Antagonism of the Antinociceptive and Discriminative Stimulus Effects of Heroin and Morphine by 3-Methoxynaltrexone and Naltrexone in Rhesus Monkeys

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ABSTRACT

It has been suggested that heroin and morphine may act on different opioid receptor populations in rodents. In support of this hypothesis, the opioid antagonist 3-methoxynaltrexone was reported to be more potent as an antagonist of the antinociceptive effects of heroin than of morphine in mice and rats. To assess the generality of this finding across species and experimental endpoints, the present study compared the potentials of naltrexone and 3-methoxynaltrexone as antagonists of heroin and morphine in two behavioral assays in rhesus monkeys. In the thermal nociception study, tail-withdrawal latencies were measured from water heated to 50°C. In the heroin discrimination study, monkeys were trained to discriminate 0.1 mg/kg heroin from saline in a two-key, food-reinforced drug discrimination procedure, and percentage of heroin-appropriate responding and response rates were measured. Both heroin and morphine produced dose-dependent antinociception, increases in percentage of heroin-appropriate responding, and decreases in response rates. Heroin was approximately 20-fold more potent than morphine. Naltrexone (0.032–0.1 mg/kg) was equipotent in antagonizing all effects of heroin and morphine (pA2 values = 7.90–8.22). 3-Methoxynaltrexone (0.1–3.2 mg/kg) was also equipotent in antagonizing the antinociceptive, discriminative stimulus, and rate-suppressant effects of heroin and morphine; however, 3-methoxynaltrexone was approximately 100-fold less potent than naltrexone (pA2/pKp values = 5.96–6.36). These results suggest that heroin and morphine act on pharmacologically similar populations of opioid receptors in rhesus monkeys, and also indicate that 3-methoxynaltrexone does not differentially antagonize the effects of heroin and morphine in rhesus monkeys.

Heroin (3,6-diacetylmorphine) is an opioid agonist that produces a morphine-like profile of physiological and behavioral effects, including analgesia and abuse-related effects (Gutstein and Akil, 2001). Although heroin itself has low affinity for opioid receptors, it is rapidly metabolized to 6-acetylmorphine and morphine, which have high affinity for μ-opioid receptors (Way et al., 1960; Inturrisi et al., 1983, 1984; Bertalmio et al., 1992). Morphine is further metabolized to morphine-6-glucuronide (M6G), which also has relatively high affinity for μ-opioid receptors (Pasternak et al., 1987; Abbott and Palmour, 1988; Paul et al., 1989). On the basis of these and related findings, it has been suggested that heroin may function as a highly lipophilic prodrug for the active metabolites 6-acetylmorphine, morphine, and M6G (Way et al., 1960; Inturrisi et al., 1983, 1984; Paul et al., 1989).

Although all these heroin metabolites have high affinity for μ-opioid receptors, accumulating evidence suggests that different receptor populations may mediate the antinociceptive effects of heroin, 6-acetylmorphine, and M6G compared with the effects of morphine in rodents. An early behavioral study that supported this position reported a lack of antinociceptive cross-tolerance to heroin in morphine-tolerant mice (Lange et al., 1980). This finding was confirmed and extended to include a lack of cross-tolerance to 6-acetylmorphine and M6G in morphine-tolerant mice (Rossi et al., 1996). Manipulation of the expression or structure of the MOR-1 gene, using antisense probes and knockout models, also resulted in differential blockade of the antinociceptive effects of heroin, 6-acetylmorphine, and M6G compared with morphine in rodents (Rossi et al., 1995a,b, 1996, 1997; Schuller et al., 1999). In an effort to identify the molecular mechanisms underlying these differences, it was found that [3H]M6G bound with higher affinity to a novel site than to the site labeled by μ-radioligands, such as [3H]morphine, and it was suggested that this novel site corresponded to a novel M6G opioid receptor (Brown et al., 1997). A role for these receptors in heroin antinociception was suggested by studies in exon-1 MOR-1 knockout mice because these mice retained high-
affinity \([^{3}H]M6G\) binding in brain and sensitivity to the antinociceptive effects of heroin but not of morphine (Schul-ler et al., 1999).

Studies with the opioid antagonist 3-methoxynaltrexone have provided additional evidence to suggest a role for M6G receptors in mediating the antinociceptive effects of heroin in rodents. 3-Methoxynaltrexone bound with higher affinity to the site labeled with \([^{3}H]M6G\) than to \([^{3}H]\)morphine-labeled sites in membranes from both mouse brain and Chinese hamster ovary cells transfected with the MOR-1 receptor, and these results suggested that 3-methoxynaltrexone may act as a relatively selective ligand for the M6G receptor (Brown et al., 1997a,b). In behavioral studies in CD-1 mice, 3-methoxynaltrexone was more potent as an antagonist of the antinociceptive effects of heroin and M6G than of morphine (Brown et al., 1997a; Rady et al., 2000). 3-Methoxy- naltrexone was also more potent in antagonizing the antino- ciceptive effects of heroin and 6-acetylmorphine compared with those of morphine in rats (Walker et al., 1999). Overall, these results have been interpreted to suggest that 1) a novel M6G opioid receptor may mediate, at least in part, the antinociceptive effect of heroin, 6-acetylmorphine, and M6G but not of morphine in rodents; and 2) 3-methoxynaltrexone may serve as a moderately selective antagonist of heroin, 6-acetyl- morphine, and M6G under some conditions. On the basis of these in vitro studies and behavioral studies of antinocicep- tion, it also has been suggested that the selectivity of 3-me- thoxynaltrexone may lead to new approaches to the treat- ment of opioid abuse (Brown et al., 1997a). However, it is of interest to note that 3-methoxynaltrexone was equipotent in altering the self-administration of heroin and morphine in rats (Walker et al., 1999).

