Asymmetric Generalization and Interaction Profiles in Rhesus Monkeys Discriminating Intravenous Cocaine or Intravenous Heroin from Vehicle

Donna M. Platt, James K. Rowlett, and Roger D. Spealman

New England Primate Research Center, Harvard Medical School, Southborough, Massachusetts

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ABSTRACT

Many polydrug abusers combine cocaine with heroin in the form of a “speedball.” This study investigated the discriminative stimulus (DS) effects of speedballs in rhesus monkeys trained to discriminate either intravenous cocaine or intravenous heroin from vehicle. Initial substitution tests revealed an asymmetry in the generalization profile of dopamine and opioid agonists such that μ agonists partially substituted for cocaine, but direct and indirect dopamine agonists did not substitute for heroin. Subsequent speedball tests in which drug mixtures were administered by coinjecting the component drugs while keeping the dose-ratio constant revealed an additional asymmetry. In cocaine-trained monkeys, coadministration of cocaine and heroin produced leftward shifts in the cocaine dose-response function. Heroin’s cocaine-enhancing effects were mimicked by the μ agonists fentanyl and methadone and less consistently by the δ agonist (+)-4-[(αR)- α -(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-*N,N*-diethylbenzamide (SNC 80) and

reversed by the μ antagonist naltrexone and the δ antagonist naltrindole. In heroin-trained monkeys, coadministration of cocaine and heroin attenuated the DS effects of heroin. Cocaine’s heroin-attenuating effects were mimicked by the D1-like agonist 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine (SKF 81297) and the D2-like agonist *R*-(-)-propylnorapomorphine and reversed by the D1-like antagonist (6*aS-trans*)-11-chloro-6,6*a*,7,8,9,13*b*-hexahydro-7-methyl-5*H*-benzo[*d*]aphtha[2,1-*b*]azepin-12-ol hydrobromide (SCH 39166) and the D2-like antagonist raclopride. Attenuation of the effects of heroin was accompanied by decreases in response rate. These results suggest that heroin enhances the DS effects of cocaine via μ , and to a lesser extent δ , receptor mechanisms; whereas cocaine-induced inhibition of the DS effects of heroin probably was due at least in part to masking of the heroin DS presumably via stimulation of both D1- and D2-like receptors.

Many polydrug abusers take cocaine combined with heroin by self-administering the drugs together in the form of “speedballs.” Between 30 and 80% of heroin abusers also use cocaine (Kosten et al., 1986), and cocaine abuse has been reported with high frequency in heroin-dependent individuals maintained on methadone (Levin et al., 1996). Likewise, Lauzon et al. (1994) found that 50% of intravenous cocaine users reported using heroin regularly.

Speedball abusers often report that the combined use of cocaine and heroin produces a more pleasurable subjective

experience than either drug alone and that the combination may ameliorate perceived undesirable effects of the individual drugs (Kosten et al., 1986). These self-reports have been largely confirmed in laboratory studies with human volunteers (Foltin and Fischman, 1992; Foltin et al., 1995; Preston et al., 1996; Walsh et al., 1996). In the studies by Foltin and Fischman (1992) and Walsh et al. (1996), for example, the subjective effects of cocaine combined with the heroin-like opioids morphine and hydromorphone were greater than those induced by the individual drugs on measures of “drug liking” and “high.” Similarly, ratings of “magnitude of drug effect” and “good effect” induced by cocaine were higher in methadone-maintained subjects than in control subjects not receiving methadone (Foltin et al., 1995; Preston et al., 1996).

Complementary findings have been reported in laboratory animals trained to discriminate cocaine from vehicle. In

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ABBREVIATIONS: DS, discriminative stimulus; DA, dopamine; FR, fixed ratio; *R*-(-)-NPA HCl, *R*-(-)-propylnorapomorphine, *R*-(-)-10,11-dihydroxy-*N*-*n*-propylnorapomorphine; SKF 81297 HCl, 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine HCl; GBR 12909 dihydrochloride, 1-[2-[bis-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride, fentanyl citrate salt; SNC 80, (+)-4-[(αR)- α -(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-*N,N*-diethylbenzamide; SCH 39166 HCl, [(6*aS-trans*)-11-chloro-6,6*a*,7,8,9,13*b*-hexahydro-7-methyl-5*H*-benzo[*d*]aphtha[2,1-*b*]azepin-12-ol hydrobromide; ANOVA, analysis of variance.

squirrel monkeys, for example, heroin and related opioids can enhance the discriminative stimulus (DS) effects of cocaine and produce dose-dependent leftward shifts in the cocaine dose-response function (Spealman and Bergman, 1994; Rowlett and Spealman, 1998; Rowlett et al., 2001). Heroin and other opioids also have been found to enhance the DS effects of cocaine in rhesus monkeys (Negus et al., 1998b; Negus and Mello, 2002) and rodents (Suzuki et al., 1995, 1997; Kantak et al., 1999). Although negative results have been reported in some studies (Broadbent et al., 1995; Lamas et al., 1998), the bulk of the evidence points to a correspondence between opioid-induced modulation of the DS effects of cocaine in animals and the subjective effects of cocaine in humans.

