Opioid Partial Agonist Effects of 3-O-Methylnaltrexone in Rhesus Monkeys

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ABSTRACT

3-O-Methylnaltrexone (3-MNTX), a putative antagonist of morphine-6-β-D-glucuronide (M6G) receptors, has been reported to block the behavioral effects of heroin at doses that do not block those of morphine, suggesting that M6G receptors may play a unique role in the properties of heroin. This study investigated the effects of 3-MNTX in monkeys trained to discriminate i.v. heroin from vehicle or to self-administer i.v. heroin under a progressive-ratio schedule. Additional in vitro studies determined the effects of 3-MNTX and reference drugs on adenylyl cyclase activity in caudate-putamen membranes of monkeys and rats. In drug discrimination experiments, heroin, morphine, and M6G substituted for heroin in all subjects, whereas 3-MNTX substituted for heroin in one-half the monkeys tested. In these latter monkeys, the effects of 3-MNTX were antagonized by naltrexone, and pretreatment with 3-MNTX enhanced the effects of heroin, M6G, and morphine, indicative of μ-agonist activity. In monkeys showing no substitution of 3-MNTX for heroin, 3-MNTX antagonized the effects of heroin, M6G, and morphine. In self-administration experiments, heroin and 3-MNTX maintained injections per session significantly above those maintained by vehicle when the initial response requirement (IRR) was low; only heroin maintained significant self-administration when the IRR was high. In vitro, 3-MNTX inhibited adenylyl cyclase activity in both monkey and rat brain membranes. The degree of inhibition produced by 3-MNTX was less than that produced by the full agonist [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO). The results suggest that 3-MNTX functions primarily as a partial agonist at μ-receptors in monkeys and do not support a singular role for M6G receptors in the abuse-related effects of heroin.

Heroin is rapidly metabolized by sequential deacetylation to yield the active metabolites 6-monoacetylmorphine (6MAM) and morphine (Kamendulis et al., 1996). Morphine, in turn, is metabolized via glucuronidation to yield morphine-6-β-D-glucuronide (M6G) and morphine-3-β-D-glucuronide (M3G) (Glare and Walsh, 1991; Milne et al., 1996). Although the in vivo effects of heroin and its metabolites have been attributed predominantly to activity at the μ-opioid receptor (Bertalmio et al., 1992; Rady et al., 1994; Migmat et al., 1995; Uchihashi et al., 1996; Platt et al., 2001), qualitative differences in the effects of heroin, morphine, 6MAM, and M6G have been observed in some situations. In morphine-tolerant mice, for example, heroin, 6MAM, and M6G do not exhibit cross-tolerance to the analgesic or locomotor-activating effects of morphine (Lange et al., 1980; Rossi et al., 1996; Grung et al., 2000). Moreover, in CXBK mice (which are notably insensitive to the analgesic effects of morphine), heroin, 6MAM, and M6G retain analgesic effects (Rossi et al., 1996). Studies using antisense and knockout techniques also have revealed differences in the analgesic effects of heroin and morphine. Antisense probes or knockouts targeting exon 1 of the cloned μ-opioid receptor result in reduced heroin- or M6G-induced analgesia (Rossi et al., 1996, 1997; Schuller et al., 1999). In contrast, antisense probes or knockouts targeting exon 2 of the cloned μ-opioid receptor result in diminished morphine-induced, but not heroin- or M6G-induced analgesia (Rossi et al., 1996, 1997; Schuller et al., 1999). Together, these findings raise the possibility that heroin and its metabolites may interact differentially with distinct subtypes of the μ-opioid receptor.

Consistent with these in vivo observations, radioligand binding studies have revealed an apparently distinct subtype of opioid binding site with high affinity for M6G, as well as compounds that bind selectively to this site. One such drug is

ABBREVIATIONS: 6MAM, 6-monoacetylmorphine; M6G, morphine-6-β-D-glucuronide; M3G, morphine-3-β-D-glucuronide; 3-MNTX, 3-O-methylnaltrexone; DS, discriminative stimulus; PR, progressive-ratio; IRR, initial response requirement; FR, fixed-ratio; BP, break point; DAMGO, [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin.
the putative M6G receptor antagonist 3-O-methylaltrexone (3-MNTX, also 3-methoxynaltrexone) (Brown et al., 1997a,b). In rodents, 3-MNTX has been reported to preferentially antagonize the analgesic effects of M6G, heroin, and 6MAM compared with morphine (Brown et al., 1997a; Walker et al., 1999), suggesting that 3-MNTX may be a tool for evaluating the role of M6G receptors in the behavioral effects of opioid agonists.