The present study was designed to extend these previous findings in two ways. First, this study compared the ability of 3-methoxynaltrexone to antagonize the antinociceptive ef- fects of heroin and morphine in rhesus monkeys. Although opioid receptor populations in rodents and monkeys are sim- ilar, there are known species differences in both the relative proportions and distributions of \(\mu\)-, \(\kappa\)-, and \(\delta\)-opioid receptors (Mansour et al., 1988). Furthermore, it is not known whether primates express M6G receptors or whether these receptors play a role in mediating the antinociceptive effects of heroin. Second, this study compared the ability of 3-methoxynaltrexone to antagonize the discriminative stimulus and rate-sup- pressant effects of heroin and morphine in rhesus monkeys trained to discriminate heroin from saline. The discriminative stimulus effects of drugs in animals are believed to model the subjective effects of drugs in humans (Schuster and Johanson, 1987), and these effects may contribute to the abuse liability of drugs such as heroin. Thus, drug discrimi- nation procedures afford an opportunity to assess the ability of 3-methoxynaltrexone to antagonize an abuse-related effect of heroin and morphine. To provide a context for interpreting the effects of 3-methoxynaltrexone, naltrexone antagonism of heroin- and morphine-induced antinociception and discrimi- native stimulus effects was also examined.

Materials and Methods

Subjects

Six male rhesus monkeys (Macaca mulatta) weighed 8.0 to 14.0 kg and were maintained on a diet of biscuits (Lab Diet Jumbo Monkey Biscuits, PMI Feeds, Inc., St. Louis, MO), fresh fruit and vegetables, and multiple vitamins. Monkeys in the discrimination experiment could obtain up to 50 1-g banana pellets (Precision Primate Pellets Formula L/I Banana Flavor; P.J. Noyes Co., Lancaster, NH) during operant sessions. Water was available continuously. A 12-h light/ dark cycle was in effect with lights on at 7:00 AM.

Animal maintenance and research were conducted according to the guidelines provided by the National Institutes of Health Committee on Laboratory Animal Resources. The research facility was licensed by the United States Department of Agriculture. Research protocols were approved by the McLean Hospital Institutional Animal Care and Use Committee. A staff veterinarian monitored the health of the monkeys on a regular basis. Monkeys had visual, auditory, and olfactory contact with other monkeys throughout the study. Environmental enrichment was provided by toys, music, and nature videotapes. For monkeys in the discrimination experiment, the operant procedure provided additional opportunities for environ- mental manipulation.

Thermal Nociception

General Procedure. Three monkeys were used in the thermal nociception study, and all of the monkeys had prior experience in this procedure (Brandt et al., 2001). During experimental sessions, mon- keys were seated in standard primate chairs, and the lower 10 cm of the shaved tail of each monkey was immersed in water heated to 42 or 50°C. An Apple IIe microcomputer was used to measure and record the latency (in seconds) for monkeys to remove their tails from warm water. If a monkey failed to remove its tail within 20 s, the timer was stopped, the monkey’s tail was removed from the warm water, and a latency of 20 s was assigned to that measurement.

Test Procedure. Test sessions were conducted on Mondays and Thursdays, and they consisted of multiple 30-min cycles. After the monkeys were seated in chairs, the experimental session began with determination of baseline tail-withdrawal latencies from water heated to 42 or 50°C. Water heated to 42°C is an innocuous stimulus in this procedure (Negus et al., 1993b), and this stimulus was in- cluded herein only during baseline determinations to ensure that tail immersion alone did not elicit the tail-withdrawal response. Mon- keys in this study never withdrew their tails from 42°C water during these baseline determinations. For the remainder of each session, tail-withdrawal latencies were evaluated only from water heated to 50°C. After baseline measurements, the first cycle began with an intramuscular injection of vehicle (sterile water) or a dose of an antagonist. The remaining cycles began with i.m. administration of cumulative doses of heroin or morphine, and each dose increased the total cumulative dose by one-quarter or one-half log units. Tail- withdrawal latencies from 50°C water were determined beginning 25 min after each injection. Heroin or morphine was administered up to doses resulting in at least 50% of the maximum possible effect (see below) in each monkey. Heroin doses ranged from 0.01 to 10.0 mg/kg, and morphine doses ranged from 0.32 to 100.0 mg/kg. The drugs and doses tested in pretreatment experiments were naltrexone (0.01–0.1 mg/kg) and 3-methoxynaltrexone (0.1–3.2 mg/kg).

Tail-withdrawal latencies were converted to percentage of maxi- mum possible effect (%MPE) using the equation \(\%MPE = \frac{[[test\ latency - baseline\ latency]/20 - baseline\ latency] \times 100}{test\ latency - baseline\ latency}\), where test latency was the tail-withdrawal latency in seconds from water heated to 50°C during a test cycle, baseline latency was the baseline tail-withdrawal latency in seconds observed at the beginning of the test session, and 20 was the maximum number of seconds that could be assigned to any tail-withdrawal latency measurement. Mean val- ues for %MPE (± S.E.M.) were calculated and plotted as a function of drug dose.

Heroin Discrimination

Three monkeys were trained to discriminate 0.1 mg/kg heroin (i.m.) from saline (i.m.) in a two-key, food-reinforced drug discrimi-
nation procedure. All of the monkeys were trained previously to discriminate a cocaine/heroin mixture (i.e., “speedball”; 0.4 mg/kg cocaine + 0.04 mg/kg heroin i.m.; Negus et al., 1998a), and this stimulus maintained responding for approximately 2 to 3 years. Speedball discrimination training was stopped 2 weeks to 2 months before training of the heroin discrimination began.