Whereas opioid modulation of the DS effects of cocaine has received considerable attention preclinically, only a few studies have specifically investigated the ability of cocaine to modulate the DS effects of heroin or related opioids. For example, in previous experiments using morphine or butorphanol as a DS, combined administration of cocaine and either opioid resulted in infra-additive interactions that depended at least in part on the species and the training dose (Suzuki et al., 1995; Cook and Picker, 1998; Platt et al., 1999). Likewise, coadministration of cocaine with heroin produced dose-dependent increases in the ED_{50} value for the DS effects of heroin in rats (Lamas et al., 1998). These preclinical findings may correspond to clinical reports that cocaine can reduce some of the characteristic opioid effects in human drug abusers.

The mechanisms underlying the DS effects of speedball combinations have not yet been elucidated fully but could reflect interactions mediated via subtypes of both dopamine (DA) and opioid receptors (Spealman and Bergman, 1994; Suzuki et al., 1997; Cook and Picker, 1998; Negus et al., 1998a; Rowlett and Spealman, 1998; Rowlett et al., 2001; Negus and Mello, 2002). Thus, the purpose of the present study was to investigate systematically the DS effects of cocaine-heroin combinations in rhesus monkeys trained to discriminate either cocaine or heroin from vehicle. As a necessary first step, we also determined the substitution profile of several DA and opioid receptor ligands in these two groups of monkeys. We then assessed the contribution of μ and δ opioid receptor, as well as D1- and D2-like DA receptor, mechanisms in the DS effects of cocaine-heroin combinations in both cocaine- and heroin-trained monkeys.

Materials and Methods

Subjects and Surgical Procedure. Ten adult rhesus monkeys (*Macaca mulatta*), eight male and two female, weighing 6.4 to 12.1 kg, were studied in daily experimental sessions (Monday to Friday). Three of the monkeys had been involved in another study characterizing the DS effects of heroin (cf. Platt et al., 2001); the remaining monkeys were experimentally naive at the beginning of the study. Between sessions, monkeys lived in individual home cages where they had unlimited access to water and were maintained at ~90% of their free-feeding body weight by adjusting their daily access to food [Teklad (Harlan Teklad, Madison, WI) 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.

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 polyvinyl chloride catheters (0.64 mm i.d.; 1.35 mm o.d.) using the general surgical procedures described by Platt et al. (2005). 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 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 (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 minimal downward force of ~0.25 N was recorded as a response. Food pellets (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.

Drug Discrimination Procedure. Monkeys were trained to discriminate intravenously administered drug (cocaine, 0.3 mg/kg or heroin, 0.056 mg/kg) from saline under a 10-response fixed ratio (FR 10) schedule of food reinforcement. After an intravenous injection of drug, 10 consecutive responses on one lever (counterbalanced across monkeys) produced a food pellet, whereas after an intravenous injection of vehicle (0.9% saline solution), 10 consecutive responses on the other lever produced a food pellet. Each response on the incorrect lever (e.g., the vehicle-appropriate lever after drug injection) reset the FR requirement to 10. Delivery of each food pellet was followed by a 10-s time-out period, during which the lights were off and responses had no scheduled consequences.

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, vehicle was injected during time-out periods preceding the first $n - 1$ components, and drug was injected before the n th component of the session. Periodically, vehicle was injected before all components of a training session to prevent an invariant association between the fourth component and drug injection. Injections of drug or vehicle were administered from outside the chamber via a catheter extension during the 5th min of the 10-min time-out periods that separated components. Each injection was followed by a 2-ml infusion of saline solution to flush the catheter of any residual drug solution.

Once consistent stimulus control was achieved, test sessions were conducted once or twice per week with training sessions on intervening days. Test sessions were conducted only if $\geq 80\%$ 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 in which incremental doses ($1/4-1/2$ log increments) of drug were administered intravenously 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 the

subject's ability to respond usually were tested only once in each subject.

Substitution Studies. Initially, dose-response functions were determined for test drugs using a cumulative dosing procedure. The same DA and opioid receptor ligands were studied in both cocaine- and heroin-trained monkeys and included: the D2-like receptor agonist *R*-(−)-NPA (0.0003–0.003 mg/kg), cocaine (0.03–1.0 mg/kg), the D1-like receptor agonist SKF 81297 (0.01–0.3 mg/kg), the DA transport inhibitor GBR 12909 (0.03–1.8 mg/kg), the μ receptor agonists fentanyl (0.0003–0.03 mg/kg), heroin (0.01–0.3 mg/kg), and methadone (0.1–1.8 mg/kg), and the δ receptor agonist SNC 80 (0.01–0.3 mg/kg).

