Discriminative Stimulus Effects of Intravenous Heroin and Its Metabolites in Rhesus Monkeys: Opioid and Dopaminergic Mechanisms

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ABSTRACT

Heroin has characteristic subjective effects that contribute importantly to its widespread abuse. Drug discrimination procedures in animals have proven to be useful models for investigating pharmacological mechanisms underlying the subjective effects of drugs in humans. However, surprisingly little information exists concerning the mechanisms underlying the discriminative stimulus (DS) effects of heroin. This study characterized the DS effects of heroin in rhesus monkeys trained to discriminate i.v. heroin from saline. In drug substitution experiments, heroin, its metabolites 6-monoacetylmorphine, morphine, morphine-6-glucuronide, and morphine-3-glucuronide, and the \( \mu \)-agonists fentanyl and methadone engendered dose-dependent increases in heroin-appropriate responding, reaching average maximums of >80% (full substitution) at doses that did not appreciably suppress response rate. In contrast, the \( \delta \)-agonist SNC 80, the \( \kappa \)-agonist spiradoline, and the dopamine uptake blockers/releasers cocaine, methamphetamine, and GBR 12909 did not engender heroin-like DS effects regardless of dose. In antagonism studies, in vivo apparent \( \mathrm{pA}_2 \) and \( \mathrm{pK}_B \) values for naltrexone combined with heroin, morphine, and 6-monoacetylmorphine (8.0–8.7) were comparable with those reported previously for naltrexone antagonism of prototypical \( \mu \)-agonists. The results show that the DS effects of heroin are pharmacologically specific and mediated primarily at \( \mu \)-opioid receptors. Moreover, the acetylated and glucuronated metabolites of heroin appear to play significant roles in these effects. Despite previous speculation that morphine-3-glucuronide lacks significant opioid activity, it substituted fully for heroin in our study, suggesting that it can exhibit prominent \( \mu \)-agonist effects in vivo.

Heroine is the most widely abused opioid and is associated with the highest mortality of all illicit drugs (United Nations International Drug Control Programme, 1997; Bammer et al., 1999). Although heroin can be smoked or snorted, i.v. injection continues to be the predominant method of use. After i.v. administration, addicts typically describe the subjective experience of heroin as a “rush”, followed by a sense of tranquility, reduced apprehension, and euphoria (Jasinski and Preston, 1986; Comer et al., 1999). Drug discrimination procedures in laboratory animals provide a useful experimental counterpart to measures of subjective effects in humans. Despite the prevalence of heroin abuse, surprisingly few studies have assessed the discriminative stimulus (DS) effects of heroin, and to date, there are no reported investigations of the DS effects of heroin when administered by the i.v. route.

Heroin can be considered a prodrug that is transformed to several metabolites that likely contribute to its behavioral effects (Umans and Inturrisi, 1981; Corrigall and Coen, 1990). After i.v. or other peripheral routes of administration, heroin is rapidly metabolized by sequential deacetylation to 6-monoacetylmorphine (6 MAM) and morphine (Kamendulis et al., 1996). Morphine, in turn, is metabolized via glucuronidation to morphine-6-\( \beta \)-o-glucuronide (M6G) and morphine-3-\( \beta \)-o-glucuronide (M3G) (Glare and Walsh, 1991; Milne et al., 1996). Previous studies have shown that some of these metabolites may contribute to the subjective effects of heroin. For example, both 6 MAM and morphine have been shown to engender heroin-like DS effects in rats trained to discriminate heroin from vehicle by the s.c. route (Corrigall and Coen, 1990). Similarly, M6G has been found to engender drug-appropriate responding in rats trained to discriminate s.c. morphine from vehicle (Easterling and Holtzman, 1998).