**Apparatus.** The drug discrimination procedure used in the present study was similar to that used in a previous experiment (Negus et al., 1998a). Each monkey was housed in a ventilated, stainless steel cage (56 × 71 × 69 cm). The front wall of each cage was adapted to fit an operant panel (28 × 28 cm) that included three square translucent response keys (6.4 × 6.4 cm) arranged 2.54 cm apart horizontally and 3.2 cm from the top of the panel. Each response key could be transilluminated by red or green stimulus lights (Superbright LEDs; Fairchild Semiconductor, San Jose, CA). A food pellet dispenser (model G5210; Ralph Gerbrands Co., Arlington, MA) was mounted on the top of the operant panel, and delivered 1-g banana-flavored food pellets to a receptacle beneath the panel. Operant panels were controlled, and data were collected, with an IBM-compatible computer interface and power supply obtained from MED Associates (Georgia, VT).

**Discrimination Training.** Training sessions consisted of one to five cycles, and each cycle consisted of a 15-min time-out period followed by a 5-min response period. Monkeys were given an intramuscular injection of either vehicle (saline) or the heroin-training dose (0.1 mg/kg) at the beginning of the 15-min time-out period. All stimulus lights were turned off and responding had no scheduled consequences during the time-out period. During the response period, the right and left response keys were transilluminated red or green, and the positions of the red and green keys were counterbalanced across monkeys. Depending upon the training condition, monkeys could respond on the stimulus-appropriate key under a fixed ratio 30 (monkeys 90B164 and 163F) or 40 (monkey 90B147) schedule to obtain up to 10 food pellets per cycle. After vehicle administration, responding on only the green key resulted in the delivery of a food pellet. After heroin administration, responding on only the red key resulted in the delivery of food. Inappropriate responses reset the fixed ratio requirement on the stimulus-appropriate key. The center key was not illuminated during operant sessions, and responses on the center key had no scheduled consequences. If all of the available food pellets were delivered in less than 5 min then the stimulus lights were extinguished, and responses had no scheduled consequences for the remainder of the 5-min response period. Training sessions consisted of zero to five saline cycles followed by zero to one drug cycles. If the training drug was administered, it was given only during the last cycle. This design ensured a constant interval between drug administration and the onset of response periods during which responding on the drug-appropriate key produced food. Monkeys were considered to have acquired the discrimination when the following criteria were met for seven of eight consecutive training sessions: 1) the percentage of injection-appropriate responding before the delivery of the first reinforcer was greater than or equal to 80% for all cycles; 2) the percentage injection-appropriate responding over the entire response period was greater than or equal to 90% for all cycles; and 3) response rates during vehicle training cycles were greater than 0.5 responses/s, which required between 30 and 90 training sessions, depending upon the animal. Experimental sessions were conducted 5 days/week.

**Discrimination Testing.** Once monkeys met criterion levels of heroin discrimination, testing began. Test sessions were conducted only if the three criteria listed above were met during the training session immediately preceding the test session. If responding did not meet criterion levels of discrimination performance then training was continued until criterion levels of performance were obtained for at least two consecutive sessions. In general, test sessions were conducted on Tuesdays and Fridays, and training sessions were conducted on Mondays, Wednesdays, and Thursdays.

Test sessions were identical to training sessions except that responding on either key produced food, and test drugs were administered using either a substitution protocol or a pretreatment protocol. In the substitution protocol, drugs were administered alone, instead of either saline or the training dose of heroin, using a cumulative dosing procedure. Heroin (0.0032–0.32 mg/kg) and morphine (0.32–10.0 mg/kg) were tested for substitution for the training stimulus. The opioid agonist U50,488 (0.0056–0.32 mg/kg) and the noncompetitive N-methyl-D-aspartate receptor antagonist ketamine (0.1–10.0 mg/kg) were also tested to evaluate the selectivity of the discrimination. Monkeys received an injection of the test compound at the beginning of each cycle of a multiple cycle session, which increased the cumulative test drug dose by either one-quarter or one-half log unit. Dose-effect curves for each compound were determined at least twice in each monkey. Each drug was tested up to doses that eliminated responding in at least two of the three monkeys.

In the pretreatment protocol, a dose of naloxone (0.0032–0.1 mg/kg) or 3-methoxynaltrexone (0.1–3.2 mg/kg) was administered 30 min before determination of a cumulative heroin or morphine dose-effect curve. After administration of an antagonist, heroin or morphine was administered up to doses that decreased response rates to less than 50% of saline control values. When response rates were suppressed during the final cycle, another response cycle was added to test a sixth dose of heroin or morphine, or the test session was repeated with a higher heroin or morphine dose range. Pretreatment tests were conducted at least once in each animal.

The percentage of heroin-appropriate responding was determined and reported only if a monkey emitted enough responses to earn at least one reinforcer (i.e., 30 or 40 responses, equivalent to a response rate of 0.1–0.13 responses/s). Percentage of heroin-appropriate responding was plotted as a function of drug dose only if at least two monkeys met the response rate criterion. Complete substitution of a test drug for the heroin-training stimulus was defined as 90% or greater heroin-appropriate responding. Response rates were calculated for all of the response periods.

**Data Analysis.** ED50 values were used to evaluate the effects of opioid agonists in both antinociception and drug discrimination procedures. In the antinociception procedure, ED50 values were defined as the doses of heroin or morphine that produced 50% MPE. In the drug discrimination procedure, ED50 values were determined for both heroin-appropriate responding and response rate-suppression, and were defined as the doses of heroin or morphine that produced 50% heroin-appropriate responding and response rate-suppression, and were defined as the doses of heroin or morphine that produced 50% heroin-appropriate responding and a 50% reduction in response rates compared with saline control values, respectively. ED50 values were calculated by interpolation when only two data points were available (one below and one above 50%) or by linear regression when at least three data points were available on the linear portion of the dose-effect curve. Individual ED50 values were calculated and averaged to yield a mean ED50 value (± S.E.M.). Individual substitution ED50 values were calculated only if a monkey responded during at least the first three cycles of a test session. Because drug doses were incremented on a logarithmic scale, ED50 values were converted to their log values for calculation of mean and S.E.M. and for statistical analysis. Mean ED50 values (± S.E.M.) were converted back to their linear values for presentation in Table 1.