The initial goal of the present study was to investigate the role of M6G receptors in the discriminative stimulus (DS) effects of heroin in rhesus monkeys trained to discriminate i.v. injections of heroin from vehicle, by determining the ability of 3-MNTX to differentially antagonize the DS effects of heroin compared with those of M6G and morphine. Unexpectedly, the effects of 3-MNTX differed qualitatively among individual subjects, showing antagonism of heroin, M6G, and morphine in some cases and enhancement of the effects of the three opioid agonists in others, a profile of effects often exhibited by opioid partial agonists (Ariëns, 1983; Colpaert and Janssen, 1984; Young et al., 1992; Morgan and Picker, 1996). Consequently, additional studies were conducted to evaluate the apparent partial agonist effects of 3-MNTX in rhesus monkeys using a progressive-ratio (PR) schedule of i.v. drug self-administration with differing initial response requirements (IRR) (Rowlett et al., 1996, 2002), and in both monkey and rat brain tissue using a quantitative in vitro assay of adenyl cyclase inhibition (Cote et al., 1993; Izenwasser et al., 1993).

Materials and Methods

Drug Discrimination

Subjects and Surgical Procedure. Four adult male rhesus monkeys (Macaca mulatta), weighing 8.4 to 12.1 kg, were studied in daily experimental sessions (Monday to Friday). Due to circumstances unrelated to the present experiment, one monkey (M-164) died during the course of the study and did not receive all drug treatments. Between sessions, monkeys lived in individual home cages where they had unlimited access to water. The monkeys were maintained at 85 to 90% of their free-feeding body weight by adjusting their access to food in the home cage (Teklad, supplemented with fresh fruit and vegetables). All animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education, and Welfare Publication No. (NIH) 85-23, revised 1996. Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Monkeys were prepared with chronic indwelling venous catheters (polyvinyl chloride; i.d. 0.64 mm, o.d. 1.35 mm) using the general surgical procedures described by Carey and Spealman (1998). Under isoflurane anesthesia and aseptic conditions, one end of a catheter was passed to the level of the right atrium by way of a brachial, femoral, or jugular vein. The distal end of the catheter was passed subcutaneously and exited in the mid-scapular region. Catheters were flushed daily with heparinized saline (150–200 U/ml) and were sealed with stainless steel obturators when not in use. Monkeys wore custom-made nylon-mesh jackets (Lomir Biomedical, Toronto, ON, Canada) at all times to protect the catheter.

Apparatus. Experimental sessions were conducted in ventilated and sound-attenuating chambers. Monkeys were seated in custom-made primate chairs (Crist Instrument Co., Hagerstown, MD). Two response levers (model ENV-610M; MED Associates, Georgia, VT) were mounted 16 cm apart on the wall of the chamber in front of the monkey. Each press of a lever with a minimum downward force of approximately 0.25 N produced an audible click and was recorded as a response. Food pellets (Formula 0094, 1 g; Bioserve, Frenchtown, NJ) could be delivered to a tray located between the levers. Colored lights mounted above the levers could be illuminated to serve as visual stimuli.

Procedure. Monkeys were trained to discriminate heroin from saline under a 10-response fixed-ratio (FR 10) schedule of food reinforcement (Platt et al., 2001). The training dose of heroin in the present study was 0.056 mg/kg for all monkeys. After an i.v. injection of heroin, 10 consecutive responses on one lever produced a food pellet, whereas after an i.v. injection of saline, 10 consecutive responses on the other lever produced a pellet. For two of the monkeys, responding on the right lever after an injection of heroin resulted in delivery of a food pellet. For the other two monkeys, responding on the left lever after injection of heroin resulted in pellet delivery. Delivery of each pellet was followed by a 10-s time-out period. Responses on the incorrect lever (e.g., the saline-appropriate lever after heroin injection) reset the FR requirement.

Training sessions consisted of a variable number of components (n = 1–4) of the FR schedule. Each component ended after the completion of the 10th FR 10 or after 5 min had elapsed, whichever occurred first. A 10-min time-out period, during which the lights were off and responses had no programmed consequences, preceded each component. During most training sessions, saline was injected during time-out periods preceding the first n = 1 components, and heroin was injected after the nth component of the session. Periodically, saline was injected before all components of a training session to prevent an invariant association between the last component and heroin injection. Injections of heroin or saline were administered from the same catheter extension via a catheter extension during the 5th min of the 10-min time-out periods. Each injection was followed by a 2-ml infusion of saline to flush the catheter of any residual drug solution.

Once consistent stimulus control was achieved, drug test sessions were conducted once or twice per week with training sessions on intervening days. Test sessions were conducted only if ≥80% of responses were made on the injection-appropriate lever during at least four of the preceding five training sessions. Test sessions consisted of four FR components, each preceded by a 10-min time-out period. During each component, completion of 10 consecutive responses on either lever produced food. Dose-response functions were determined for test drugs using a cumulative dosing procedure. The effects of heroin (1 mg/kg), M6G (0.5–1.0 mg/kg), and morphine (0.1–3.0 mg/kg) alone were determined by injecting incremental doses (0.25–0.50 log increments) i.v. during time-out periods that preceded sequential FR components. This procedure permitted a four-point cumulative dose-response function to be determined in a single session. When appropriate, five or more different doses of a drug were studied by administering overlapping ranges of cumulative doses during test sessions on different days. The effects of most doses were determined twice, although low, inactive doses and high doses that disrupted behavior usually were tested only once in each subject. Subsequently, antagonism studies were conducted with 3-MNTX (0.1 and 1.0 mg/kg i.m.) administered as a pretreatment 10 min before cumulative dosing with heroin, M6G, and morphine.