Opioid receptors traditionally have been classified into \( \mu \)-, \( \kappa \)-, and \( \delta \)-subtypes (Martin et al., 1976). Although heroin displays little selectivity at these receptor subtypes, the in vivo effects of heroin have been attributed predominantly to \( \mu \)-opioid receptor activation (Bertalmio et al., 1992). In some situations, however, \( \delta \)-opioid receptors also may play a role in

ABBREVIATIONS: DS, discriminative stimulus; 6 MAM, 6-monoacetylmorphine; M6G, morphine-6-\( \beta \)-o-glucuronide; M3G, morphine-3-\( \beta \)-o-glucuronide; DA, dopamine; FR, fixed ratio; DR, drug ratio; CI, confidence interval.
the effects of heroin (Rady et al., 1994; Uchihashi et al., 1996). Rady et al. (1994), for example, demonstrated that the δ-opioid receptor antagonist naltirindole blocked the antinoceptive and locomotor stimulant effects of heroin in rodents. Thus, the first purpose of the present study was to evaluate the role of opioid receptor mechanisms in the DS effects of i.v. heroin. This was accomplished by conducting substitution tests with selective μ-, κ-, and δ-opioid agonists. Additional antagonism studies, in conjunction with in vivo apparent pA2 analysis, were conducted with the opioid antagonist naltrexone.

Compared with other heroin metabolites, M3G binds weakly to opioid receptors (Mignat et al., 1995; Löser et al., 1996). Moreover, M3G exhibits little analgesic activity (Gong et al., 1991; Easterling and Holtzman, 1998) and may have effects on respiration opposite to those of conventional μ-opioid agonists (Gong et al., 1991). For example, Gong et al. (1991) found that administration of morphine or M6G reduced respiratory frequency in anesthetized rats, whereas M3G increased respiratory frequency. Our second purpose, then, was to characterize the role of heroin’s metabolites in its subjective effects by assessing the capacity of the heroin metabolites 6-MAM, morphine, M6G, and M3G to engender heroin-like responding.

Although opioid receptor stimulation undoubtedly plays a key role in the behavioral effects of heroin, several μ-opioid agonists have been shown to stimulate release of dopamine (DA) in mesolimbic brain regions, a mechanism that may also contribute importantly to the behavioral effects of these drugs (Di Chiara and North, 1992; Wise, 1998). Moreover, other compounds that stimulate DA release or block its uptake such as cocaine and methamphetamines have been shown to share DS effects with μ-opioid agonists under certain conditions (Lamas et al., 1998; Platt et al., 1999; Rowlett et al., 2000), raising the possibility that stimulation of DA activity contributes at least indirectly to the DS effects of heroin. Along these lines, Platt et al. (1999) previously found the morphine DS to generalize to cocaine, amphetamine, and the selective DA uptake blocker GBR 12909 in the majority of squirrel monkeys tested. The final purpose of our study, then, was to determine the contribution of DA receptor stimulation to the DS effects of heroin by investigating the ability of prototypic DA releasers and uptake blockers to mimic the DS effects of heroin.

**Materials and Methods**

**Subjects and Surgical Procedure.** Four male rhesus monkeys (Macaca mulatta), weighing 5.7 to 6.8 kg, were studied in daily experimental sessions (Monday to Friday). All were experimentally naive at the beginning of the study. Between sessions, monkeys lived in individual home cages where they had unlimited access to water. Monkeys were maintained at 85 to 90% of their free-feeding body weight by adjusting their access to food in the home cage (Lab Diet 5038; PMI Nutrition International, Inc., Brentwood, MO, supplemented with fresh fruit). 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. National Institutes of Health 85-23, revised 1986. Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Monkeys were prepared with a chronic indwelling venous catheter (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 Polycarbonate primate chairs (Crist Instrument Co., Hagerstown, MD). Two response levers (model ENV-610 M, 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.