Antinociception, substitution, and rate-suppression ED50 values for morphine and heroin administered alone and after opioid antagonist pretreatments were compared using one-way repeated measures analysis of variance (SuperAnova; Abacus Concepts, Berkeley, CA) with antagonist dose as the within-subjects factor. The statistical analyses included only antagonist doses for which there were ED50 values for all three of the monkeys. A significant analysis of variance was followed by linear contrasts comparing individual means. For all statistical analyses, the criterion for significance was set a priori at \( p < 0.05 \).

For each monkey, dose ratios were calculated as the ED50 of an opioid agonist in the presence of some dose of antagonist divided by
TABLE 1
Mean ED\textsubscript{50} values in mg/kg (±S.E.M.) for morphine or heroin administered alone or after pretreatment with naltrexone or 3-methoxynaltrexone in monkeys trained in a warm water tail-withdrawal assay (n = 3) and monkeys trained to discriminate heroin (n = 3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antinociception ED\textsubscript{50}</th>
<th>Substitution ED\textsubscript{50}</th>
<th>Rate Suppression ED\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naltrexone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine alone</td>
<td>5.82 (2.02)</td>
<td>0.87 (0.27)</td>
<td>2.28 (0.82)</td>
</tr>
<tr>
<td>+0.0032 Naltrexone</td>
<td>11.37 (2.50)</td>
<td>2.52 (0.68)*</td>
<td>5.52 (0.89)*</td>
</tr>
<tr>
<td>+0.01 Naltrexone</td>
<td>43.12 (0.53)*</td>
<td>6.79 (0.21)*</td>
<td>12.57 (2.69)*</td>
</tr>
<tr>
<td>+0.032 Naltrexone</td>
<td>72.52 (6.55)*</td>
<td>16.69 (3.22)</td>
<td>24.59 (5.54)*</td>
</tr>
<tr>
<td>Heroin alone</td>
<td>6.30 (0.06)</td>
<td>0.05 (0.02)</td>
<td>0.14 (0.03)</td>
</tr>
<tr>
<td>+0.0032 Naltrexone</td>
<td>3.90 (0.15)</td>
<td>0.11 (0.05)</td>
<td>0.18 (0.04)</td>
</tr>
<tr>
<td>+0.01 Naltrexone</td>
<td>1.33 (0.01)*</td>
<td>0.18 (0.04)*</td>
<td>0.45 (0.16)*</td>
</tr>
<tr>
<td>+0.032 Naltrexone</td>
<td>1.70 (0.25)*</td>
<td>1.01 (0.55)</td>
<td>0.87 (0.24)*</td>
</tr>
<tr>
<td>+0.1 Naltrexone</td>
<td>7.16 (0.26)</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>3-Methoxynaltrexone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine alone</td>
<td>5.82 (2.02)</td>
<td>0.88 (0.26)</td>
<td>3.33 (1.82)</td>
</tr>
<tr>
<td>+0.13 3-Methoxynaltrexone</td>
<td>4.90 (0.88)</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>+0.32 3-Methoxynaltrexone</td>
<td>22.39 (0.74)*</td>
<td>2.77 (1.92)</td>
<td>6.27 (0.67)</td>
</tr>
<tr>
<td>+1.0 3-Methoxynaltrexone</td>
<td>31.90 (7.90)*</td>
<td>6.31 (3.85)†</td>
<td>18.14 (3.97)*</td>
</tr>
<tr>
<td>+3.2 3-Methoxynaltrexone</td>
<td>62.13 (10.27)†</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Heroin alone</td>
<td>0.90 (0.06)</td>
<td>0.05 (0.02)</td>
<td>0.16 (0.01)</td>
</tr>
<tr>
<td>+0.32 3-Methoxynaltrexone</td>
<td>0.50 (0.03)*</td>
<td>0.13 (0.04)</td>
<td>0.33 (0.05)</td>
</tr>
<tr>
<td>+1.0 3-Methoxynaltrexone</td>
<td>1.66 (0.24)*</td>
<td>0.37 (0.15)†</td>
<td>0.63 (0.15)*</td>
</tr>
<tr>
<td>+3.2 3-Methoxynaltrexone</td>
<td>1.85 (0.25)*</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not determined; N.T., not tested.
* Significantly different from morphine or heroin alone (p values < 0.05).
† Data not submitted to statistical analyses (see text). However, 1.0 mg/kg 3-methoxynaltrexone produced rightward or downward shifts in heroin and morphine substitution dose-effect curves in all three monkeys.

3-Methoxynaltrexone Antagonism of Heroin’s Effects

TABLE 1

- Naltrexone pretreatments produced rightward shifts of the antagonism dose-effect curves of heroin and morphine. The pretreatment with naltrexone significantly increased the morphine and heroin ED\textsubscript{50} values.
- 3-Methoxynaltrexone pretreatments also produced rightward shifts of the antagonism dose-effect curves for morphine and heroin. The pretreatment with 3-methoxynaltrexone significantly increased the morphine and heroin ED\textsubscript{50} values.

Thermal Nociception

Under baseline conditions, the mean baseline tail-withdrawal latency (± S.E.M.) from 50°C water was 2.20 ± 0.21 s. Both morphine and heroin administered alone produced dose-dependent antinociception (Fig. 1), and the mean antinociception ED\textsubscript{50} values of morphine and heroin are shown in Table 1. Heroin was approximately 10- to 20-fold more potent than morphine in this warm water tail-withdrawal assay. Neither naltrexone nor 3-methoxynaltrexone alone altered baseline tail-withdrawal latencies (Fig. 1, points above PT).