Based on the results of these studies, an additional experiment was conducted to evaluate the effects of 3-MNTX (0.01–3.0 mg/kg) alone and in combination with the opioid antagonist naltrexone. Naltrexone (0.01 mg/kg i.m.) was administered 5 min before the session, followed by cumulative doses of 3-MNTX. Naltrexone dose and pretreatment time were chosen based on the results from an earlier study showing that this dose of naltrexone, administered under these conditions, antagonized the DS effects of heroin and other μ-opioid agonists (Platt et al., 2001).

Data Analysis. Percentage of heroin-lever responding was computed for individual subjects in each component of a test session by
dividing the number of responses on the heroin lever by the total number of responses on both levers and multiplying by 100, with the restriction that response rate was ≤ 0.1 responses/s during the component. Mean percentage of heroin-lever responding and S.E.M. were then calculated for the group at each dose. A drug was considered to substitute fully for the training dose of heroin if the maximum percentage of drug-lever responding was ≥ 80%. The doses of drug estimated to engender 50% heroin-appropriate responding (ED50) were determined for individual subjects by linear regression analysis in cases where the ascending limb of the log dose-response function was defined best by three or more data points or by linear interpolation in cases where the ascending limb was defined best by two points (cf. Holtzman, 1993).

The overall rate of responding in each component was computed by dividing the total number of responses in a component (regardless of lever) by the total component duration. Rate of responding was converted to percentage of control by dividing an individual animal’s response rate after drug or vehicle test by that animal’s average response rate after the last two saline training sessions before the test, and multiplying by 100. Mean response rate (% control ± S.E.M.) was then calculated for the group at each dose.

### Intravenous Drug Self-Administration

#### Subjects and Surgical Procedure.
Four adult female rhesus monkeys, weighing 5.3 to 7.8 kg, were studied in daily experimental sessions. The monkeys lived in individual home cages, which also served as experimental chambers and had unrestricted access to food and water. Monkeys were prepared with venous catheters and wore nylon mesh jackets as previously described (Carey and Spealman, 1998).

**Apparatus.** Cages were equipped with a removable panel on the front of each cage that contained stimulus lights and a response lever (model ENV-610M; MED Associates). Each monkey’s jacket was connected to a 1-m stainless steel flexible tether (Lomir Biomedical, Toronto, Canada). The monkey’s catheter was routed through the tether and attached to a fluid swivel (Lomir Biomedical, Toronto, ON, Canada) on top of the cage. The swivel was attached to an injection pump (MED Associates) that could infuse drug solutions at a rate of 0.2 ml/s.

**Procedure.** All monkeys had been trained previously to self-administer opioids and cocaine under a PR schedule of i.v. drug injection, a procedure shown previously to be sensitive to the reinforcing effects of opioid full and partial agonists (Rowlett et al., 2002). At the beginning of the self-administration session, white stimulus lights above the lever were illuminated to signal the start of a trial. Upon completion of the response requirement, the white lights were extinguished and red stimulus lights were illuminated for 1 s, coinciding with a 1-s infusion of drug or saline. Each trial ended with either an injection or the expiration of a 30-min limited hold. Trials were separated by a 30-min time-out period, during which all lights were extinguished and responding had no programmed consequences.

Experimental sessions consisted of five components made up of four trials each (maximum 20 trials per session). The response requirement remained constant during each of the four trials within a component, and doubled at the beginning of each successive component. For example, a session with an IRR of 100 was comprised of the following four components with increasing response requirements (four trials each): 100, 200, 400, 800, and 1600. The session ended when a monkey self-administered a maximum of 20 injections or when the response requirement was not completed for two consecutive trials.

On alternate training days, either cocaine (0.1 mg/kg/injection) or saline was available for injection under a PR schedule with an IRR of 100. Once stable self-administration was maintained (i.e., the number of injections per session exceeded 10 for at least three consecutive drug (D) sessions, the number of injections/session was less than 5 for at least three consecutive vehicle (V) sessions, and no upward or downward trends were observed across consecutive sessions), test sessions (T) were introduced according to the following sequence: DVTIDVTIDVT, etc. During test sessions, 3-MNTX (0.005–0.1 mg/kg/injection) and heroin (0.0003–0.03 mg/kg/injection) were evaluated for their ability to maintain responding under the PR schedule using two different IRRs, 100 and 400. Drugs, doses, and IRRs were tested in an irregular order across subjects, with the restriction that all doses of one drug were studied at both IRRs before testing the other drug. Each dose of 3-MNTX and heroin was determined twice in each monkey.

#### Data Analysis.

The number of injections per session was determined for individual monkeys under each test condition. Reliability of drug self-administration was determined by comparing the mean number of injections per session for each dose to the corresponding vehicle control value (Dunnett’s test, α level equal to p < 0.05).

Break point (BP), defined as the highest response requirement completed during a test session, was calculated for individual monkeys under each test condition. The maximum BP (BPmax) was calculated for each drug as the highest median BP for a drug, irrespective of dose. Medians rather than means were used for this analysis because BP values characteristically have distributions that do not meet the assumption of homogeneity of variance (cf. Rowlett et al., 1996).