**Heroin Discrimination Procedure.** Monkeys were trained to respond on each of two levers under a 10-reinforcer fixed ratio (FR 10) schedule of food reinforcement. Once consistent lever pressing was established, the monkeys were implanted with i.v. catheters, and drug discrimination training was started 2 to 4 days later. The training dose of heroin initially was 0.03 mg/kg for all subjects, but subsequently was increased to 0.1 mg/kg for monkeys M-163 and M-426 to achieve consistent stimulus control of behavior. 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 half of the monkeys, responding on the right lever after an injection of heroin resulted in pellet delivery. For the other monkeys, responding on the left lever after injection of heroin was reinforced. Delivery of each pellet was followed by a 10-s timeout 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 timeout period, during which the lights were off and responses had no programmed consequences, preceded each component. During most training sessions, saline was injected during timeout periods preceding the first n – 1 components, and heroin was injected before 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 outside the chamber via a catheter extension during the 5th min of the 10-min timeout periods. Each injection was followed by a 2-ml infusion of saline to flush the catheter of any residual drug solution.

**Drug Testing Procedure.** Once consistent stimulus control was achieved, drug test sessions were conducted once or twice per week with training sessions scheduled 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 timeout 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 drugs studied were heroin (0.001–0.1 mg/kg), 6-MAM (0.003–0.3 mg/kg), morphine (0.01–0.56 mg/kg), M3G (0.1–10.0 mg/kg), M6G (0.1–10.0 mg/kg), fentanyl (0.0003–0.018 mg/kg), methadone (0.03–1.0 mg/kg), SNC 80 (0.003–0.3 mg/kg), spiradoline (0.0003–0.03 mg/kg), cocaine (0.01–1.0 mg/kg), GBR 12909 (0.001–0.1 mg/kg), naloxone (0.003–0.3 mg/kg), naltrexone (0.03–1.0 mg/kg), and fentanyl (0.0003–0.018 mg/kg).
12909 (0.1–1.0 mg/kg), and methamphetamine (0.01–0.3 mg/kg). Under the cumulative dosing procedure, incremental doses of each drug (1/4–1/2 log increments) were injected i.v. during timeout periods that preceded sequential FR components, permitting a four-point cumulative dose-response function to be determined in a single session. When warranted, 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 produced adverse effects were usually studied only once in each subject. Antagonist studies were conducted by administering naltrexone (0.003–0.1 mg/kg i.m.) 5 min before the session, followed by cumulative doses of heroin, 6 MAM, morphine, M6G, and M3G as described above. In addition to establishing a cumulative dose-response function for heroin, a conventional single-dose testing procedure with varying pretreatment times (5, 20, 60, 160, and 320 min) was used to determine the time course of the DS effects of heroin (M-164 and M-216, 0.03 mg/kg heroin; M-163 and M-426, 0.1 mg/kg heroin).

Analysis of Drug Effects. 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. Percentage of heroin-lever responding was calculated for an individual monkey only if the 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 of monkeys at each dose. A drug was considered to substitute fully for heroin if the maximum percentage of drug-lever responding was ≥80%.

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 (percentage of control ± S.E.M.) was then calculated for the group at each dose.

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. In experiments involving interactions of heroin and naltrexone, in vivo apparent pA2 analysis was conducted using Schild analysis, with drug dose (mol/kg) substituted for drug concentration (Takemori, 1974). A Schild function was constructed by plotting the relationship between dose of naltrexone (expressed as \(-\log[\text{mol/kg}]\)) and log(DR − 1), where DR is the dose ratio (ED50 for heroin plus naltrexone/ED50 for heroin alone). Linear regression analysis was performed to test whether the slope differed reliably from −1.0, which would imply a violation of the assumption of unity (Tallarida et al., 1979). The apparent pA2 was defined as the x-intercept of the function if the slope did not differ reliably from −1.0.

In experiments involving interactions between the heroin metabolites and naltrexone, a single dose of naltrexone (0.01 mg/kg) was tested in combination with varying doses of each metabolite due to limited amounts of the latter drugs. In these cases, apparent pKb values for naltrexone were determined by the method of Tallarida et al. (1979) as modified by Negus et al. (1993). Like pA2, apparent pKb provides an estimate of the apparent in vivo dissociation constant for the antagonist and is defined by the equation pKb = −log[B/DR − 1], where B is the dose of the antagonist in moles per kilogram.

