Self-Administration of Cocaine-Heroin Combinations by Rhesus Monkeys: Antagonism by Naltrexone

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ABSTRACT

Low, nonreinforcing doses of heroin have been shown to shift the dose-response function of cocaine leftward in rhesus monkeys trained under a progressive-ratio schedule of i.v. drug injection. Our study sought to determine 1) whether a reciprocal enhancement of heroin self-administration would be observed when heroin was combined with low, nonreinforcing doses of cocaine, and 2) whether self-administration of cocaine-heroin combinations could be antagonized by the opioid antagonist naltrexone. Rhesus monkeys (n = 4) were prepared with i.v. catheters and trained to self-administer cocaine under a progressive-ratio schedule. The initial response requirement of this schedule was fixed-ratio 120, which doubled across the session to a maximum of 1920. Injections were separated by a time-out of 30 min. A maximum of 1920 injections/session was allowed. Injections/session produced by drug alone; FR, fixed-ratio; I max, maximum injections/session, reinforcing efficacy; LH, limited hold; PR, progressive-ratio; TO, time-out.

Injections of 2.5 mg/kg/injection of heroin (4.6–25 mg/kg/injection) either increased to a peak and then decreased or reached an asymptote. When nonreinforcing doses of cocaine (3.2–25 μg/kg/injection) were combined with heroin, the heroin dose-response function was shifted to the left, without change in maximum injections/session. Presession treatments with naltrexone (3.2–1600 μg/kg, i.m., 10-min presession) antagonized self-administration of heroin and heroin + cocaine combinations in a dose-dependent fashion. However, naltrexone treatment had no effect on cocaine self-administration. Antagonism by naltrexone of self-administration of heroin and heroin + cocaine was surmounted by increasing the dose of heroin alone or in the heroin + cocaine combination. In vivo apparent pA2 and pKb analyses of these data revealed values of approximately 8.0, consistent with a role for mu opioid receptors in the self-administration of heroin and cocaine-heroin (i.e., “speedball”) combinations.

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Many polydrug abusers inject cocaine in combination with heroin by mixing the drugs in solution and injecting them simultaneously. This combination is referred to as a “speedball,” and the abuse of speedballs has increased worldwide along with the rise in cocaine and heroin abuse (Darke and Hall, 1995; Frank and Galea, 1996). In the United States, recent epidemiological findings show that up to 92% of polydrug abusers also inject cocaine (Office of National Drug Control Policy, 1997). Clinical observations have revealed that speedball abuse is associated with a higher incidence of psychopathology and a greater risk for contracting AIDS than either cocaine or heroin abuse alone (Battjes et al., 1994; Meandzija et al., 1994; Brooner et al., 1997).

Despite the prevalence and detrimental consequences of speedball abuse, relatively little is known concerning the pharmacological mechanisms underlying this form of polydrug abuse. Mechanisms that have been advanced to account for combined cocaine-opioid abuse have been based primarily on anecdotal observations from polydrug abusers, and include mutual enhancement of effects of the individual drugs or reduction in aversive effects of the drugs when combined (Tutton and Crayton, 1993). Empirically based hypotheses have been developed from recent controlled clinical studies evaluating the subjective effects of cocaine in combination with mu opioid agonists. The subjective effects of various dose combinations of cocaine and mu agonists were greater than those produced by either drug alone on key measures, such as positive ratings of “drug liking” and “high” (e.g., Foltin and Fischman, 1992; Walsh et al., 1996). Consistent with these findings, results with drug discrimination procedures, generally considered to be predictive of subjective

ABBREVIATIONS: AIDS, acquired-immune deficiency syndrome; CI, confidence interval; ED50, dose that produced 50% of the maximum injections/session produced by drug alone; FR, fixed-ratio; Imax, maximum injections/session, reinforcing efficacy; LH, limited hold; PR, progressive-ratio; TO, time-out.
effects in people, have shown that morphine and similar drugs can enhance the discriminative stimulus effects of cocaine in monkeys and rats (Speelman and Bergman, 1992, 1994; Suzuki et al., 1997; but see Broadbent et al., 1995). Taken together, these findings suggest that administration of mu agonists enhance the subjective and discriminative stimulus effects of cocaine, which in turn may play a role in the high prevalence of speedball abuse.

A preclinical approach for studying self-administration of cocaine-opioid combinations is to mimic the typical pattern of speedball abuse by people, who characteristically mix the two drugs and inject them i.v. Using this approach, Rowlett and Woolverton (1997) demonstrated a leftward shift in the dose-response function of cocaine when combined with relatively low, nonreinforcing doses of heroin in monkeys trained under a PR schedule of i.v. drug injection. In another study, Mello et al. (1995) demonstrated that combination of relatively high heroin doses with lower, nonreinforcing doses of cocaine resulted in an increase in self-administration. Both Mello et al. (1995) and Hemby et al. (1996), however, demonstrated that self-administration of higher dose combinations of cocaine and heroin was reduced relative to cocaine alone. Collectively, the results of these studies suggest that combining cocaine and heroin results in an enhancement of self-administration relative to the component drugs alone, at least when relatively low doses are combined, probably via enhanced reinforcing effects.