### Adenylyl Cyclase Assays

#### Tissue.
Caudate putamen tissue was obtained from drug-naive, male Sprague-Dawley rats weighing 200 to 225 g (Charles River Laboratories, Inc., Wilmington, MA) and drug-naive, male rhesus monkeys weighing 8.4 to 10.3 kg (Division of Comparative Pathology, New England Primate Research Center, Southborough, MA), frozen on dry ice and stored at −70°C until use.

**Procedure.** Membrane preparations and adenylyl cyclase assays were conducted as described previously by Cote et al. (1993) and Izenwasser et al. (1993). Briefly, to prepare membranes, dissected tissue from either the caudate putamen of one rat or a section of the caudate putamen of one rhesus monkey was homogenized in a Teflon/glass homogenizer, diluted in 25 ml of buffer [20 mM Tris-HCl (pH 7.4), 2 mM EGTA, 1 mM MgCl2, and 250 mM sucrose] and centrifuged at 27,000g for 15 min at 4°C. The pellet was resuspended in 25 ml of fresh buffer and centrifuged again for 15 min. The supernatant was discarded and the tissue homogenized in 30 volumes (w/v) for rat and 20 volumes (w/v) for monkey of ice-cold buffer [2 mM Tris-HCl (pH 7.4) and 2 mM EGTA].

To determine adenylyl cyclase activity, tissue homogenate (20–50 mg of protein in 10 μl) was added to ice to assay tubes (final volume 50 μl) containing 80 mM Tris-HCl (pH 7.4), 10 mM theophylline, 1 mM MgSO4, 0.8 mM EGTA, 30 mM NaCl, 0.25 mM ATP, 0.01 mM GTP, and either the test drug (3-MNTX, heroin, morphine, M6G, and [d-Ala2,N-Me-Phe4,Gly5-ol]-enkephalin (DAMGO)) or sterile water. Each sample was then incubated at 30°C for 5 min. Adenylyl cyclase activity was terminated by placing the tubes into boiling water for 2 min. The amount of cAMP formed was determined by a [3H]cAMP protein binding assay, as described previously (Cote et al., 1993; Izenwasser et al., 1993). Briefly, [3H] cAMP (final concentration 17 nM) was added to each test tube followed by a binding protein prepared from bovine adrenal glands. The samples were incubated on ice for 90 min, and the assay was terminated by the addition of charcoal and centrifugation to separate the free [3H] cAMP from that which was bound to the binding protein. Aliquots of the supernatant containing bound cAMP were placed into scintillation vials to which Beckman Ready Value scintillation cocktail was added. Radioactivity was determined by liquid scintillation spectrometry. Each sample was run in triplicate, and each assay was repeated a minimum of three times.

#### Data Analysis.

The amount of cAMP formed as a function of agonist concentration was determined using linear regression analysis by comparison to known standards. Based on the maximum inhibition induced by a particular drug, the concentration of drug estimated to inhibit adenylyl cyclase by 50% of the maximum (IC50)
was derived using nonlinear curve-fitting techniques analyzed with GraphPad Prism software. Significance of adenylyl cyclase inhibition was determined by comparing the mean inhibition at the 100 μM concentration of each drug to the corresponding control value for basal activity (Dunnett’s test, α level equal to p < 0.05). Planned comparisons between the maximum inhibition induced by 3-MNTX and DAMGO were conducted using independent t tests (α level equal to p < 0.05).

Drugs, Chemicals, and Reagents. Heroin HCl, morphine-6-β-D-glucoronide base, and 3-O-methylnaloxone HCl were provided by the National Institute of Drug Abuse (Rockville, MD). Morphine SO4, DAMGO, and naltrexone HCl were purchased from Sigma-Aldrich (St. Louis, MO). All drugs were dissolved in small amounts of 0.1 N HCl as required and diluted to the desired concentrations with sterile water or 0.9% saline solution. Other chemicals and reagents were obtained from the following sources: [3H]cAMP (ammonium salt; specific activity 37.2 Ci/mmol) from PerkinElmer Life Sciences (Boston, MA); and ATP, GTP, cAMP, theophylline, and EGTA from Sigma-Aldrich.

Results

Discriminative Stimulus Effects of Heroin Alone and Combined with 3-MNTX. Heroin engendered dose-dependent increases in the percentage of responses on the heroin-associated lever (Fig. 1, solid line) with full substitution occurring at doses ≥0.03 mg/kg in each monkey. Individual response rates were not affected systematically by heroin over the range of doses tested, and no dose of heroin decreased the response rate to less than 85% of the control rate (data not shown).