Drugs. Heroin hydrochloride, 6-monacetylmorphine base, morphine-6-β-d-glucuronide base, and morphine-3,6-β-d-glucuronide base were provided by the National Institute of Drug Abuse (Rockville, MD). Other drugs were purchased from commercial sources: morphine sulfate, fentanyl citrate, spiradoline mesylate, (+)-methamphetamine hydrochloride, naltrexone hydrochloride, GBR 12909 di-hydrochloride, and cocaine hydrochloride (Sigma Chemical, St. Louis, MO); (±)-methadone hydrochloride (Sandoz, Basel, Switzerland); and SNC 80 base (Tocris Cookson, Ballwin, 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.

Results

Intravenous Heroin Discrimination. Two monkeys (M-164 and M-216) acquired the i.v. heroin (0.03 mg/kg) discrimination after 50 and 98 sessions, respectively. The remaining two subjects (M-163 and M-426) initially acquired the i.v. heroin discrimination after 54 and 97 sessions, respectively, but then required additional training, for an average of at least 15 sessions, with 0.1 mg/kg heroin to maintain consistent stimulus control of behavior. Despite the different training doses (0.03 versus 0.1 mg/kg), no systematic differences in drug effects were noted during the study, and consequently, all data are presented as means for the group of four subjects.

During training sessions on days immediately before test sessions, individual monkeys made between 80 to 100% of responses (mean ± S.E.M. = 97 ± 1) on the heroin-associated lever after injections of heroin and 0 to 20% (mean ± S.E.M. = 2 ± 1) of responses on the heroin-associated lever after injections of saline. Rates of responding during training sessions were similar after injections of heroin (mean responses/s = 0.91 ± 0.16) and injections of saline (mean responses/s = 0.93 ± 0.12).

Experiments in which the heroin pretreatment time was varied showed that the percentage of heroin-lever responding after 0.03 and 0.1 mg/kg heroin was maximal (99–100%) 5 min after i.v. injection. In addition, both doses of heroin engendered >80% (i.e., full substitution) heroin-lever responding up to 60 min after i.v. injection. By 160 min postinjection, however, both doses of heroin engendered <20% heroin-lever responding (saline-like levels). No consistent effects of either dose of heroin on response rate were observed at any pretreatment time (data not shown).

In substitution experiments using the cumulative dosing procedure, heroin (0.001–0.1 mg/kg) engendered dose-dependent increases in the percentage of responses on the heroin-associated lever (Fig. 1, top; filled circles) with full substitution occurring at doses ≥0.03 mg/kg. The average response rate was not affected systematically by heroin over the range of doses tested, and no dose of heroin decreased the response rate to <50% of the control rate (Table 1).

Pretreatment with naltrexone resulted in dose-dependent shifts to the right in the dose-response function for the DS effects of heroin (Fig. 1, top; open symbols). Increasing the dose of heroin could surmount the antagonism produced by each dose of naltrexone. Response rate was unaffected by any heroin/naltrexone combination (data not shown). In vivo apparent pA2 analysis revealed values of 8.3 (lower CI = 7.5, upper CI = 9.2) for antagonism of the DS effects of heroin by naltrexone (Fig. 1, bottom). The slope of the Schild plot (−1.2; lower CI = −2.1, upper CI = −0.28) was not reliably different from −1.0, indicating that the assumption of unity was not violated. The slope, however, was reliably different from zero, indicating a statistically reliable relationship between log(DR − 1) and the dose of naltrexone.
Fig. 1. Percentage of heroin-lever responding (mean ± S.E.M.) engendered by heroin in the presence and absence of naltrexone in rhesus monkeys (n = 4) trained to discriminate heroin from saline (top). Naltrexone was administered i.m. 5 min before sessions in which cumulative doses of heroin were tested. Points above Sal show the effects of saline alone or combined with different doses of naltrexone. The bottom panel shows a Schild plot for naltrexone antagonism of the DS effects of heroin. DR is the E_{D50} for heroin plus naltrexone divided by the E_{D50} for heroin alone. ●, heroin alone; □, +0.003 naltrexone; △, +0.01 naltrexone; ▽, +0.03 naltrexone; ○, +0.1 naltrexone.