Naltrexone pretreatments produced rightward shifts of the antinociception dose-effect curves for morphine and heroin (Fig. 1, top) and dose-dependent increases in the morphine \[ F = 24.588, p = 0.0009 \] and heroin \[ F = 43.723, p = 0.0001 \] ED\textsubscript{50} values (Table 1). Doses of 0.01 to 0.1 mg/kg naltrexone significantly increased the morphine and heroin ED\textsubscript{50} values.

3-Methoxynaltrexone pretreatments also produced rightward shifts of the antinociception dose-effect curves for morphine and heroin (Fig. 1, bottom) and dose-dependent increases in the morphine \[ F = 47.352, p = 0.0001 \] and heroin \[ F = 40.408, p = 0.0002 \] ED\textsubscript{50} values (Table 1). Doses of 0.32 to 3.2 mg/kg 3-methoxynaltrexone significantly increased morphine and heroin ED\textsubscript{50} values. A lower dose of 0.1 mg/kg 3-methoxynaltrexone was tested as a pretreatment to morphine, and this low dose of 3-methoxynaltrexone did not significantly alter the morphine ED\textsubscript{50} value (Table 1).

Heroin Discrimination

Figs. 2 and 3 show the discriminative stimulus effects of heroin and morphine alone in individual monkeys. The cumulative administration of heroin (open circles, top) or morphine (open circles, bottom) alone produced dose-dependent and complete substitution for the training dose of heroin in each of the three subjects. Both

Drugs

Heroin hydrochloride, morphine sulfate, naltrexone hydrochloride, and 3-methoxynaltrexone were supplied by the National Institute on Drug Abuse (Bethesda, MD). USP488 was purchased from Sigma/RBI (Natick, MA). Ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) was purchased as a 100-μg/ml stock solution. Unless otherwise noted, drugs were dissolved in sterile water. To get 3-methoxynaltrexone into solution, 1% lactic acid was added. Drug stock solutions were diluted to the appropriate concentrations with sterile saline. Doses were based on the salt forms of the drugs, and i.m. injections were administered in volumes of 0.1 to 3.8 ml.

Results

Thermal Nociception

Under baseline conditions, the mean baseline tail-withdrawal latency (± S.E.M.) from 50°C water was 2.20 ± 0.21 s. Both morphine and heroin administered alone produced dose-dependent antinociception (Fig. 1), and the mean antinociception ED\textsubscript{50} values of morphine and heroin are shown in Table 1. Heroin was approximately 10- to 20-fold more potent than morphine in this warm water tail-withdrawal assay. Neither naltrexone nor 3-methoxynaltrexone alone altered baseline tail-withdrawal latencies (Fig. 1, points above PT).

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Heroin Discrimination

Figs. 2 and 3 show the discriminative stimulus effects of heroin and morphine alone in individual monkeys. The cumulative administration of heroin (open circles, top) or morphine (open circles, bottom) alone produced dose-dependent and complete substitution for the training dose of heroin in each of the three subjects. Both
morphine and heroin also produced dose-dependent decreases in response rates (Fig. 4). Responding was eliminated in all of the monkeys by 0.32 mg/kg heroin, and a dose of 3.2 mg/kg morphine eliminated responding in two monkeys and decreased response rates in the third monkey. The mean substitution and rate-suppression ED$_{50}$ values of morphine and heroin are shown in Table 1. With respect to both discriminative stimulus and rate-suppressant effects, heroin was approximately 10- to 20-fold more potent than morphine. The pharmacological selectivity of this discrimination was evaluated by administering the $\kappa$-opioid receptor agonist U50,488 and the noncompetitive N-methyl-D-aspartate glutamate receptor antagonist ketamine. Both U50,488 and ketamine produced less than 10% heroin-appropriate responding up to doses that eliminated responding in at least two of the three monkeys (data not shown).

Figure 2 also shows the discriminative stimulus effects of heroin and morphine after pretreatment with three doses of naltrexone in individual monkeys. Naltrexone produced rightward shifts of the heroin (top) and morphine (bottom) substitution dose-effect curves and dose-dependent increases in morphine [F = 54.526, p = 0.0013] and heroin [F = 103.0, p = 0.0096] substitution ED$_{50}$ values (Table 1). However, at some doses, naltrexone pretreatments completely eliminated heroin-appropriate responding, and individual substitution ED$_{50}$ values could not be determined (Fig. 2; monkey 163F, top right and monkey 90B147, bottom center). Naltrexone also produced rightward shifts of the heroin and morphine rate-suppression dose-effect curves (Fig. 4, top) and dose-dependent increases in morphine [F = 18.580, p = 0.0019] and heroin [F = 6.660, p = 0.0245] rate-suppression ED$_{50}$ values (Table 1). Doses of 0.0032 to 0.032 mg/kg naltrexone significantly increased morphine ED$_{50}$ values, and 0.01 to 0.032 mg/kg naltrexone significantly increased heroin ED$_{50}$ values. In the three monkeys, naltrexone maximally increased substitution ED$_{50}$ values of morphine and heroin 7- to 31-fold and 3- to 13-fold, respectively. Rate-suppression ED$_{50}$ values of morphine and heroin were increased maximally 8- to 23-fold and 4- to 19-fold, respectively.