Preclinical characterization of the abuse-related effects of cocaine-opioid combinations should allow collection of meaningful data regarding the understanding of the pharmacological basis of speedball abuse. A useful method for investigating pharmacological mechanisms underlying behavioral effects is to conduct antagonism studies (see Mello and Negus, 1996, for review). Few studies have assessed antagonism of the abuse-related effects of cocaine-opioid combinations. Recently, Walsh et al. (1996) examined the ability of the general opioid antagonist naltrexone to attenuate subjective effects of cocaine-hydromorphone combinations. Naltrexone blocked the subjective effects of hydromorphone, but not cocaine, and attenuated hydromorphone-induced enhancement of cocaine’s effects (Walsh et al., 1996). Regarding self-administration of speedballs, Hemby et al. (1996) demonstrated a reduction of heroin-induced changes in cocaine self-administration by naltrexone. As mentioned, however, cocaine-heroin interactions were studied at relatively high doses that resulted in decreased self-administration compared to the constituent drugs alone. At this time, the extent to which opioid receptors are involved in the self-administration of low dose cocaine-heroin combinations in which self-administration is enhanced is not known. In addition, no studies have included quantitative analysis of naltrexone antagonism (i.e., in vivo apparent pA2 analysis) to determine the possible involvement of mu vs. other subtypes of opioid receptors.

Because our earlier experiments revealed an enhancement of cocaine self-administration by otherwise inactive doses of heroin (Rowlett and Woolverton, 1997), the present study sought to extend this finding by determining whether a reciprocal enhancement of heroin self-administration would result when combined with ineffective doses of cocaine. The role of opioid receptors in the self-administration of cocaine-heroin combinations was assessed by conducting antagonism studies with the opioid receptor blocker naltrexone. The present experiments extended the results of Hemby et al. (1996) by evaluating low instead of high dose combinations and by quantitatively assessing the role of opioid receptor subtypes by use of in vivo apparent pA2/pK2 analyses (cf., Negus et al., 1993). A PR schedule of drug injection similar to the one used by Rowlett and Woolverton (1997) was used, which allowed examination of potential changes in the relative reinforcing potency (lateral shifts in dose-response functions) and reinforcing efficacy (maximum responding maintained by a drug) of heroin after combination with cocaine and/or naltrexone (Rowlett et al., 1996; Rowlett and Woolverton, 1997).

Materials and Methods

Animals and apparatus. Subjects were one adult female (monkey 11084) and three adult male (monkeys RLk2, 9002, AP13) rhesus monkeys (Macaca mulatta) with body weights between 6.1 and 12 kg during the course of the experiments. One monkey (AP13) was experimentally naive at the beginning of the studies. Monkeys RLk2 and 9002 participated in the previous study involving self-administration of cocaine and heroin combinations (Rowlett and Woolverton, 1997), whereas monkey 11084 had a history of self-administration of i.v. cocaine and selective D1 dopamine agonists under a fixed-ratio schedule. For the present experiments, each monkey was fitted with a stainless-steel restraint harness and tether which was attached to the rear of an experimental cubicle (90 cm wide × 90 cm deep × 90 cm high) in which the monkey lived for the duration of the studies. Two response levers (BRS/LVE, PRL-001, Beltsville, MD) were mounted on the inside of the transparent front of each cubicle, 10 cm above the floor. Four jeweled stimulus lights, two red and two white, were mounted directly above each lever. Drug injections were delivered by a peristaltic infusion pump (Cole-Parmer Co., Chicago, IL) located outside the cubicle. All programming and recording of experimental events was accomplished using a Macintosh II computer and associated interfaces located in an adjacent room. Water was available continuously and each monkey was fed, between 7:00 and 7:30 A.M. each morning, a sufficient amount of monkey chow (Ralston-Purina Co., St. Louis, MO), fresh fruit and vegetables to maintain a stable body weight. In addition, each monkey was given a chewable vitamin tablet 3 days per week. Experiments were conducted during the light cycle (lights on 7:00 A.M. to 10:00 P.M.), 7 days per week.

General procedure. After adapting to the cubicle and restraint system, each monkey received injections of a combination of ketamine (1.0 mg/kg, i.m.) and atropine (.04 mg/kg, i.m.) followed in 20 to 30 min by inhaled isoflurane. When anesthesia was adequate, a catheter was surgically implanted into a major vein. For femoral and jugular (internal and external) veins, a silicone catheter (.076 cm, i.d., .26 cm o.d.; Cole-Parmer Co.) was used. For brachial veins, the catheter was Micro-Renethane (.1 cm i.d., .2 cm o.d.; Braintree Scientific, Braintree, MA) drawn to a tapered tip after heating. An antibiotic was injected locally to the incision site and was administered i.m. twice daily for 7 days to prevent infection.