**Effects of Metabolites of Heroin.** The deacetylated metabolites of heroin, 6-MAM and morphine, had DS effects that were qualitatively similar to those of heroin (Fig. 2, top, closed symbols). Increasing cumulative doses of both 6-MAM and morphine engendered dose-related increases in the percentage of responses on the heroin-associated lever, with one or more doses substituting fully for heroin in 3 of 4 monkeys (6 MAM) and 4 of 4 monkeys (morphine). As in the case of heroin, these effects were observed after administration of doses of 6 MAM and morphine that did not markedly alter response rate (Table 1).

Pretreatment with naltrexone (0.01 mg/kg) antagonized the DS effects of both 6-MAM and M6G (Fig. 2, bottom; open symbols). It was, however, not possible to determine the extent to which antagonism of the DS effects of M3G and M6G could be surmounted. In the case of M3G, higher doses were not tested due to the possibility of inducing seizures (Pasternak et al., 1987). Higher doses of M6G could not be tested due to insufficient quantities of the compound available at the time of the study. In combination with naltrexone, M3G, M6G, and M6G had little or no effect on response rate (data not shown).

**Effects of Other Opioids, DA Release, and DA Uptake Blockers.** Fentanyl, an agonist with high selectivity for μ- and κ-opioid receptors, as well as methadone, a moderately selective μ-opioid receptor agonist, had DS effects that were qualitatively similar to those of heroin (Fig. 3). Increasing cumulative doses of fentanyl and methadone engendered dose-related increases in the percentage of responses on the heroin-associated lever, with full substitution for heroin observed in each subject at doses that did not markedly alter response rate (Table 1). In contrast, the selective κ-agonist spiradoline and the selective δ-agonist SNC 80 did not engender consistent responding on the heroin-associated lever in any subject, regardless of dose (Fig. 3). Spiradoline produced an average maximum of 33% heroin-lever responding, and SNC 80 engendered an average maximum of 19% heroin-appropriate responding at doses that severely reduced responding (Table 1).

The DA transport blockers cocaine and GBR 12909, as well as the DA releaser methamphetamine, did not share DS effect with heroin. Across the dose ranges tested, none of these compounds engendered more than 13% heroin-lever responding in any subject (Table 1). The highest dose of cocaine (1.0 mg/kg) reduced responding to approximately 5% of control in all animals. The highest doses of methamphetamine (0.3 mg/kg) and GBR 12909 (1.0 mg/kg) were associated with more modest reductions in average response rate (71 and 54%, respectively; Table 1). Higher doses of the latter drugs were not tested, however, because of adverse effects (including rapid eye, head, and limb movements) and the elimination of lever pressing in at least one monkey.

**Discussion**

Despite the well-characterized subjective effects of heroin in humans and the recognized relevance of these effects for heroin addiction, comparatively little is known about the mechanism of action and time course of the DS effects of heroin in animals. In the present study, i.v. heroin was...
successfully established as a DS in nonhuman primates. Consistent with clinical reports of peak plasma levels of heroin occurring 5 min postinjection (Jenkins et al., 1994), the onset of the DS effects of heroin in monkeys was rapid and endured for 1 to 2 h.