Cocaine-Heroin Combination Studies. The cumulative dosing procedure described above was adapted so that incremental doses of cocaine and heroin could be coadministered during the test session while keeping the dose-ratio constant (e.g., cocaine-heroin dose ratios of 1:0.1 or 1:1 in cocaine-trained monkeys, heroin-cocaine dose ratios of 1:0.3 or 1:1 in heroin-trained monkeys). In general, dose ratios were selected to encompass the range of doses of the component drugs that did not induce untoward side effects or eliminate response rates when studied alone. Different dose ratios were evaluated in separate test sessions to provide data on a full range of doses of drug combinations.

Opioid Receptor Mechanisms in Speedball Combinations in Cocaine-Trained Monkeys. To evaluate potential opioid receptor mechanisms underlying heroin-induced modulation of the DS effects of cocaine in cocaine-trained monkeys, incremental doses of cocaine were coadministered cumulatively with the μ receptor agonists methadone and fentanyl or the δ receptor agonist SNC 80, while keeping the dose ratio constant. Different dose ratios were evaluated in separate test sessions to provide data on a full range of doses for the drug combinations. Subsequently, antagonism studies were conducted with the μ receptor antagonist naltrexone and the δ receptor antagonist naltrindole. Naltrexone (0.01 mg/kg) or naltrindole (1.0 mg/kg) was administered intravenously in the first component of a four-component test session in which incremental doses of the maximally effective speedball combination (1 cocaine:1 heroin) or cocaine alone was administered as described above. Doses of the antagonists were chosen on the basis of previous studies that showed at least a 10-fold shift in the DS effects of either heroin or SNC 80 alone (cf. Brandt et al., 1999; Platt et al., 2001).

DA Receptor Mechanisms in Speedball Combinations in Heroin-Trained Monkeys. To evaluate possible DA receptor mechanisms underlying cocaine-induced modulation of the DS effects of heroin in heroin-trained monkeys, incremental doses of heroin were coadministered cumulatively with either the D1-like receptor agonist SKF 81297 or the D2-like receptor agonist *R*-(−)-NPA while keeping the dose ratio constant. Different dose ratios were evaluated in separate test sessions to provide data on a full range of doses of the drug combinations. Antagonism studies then were conducted with the D1-like receptor antagonist SCH 39166 and the D2-like receptor antagonist raclopride. SCH 39166 (0.001 mg/kg) or raclopride (0.01 mg/kg) was administered intravenously in the first component of a four-component test session in which incremental doses of the maximally effective speedball combination (1 heroin:1 cocaine) or heroin alone was tested as described above. Doses of the antagonists were chosen on the basis of their ability to block the effects of the DA agonists without producing behavioral disruptions when administered alone (cf. Weed and Gold, 1998; Platt et al., 2000).

Data Analysis. The percentage of drug-lever responding was computed for individual subjects in each component of a test session, with the restriction that response rate was ≥ 0.1 responses/s during the component, by dividing the number of responses on the drug-associated lever by the total number of responses on both levers and multiplying by 100. Mean percentage of drug-lever responding and S.E.M. were then calculated for the group at each dose. A test drug was considered to substitute fully for the training drug if the maximal percentage of drug-lever responding was $\geq 80\%$. A test drug was

considered to substitute partially for the training drug if the maximal percentage of drug-lever responding differed significantly from both vehicle and the training dose. In addition, the dose of a drug estimated to engender 50% cocaine- or heroin-appropriate responding (ED_{50}) was estimated for individual subjects by linear regression analysis in cases where the ascending portion of the log dose-response function was defined by three or more data points or by linear interpolation in cases where the log dose-response function was defined best by two points. The mean (S.E.M.) ED_{50} value for each drug was determined by averaging ED_{50} values for individual subjects.

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 $\pm$ S.E.M.) was then calculated for the group at each dose. When appropriate, data were analyzed using separate one-way or two-way repeated measures ANOVAs followed by Bonferroni *t* tests. The α level for all statistical tests was $P \leq 0.05$.

Drugs. Cocaine HCl, *R*-(−)-propylorapomorphine (*R*-(−)-NPA) HCl; *R*-(−)-10,11-dihydroxy-*N*-*n*-propylorapomorphine, SKF 81297 HCl, GBR 12909 dihydrochloride, fentanyl citrate salt, SNC 80, methadone HCl, naltrexone HCl, naltrindole HCl, and *S*-(−)-raclopride (+)-tartrate salt were purchased from Sigma-Aldrich (St. Louis, MO). SCH 39166 HCl was provided by Schering-Plough Research Institute (Kenilworth, NJ). Heroin HCl was provided by the National Institute of Drug Abuse (Rockville, MD). All drugs were dissolved in small amounts of 0.1 N HCl if required and diluted to the desired concentrations with sterile water or 0.9% saline solution.