After surgery the monkey was returned to the cubicle and the catheter was threaded through the tether, out the back of the cubicle and connected to the infusion pump. If a catheter became nonfunctional during the experiment, a new catheter was implanted as described above following a 1- to 2-wk period to allow any infection to clear. Catheters were filled with drug solution 30 min before the session and with a solution of 20 μl heparin after the session to prevent clotting at the catheter tip. A short-acting barbiturate (methohexital, 3.0 mg/kg, i.v.) was administered periodically to assess catheter patency. Catheters were considered to be patent if evidence of anesthesia occurred within 30 sec of the injection.

Details of the PR procedure have been reported previously (Rowlett et al., 1996). Experimental sessions, signaled by the illumination of the white lever lights, started at noon each day. During
initial sessions, one lever press on the right lever resulted in an injection of cocaine (100 μg/kg/injection administered over 10 sec). The white lights were turned off and the red lights were illuminated during drug infusion. Once the monkey began pressing the lever, the PR schedule was initiated with a TO, in which the lights were extinguished and responding had no programmed consequences, and a LH, in which a preset length of time was allowed to complete a response requirement, equal to 60 sec. The response requirements and TO/LHs were gradually increased to the terminal conditions of the PR schedule. The PR schedule consisted of five components, each made up of four trials (i.e., 20 trials total). Each trial within a component had the same response requirement. Thus, the response requirement in the first component was PR 120 and doubled in successive components to a maximum of PR 1920 in the fifth component. A trial ended with an injection or the expiration of a 15-min LH. After an injection or expiration of the LH, all stimulus lights were extinguished and responding had no consequences during a 30-min TO. A session ended when the response requirement was not met within the LH for two consecutive trials or when all 20 trials had been completed. Thus, in order to complete a component and progress to the next FR, at least two trials had to be completed within the component.

Once the final conditions of the PR schedule were reached, a single-alternation procedure was initiated in which the maintenance dose of cocaine (100 μg/kg/injection) was available alternating with sessions of saline availability. Training under this single-alternation sequence occurred until a 6-day sequence was achieved in which 1) injections/session for saline were 5 or less for three saline sessions, 2) mean injections/session for saline were ≥ one injection/session with no upward or downward trends for three saline sessions in the sequence, and 3) mean injections/session for cocaine were ≥ one injection/session with no upward or downward trends for three cocaine sessions in the sequence. Once the above criteria were met, testing began according to the following four-session cycles: DSTD, SDTD; where “D” represents the maintenance drug, “S” equals saline and “T” represents a test session. The sequences for the four-session cycles were chosen so that each test session was preceded by either drug or saline with equal frequency and so that sessions with the maintenance drug occurred after the test session. This latter condition was used to assess if any effects of the treatments incurred during the test session carried over to the day after testing. Test sessions were conducted if injections/session during the two preceding sessions were within the range of the previously determined stable performance. If these criteria were not met, testing was stopped and the monkey was returned to the single-alternation sequence until the 6-day criteria described above were again met. Each dose and dose combination condition was determined twice. Second determinations occurred in consecutive 4-session cycles for approximately half of the conditions, for the remaining conditions the second determination occurred after intervening test sessions with different doses or dose combinations.

Cocaine-heroin combination experiments. To compare self-administration of cocaine-heroin combinations to self-administration of the drugs singly, dose-response functions for cocaine and heroin alone were determined initially for each monkey. On intervening sessions (i.e., sessions 1, 2 and 4 of the four-session cycle), saline, doses of cocaine (100 μg/kg/injection) or heroin (6.4 μg/kg/injection) were available. The doses of cocaine and heroin were determined previously to maintain maximum injections/session and breakpoints under the PR sequence used in this study (Rowlett and Woolverton, 1997). Saline or various doses of cocaine alone (6.4–100 μg/kg/injection) or heroin alone (1.6–25 μg/kg/injection) were made available during test sessions (session 3 of the four-session cycle). These doses were chosen based on our previous study to represent primarily ascending limb doses of both drugs under the conditions of this study (Rowlett and Woolverton, 1997).

To assess the effects of combining cocaine with heroin, dose-response functions for heroin were redetermined in the presence of low, non-reinforcing doses of cocaine. On intervening sessions, behavior was maintained by saline, 6.4 or 13 μg/kg/injection heroin (the dose of heroin depended on the monkey). Because marked interanimal differences were found in our previous study for combinations of cocaine and heroin that resulted in enhanced self-administration (Rowlett and Woolverton, 1997), the dose of cocaine to be mixed with heroin was determined in the monkeys on an individual basis. Initially, a dose of cocaine was chosen, based on initial dose-response determinations, that was 4-fold below the lowest dose that maintained behavior above saline levels. This cocaine dose then was combined with the lowest dose of heroin tested that did not maintain behavior above saline levels. Next, the dose of cocaine in the mixture was either increased or decreased on an individual basis in each monkey until responding maintained by the combination was above saline levels for two test sessions. Finally, holding the nonreinforcing dose of cocaine constant, the dose of heroin in the combination was decreased until responding was at saline levels and increased until responding reached a peak or asymptote. In this fashion, a dose-response function for the cocaine-heroin combination was constructed in each monkey with fixed and relatively low doses of cocaine plus varying doses of heroin.