Role of Metabolites in DS Effects of Heroin. The deacetylated metabolites of heroin, 6 MAM and morphine, as well as the glucuronated metabolites, M6G and M3G, substitute fully for the DS effects of heroin. The rank order of potency for heroin and its metabolites to engender heroin-lever responding was heroin > 6 MAM > morphine > M3G ≥ M6G. This order is consistent with the rank order of potency observed for these opioids in other in vivo procedures (e.g., antinociception, respiratory depression; Umans and Inturrisi, 1981; Corrigall and Coen, 1990; Easterling and Holtzman, 1998). The observed potency differences, however, do not correspond to differences in binding affinity at the μ-opioid receptor because 6 MAM, morphine, and M6G have higher affinities at this receptor than does heroin (Bertalmio et al., 1992; Mignat et al., 1995). More likely, the potency differences reflect the ability of these compounds to penetrate the central nervous system. For example, in contrast to heroin and 6 MAM, morphine, M6G, and M3G penetrate the central nervous system more slowly after peripheral administration (Umans and Inturrisi, 1981; Bickel et al., 1996; Wu et al., 1997). The observation that several of the metabolites of heroin exhibit relatively slow brain penetration, yet high affinity at μ-opioid receptors, may explain why the DS effects of heroin persist for 1 to 2 h, even though it is metabolized rapidly itself (Jenkins et al., 1994).

Heroin-Like Effects of Morphine-3-β-D-Glucuronide. M3G engendered full heroin-like DS effects in all animals. Moreover, the heroin-like DS effects of M3G could be blocked by the opioid antagonist naltrexone, indicating that these effects were mediated via stimulation of opioid receptors. These findings were unexpected in light of previous reports that M3G binds only weakly at μ-opioid receptors (Löser et al., 1996) and exhibits limited μ-agonist activity. For example, M3G has been shown to engender only low levels of drug-lever responding in rats discriminating i.m. morphine from saline (Easterling and Holtzman, 1998). However, recent studies have provided evidence for μ-agonist effects of M3G in vitro. For example, in human neuroblastoma cells, M3G has been shown to inhibit cAMP formation to the same extent as morphine, and this inhibitory effect of M3G was antagonized by the opioid antagonist naltrexone (Baker et al., 2000a). In a related study, Baker et al. (2000b) also demonstrated μ-agonist effects of M3G using voltage-clamp techniques to measure opioid receptor-activated channel responses. Collectively, these results suggest that M3G possesses significant μ-agonist activity in functional assays.

The substitution of M3G for heroin was not due to lack of specificity of the assay because selective δ- and κ-opioid agonists, as well as DA releasers and uptake blockers did not engender appreciable levels of heroin-lever responding. It remains possible, however, that the heroin-like DS effects of M3G were the result of biotransformation of M3G to morphine. It has been shown, for example, that M3G can undergo biotransformation via enterohepatic recirculation whereby intestinal microflora cleave the glucuronide conjugate bond and release morphine back into circulation (Bartlett and Smith, 1995). In the present study, it is likely that only a portion of M3G was reverse-metabolized to morphine because M3G was about 6-fold less potent than morphine with respect to producing heroin-like DS effects. If M3G were biotransformed completely into morphine, based on relative binding affinities, one would expect M3G to exhibit a similar potency as morphine.

Opioid Mechanisms in DS Effects of Heroin. Although some of the effects of heroin have been attributed to δ-opioid receptor stimulation (Rady et al., 1994; Uchihashi et al., 1996), heroin is thought to exert its behavioral effects primarily via stimulation of μ-opioid receptors. In the present study, several lines of evidence support a primary role for μ-opioid receptors in the transduction of the DS effects of heroin. First, the DS effects of heroin were antagonized in a dose-dependent manner by relatively low doses of naltrexone. Increasing the dose of heroin surmounted this antagonism, resulting in rightward shifts in the heroin dose-response function. Schild analysis of these studies was consistent with competitive antagonism at a single receptor population (Tallarida et al., 1979). Moreover, the in vivo apparent pA2 value of 8.3 obtained in the present study is similar to apparent pA2 values obtained previously in rhesus

### Table 1

Effects of opioids and DA uptake blockers/releasers in rhesus monkeys trained to discriminate i.v. heroin from saline