Pretreatment with 3-MNTX altered the DS effects of heroin in different ways in individual subjects (Fig. 1, bottom). Pretreatment with the low dose of 3-MNTX (0.1 mg/kg; Fig. 1, bottom left) had little or no effect on the DS effects of heroin in monkeys M-163 and M-164 (open diamonds and open triangles, respectively), whereas in monkeys M-216 and M-426 (open circles and open squares, respectively) this same dose of 3-MNTX produced an enhancement of the DS effects of heroin such that doses of heroin <0.01 mg/kg engendered a larger percentage of heroin-lever responses compared with heroin alone. Averaged for the group of four subjects, 0.1 mg/kg 3-MNTX produced a slight leftward shift in the dose-response function for the DS effects of heroin (Fig. 1, bottom left, dashed line). Pretreatment with a higher dose of 3-MNTX (1.0 mg/kg; Fig. 1, bottom right) attenuated the DS effects of heroin in monkeys M-163 and M-164 such that doses of heroin ≥0.03 mg/kg engendered a reduced percentage of heroin-lever responses compared with heroin alone. In monkeys M-216 and M-426, pretreatment with 1.0 mg/kg of 3-MNTX resulted in a striking enhancement of the DS effects of heroin such that responding occurred exclusively on the heroin-paired lever, even at doses of heroin 50 times lower than the training dose. When averaged over the group of four monkeys, 1.0 mg/kg 3-MNTX resulted in a marked change in the shape of the heroin dose-response function characterized by increased drug-lever responding at doses of heroin <0.01 mg/kg and reduced drug-lever responding at doses of heroin ≥0.1 mg/kg (Fig. 1, bottom right, dashed line). Response rates engendered by heroin after pretreatment with 3-MNTX were not different from response rates for heroin alone in most monkeys. The single exception was in monkey M-163 for which 1.0 mg/kg 3-MNTX combined with 1.0 mg/kg heroin reduced response rate to approximately 20% of control (data not shown).

Discriminative Stimulus Effects of M6G and Morphine Alone and Combined with 3-MNTX. Both M6G and morphine displayed DS effects that were qualitatively similar to those of heroin (Figs. 2 and 3, respectively; solid lines). Increasing cumulative doses of each drug engendered dose-related increases in the heroin-associated lever, with

Fig. 1. Percentage of heroin-lever responding engendered by heroin alone (top) and combined with 3-MNTX (bottom) in rhesus monkeys trained to discriminate heroin from saline. Each symbol represents data from an individual monkey. Solid line, mean heroin-lever responding engendered by heroin alone; dashed line (left), mean heroin-lever responding engendered by heroin combined with 0.1 mg/kg 3-MNTX; and dashed line (right), mean heroin-lever responding engendered by heroin combined with 1.0 mg/kg 3-MNTX (in this panel, point associated with 1.0 mg/kg heroin reflects mean from two animals).
one or more doses substituting fully for heroin in each subject. As in the case of heroin, these effects were observed after administration of doses of M6G and morphine that did not markedly alter the rate of responding (data not shown).

In general, when 3-MNTX was combined with M6G, a pattern of results similar to that of 3-MNTX combined with heroin was evident (Fig. 2). Although pretreatment with 0.1 mg/kg 3-MNTX (Fig. 2, bottom left, dashed line) had little or no effect on the heroin-like DS effects of M6G in most monkeys, pretreatment with the higher dose of 3-MNTX (1.0 mg/kg) altered the overall shape of the M6G dose-response function for individual monkeys (Fig. 2, bottom right). Compared with M6G alone, pretreatment with 1.0 mg/kg 3-MNTX resulted in enhanced drug-lever responding after a dose of 1.0 mg/kg M6G and reduced drug-lever responding after a dose of 10.0 mg/kg M6G in both M-163 and M-426.

**Fig. 2.** Percentage of heroin-lever responding engendered by M6G alone (top) and combined with 3-MNTX (bottom) in rhesus monkeys trained to discriminate heroin from saline. Each symbol represents data from an individual monkey. Solid line, mean heroin-lever responding engendered by M6G alone; dashed line (left), mean heroin-lever responding engendered by M6G combined with 0.1 mg/kg 3-MNTX; and dashed line (right), mean heroin-lever responding engendered by M6G combined with 1.0 mg/kg 3-MNTX.

**Fig. 3.** Percentage of heroin-lever responding engendered by morphine alone (top) and combined with 3-MNTX (bottom) in rhesus monkeys trained to discriminate heroin from saline. Each symbol represents data from an individual monkey. Solid line, mean heroin-lever responding engendered by morphine alone; dashed line (left), mean heroin-lever responding engendered by morphine combined with 0.1 mg/kg 3-MNTX; and dashed line (right), mean heroin-lever responding engendered by morphine combined with 1.0 mg/kg 3-MNTX (in this panel, point associated with 10.0 mg/kg morphine reflects mean from two animals).
(open diamonds and open squares, respectively). In monkey M-216 (open circles), 1.0 mg/kg 3-MNTX enhanced the DS effects of all doses of M6G. Averaged over the group of three monkeys, 1.0 mg/kg 3-MNTX produced an overall flattening of the M6G dose-response function, with most doses of M6G engendering between 60 and 90% drug-lever responding (Fig. 2, bottom right, dashed line). Response rates engendered by M6G after pretreatment with 3-MNTX did not differ substantially from response rates engendered by M6G alone in any subject (data not shown).