Figure 3 shows the discriminative stimulus effects of heroin and morphine after pretreatment with three doses of 3-methoxynaltrexone in individual monkeys. In general, the lower doses of 0.32 and 1.0 mg/kg 3-methoxynaltrexone dose dependently attenuated the discriminative stimulus effects of heroin and morphine in all three monkeys and produced rightward shifts in the heroin and morphine discrimination dose-effect curves. In some cases, the antagonist effects of 1.0 mg/kg 3-methoxynaltrexone were not surmounted by increasing the heroin or morphine dose, which resulted in downward shifts in discrimination dose-effect curves (i.e., heroin in monkey 163F and morphine in monkey 90B164). In contrast to the orderly effects of these lower 3-methoxynaltrexone doses, a higher dose of 3.2 mg/kg 3-methoxynaltrexone produced variable effects across subjects. In monkey 90B164, 3.2 mg/kg 3-methoxynaltrexone produced further rightward shifts in both the heroin and morphine discrimination dose-effect curves. However, after pretreatment with 3.2 mg/kg 3-methoxynaltrexone in monkey 90B147, high levels of heroin-appropriate responding were observed during the initial test cycles of the heroin and morphine dose-effect curves, when the first cumulative dose of heroin (0.032 mg/kg) or morphine (1.0 mg/kg) was administered. This monkey then responded exclusively on the saline-appropriate key during the second and third test cycles (0.1 and 0.32 mg/kg heroin or 3.2 and 10 mg/kg morphine) and, in the fourth test cycle, monkey 90B147 either stopped responding (1.0 mg/kg heroin) or switched back to the heroin-appropriate key (32 mg/kg morphine). Finally, after pretreatment with 3.2 mg/kg 3-methoxynaltrexone in monkey 163F, an intermediate level (66%) of heroin-appropriate responding was observed during the initial test cycle of the heroin dose-effect curve (0.032 mg/kg), and this monkey did not respond during the subsequent test cycle (0.1 mg/kg heroin). However, 3.2 mg/kg 3-methoxynaltrexone produced a rightward shift in the morphine dose-effect curve, and the magnitude of the shift was similar to that produced by a lower dose of 1.0 mg/kg 3-methoxynaltrexone. Because substitution ED$_{50}$ values could not be determined in many cases after pretreatment with 3-methoxynaltrexone, these data were not submitted to repeated measures statistical analyses. However, 1.0 mg/kg 3-methoxynaltrexone produced rightward or downward shifts in the heroin and morphine discrimination dose-effect curves in all three monkeys.

3-Methoxynaltrexone also produced rightward shifts of the heroin and morphine rate-suppression dose-effect curves (Fig. 4, bottom) and increased morphine [F = 7.417, p = 0.0451] and heroin [F = 8.635, p = 0.0354] rate-suppression ED$_{50}$ values (Table 1). A dose of 1.0 mg/kg 3-methoxynaltrex-
one significantly increased ED$_{50}$ values for both morphine and heroin. A higher dose of 3.2 mg/kg 3-methoxynaltrexone produced rate-decreasing effects that precluded determination of rate-suppression ED$_{50}$ values for morphine and heroin. In the three monkeys, 3-methoxynaltrexone maximally increased rate-suppression ED$_{50}$ values of morphine and heroin 4- to 25-fold and 4- to 7-fold, respectively.

**In Vivo Apparent pA$_2$ and pK$_B$ Analysis of Antagonist Effects.** Fig. 5 shows the Schild plots and Table 2 shows Schild plot slopes and in vivo affinity estimates for naltrexone and 3-methoxynaltrexone antagonism of the antinociceptive, discriminative stimulus, and rate-suppressant effects of morphine and heroin. For naltrexone, all Schild plot slopes included the value of −1 and did not include positive values, so pA$_2$ values also were determined with slopes constrained to −1. The constrained in vivo apparent pA$_2$ values were similar for naltrexone antagonism of the antinociceptive, discriminative stimulus and rate-suppressant effects of both morphine and heroin, as determined by overlapping 95% confidence limits.

For 3-methoxynaltrexone antagonism of the antinociceptive effects of morphine and heroin, Schild plot slopes included the value of −1 and did not include positive values, so pA$_2$ values also were determined with slopes constrained to −1. The constrained pA$_2$ values for 3-methoxynaltrexone antagonism of the antinociceptive effects of morphine and heroin were sim-
monkeys.

E

heroin, Schild plot slopes included the value of tive stimulus and rate-suppressant effects of morphine and heroin. For 3-methoxynaltrexone antagonism of the discriminative stimulus effects of heroin and morphine, although naltrexone was approximately 100-fold more potent than 3-methoxynaltrexone. These findings are consistent with the conclusion that the effects of heroin and morphine are mediated by pharmacologically similar populations of μ-opioid receptors in rhesus monkeys. These results also suggest that some of the differences in the pharmacology of heroin and morphine that have been observed in rodents may not extend to studies in non-human primates.

**Discussion**

The main finding of this study was that 3-methoxynaltrexone was equipotent in antagonizing the effects of heroin and morphine in thermal nociception and heroin discrimination procedures in rhesus monkeys. The opioid antagonist naltrexone also was equipotent in antagonizing the effects of heroin and morphine, although naltrexone was approximately 10-fold more potent than 3-methoxynaltrexone. These findings agree with a recent study in which heroin was 10- to 20-fold more potent than morphine (Dykstra et al., 1987; Negus et al., 1998a,b). We also demonstrated previously that heroin has a more rapid rate of onset and a shorter duration of action than morphine in this procedure (Negus et al., 1998b).