<table>
<thead>
<tr>
<th>Compound</th>
<th>Maximum Heroin-Lever Responding %</th>
<th>ED50 mg/kg</th>
<th>Maximum Suppression of Response Rate % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heroin</td>
<td>100.0 ± 0</td>
<td>0.02 ± 0.01</td>
<td>86.0 ± 10.0 [0.1]a</td>
</tr>
<tr>
<td>Metabolites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 MAM</td>
<td>87.1 ± 12.5</td>
<td>0.09 ± 0.05</td>
<td>72.7 ± 13.7 [0.3]</td>
</tr>
<tr>
<td>Morphine</td>
<td>95.5 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>71.9 ± 16.1 [0.56]</td>
</tr>
<tr>
<td>M3G</td>
<td>99.5 ± 0.4</td>
<td>3.4 ± 1.7</td>
<td>87.2 ± 12.0 [10.0]</td>
</tr>
<tr>
<td>M6G</td>
<td>80.0 ± 20.0</td>
<td>5.5 ± 2.4</td>
<td>91.3 ± 8.8 [10.0]</td>
</tr>
<tr>
<td>Selective opioids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fentanyl</td>
<td>100.0 ± 0</td>
<td>0.003 ± 0.003</td>
<td>77.4 ± 1.4 [0.018]</td>
</tr>
<tr>
<td>Methadone</td>
<td>100.0 ± 0</td>
<td>0.02 ± 0.01</td>
<td>90.6 ± 2.6 [1.0]</td>
</tr>
<tr>
<td>Spiradoline</td>
<td>33.2 ± 33.2</td>
<td>—b</td>
<td>8.3 ± 5.1 [0.03]</td>
</tr>
<tr>
<td>SNC 80</td>
<td>18.6 ± 15.5</td>
<td>—b</td>
<td>20.7 ± 1.5 [0.3]</td>
</tr>
<tr>
<td>DA uptake blockers/releasers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>0 ± 0</td>
<td>—b</td>
<td>71.0 ± 33.0 [0.3]</td>
</tr>
<tr>
<td>Cocaine</td>
<td>13.0 ± 1.0</td>
<td>—b</td>
<td>5.2 ± 4.8 [1.0]</td>
</tr>
<tr>
<td>GBR 12909</td>
<td>0 ± 0</td>
<td>—b</td>
<td>54.2 ± 11.5 [1.0]</td>
</tr>
</tbody>
</table>

a Numbers in square brackets indicate dose (mg/kg) that induced maximum suppression of responding.
b Heroin-lever responses <50%.
monkeys treated with naltrexone combined with either morphine or heroin (8.3, France et al., 1990; 8.0, Rowlett et al., 1998). Finally, in substitution studies, the DS effects of heroin were mimicked fully by the \textsuperscript{\textbeta}opioid agonists fentanyl and methadone at doses that did not alter rates of responding. Another \textsuperscript{\textmu}-opioid agonist, dihydroetorphine, also has been found to substitute fully for the DS effects of heroin in rats (Beardsley and Harris, 1997). In contrast to these consistent findings, neither the \textsuperscript{\textkappa}-selective agonist SNC 80 nor the \textsuperscript{\textdelta}-opioid agonist spiradoline engendered heroin-like DS effects at any dose. These findings suggest that \textsuperscript{\textmu}- but not \textsuperscript{\textdelta}- or \textsuperscript{\textkappa}-opioid receptor mechanisms play a principal role in transduction of the interoceptive effects of heroin.

A comparison of in vivo apparent pK\textsubscript{B} values for 6 MAM (8.7) and morphine (8.0) with the apparent pA\textsubscript{2} value for heroin (8.3) in our study suggests that the heroin-like DS effects of these metabolites also were mediated predominately via \textsuperscript{\textmu}-opioid receptors and corroborates existing evidence that the DS effects of morphine are mediated principally at these sites. For example, in squirrel monkeys trained to discriminate morphine from vehicle, \textsuperscript{\textmu}-opioid agonists such as fentanyl fully mimic the DS effects of morphine, whereas \textsuperscript{\textkappa}- and \textsuperscript{\textdelta}-opioid agonists fail to engender substantial morphine-lever responding (Platt et al., 1999).