As in the case of M6G, pretreatment with 0.1 mg/kg 3-MNTX had little or no effect on the DS effects of morphine in any monkey (Fig. 3, bottom left, dashed line). Pretreatment with 1.0 mg/kg 3-MNTX, however, attenuated the effects of all doses of morphine up to 3.0 mg/kg in M-163 and M-164 (open diamonds and open triangles, respectively) and enhanced the effects of all doses of morphine up to 1.0 mg/kg in monkeys M-216 and M-426 (open circles and open squares, respectively). Averaged over the group of four monkeys, pretreatment with 3-MNTX resulted in a markedly altered morphine dose-response function characterized by enhancement of the effects of low doses of morphine and attenuation of the effects of high doses of morphine (Fig. 3, bottom right, dashed line). Response rates engendered by morphine after pretreatment with 3-MNTX did not differ systematically from response rates engendered by morphine alone in any monkey (data not shown).

Fig. 4. Percentage of heroin-lever responding engendered by 3-MNTX alone (all subjects) and combined with naltrexone (subjects M-216 and M-426) in rhesus monkeys trained to discriminate heroin from saline. Each panel represents data from an individual monkey, identified at the top of each panel.

**Discriminative Stimulus Effects of 3-MNTX Alone and Combined with Naltrexone.** When tested alone, 3-MNTX had qualitatively different effects in different subjects. For the two monkeys (M-163 and M-164) in which 3-MNTX attenuated the DS effects of heroin, M6G, and morphine, 3-MNTX engendered little or no drug-lever responding (0–32% heroin-lever responding) up to doses that reduced response rate by >50% of control (Fig. 4, top). On the other hand, for monkeys M-216 and M-426 in which 3-MNTX consistently enhanced the DS effects of heroin, M6G, and morphine, 3-MNTX substituted fully for the DS effects of heroin (Fig. 4, bottom, closed circles). Full substitution occurred at doses of 3-MNTX (0.1 or 0.3 mg/kg) that did not alter appreciably the response rate in either subject (data not shown).

For these same two monkeys, pretreatment with naltrexone (0.01 mg/kg) attenuated the effects of 3-MNTX, resulting in an overall rightward shift in the dose-response function (Fig. 4, bottom, open squares) and a 12- to 18-fold increase in the ED50 for 3-MNTX (ED50 for M-216 = 0.03 mg/kg 3-MNTX alone, 0.55 mg/kg 3-MNTX + naltrexone; M-426 = 0.03 mg/kg 3-MNTX alone, 0.35 mg/kg 3-MNTX + naltrexone).

**Self-Administration of Cocaine, Heroin, and 3-MNTX.** All monkeys exhibited stable self-administration baselines under the PR schedule with alternating training sessions of cocaine and saline availability. The average number of injections per session (+ S.E.M.) maintained by cocaine were 15 ± 2 under the IRR 100 condition and 12 ± 4 under the IRR 400 condition.
The average number of injections/session (±S.E.M.) maintained by saline were 3 ± 1 under the IRR 100 condition and 1 ± 0 under the IRR 400 condition.

During test sessions, heroin maintained reliable self-administration at one or more doses in each monkey (Fig. 5, left). When the IRR was 100 responses, the average number of injections per session maintained by heroin was significantly different from the number of injections per session maintained by saline at all but the lowest dose tested (Fig. 5, upper left; Dunnett’s test, p < 0.05). When the IRR was increased to 400 responses, the number of injections per session maintained by heroin was significantly different from the number of injections per session maintained by saline at all but the lowest dose tested (Fig. 5, upper left; Dunnett’s test, p < 0.05). Increasing the IRR from 100 to 400 responses resulted in a slight reduction in the maximum number of heroin injections per session, from an average (±S.E.M.) of 11 ± 1 at IRR 100 to an average of 8 ± 2 at IRR 400. The BP_max maintained by heroin, however, increased from 400 responses under the IRR 100 condition to 800 responses under the IRR 400 condition, as the increased demands of the latter condition were met.

In contrast to heroin, 3-MNTX maintained consistent self-administration only under the IRR 100 condition. Under this condition, one or more doses of 3-MNTX maintained responding above saline levels in all monkeys (Fig. 5, upper right). For the group, the average number of injections per session maintained by 0.03 mg/kg/injection of 3-MNTX (10 ± 1) was significantly greater than the number of injections per session maintained by saline (Fig. 5, upper right; Dunnett’s test, p < 0.05). When the IRR was increased to 400 responses, however, 3-MNTX rarely maintained self-administration above the level maintained by saline in any subject (Fig. 5, lower right). For the group, the number of injections per session maintained by 3-MNTX was not significantly greater than the number of injections per session maintained by saline, regardless of dose. The BP_max for 3-MNTX at IRR 100 was 400 responses, identical to the value observed for heroin at IRR 100. Because no dose of 3-MNTX maintained responding significantly above the level maintained by saline under the IRR 400 condition, a meaningful BP_max value could not be calculated.