Results

Discriminative Stimulus Effects of DA Receptor Ligands in Cocaine- and Heroin-Trained Monkeys. In general, an asymmetrical substitution pattern was observed for the DS effects of DA receptor ligands in cocaine- versus heroin-trained monkeys (Fig. 1). In cocaine-trained monkeys, cocaine engendered dose-dependent increases in the percentage of responses on the cocaine-associated lever (Fig. 1, top left, open circles) with partial-to-full substitution occurring at doses ≥ 0.1 mg/kg ($F_{4,16} = 83.9$; $P < 0.001$). Response rates were not affected significantly by cocaine over the range of doses tested, and no dose of cocaine decreased the response rate to less than 80% of the control rate (Fig. 1, bottom left). GBR 12909 (Fig. 1, open squares), at dose of ≥ 1 mg/kg, also partially or fully substituted for cocaine in the cocaine-trained monkeys, engendering a maximum of 83% cocaine-lever responding ($F_{5,15} = 29.7$; $P < 0.001$). At the highest dose of GBR 12909, response rates were decreased to 66% of control rates. Partial substitution for cocaine also was observed with *R*-(−)-NPA (Fig. 1, filled triangles) in cocaine-trained subjects, engendering a maximum of 38% cocaine-lever responding at a dose of 0.003 mg/kg ($F_{4,10} = 34.2$; $P < 0.001$). SKF 81297 did not substitute for cocaine at any dose in the cocaine-trained monkeys (Fig. 1, filled diamonds). Although these latter compounds only reduced responding to 30 to 54% of control rates, higher doses of *R*-(−)-NPA and SKF 81297 were not tested due to the emergence of self-injurious behavior [*R*-(−)-NPA] or the elimination of responding in at least one monkey (SKF 81297).

In heroin-trained monkeys, none of the DA receptor ligands substituted for the DS effects of heroin (Fig. 1, top

Fig. 1. Discriminative stimulus effects of *R*(-)-NPA, cocaine, SKF 81297, and GBR 12909 in rhesus monkeys ($n = 4$ or 5) trained to discriminate either cocaine or heroin from saline. Top, percentage of cocaine- (left) or heroin-lever (right) responding (mean $\pm$ S.E.M.) engendered by the dopaminergic ligands. Bottom, effects of dopaminergic ligands on response rate in cocaine- (left) or heroin-trained (right) monkeys. *, significant increase in percentage of drug-lever responding compared with vehicle (top) or significant reduction in response rate (bottom). $P < 0.05$, Bonferroni t test.

right). The greatest amount of heroin-lever responding (25%) was elicited by 1 mg/kg GBR 12909. Cocaine, SKF 81297, and *R*(-)-NPA engendered maximums of only 13 to 16% heroin-lever responding. Although all of the DA receptor ligands reduced response rates to some degree in the heroin-trained group, for SKF 81297 and cocaine, these reductions were significant (SKF 81297: $F_{3,4} = 10.8$; $P < 0.05$; and cocaine: $F_{3,7} = 70.9$; $P < 0.001$; Bonferroni t tests, $P < 0.05$). Higher doses of *R*(-)-NPA and GBR 12909 were not tested due to emergence of side effects (e.g., self-injurious behavior, hair pulling) or the elimination of responding in at least one monkey.

Discriminative Stimulus Effects of Opioid Receptor Ligands in Cocaine- and Heroin-Trained Monkeys. All of the opioid receptor ligands substituted partially for cocaine in the cocaine-trained monkeys, although full substitution ($\geq 80\%$) was never achieved (Fig. 2; fentanyl: $F_{6,17} = 23.0$; $P < 0.001$; heroin: $F_{5,15} = 17.5$; $P < 0.001$; SNC 80: $F_{5,13} = 17.2$, $P < 0.001$; and methadone: $F_{5,11} = 29.1$; $P < 0.001$). In addition, all of the opioid receptor ligands reduced response rates to some degree (Fig. 2, bottom left), although the reductions were significant only for SNC 80 ($F_{3,8} = 9.5$; $P < 0.01$) and methadone ($F_{3,8} = 5.3$; $P < 0.05$). Higher doses of fentanyl and heroin were not evaluated due to the virtual elimination of responding in at least one monkey, as well as concerns regarding potential respiratory depression.