**DA Mechanisms in DS Effects of Heroin.** Although \textsuperscript{\textmu}-opioid agonists have been found to augment release of DA in the nucleus accumbens and other brain regions implicated in the effects of abused drugs (Di Chiara and North, 1992; Wise, 1998), we found no evidence that either blockade of DA reuptake or enhancement of DA release by cocaine, GBR 12909, or methamphetamine played a major role in the DS effects of i.v. heroin. In this regard, cocaine, methamphetamine, and GBR 12909 consistently failed to engender heroin-lever responding regardless of dose in any subject. This finding differs from other studies (Lamas et al., 1998; Platt et al., 1999) that have found cocaine to substitute for the DS effects of heroin or morphine in some individual rats or squirrel monkeys. Collectively, these findings imply a possible role for dopaminergic mechanisms underlying the DS effects of i.m. morphine and i.p. heroin in at least some

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**Fig. 2.** Percentage of heroin-lever responding (mean ± S.E.M.) for 6 MAM, morphine, M3G, and M6G in the presence and absence of 0.01 mg/kg naltrexone in rhesus monkeys (n = 4) trained to discriminate heroin from saline. Naltrexone was administered i.m. 5 min before sessions in which cumulative doses of 6 MAM, morphine, M3G, and M6G were tested: ●, alone; ○, +0.01 naltrexone.
Fig. 3. Percentage of heroin lever responding (mean ± S.E.M.) for fentanyl, spiradoline, SNC 80, and methadone in rhesus monkeys (n = 4) trained to discriminate heroin from saline. Points above Sal show the effects of saline, †, methadone; ‡, fentanyl; ‡‡, spiradoline; ◊, SNC 80.

subjects, but not the DS effects of i.v. heroin. Although the factors underlying these different findings are not clear, one cannot rule out species or procedural differences (route of administration). Interestingly, Negus et al. (1998) recently reported that several μ-opioids including heroin substituted for the DS effects of cocaine in about one-half of the rhesus monkeys trained to discriminate cocaine from vehicle. Taken together, our findings and those of Negus et al. (1998) raise the possibility that there exists an asymmetrical generalization profile for stimulants and opioids in rhesus monkeys. It is not unprecedented that we should observe asymmetrical generalization. For example in other species, the DS effects of barbiturates generalize to benzodiazepines, but the DS effects of benzodiazepines do not generalize to barbiturates (Astor and Griffiths, 1989).

Summary. To our knowledge, the present study provides the first demonstration that intravenously administered heroin can be established as a DS in nonhuman primates. Because this route of administration is the predominant method of abuse by heroin addicts, the i.v. heroin discrimination may be a useful procedure for establishing mechanisms of action underlying the subjective effects of heroin in people. In the present study, the DS effects of i.v. heroin were largely attributable to stimulation of μ-opioid receptors, rather than stimulation of δ- or κ-opioid receptors, or DA activity. Unexpectedly, M3G, a metabolite of heroin previously thought to have little opioid activity, occasioned full heroin-like DS effects. Similarly, 6-MAM, morphine, and M6G also shared DS effects with heroin, suggesting that the acetylated and glucuronide metabolites of heroin contribute significantly to the transduction of the DS effects of heroin.

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Martin WR, Eades CG, Thompson JA, Huppler RE, and Gilbert PE (1976) The effects of fentanyl, SNC 80, and methadone in rhesus monkeys (n = 4) trained to discriminate heroin from saline. Points above Sal show the effects of saline, †, methadone; ‡, fentanyl; ‡‡, spiradoline; ◊, SNC 80.

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References


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