**Inhibition of Adenylyl Cyclase by μ-Opioid Agonists.**

3-MNTX induced a significant reduction in adenylyl cyclase activity in monkey and rat caudate-putamen homogenates, indicative of agonist activity (Fig. 6; Dunnett’s test, p < 0.05). Significant inhibition of adenylyl cyclase activity also was observed at one or more concentrations of DAMGO, morphine, and M6G (Fig. 6; Dunnett’s test, p < 0.05). Table 1 compares the maximum inhibition induced by the drugs in rhesus monkey and rat caudate-putamen homogenates. The maximum inhibitory effect of 3-MNTX (~24%) did not differ from that of DAMGO (~32%) in the monkey [t(8) = −1.2, N.S.]. In the rat, the maximum inhibitory effect of 3-MNTX (~16%), although significant, was less than that of the prototypical full agonist DAMGO (~37%) [t(7) = 5.9, p < 0.05].

**Discussion**

In the present study, 3-MNTX modulated the DS effects of heroin and the heroin-like DS effects of M6G and morphine in a similar manner. These results corroborate and extend the findings of Bowen et al. (2002), suggesting no unique role for M6G receptors in the DS effects of heroin or M6G. Additional support for this conclusion includes the finding that M6G, like morphine, fully reproduced the DS effects of heroin in monkeys trained to discriminate heroin from vehicle (present study; Platt et al., 2001). M6G also has been found to reproduce the DS effects of morphine in rats trained to discriminate morphine from vehicle (Easterling and Holtzman, 1998). Shared DS effects may indicate pharmacological effects at a common site of action, implying that similar receptor populations mediate the DS effects of heroin, morphine, and M6G.
In an earlier study in rodents, 3-MNTX did not differen-
tially modulate self-administration of heroin compared with
morphine (Walker et al., 1999). The dose of 3-MNTX that
altered self-administration in that study, however, was lower
than the dose required to antagonize the analgesic effects of
morphine. One interpretation of these results is that, in
contrast to analgesia, the reinforcing effects of heroin and
morphine are mediated at least in part at M6G receptors.
Extending this interpretation to the present study, our find-
inings suggest that M6G receptors could contribute equally
to the DS effects of heroin, M6G, and morphine. Bowen et al.
(2002), however, found that similar doses of 3-MNTX atten-
uated both the DS and analgesic effects of heroin and mor-
phine in rhesus monkeys. Collectively, these findings suggest
that the DS and antinoceptive effects of heroin, M6G, and
morphine are mediated predominantly at a common popula-
tion of (presumably \( \mu \))-opioid receptors in rhesus monkeys.

Differences in the effects of 3-MNTX were observed among
individual subjects in our study. In general, in two of four
monkeys, 3-MNTX attenuated the DS effects of heroin, M6G,
and morphine and did not mimic the DS effects of heroin
when tested alone. In the other two monkeys, 3-MNTX en-
hanced the DS effects of heroin, M6G, and morphine and
fully reproduced the DS effects of heroin when tested alone.
Furthermore, in these latter two monkeys, the DS effects of
3-MNTX could be antagonized by a low dose of naltrexone. It
is likely that the behavioral effects of 3-MNTX, like those of
heroin, are mediated at the \( \mu \)- opioid receptor. For example,
in these same monkeys, we have shown previously that the
DS effects of heroin were mimicked by the \( \mu \)-receptor ago-
nants fentanyl and methadone, but not by the \( \delta \)-receptor
agonist SNC 80 nor the \( \kappa \)-receptor agonist spiradoline (Platt
et al., 2001). Additionally, in the two monkeys for which
3-MNTX mimicked the effects of heroin, naltrexone shifted
rightward to approximately the same degree the dose-re-
sponse curves for the heroin-like DS effects of 3-MNTX and
the DS effects of heroin (on average, 15-fold versus 19-fold,
respectively; cf. Platt et al., 2001). Moreover, the observed
individual differences may reflect partial agonist activity of
3-MNTX. A consistent finding with \( \mu \)-opioid agonists is that
the lower the agonist efficacy of a drug, the greater the
individual differences produced by this drug (Colpaert and
Janssen, 1984; Young et al., 1992). Several studies, for example,
have shown that the opioid partial agonists nalbuphine and
nalorphine vary greatly in their ability to reproduce the DS
effects of higher efficacy agonists among individual subjects
trained to discriminate either fentanyl or morphine (Colpaert
and Janssen, 1984; Young et al., 1992; Morgan and Picker,
1996). Moreover, in these studies, it was more likely that a
partial agonist would antagonize the effects of a high-efficacy
agonist if it did not share DS effects with the high-efficacy
agonist. The opposite also was true; a partial
agonist would more likely enhance the effects of a high-
efficacy agonist if it shared DS effects with this high-efficacy