In heroin-trained monkeys, heroin engendered dose-dependent increases in the percentage of responses on the heroin-associated lever (Fig. 2, top right) with full or partial substitution for the training dose occurring at ≥ 0.03 mg/kg ($F_{5,20} = 108.6$; $P < 0.001$). Fentanyl, at doses of 0.001 to 0.01 mg/kg,

and methadone, at a dose of 0.3 and 1 mg/kg, also substituted fully or partially for heroin in the heroin-trained group (fentanyl: $F_{5,13} = 9.9$; $P < 0.001$; methadone: $F_{4,8} = 18.9$; $P < 0.001$). Heroin, fentanyl, and methadone did not significantly affect response rates across the range of doses tested (Fig. 2, bottom right). No substitution for heroin was observed for SNC 80 (maximal heroin-lever responses, 19%) up to a dose (0.3 mg/kg) that significantly reduced rate of responding ($F_{3,7} = 65.5$; $P < 0.01$).

Discriminative Stimulus Effects of Cocaine-Heroin Combinations in Cocaine- and Heroin-Trained Monkeys. A marked asymmetry was observed for the interaction between cocaine and heroin in cocaine- versus heroin-trained monkeys (Fig. 3). In the cocaine-trained group, combined administration of cocaine and heroin resulted in an overall enhancement of the DS effects of cocaine (Fig. 3, top left). Dose-dependent leftward shifts in the cocaine dose-response function along with a 2- to 4-fold decrease in the ED_{50} value for the DS effects of cocaine (Table 1) were observed as the ratio of cocaine to heroin was increased from 1:0.1 to 1:1. A two-way repeated measures ANOVA identified significant main effects of both cocaine dose ($F_{1,8} = 352.7$; $P < 0.001$) and heroin dose ($F_{2,8} = 43.6$; $P < 0.001$). Bonferroni t tests further showed that combination of cocaine with heroin at both dose ratios augmented the DS effects of cocaine compared with the effects of cocaine alone (asterisks in Fig. 3, top left). Combining cocaine with heroin had little effect on response rate except at the highest dose combination evaluated at the 1:1 dose ratio, which virtually eliminated responding (Fig. 3, bottom left; $F_{2,16} = 14.4$, $P < 0.01$ for cocaine; $F_{2,16} =$

Fig. 2. Discriminative stimulus effects of fentanyl, heroin, SNC 80, and methadone in rhesus monkeys ($n = 4$ or 5) trained to discriminate either cocaine or heroin from saline. Top, percentage of cocaine- (left) or heroin-lever (right) responding (mean + S.E.M.) engendered by the opioid receptor agonists. Bottom, effects of the opioid receptor agonists on response rate in cocaine- (left) or heroin-trained (right) monkeys. *, significant increase in percentage of drug-lever responding compared with vehicle (top) or significant reduction in response rate (bottom). $P < 0.05$, Bonferroni t test.

6.0, $P < 0.05$ for heroin; $F_{4,16} = 2.9$; $P < 0.05$ for cocaine $\times$ heroin).

In contrast to the findings in cocaine-trained monkeys, combined administration of cocaine and heroin resulted in an

attenuation of the DS effects of heroin in heroin-trained monkeys (Fig. 3, top right). Dose-dependent rightward and downward shifts in the heroin dose-response function, accompanied by at least a 2-fold increase in the ED_{50} value for

Fig. 3. Discriminative stimulus effects of cocaine-heroin combinations in rhesus monkeys ($n = 4$ or 5) trained to discriminate either cocaine or heroin from saline. Left, percentage of cocaine-lever responding (top) and response rate (bottom) engendered by cocaine, alone and combined with heroin at dose ratios of 1 cocaine:0.1 heroin and 1 cocaine:1 heroin. Right, percentage of heroin-lever responding (top) and response rate (bottom) engendered by heroin, alone and combined with cocaine at dose ratios of 1 heroin:0.3 cocaine and 1 heroin:1 cocaine. *, significant alteration of percentage of drug-lever responding compared with cocaine or heroin alone (top) or significant reduction in response rate compared with cocaine or heroin alone (bottom). $P < 0.05$, Bonferroni t test.

TABLE 1

Effects of cocaine-heroin, cocaine-opioid agonist, and heroin-DA agonist combinations with and without opioid or DA antagonist pretreatments in rhesus monkeys trained to discriminate either intravenous cocaine or intravenous heroin from saline

Data are means $\pm$ S.E.M. ($n = 3-5$).