Naltrexone antagonism experiments. Various doses of naltrexone were administered prior to maintenance doses of cocaine, heroin or their combination to establish naltrexone’s ability to antagonize self-administration of cocaine-heroin combinations relative to the constituent drugs alone. Either saline, cocaine (100 μg/kg/injection), heroin (6.4 or 13 μg/kg/injection) or a cocaine-heroin combination was used as the maintenance drug condition on sessions 1, 2 and 4 of the four-session cycle. The cocaine-heroin combination was chosen as the lowest dose combination that maintained responding above saline levels when combined but not when the constituent drugs were tested alone. Each maintenance drug condition was in effect until the 6-day stability criteria described above were met. The maintenance condition was then tested with presession treatments of various doses of naltrexone (6.4–1600 μg/kg) or saline (0.1 ml/kg), injected i.m. 10 min before the beginning of session 3 of the four-session cycle. The order of naltrexone test dose was counter-balanced across the four monkeys and all test conditions were determined twice.

After the establishment of effective naltrexone doses, heroin and cocaine-heroin dose-response functions were redetermined in monkeys RK2, 11084 and AP13 in the presence of various doses of naltrexone (1.6–50 μg/kg, i.m., 10 min presession). Higher doses of heroin (50 and 100 μg/kg/injection), alone or in the cocaine-heroin combinations, also were added to the redeterminations of dose-response functions to assess whether blockade by naltrexone was mountable. Monkey 9002 was not available for this phase of the study.

Determination of dose-response functions were repeated for monkeys RK2, 11084 and AP13 after completion of all test conditions. This second determination was conducted to control for baseline changes for heroin alone, which were evident in our initial study (Rowlett and Woolverton, 1997). The second determinations were conducted with submaximal and peak doses of cocaine and heroin only, because the only change in sensitivity to heroin previously observed was with low-to-intermediate doses of heroin.

Drugs. Cocaine HCl, heroin HCl and naltrexone HCl were provided by the National Institute on Drug Abuse, mixed in 0.9% saline solution and sterilized by filtration (0.2-µm filter, Naïge Co., Rochester, NY). Combinations of cocaine and heroin were prepared in the same saline solution. Cocaine, heroin and cocaine-heroin injections occurred over a 10-sec period in a volume of approximately 1 ml. Naltrexone was injected into a quadriceps muscle at a volume of 0.1 ml/kg. All drug doses are expressed as the salt form of the drug.

Data analysis. Injections/session, averaged for the two determinations, was the dependent measure, with the corresponding FR breakpoint also noted. Previous research has indicated that injections/session provides a reliable measure of self-administration of a
drug that, in contrast to breakpoint, does not violate assumptions of standard statistical tests (Depoortere et al., 1993; Rowlett et al., 1996). Each monkey served as its own control, and doses of cocaine, heroin and cocaine-heroin combinations were considered to maintain self-administration if the mean injections/session for the two determinations was above the range of an individual monkey’s mean injections/session after saline determination.

To analyze shifts in heroin dose-response functions produced by combination with cocaine and/or pretreatment with naltrexone, potencies (ED50, dose that produced 50% of the maximum injections/session produced by drug alone) were obtained. For these calculations, the maximum number of injections/session for heroin alone (I\text{max}) was determined and considered 100% for an individual monkey. ED50 values were calculated by log-linear regression analysis, according to the following: ED50 = 10^[y-y intercept/slope], where y = 0.5(I\text{max}). Group means and 95% CI were calculated for the ED50 values under each condition. To test for reliable differences in the reinforcing potency (i.e., the ED50 value) and the reinforcing efficacy (i.e., the I\text{max} value), changes in ED50 values and I\text{max} values regardless of dose combinations were analyzed among the conditions (heroin alone vs. cocaine-heroin combinations) using a repeated measures t test. For these tests, the alpha level was constrained to P ≤ .05. ED50 values for cocaine alone were calculated as described above except that the maximum injections/session maintained by cocaine was used as the I\text{max} value in the calculation.

For naltrexone antagonism of cocaine-heroin self-administration, apparent pA2 analysis was conducted. The method of Arunlakshana and Schild (1959) was used with drug doses in mol/kg substituted for drug concentrations. Linear regression analysis was conducted to obtain the slope values (to test whether the slope differed from –1.0 using a one-sample t test, Tallarida et al., 1979), and the x-intercept (apparent pA2 value). For naltrexone antagonism of heroin self-administration, apparent pKb analysis was conducted according to the method described by Negus et al. (1993). Apparent pKb values were calculated according to the equation: pKb = -log[B/X-1]. In this equation, “B” is the dose in mol/kg and “X” is the dose ratio (ED50 of the agonist combined with antagonist divided by the ED50 of the agonist alone).

