrexone reflects a different state that occurs with a combination of the two compounds that does not occur with the antagonist alone. This would be consistent with discrimination studies. Saline-naltrexone did not substitute for morphine-naltrexone, even at a dose of naltrexone 2000-fold higher than the naltrexone ED<sub>50</sub> after pretreatment with morphine. This also would be consistent with results from clinical studies (Bickel et al., 1988) and with antagonist-induced jumping in mice (Ramabhadran, 1983). Based on the findings presented here, we cannot clearly determine which is the case. Nevertheless, the results are consistent with the behavioral effects associated with withdrawal, implying a state of acute physical dependence.

Differences across studies might well be due to differences in dependent measures. Different signs of withdrawal can emerge, depending upon magnitude of dependence (Cicero and Meyer, 1973; Blasig et al., 1973). It is possible that the rate and/or magnitude of acute dependence development differs across physiological systems, which could be reflected in differing time courses for different measures of withdrawal. In mice, antagonist-induced jumping, a reliable withdrawal sign in that species, is maximum 4 h after an acute dose of morphine and occurs to a much lesser extent at shorter intervals (Wang et al., 1994). On the other hand, antagonist-induced increases in plasma corticosterone in rats occur at relatively short intervals after a morphine-like agonist (Eisenberg, 1982a).

Potentiation of naltrexone was still apparent at time points when the agonist action had dissipated: at 6 h for all drugs and at 4 h for fentanyl. At the 4- and 6-h intervals, meperidine and fentanyl induced potentiation that was comparable with that induced by morphine, despite having total analgesic effects that were significantly less than the effect of morphine. Analgesia is only one of the many agonist effects of these drugs. Different relationships with sensitization might have been observed had other agonist effects been studied. During chronic morphine administration, development of tolerance to the analgesic effect and development of sensitization to antagonists have a similar time course (Cicero and Meyer, 1973; Adams and Holtzman, 1990b), suggesting a common underlying mechanism.

In the discrimination and mouse-jumping studies, peak sensitization to antagonists occurred at 4 h, when morphine tissue levels were declining, based upon the half-life of morphine in those species (e.g., morphine t<sub>1/2</sub> in rat = 0.9 ± 0.2 h) (Barjavel et al., 1995). In clinical studies, significant antagonist-induced withdrawal changes were observed 12 to 24 h after a single dose of morphine (Kirby et al., 1990), when morphine tissue levels must have been negligible, based upon the half-life of morphine in humans (t<sub>1/2</sub> = 3–4 h) (Berkowitz, 1976). The results suggest that agonist occupancy of mu-opioid receptors results in changes that persist (and in some cases first become manifest) long after the peak of agonist tissue levels. These changes must involve the opioid receptor because that is where antagonists act. One proposed explanation is that the agonist causes the receptor to become constitutively active (Wang et al., 1994). Another possibility is that morphine causes the release of endogenous ligands that continue to occupy the receptor in the absence of morphine. It would be interesting to determine whether these “changes” that occur during acute dependence are the same as those that underlie the state of chronic dependence.

In summary, these data extend the range of behavioral effects of opioid antagonists enhanced by opioid agonist pretreatment to suppression of drinking. The lack of an obvious relationship between agonist intrinsic efficacy and the pretreatment interval needed for potentiating the effects of naltrexone suggests that efficacy is not a determinant of the rate at which an agonist induces sensitization to an antagonist. The data also suggest that acute dependence upon morphine-like opioids is mediated by mechanisms that are temporally distinct from those mediating analgesia.

Acknowledgments

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Address correspondence to: David A. White, Ph.D., Dept. of Pharmacology, Emory University School of Medicine, O. Wayne Rollins Research Building, 1510 Clifton Rd. NE, Atlanta, GA 30322. E-mail: dwhite4@emory.edu