	ED ₅₀ mg/kg
Cocaine-trained monkeys	
Cocaine alone	0.091 $\pm$ 0.011
Cocaine/heroin (1:0.1)	0.048 $\pm$ 0.012
Cocaine/heroin (1:1)	0.022 $\pm$ 0.006
μ Mechanisms	
Cocaine/methadone (1:1)	0.020 $\pm$ 0.003
Cocaine/fentanyl (1:0.01)	0.040 $\pm$ 0.009
Cocaine/fentanyl (1:0.1)	0.021 $\pm$ 0.005
Cocaine/heroin (1:1) + naltrexone	0.120 $\pm$ 0.065
δ Mechanisms	
Cocaine/SNC 80 (1:1)	0.052 $\pm$ 0.013
Cocaine/SNC 80 (1:3)	—
Cocaine/heroin (1:1) + naltrindole	0.103 $\pm$ 0.025
Heroin-trained monkeys	
Heroin alone	0.022 $\pm$ 0.006
Heroin/cocaine (1:0.3)	0.036 $\pm$ 0.018
Heroin/cocaine (1:1)	—
Dopamine D1 mechanisms	
Heroin/SKF 81297 (1:3)	0.070 $\pm$ 0.008
Heroin/SKF 81297 (1:10)	—
Heroin/cocaine (1:1) + SCH 39166	0.028 $\pm$ 0.015
Dopamine D2 mechanisms	
Heroin/R(-)-NPA (1:0.01)	0.062 $\pm$ 0.023
Heroin/R(-)-NPA (1:0.1)	—
Heroin/cocaine (1:1) + raclopride	0.025 $\pm$ 0.015

—, drug-lever responses <50%.

the DS effects of heroin (Table 1), were observed as the ratio of heroin to cocaine was increased from 1:0.3 to 1:1. A two-way repeated measures ANOVA identified significant main effects of both heroin dose ($F_{3,18} = 10.6$; $P < 0.005$) and

cocaine dose ($F_{2,18} = 8.4$; $P < 0.05$). Bonferroni t tests further showed that combination of heroin with cocaine at the highest dose ratio (1:1) significantly attenuated the DS effects of heroin compared with the effects of heroin alone (asterisks in Fig. 3, top right). Combining heroin with cocaine had little systematic effect on response rate except at the highest dose combinations tested (Fig. 3, bottom right; $F_{3,18} = 31.4$, $P < 0.001$ for heroin; $F_{2,18} = 6.1$, $P < 0.05$ for cocaine; $F_{6,18} = 3.2$; $P < 0.05$ for heroin $\times$ cocaine).

Opioid Receptor Mechanisms Underlying Heroin-Induced Enhancement of the Discriminative Stimulus Effects of Cocaine in Cocaine-Trained Monkeys. Combined administration of cocaine with either methadone or fentanyl in cocaine-trained subjects enhanced the DS effects of cocaine (Fig. 4). Leftward shifts in the cocaine dose-response function along with a 2- to 5-fold decrease in the ED₅₀ value for the DS effects of cocaine (Table 1) were evident at dose ratios of 1 cocaine:1 methadone and 1 cocaine:0.1 fentanyl. Separate two-way repeated measures ANOVAs identified significant main effects of cocaine, methadone, and fentanyl dose ($F_{1,3} = 34.38$ and $F_{1,3} = 39.90$, $P < 0.05$ for cocaine in the separate ANOVAs, $F_{1,3} = 24.58$, $P < 0.05$ for methadone; $F_{2,6} = 10.15$, $P < 0.05$ for fentanyl). Bonferroni t tests showed that combination of cocaine with either methadone or fentanyl augmented the DS effects of cocaine compared with the effects of cocaine alone (asterisks in Fig. 4, top left and middle). Combining cocaine with either methadone or fentanyl had little effect on response rate with the exception of the highest dose combination of cocaine and fentanyl, which significantly reduced the rate of responding (Fig. 4, bottom middle).

When cocaine was combined with SNC 80 at a dose ratio of 1:1, a portion of the cocaine dose-response function was

Fig. 4. Discriminative stimulus effects of cocaine-opioid agonist combinations in rhesus monkeys ($n = 3$ or 4) trained to discriminate cocaine from saline. Top, percentage of cocaine-lever responding engendered by cocaine, alone and combined with methadone (1:1), fentanyl (1:0.01 and 1:0.1) and SNC 80 (1:1 and 1:3). Bottom, effects of cocaine-opioid agonist combinations on response rate. *, significant enhancement of percentage of cocaine-lever responding compared with cocaine alone (top) or significant reduction in response rate compared with cocaine alone (bottom). $P < 0.05$, Bonferroni t test.

shifted leftward, and the ED_{50} value for the DS effects of cocaine was decreased by 2-fold (Fig. 4; Table 1). However, when the ratio of SNC 80 to cocaine was increased, the cocaine dose-response function did not shift further to the left. Rather, at this higher ratio (1 cocaine:3 SNC 80), the dose-response function appeared largely unchanged from cocaine alone, at least for the two lower cocaine doses (0.03 and 0.1 mg/kg). Two-way repeated measures ANOVA showed a significant main effect of cocaine ($F_{1,6} = 39.79$; $P < 0.01$), but no significant effect of SNC 80 dose or the cocaine $\times$ SNC 80 interaction. Higher dose combinations were not studied at the higher dose ratio because of marked reductions in response rate (Fig. 4, bottom right; Bonferroni t tests, $P < 0.05$).