## Results

### Self-administration of cocaine and heroin, alone and combined.

During the course of the study, all monkeys completed one to four injections/session (breakpoint = 120) when saline was available (table 1). When doses of cocaine were available, injections/session and breakpoints increased as the dose of cocaine increased, with saline-like levels maintained from 6.4 to as high as 25 μg/kg/injection (table 1). At the largest dose of cocaine tested (100 μg/kg/injection), injections/session were 16 to 19 with breakpoints of 960 and 1920 responses. Redetermination of the cocaine dose-response functions for monkeys Rlk2, 11084 and AP13 at the end of the study revealed injections/session that varied by approximately one injection/session at every dose with no consistent trend for an increase or decrease in responding (data not shown). Comparison of the mean ED50 values (table 2) revealed no apparent difference from the first to second determinations [first determination (mean, 95% CI, n): 36, 8.1, 4; second determination: 38, 14, 3].

### Injections/session and breakpoints for heroin alone also increased with dose in all monkeys tested (fig. 1, filled symbols). Saline-like levels of responding were maintained from 1.6 to 3.2 μg/kg/injection in the four monkeys tested. Injections/session and breakpoints reached a maximum of 8 to 14 and 240 to 960, respectively, at a dose of either 6.4 μg/kg/injection for monkeys Rlk2, 9002 and 11084 or 13 μg/kg/injection for monkey AP13, who showed the lowest injections/session and breakpoints. Redetermination of the heroin dose-response functions for monkeys Rlk2, 11084 and AP13 at the end of the study revealed no consistent trend for an increase or decrease in responding (data not shown). Comparison of the mean ED50 values (table 2) revealed no apparent difference from the first to second determinations [first determination (mean, 95% CI, n): 4.8, 2.0, 4; second determination: 5.1, 2.3, 3].

The lowest dose of cocaine that increased injections/session and breakpoints for subthreshold doses of heroin was as follows: Rlk2, 25 μg/kg/injection cocaine; 9002, 6.4 μg/kg/injection cocaine; 11084, 3.2 μg/kg/injection cocaine; AP13, 1.6 μg/kg/injection cocaine. Except for monkey Rlk2, these doses were below the range of cocaine doses that maintained responding at saline-like levels when tested alone. When these cocaine doses were combined with heroin, the dose-response functions for the combinations were shifted to the left relative to heroin dose-response functions in an approximately parallel fashion (fig. 1). Determination of individual ED50 values revealed decreases in the ED50s for heroin of 2.9- to 4.1-fold (fig. 1). Repeated measures t tests of the effect of combining cocaine, regardless of dose, on heroin ED50 values revealed a reliable decrease [t(3) = 3.6, P < .05]. The group mean for heroin alone was 4.9 (95% CI = 1.8) and the group mean for heroin plus cocaine was 1.4 (95% CI = 0.5). For each of the four monkeys, the maximum injections/session and breakpoints were similar for heroin alone compared to heroin plus cocaine (fig. 1). Consistent with this observation, anal-

<table>
<thead>
<tr>
<th>猴 Identification</th>
<th>Rlk2</th>
<th>9002</th>
<th>11084</th>
<th>AP13</th>
</tr>
</thead>
<tbody>
<tr>
<td>剂量 (μg/kg/次)</td>
<td>6.4</td>
<td>3</td>
<td>2 (1–3)</td>
<td>2 (1–2)</td>
</tr>
<tr>
<td>注射次数/时段（范围）</td>
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<td>3 (2–3)</td>
<td>3 (2–3)</td>
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<td>2 (120)</td>
<td>2 (120)</td>
<td>2 (120)</td>
</tr>
</tbody>
</table>

*Saline values represent means taken from saline tests conducted at the beginning and end of the study.

*Breakpoint* represents the last completed response requirement.

### Table 2

Potency (ED50) for self-administration of cocaine and heroin in four rhesus monkeys responding under a progressive-ratio schedule

<table>
<thead>
<tr>
<th>Drug and determination</th>
<th>Rlk2</th>
<th>9002</th>
<th>11084</th>
<th>AP13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First determination</td>
<td>45</td>
<td>38</td>
<td>37</td>
<td>25</td>
</tr>
<tr>
<td>Second determination</td>
<td>46</td>
<td>43</td>
<td>43</td>
<td>26</td>
</tr>
<tr>
<td>Heroin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First determination</td>
<td>3.6</td>
<td>4.1</td>
<td>3.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Second determination</td>
<td>3.7</td>
<td>4.2</td>
<td>7.5</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*ED50 values were calculated by log-linear regression analysis, according to the following: ED50 = 10^[y-y intercept/slope], where y = 0.5(I\text{max}).

Not determined.
Saline levels in all monkeys at either 13 or 25 injections/session (S.E.M. 5.1) was tested with increasing doses of heroin or heroin injections/session (S.E.M. 5.1) was tested with increasing doses of heroin and heroin combinations resulted in rightward shifts in the dose-response functions for all three monkeys tested (fig. 4). These rightward shifts were observed with naltrexone doses ranging from 3.2 to 50 μg/kg, in a naltrexone dose-dependent fashion in the three monkeys (fig. 4). Schild analyses of the data shown in figure 4 revealed apparent pKB values for antagonism of heroin alone (mean = 8.0, S.E.M. = 0.12) were nearly identical to the pKB values obtained after antagonism of cocaine-heroin combinations with 6.4 μg/kg naltrexone (mean = 8.1, S.E.M. = 0.12).