The effects of heroin and morphine also were compared in monkeys trained to discriminate heroin (i.m.) from saline. The discriminative stimulus effects of drugs are often pharmacologically selective, such that only drugs sharing pharmacological mechanisms of action with the training drug elicit training drug-appropriate responding (Holtzman, 1985). Cumulative administration of heroin or morphine dose dependently substituted for the heroin training stimulus and produced high levels of heroin-appropriate responding. In contrast, the κ-agonist U50,488 and the N-methyl-D-aspartate glutamate receptor antagonist ketamine produced primarily saline-appropriate responding up to doses that decreased response rates. These results provide one line of evidence to suggest that the discriminative stimulus effects of heroin and morphine were mediated by pharmacologically similar populations of μ-opioid receptors in rhesus monkeys. Also, as in the thermal nociception study, heroin was approximately 10- to 20-fold more potent than morphine in producing both discriminative stimuli and rate-suppressant effects. These findings agree with a recent study in which rhesus monkeys were trained to discriminate heroin (i.v.) from saline (Platt et al., 2001). Heroin and morphine also produced similar discriminative stimulus effects in rhesus monkeys trained to discriminate codeine from saline (Bertalmio et al., 1993) and in rats trained to discriminate either heroin or morphine from saline (Shannon and Holtzman, 1977; Corrigan and Coen, 1990). The heroin discrimination monkeys in the present study had been trained previously to

![Fig. 4](image)

**Fig. 4.** Effects of naltrexone (NTX; top) and 3-methoxynaltrexone (3MeONTX; bottom) on the rate-suppressant effects of morphine (left) and heroin (right). Abscissae, dose of drug in milligrams per kilogram (log scale). Ordinates, response rates in responses per second. All of the points show mean data (± 1 S.E.M.) from three monkeys.

![Fig. 5](image)

**Fig. 5.** Schild plots for naltrexone (NTX) and 3-methoxynaltrexone (3MeONTX) antagonism of the antinociceptive, discriminative stimulus, and rate-suppressant effects of morphine and heroin. Abscissae, negative log of the antagonist dose in moles per kilogram. Ordinates, log (dose ratio - 1). All of the points show mean data (± 1 S.E.M.) from two to three monkeys. ○, NTX + morphine; ●, NTX + heroin; □, 3MeONTX + morphine; ■, 3MeONTX + heroin.

ilar to each other but significantly lower than the constrained pA2 values for naltrexone antagonism of morphine and heroin. For 3-methoxynaltrexone antagonism of the discriminative stimulus and rate-suppressant effects of morphine and heroin, Schild plot slopes included the value of −1 and positive values. Constrained pA2 values were not determined because of the individual differences observed in the effects of the highest dose of 3.2 mg/kg 3-methoxynaltrexone. However, in vivo apparent pKd values were determined from data obtained with 1.0 mg/kg 3-methoxynaltrexone, and these pKd values were similar to each other and to pA2 values determined for 3-methoxynaltrexone antagonism of the antinociceptive effects of heroin and morphine.
discriminate a speedball mixture of heroin and cocaine, and sequential training with different drug stimuli may result in a pharmacological broadening of the cue (McMillan et al., 1996). However, given the concordance of the present results with previous findings, it is unlikely that the speedball discrimination history of these monkeys significantly influenced the substitution profiles of the drugs tested in this experiment.

Antagonism of Heroin and Morphine Antinociception by 3-Methoxynaltrexone. 3-Methoxynaltrexone was equipotent as an antagonist of the antinociceptive effects of heroin and morphine as indicated by similar pA\textsubscript{2} values. Moreover, the slopes of the Schild plots for 3-methoxynaltrexone antagonism of heroin and morphine were similar to −1, which is the theoretical value predicted by receptor theory for a competitive agonist and a competitive antagonist interacting at a homogenous population of receptors (Kenakin, 1993). These results are consistent with the conclusion that the antinociceptive effects of heroin and morphine were mediated by a single population of pharmacologically similar opioid receptors.

These results contrast with previous findings in mice and rats that 3-methoxynaltrexone was usually at least 10-fold more potent as an antagonist of the antinociceptive effects of heroin or its primary metabolite 6-acetylmorphine than of morphine (Brown et al., 1997a; Walker et al., 1999; Rady et al., 2000). The reason for this discrepancy is not clear. It is possible that the discrepancy in 3-methoxynaltrexone effects may reflect species differences in the opioid receptor populations that mediate heroin and morphine antinociception. Although opioid receptor populations in rodents and monkeys are similar, there are species differences in the regional distributions and relative proportions of \(\mu\)-, \(\kappa\)-, and \(\delta\)-receptors (Mansour et al., 1988). It is not known whether the M6G receptor is expressed in primates. If such a receptor is present, our results suggest that it contributes equally to the antinociceptive effects of both heroin and morphine.

Antagonism of Discriminative Stimulus and Rate-Suppressor Effects of Heroin and Morphine by 3-Methoxynaltrexone. 3-Methoxynaltrexone was equipotent as an antagonist of the discriminative stimulus and rate-suppressor effects of heroin and morphine. Although previous studies in mice used an intracerebroventricular route of administration for 3-methoxynaltrexone (Brown et al., 1997a; Rady et al., 2000), studies in rats have used systemic routes of administration similar to the one used herein (Walker et al., 1999; K. D’Anci and S. Negus, unpublished observations). It is possible that the discrepancy in 3-methoxynaltrexone antagonism of the discriminative stimulus and rate-suppressor effects of heroin and morphine was similar. Moreover, these \(pK_{B}\) values were similar to the pA\textsubscript{2} values for 3-methoxynaltrexone antagonism of the antinociceptive effects of heroin and morphine.