Before assessing the ability of naltrexone (0.01 mg/kg) and naltrindole (1.0 mg/kg) to attenuate the cocaine-like DS effects of a speedball combination, the effects of the two antagonists were first assessed with cocaine alone. Neither naltrexone nor naltrindole altered percentage of drug-lever responding engendered by cocaine alone, nor did they alter the effects of cocaine on response rate (data not shown). Subsequently, the effects of a 1 cocaine:1 heroin speedball combination were redetermined in the presence of naltrexone and naltrindole (Fig. 5). Pretreatment with 0.01 mg/kg naltrexone ($F_{2,6} = 6.02$; $P < 0.05$) or 1.0 mg/kg naltrindole ($F_{2,6} = 18.44$; $P < 0.01$) completely blocked heroin-induced enhancement of the DS effects of cocaine (Fig. 5). Both drugs shifted the ED_{50} for the DS effects of the speedball combination approximately 5-fold to the right (Table 1), and the ED_{50} value after antagonist pretreatment did not differ significantly from the ED_{50} for cocaine alone. Neither cocaine nor the speedball combination alone or after antagonist pretreat-

ment significantly altered response rates, although a downward trend is apparent in both cases (Fig. 5, bottom).

DA Receptor Mechanisms Underlying Cocaine-Induced Inhibition of the Discriminative Stimulus Effects of Heroin in Heroin-Trained Monkeys. Combined administration of heroin with the D1-like receptor agonist SKF 81297 to heroin-trained subjects resulted in attenuation of the DS effects of heroin, similar to the attenuation seen with cocaine (Fig. 6, top left). Rightward and downward shifts in the heroin dose-response function along with a >3 -fold increase in the ED_{50} value for the DS effects of heroin (Table 1) were evident as the ratio of heroin to SKF 81297 was increased from 1:3 to 1:10. The significant inhibition of the DS effects of heroin by SKF 81297 was confirmed by results from a two-way repeated measures ANOVA, which identified significant main effects of heroin, SKF 81297, and their interaction ($F_{2,8} = 25.11$; $P < 0.01$ for heroin; $F_{2,8} = 13.10$, $P < 0.05$ for SKF 81297; and $F_{4,8} = 5.89$; $P < 0.05$ for the interaction). Bonferroni t tests showed that combination of heroin with SKF 81297 (1 heroin:10 SKF 81297 dose ratio) attenuated the DS effects of heroin compared with the effects of heroin alone (asterisks in Fig. 6, top left). Combining heroin with SKF 81297 at both dose ratios markedly reduced response rate at the highest dose combinations (asterisks in Fig. 6, bottom left).

Combined administration of heroin with the D2-like receptor agonist $R(-)$ -NPA to heroin-trained subjects resulted in attenuation of the DS effects of heroin (Fig. 6, top right). Rightward and downward shifts in the heroin dose-response function along with a >3 -fold increase in the ED_{50} value for the DS effects of heroin (Table 1) were evident as the ratio of heroin to $R(-)$ -NPA was increased from 1:0.01 to 1:0.1. A

Fig. 5. Antagonism by opioid antagonists of the discriminative stimulus effects of a 1 cocaine:1 heroin combination in rhesus monkeys ($n = 4$) trained to discriminate cocaine from saline. Left, percentage of cocaine-lever responding (top) and response rate (bottom) engendered by cocaine alone and cocaine + heroin alone and after pretreatment with naltrexone (0.01 mg/kg). Right, percentage of cocaine-lever responding (top) and response rate (bottom) engendered by cocaine alone and cocaine + heroin alone and after pretreatment with naltrindole (1.0 mg/kg). *, significantly different percentage of cocaine-lever responding compared with cocaine alone (top). $P < 0.05$, Bonferroni t test.

Fig. 6. Discriminative stimulus effects of heroin-DA receptor agonist combinations in rhesus monkeys ($n = 3$) trained to discriminate heroin from saline. Top, percentage of heroin-lever responding engendered by heroin, alone and combined with either SKF 81297 or *R*(-)-NPA. Bottom, effects of heroin-DA receptor agonist combinations on response rate. *, significant inhibition of percentage of heroin-lever responding compared with heroin alone (top) or significant reduction in response rate compared with heroin alone (bottom). $P < 0.05$, Bonferroni t test.

two-way repeated measures ANOVA identified significant main effects of heroin and *R*(-)-NPA ($F_{2,8} = 13.12$; $P < 0.05$ for heroin; and $F_{2,8} = 14.98$; $P < 0.05$ for *R*(-)-NPA. Bonferroni t tests showed that combination of heroin with *R*(-)-NPA (1:0.1 dose ratio) attenuated the DS effects of heroin compared with the effects of heroin alone (asterisks in Fig. 6, top right). Neither dose ratio combination of heroin and *R*(-)-NPA significantly altered rates of responding although a downward trend is apparent for the higher dose ratio combination (Fig. 6, bottom right).