Discussion

In our study, combination of low, normally ineffective doses of cocaine with a range of doses of heroin resulted in an enhancement of self-administration compared to heroin alone. The overall effect was a leftward and approximately parallel shift in the heroin dose-response function relative to the dose-response function for heroin alone. Moreover, non-reinforcing doses of heroin combined with nonreinforcing doses of cocaine resulted in injections/session greater than observed when saline was available for self-administration. These results confirm and extend our earlier data in which combining low doses of heroin with cocaine resulted in an overall shift to the left in the cocaine dose-response function (Rowlett and Woolverton, 1997). In our previous study, however, the dose-response functions for heroin alone were shifted to the left in two monkeys when tested after exposure to the various cocaine-heroin combinations, raising the possibility that the enhancement of cocaine self-administration by heroin was due, at least in part, to changes in sensitivity to heroin alone that may have developed over the course of our study. Our findings indicate that this likely is not the case, because no changes in self-administration were observed upon redetermination of dose-response functions of heroin alone. Collectively, the results of the present and our previous study indicate that combinations of low doses of cocaine and heroin result in a reciprocal enhancement of the reinforcing potency of the combinations compared to either drug alone.

In addition to changes in sensitivity to heroin, in our previous study interanimal differences were observed for the change in position and shape of the cocaine dose-response functions after combination with heroin. Specifically, one monkey showed a marginal leftward and downward shift in the cocaine dose-response function, which clearly differed from the robust parallel leftward shifts in the dose-response functions of the other animals. By contrast, in our study relatively few interanimal differences were observed, except that monkey AP13 showed higher ED50 and lower Imax values for heroin and heroin-cocaine combinations compared to the other animals. Moreover, monkey AP13 required the lowest dose of cocaine to shift the heroin dose-response function to the left in comparison to the other monkeys. It is interesting to note that this monkey was experimentally naive, whereas the other animals had extensive experience with cocaine, heroin or related drugs, raising the possibility

![Fig. 1. Self-administration of heroin alone and combined with nonreinforcing doses of cocaine in rhesus monkeys responding under a progressive-ratio schedule of i.v. drug injection. Cocaine doses were chosen as the highest dose not self-administered but enhancing self-administration after subthreshold heroin doses and were as follows: RIk2, 25 μg/kg injection cocaine; 9002, 6.4 μg/kg injection cocaine; 11084, 3.2 μg/kg injection cocaine; AP13, 1.6 μg/kg injection cocaine. Each panel represents an individual monkey, identified in the upper left of the panel. Shaded bars represent the range of saline injections/session self-administered during saline tests conducted throughout the experiment. Italicized numbers represent ED50 values for corresponding curves. X-axis: Heroin dose in μg/kg injection. Left y-axis: Injections/session after test sessions of heroin or heroin + cocaine. Right y-axis: Breakpoint corresponding to injections/session, represented by fixed-ratio response requirement. Data are means of two determinations under a single-alternation sequence.

ysis of Imax values revealed no reliable effect [t(3) = -0.53, P > .05].

Self-administration of cocaine, heroin and cocaine plus heroin after presession naltrexone. Presession naltrexone treatments did not alter self-administration of 100 μg/kg/injection of cocaine over the 250-fold dose range tested (6.4–1600 μg/kg, data not shown). At the highest dose of naltrexone tested (1600 μg/kg), the mean injections/session was 16 (S.E.M. = 1.0), which was the same as the mean of 16 injections/session (S.E.M. = 2.0) maintained by cocaine after presession i.m. injection with saline.

Presession naltrexone treatments reduced self-administration of 6.4 μg/kg/injection of heroin (monkeys RIk2, 9002, 11084) or 13 μg/kg/injection of heroin (monkey AP13) to saline levels in a dose-dependent fashion in all monkeys (fig. 2, filled symbols). When combinations of cocaine and heroin at the peak of the combination dose-response functions were tested after presession treatments with naltrexone, injections/session and breakpoints were also reduced to saline levels in a dose-dependent fashion in all monkeys (fig. 2, open symbols).

When the lowest dose of naltrexone that suppressed injections/session and breakpoints for heroin (6.4 μg/kg naltrexone in all monkeys) was tested with increasing doses of heroin, injections/session and breakpoints increased to above saline levels in all monkeys at either 13 or 25 μg/kg/injection of heroin (fig. 3). The overall effect was a rightward shift in the heroin dose-response function in the four monkeys. Similarly, increasing the dose of the heroin component of cocaine-heroin combinations resulted in rightward shifts in the dose-response functions for all three monkeys tested (fig. 4). These rightward shifts were observed with naltrexone doses ranging from 3.2 to 50 μg/kg, in a naltrexone dose-dependent fashion in the three monkeys (fig. 4).