Drug discrimination is one of several procedures used to assess the abuse-related effects of drugs. Our results indicated that similar doses of 3-methoxynaltrexone blocked the discriminative stimulus effects of heroin and morphine. These results agree with a previous study that used drug self-administration, another procedure used to assess the abuse-related effects of drugs. In that study, 3-methoxynaltrexone was equipotent in altering the self-administration of

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>pA\textsubscript{2}</th>
<th>Slope</th>
<th>(pK_{B}) or Constrained pA\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naltrexone</td>
<td>+Morphine</td>
<td>8.12 (7.58–8.66)</td>
<td>−1.18 (−2.13−−0.24)</td>
</tr>
<tr>
<td></td>
<td>+Heroin</td>
<td>7.89 (7.52–8.26)</td>
<td>−1.02 (−1.45−−0.59)</td>
</tr>
<tr>
<td>+Morphine</td>
<td>6.59 (5.99–7.20)</td>
<td>−0.72 (−1.14−−0.30)</td>
<td>6.34 (6.12–6.56)</td>
</tr>
<tr>
<td>3-Methoxynaltrexone</td>
<td>+Heroin</td>
<td>6.01 (5.55–6.46)</td>
<td>−0.91 (−1.60−−0.22)</td>
</tr>
<tr>
<td>Substitution</td>
<td>Naltrexone</td>
<td>+Morphine</td>
<td>8.40 (7.78–9.02)</td>
</tr>
<tr>
<td></td>
<td>+Heroin</td>
<td>8.17 (7.82–8.51)</td>
<td>−1.06 (−1.47−−0.65)</td>
</tr>
<tr>
<td>+Morphine</td>
<td>6.31 (5.79–7.00)</td>
<td>−0.54 (−1.11−−0.24)</td>
<td>6.21 (5.97–6.55)</td>
</tr>
<tr>
<td>3-Methoxynaltrexone</td>
<td>+Heroin</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Rate-suppression</td>
<td>Naltrexone</td>
<td>+Morphine</td>
<td>8.51 (7.91–9.1)</td>
</tr>
<tr>
<td></td>
<td>+Heroin</td>
<td>8.09 (7.57–8.61)</td>
<td>−0.68 (−1.11−−0.24)</td>
</tr>
<tr>
<td>3-Methoxynaltrexone</td>
<td>+Morphine</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>+Heroin</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not determined.
heroin and morphine by rats (Walker et al., 1999). Together, these findings suggest that 3-methoxynaltrexone does not differentially antagonize the abuse-related effects of heroin and morphine. However, in the previous study, self-administration of heroin and morphine by rats was altered by relatively low doses of 3-methoxynaltrexone that antagonized the antinociceptive effects of heroin but not of morphine. These data were interpreted to suggest that M6G receptors mediated the reinforcing effects of both heroin and morphine, and that drugs such as 3-methoxynaltrexone might be useful in blocking the abuse-related effects of morphine without blocking the antinociceptive effects of morphine (Walker et al., 1999). The results of the present study in rhesus monkeys do not support this conclusion because doses of 3-methoxynaltrexone that blocked the discriminative stimulus effects of morphine also blocked the antinociceptive effects of morphine. It is unclear whether these discrepant findings resulted from species differences or the use of different procedures to assess the abuse-related effects of drugs. This issue could be clarified by investigating the effects of 3-methoxynaltrexone in both rats and nonhuman primates using comparable procedures.

The highest dose of 3-methoxynaltrexone (3.2 mg/kg) produced high levels of heroin-appropriate responding in some monkeys during the initial test cycle of heroin and morphine dose-effect curves. These effects obscured the antagonist effects of 3-methoxynaltrexone and precluded the use of these data in determination of mean EDso or pA2 values. The reasons for these effects of 3-methoxynaltrexone are not clear, but several findings suggest that the apparent partial substitution of high-dose 3-methoxynaltrexone for heroin may not reflect partial agonist effects of 3-methoxynaltrexone at µ-opioid receptors. First, these effects were transient and were apparent only during the initial test cycle, whereas the antagonist effects of 3-methoxynaltrexone were clearly apparent throughout the 1- to 2-h thermal nociception and heroin discrimination test sessions. Second, 3-methoxynaltrexone produced pronounced rate-suppressant effects in monkey 163F on the one occasion when it also produced partial substitution for heroin, which indicated that patterns of responding were impaired in this monkey at the time when partial substitution was observed. Finally, 3-methoxynaltrexone did not produce any agonist effect in the thermal nociception study, whereas other low-efficacy µ-agonists (e.g., butorphanol and nalbuphine) produce measurable agonist effects in this procedure (Negus and Mello, 1999). Together, these findings suggest that a more plausible explanation may be that high doses of 3-methoxynaltrexone produced direct effects that sometimes disrupted stimulus control. A better understanding of these effects will require further study.

**Antagonist Effects of Naltrexone.** The Schild plot slopes for naltrexone antagonism of heroin and morphine were similar to −1, and naltrexone was equipotent as an antagonist of the antinociceptive, discriminative stimulus, and rate-suppressant effects of heroin and morphine as indicated by similar pA2 values. The in vivo apparent pA2 values for naltrexone antagonism of heroin and morphine are consistent with those reported in other studies of naltrexone antagonism of the behavioral effects of heroin and morphine in rhesus monkeys (France et al., 1990; Platt et al., 2001; Bowlett et al., 1998). Furthermore, these in vivo apparent pA2 values are similar to pA2 values obtained for naltrexone antagonism of other selective µ-agonists in behavioral studies in rhesus monkeys (France et al., 1990; Gerak et al., 1994; Ko et al., 1998). Together, the results with naltrexone and 3-methoxynaltrexone suggest that the effects of heroin and morphine assessed in this study were mediated by a single population of pharmacologically similar µ-opioid receptors. The finding that naltrexone pA2 values were consistently and significantly higher than 3-methoxynaltrexone pA4 and pKia values suggests that naltrexone has higher affinity than 3-methoxynaltrexone for the µ-opioid receptors that mediate these effects of heroin and morphine in rhesus monkeys. This in vivo finding agrees with binding studies that suggest that 3-methoxynaltrexone has relatively low affinity for µ-opioid receptors (Brown et al., 1997b).

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**References**


Negus SS, Butelman ER, and Mello NK (1998a) Discriminative stimulus effects of a


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