<table>
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<th>TABLE 1</th>
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<td>Opioid-induced inhibition of adenylyl cyclase activity in rhesus monkey and rat caudate putamen. Data are means ( \pm ) S.E.M. from three to five independent experiments, each performed in triplicate. All values are significantly different from baseline as determined by Dunnnett’s ( q ) statistic (( p &lt; 0.05 )).</td>
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<tr>
<th>Compound</th>
<th>( IC_{50} ) (nM)</th>
<th>Maximum Percentage of Inhibition( ^a )</th>
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<tr>
<td></td>
<td>Monkey</td>
<td>Rat</td>
</tr>
<tr>
<td>3-MNTX</td>
<td>49 ± 4</td>
<td>563 ± 444</td>
</tr>
<tr>
<td>DAMGO</td>
<td>59 ± 16</td>
<td>171 ± 81</td>
</tr>
<tr>
<td>Morphine</td>
<td>735 ± 226</td>
<td>425 ± 91</td>
</tr>
<tr>
<td>M6G</td>
<td>300 ± 27</td>
<td>215 ± 73</td>
</tr>
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</table>

\( ^a \) Maximum percentage of inhibition is the mean inhibition produced by a 100 \( \mu \)M concentration of each compound.

Figure 6. Inhibition of adenylyl cyclase activity by 3-MNTX, DAMGO, morphine, and M6G in rhesus monkey and rat caudate putamen homoge-
nates. Data are presented as percentage of basal activity (100%). Data are expressed as mean \( \pm \) S.E.M. from three to five independent experi-
ments, each performed in triplicate.
agonist (Colpaert and Janssen, 1984; Koek and Woods, 1989; Young et al., 1992; Picker et al., 1993). The overall similarity of our results with 3-MNTX compared to results in similar experiments with nalbuphine and nalorphine is consistent with the characterization of 3-MNTX as an opioid partial agonist. Bowen et al. (2002), however, reported that 3-MNTX had primarily opioid antagonist effects in rhesus monkeys trained to discriminate i.m. heroin from vehicle. Although the factors underlying these different findings are not clear, one cannot rule out the contribution of training history (heroin exclusively versus cocaine/heroin combinations) or procedural variables such as route of administration (i.v. versus i.m.) or training dose (0.056 versus 0.1 mg/kg).

To further characterize the effects of 3-MNTX in rhesus monkeys, self-administration of 3-MNTX was compared with that of heroin under a PR schedule with different IRRs. This strategy is particularly useful for comparing the effectiveness of drugs as reinforcers (Winger et al., 1996; Rowlett et al., 2002). We have previously shown, for example, that the partial agonist nalbuphine can maintain self-administration under low but not high IRR conditions, whereas the opioid full agonist alfentanil maintained reliable self-administration under both low and high IRR conditions. In the present study, 3-MNTX maintained levels of behavior reliably above levels maintained by saline when the IRR was relatively low (IRR 100). Moreover, the maximum injections per session and maximum breakpoint engendered by 3-MNTX under these conditions were similar to those engaged by heroin. Under the high IRR requirement, 3-MNTX engaged levels of responding not different from saline, whereas heroin continued to maintain self-administration behavior reliably above levels during saline availability. The largely comparable findings of Rowlett et al. (2002) with nalbuphine provide support for the notion that 3-MNTX can function as a partial agonist under some conditions.

In vitro, µ-opioid agonists inhibit adenylyl cyclase activity in an efficacy-dependent manner, with partial agonists displaying reduced inhibition compared to full agonists (Liu and Prather, 2001). In the present study, we compared the ability of 3-MNTX to inhibit adenylyl cyclase activity with that of prototypical µ-opioid agonists. Our results revealed that, under the specific conditions of the assay, 3-MNTX significantly inhibited adenylyl cyclase activity in both monkey and rat caudate putamen homogenates, indicating agonist activity in this assay. Moreover, as would be expected for a partial agonist, the degree of inhibition induced by 3-MNTX was less than that of the prototypical full agonist DAMGO in the rat. In monkey tissue, 3-MNTX and DAMGO induced similar degrees of adenylyl cyclase inhibition and likely reflects the fact that demonstrations of agonist efficacy frequently are dependent on the conditions of the assay.

In summary, our studies show that 3-MNTX did not selectively alter the DS effects of heroin compared with morphine or M6G, suggesting no unique role for M6G receptors in the DS effects of heroin. Moreover, 3-MNTX displayed a profile of behavioral effects similar to those of prototypical µ-opioid partial agonists. Similar to reported findings with nalbuphine and nalorphine (Colpaert and Janssen, 1984; Young et al., 1992), large individual differences were observed in the degree to which 3-MNTX reproduced the DS effects of heroin. Furthermore, under a PR schedule of i.v. self-administration, 3-MNTX was self-administered reliably when the IRR was low but not when it was high, whereas heroin was reliably self-administered under both IRR conditions. These findings extend our previous observations with nalbuphine and alfentanil and suggest that, like nalbuphine, 3-MNTX has lower reinforcing effectiveness compared to opioid full agonists. Finally, in an in vitro assay of µ-agonist efficacy, 3-MNTX significantly inhibited adenylyl cyclase indicating agonist activity; however, as expected for a µ-opioid partial agonist 3-MNTX inhibited adenylyl cyclase to a lesser degree than the prototypical full agonist DAMGO in the rat.

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References
morphine, activates delta opioid receptors to produce antinociception in Swiss-Webster mice. *J Pharmacol Exp Ther* 268:1222–1231.


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