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<td><strong>Heroin Dose (μg/kg/inj)</strong></td>
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that experimental history may be a factor in determining sensitivity to cocaine-heroin combinations. Regardless of individual differences in ED50 values, Imax values and dose of cocaine required to enhance the effects of heroin, the overall effect of a leftward and parallel shift in the heroin dose-response function after combination with cocaine was evident in all monkeys tested in our study.

As with our previous results, the shift in the heroin dose-response functions was not accompanied by a corresponding change in the maximum injections/session (Imax) or break-

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**Fig. 2.** Effects of presession naltrexone treatments on self-administration of heroin alone or combined with cocaine in rhesus monkeys responding under a progressive-ratio schedule of i.v. drug injection. Points above “S” represent self-administration after heroin or heroin + cocaine at the peak of the individual monkey’s dose-response function, preceded by a presession saline injection. Naltrexone or saline were administered by the intramuscular route (0.1 ml/kg), 10 min before the session, in a quadricep muscle. See figure 1 for other details.

**Fig. 3.** Self-administration of different doses of heroin alone and after presession naltrexone treatments in rhesus monkeys responding under a progressive-ratio schedule of i.v. drug injection. Naltrexone was administered by the intramuscular route (0.1 ml/kg), 10 min before the session, in a quadricep muscle. See figure 1 for other details.
points compared to heroin alone. If one posits that $I_{\text{max}}$ reflects the relative reinforcing efficacy of a drug, then our findings suggest that the relative reinforcing efficacy (as compared to potency) of heroin was not altered in combination with cocaine. This possibility needs to be viewed cautiously, however, because the dose-response functions for heroin tended to be biphasic, as was clearly observed in our previous study (Rowlett and Woolverton, 1997). It is conceivable, therefore, that the tendency of high doses of heroin to suppress responding may have interfered with detection of any cocaine-induced increase in $I_{\text{max}}$. Interestingly, in a recent study by Mattox et al. (1997), behavioral economic analysis of speedball interactions revealed that “demand” for cocaine-heroin combinations self-administered by smoke inhalation was increased relative to smoked heroin base, which may reflect an increase in relative reinforcing efficacy of the speedball mixture compared to heroin alone. Consistent with this view, under conditions in which the cocaine and cocaine-heroin dose-response functions were monophasic, $I_{\text{max}}$ values for cocaine-heroin combinations were reliably greater than the $I_{\text{max}}$ values for heroin alone (Rowlett and Woolverton, 1997). Together, these results raise the possibility that the reinforcing efficacy, in addition to the potency, of heroin may increase when combined with active doses of cocaine. However, only the reinforcing potency of heroin appears to be increased when combined with normally inactive doses of cocaine. The extent to which any changes in reinforcing efficacy produced by combining cocaine and heroin are determined by rate-suppressing effects, scheduling conditions, or doses in the mixture remains to be determined.

Consistent with previous research with opioid antagonists combined with $\mu$ agonists (Harrigan and Downs, 1978; Ettenberg et al., 1982; Bertalmio and Woods, 1989; Winger et al., 1992), presession administration with naltrexone reduced heroin self-administration in our study. Increasing the heroin dose surmounted naltrexone’s antagonism of heroin self-administration, resulting in a shift to the right in the dose-response function. In vivo apparent $pK_B$ analysis of this rightward shift revealed a $pK_B$ value of 8.0, consistent with apparent $pA_2$ values obtained in previous behavioral and physiological studies in rhesus monkeys treated with naltrexone combined with prototypic $\mu$ agonists (e.g., 8.3 for morphine in drug discrimination, France et al., 1990; 7.8 for alfentanil antinociception, Gerak et al., 1994). Moreover, our findings with naltrexone complement and extend a previous study by Bertalmio and Woods (1989), in which quadazocine antagonized self-administration of the $\mu$ selective agonist alfentanil with an apparent $pA_2$ value of 7.6. Evidence of surmountable antagonism of heroin self-administration by naltrexone at an apparent affinity value consistent with interaction at the $\mu$ receptor supports the view that stimulation of $\mu$ receptors is a primary mechanism underlying the reinforcing effects of heroin and other $\mu$ opioid agonists. The pharmacological activity of heroin, however, is thought to be due to the action of its metabolites 6-monoacetylmorphine and morphine (Inturrisi et al., 1983). Thus, interpretation of $pA_2/pK_B$ analyses is complicated by the possibility that self-administration was mediated by either morphine or 6-monoacetylmorphine, or both, rather than the parent compound. Although we cannot at present state definitively which metabolite modulates the reinforcing effects of heroin, our results suggest that regardless of the metabolite, $\mu$ receptor stimulation plays a prominent role in heroin self-administration.

**Fig. 4.** Self-administration of heroin-cocaine combinations after presession treatments with naltrexone in rhesus monkeys responding under a progressive-ratio schedule of i.v. drug injection. Cocaine doses were chosen as the highest dose not self-administered but enhancing self-administration after subthreshold heroin doses. Each panel represents an individual monkey, identified in the upper left of the panel. Naltrexone was administered by the intramuscular route (0.1 ml/kg), 10 min before the session, in a quadriceps muscle. See figure 1 for other details.

**Fig. 5.** Schild analysis of the naltrexone antagonism data from figure 4. Each panel represents an individual monkey, identified in the upper right of the panel. X-axis: negative logarithm of the dose of naltrexone, $B$, in mol/kg. Y-axis: logarithm of the dose ratio ($x$) minus 1.0. The dose ratio was obtained by dividing the ED_{50} of agonist combination plus antagonist by the ED_{50} of the agonist combination alone. The x-intercept (in vivo apparent $pA_2$ value) and corresponding slope shown in the lower left of each panel were derived from linear regression analysis.
In contrast to the interaction of naltrexone with heroin, presession treatments of naltrexone did not alter cocaine self-administration over an approximately 300-fold dose range. This observation is concordant with previous findings that opioid antagonists do not consistently alter the reinforcing effects of cocaine (e.g., Killian et al., 1978; Ettenberg et al., 1982; Winger et al., 1992). The lack of antagonism by naltrexone of cocaine self-administration in our study over a relatively broad dose range supports the notion that opioid receptor stimulation does not play a primary role in mediating the reinforcing effects of cocaine (cf., Ettenberg et al., 1982). Interestingly, some reports have noted decreases in cocaine self-administration after treatments with opioid antagonists in rats (De Vry et al., 1989; Corrigall and Coen, 1991), as well as opioid antagonist-induced decreases in cocaine conditioned place preference (Houdi et al., 1989) and cocaine’s effects on electrical brain stimulation (Bain and Kornetsky, 1987). The reasons for these different findings with naltrexone are not clear but may reflect species and/or procedural differences.

Evaluation of the effects of naltrexone on self-administration of heroin and cocaine alone allowed determination of whether naltrexone’s effects on cocaine-heroin combinations were similar to its effects on heroin (i.e., surmountable antagonism) or on cocaine (i.e., no effect) self-administration. Naltrexone pretreatment resulted in a dose-dependent suppression of responding maintained by cocaine-heroin combinations, at doses similar to that suppressed self-administration of heroin alone. Moreover, increasing the dose of heroin in the cocaine-heroin mixture resulted in an increase in cocaine-heroin self-administration, such that the dose-response function was shifted to the right (i.e., surmountable antagonism). When differing doses of naltrexone were administered prior to determination of speedball dose-response functions, rightward shifts were observed that increased in magnitude as the dose of naltrexone was increased. Despite individual differences in the dose combinations that maintained behavior, the observed shifts in speedball dose-response functions produced by naltrexone were similar across animals, and in vivo apparent \( p_A2 \) analysis of these data revealed apparent affinity estimates of 7.9 to 8.2 (mean \( p_A2 = 8.0 \)). This apparent \( p_A2 \) value is within the range of \( p_A2 \) values obtained with naltrexone combined with other prototypic mu agonists in behavioral studies with monkeys (France et al., 1990; Gerak et al., 1994; Gerak and France, 1996). This \( p_A2 \) value also is identical to the in vivo apparent \( p_KB \) value obtained with naltrexone combined with heroin alone in our study. Overall, our results are consistent with previous data showing attenuation of the reinforcing, discriminative stimulus and subjective effects of speedball combinations by naltrexone (Spealman and Bergman, 1992; Hemby et al., 1996; Walsh et al., 1996) and extend previous studies have shown that the reinforcing effects of cocaine and heroin reflect separate pharmacological mechanisms (cf., Ettenberg et al., 1982).

As mentioned, the finding of naltrexone-reversible enhancement of the effects of speedballs compared to the constituent drugs is consistent with results from other behavioral paradigms (Spealman and Bergman, 1992; Hemby et al., 1996; Kimmel and Holtzman, 1997). Although neuropharmacological mechanisms underlying enhancement of the behavioral effects of cocaine by opioid agonists have not been established definitively, our study provides support for the involvement of mu receptors as has been proposed previously (Brown et al., 1991; Spealman and Bergman, 1992). We cannot, however, rule out a possible role for delta receptors in mediating the enhanced self-administration of cocaine-heroin combinations. Concordant with a possible role for delta receptors in abuse-related effects of speedballs, previous studies have shown that the delta antagonist naltrindole attenuated both the discriminative stimulus effects of cocaine and cocaine-induced conditioned place preference in rats (Menkens et al., 1992; Suzuki et al., 1994). In addition, the delta agonists TAN-67 and SNC-80 have been shown to enhance the discriminative stimulus effects of cocaine in both rats and squirrel monkeys (Rowlett and Spealman, in press; Suzuki et al., 1997), and delta receptors apparently are involved in the antinociceptive effects of 6-monoacetylmorphine (Rady et al., 1997). Regardless of the exact mechanisms underlying interactions between cocaine and opioids, our results suggest that mutual enhancement of the self-administration of cocaine and heroin may serve as the pharmacological basis for the persistence of speedball abuse.

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