Before assessing the ability of the D1-like receptor antagonist SCH 39166 (0.001 mg/kg) and the D2-like receptor antagonist raclopride (0.01 mg/kg) to reverse cocaine-induced inhibition of the DS effects of heroin, the effects of the two antagonists were assessed for their effects on the DS and rate-altering effects of heroin alone. Neither SCH 39166 nor raclopride significantly altered percentage of drug-lever responding engendered by heroin alone. Likewise, neither drug significantly altered the effects of heroin on response rate (data not shown). The effects of a 1 heroin:1 cocaine speedball combination were then determined in the presence of SCH 39166 and raclopride (Fig. 7). Pretreatment with 0.001 mg/kg SCH 39166 ($F_{2,4} = 11.59$; $P < 0.05$) or 0.01 mg/kg raclopride ($F_{2,4} = 8.49$; $P < 0.05$) completely blocked inhibition of the DS effects of heroin induced by cocaine (Fig. 7). The ED_{50} value for the speedball combination after antagonist pretreatment did not differ significantly from the ED_{50} for heroin alone (Table 1). Neither heroin nor the speedball combination alone or after antagonist pretreatment significantly altered response rates (Fig. 5, bottom).

Discussion

The primary aim of the present study was to characterize the DS effects of speedball combinations in rhesus monkeys trained to discriminate either intravenous cocaine or intravenous heroin from vehicle. Before administering the drug combination, we assessed the potential cocaine- and heroin-like DS effects of the component drugs (cocaine and heroin) alone, and of other direct and indirect DA receptor agonists and μ and δ opioid receptor agonists, in both groups of monkeys. We found a marked asymmetry in the generalization profile of these compounds in cocaine- compared with heroin-trained subjects. With respect to DA substitution profiles, only cocaine, GBR 12909, and *R*(-)-NPA engendered partial-to-full substitution for the training dose of cocaine, and none of the DA agonists engendered heroin-like DS effects regardless of dose. These findings differ from some studies (Cook and Picker, 1998; Lamas et al., 1998; Platt et al., 1999) in which cocaine and other stimulants substituted for heroin or morphine in rats, pigeons, and squirrel monkeys. It is interesting to note that in nondependent humans trained in a three-choice drug discrimination procedure with saline, hydromorphone, and pentazocine as the training stimuli, amphetamine did not generalize to hydromorphone but did partially generalize to both pentazocine and saline (Bickel et al., 1989). Previous studies also have shown that D1-like receptor agonists generally substitute for cocaine in monkeys (Spealman et al., 1991; Sinnott and Nader, 2001). Although the factors underlying these different findings are not clear, we cannot rule out species or procedural differences (e.g., route of administration: intraperitoneal or intramuscular in

Fig. 7. Antagonism of the discriminative stimulus effects of a 1 heroin:1 cocaine combination in rhesus monkeys ($n = 3$) trained to discriminate heroin from saline. Left, percentage of heroin-lever responding (top) and response rate (bottom) engendered by heroin alone and heroin + cocaine alone and after pretreatment with SCH 39166 (0.001 mg/kg). Right, percentage of heroin-lever responding (top) and response rate (bottom) engendered by heroin alone and heroin + cocaine alone and after pretreatment with raclopride (0.01 mg/kg). *, significantly different percentage of heroin-lever responding compared with heroin alone (top). $P < 0.05$, Bonferroni t test.

previous studies versus intravenous in the present study; pattern of administration: single dose versus cumulative dose).

With respect to opioid substitution profiles, the μ opioid agonists heroin, methadone, and fentanyl substituted fully for the training dose of heroin and at least partially for the training dose of cocaine. The δ opioid agonist SNC 80 also substituted partially for cocaine but not for heroin. These findings are consistent with other studies (Negus et al., 1998b; Rowlett and Spealman, 1998; Kantak et al., 1999; Rowlett et al., 2004) demonstrating that heroin and other opioid agonists can share DS effects with cocaine, despite being pharmacologically distinct.

An examination of the compounds that share effects with a training drug can provide information regarding mechanisms that underlie the specificity of its DS effects. Based on the profile of compounds substituting for cocaine, it seems likely that the cocaine-trained subjects are discriminating on the basis of a DA cue. It is well established that the indirect DA agonist effects of cocaine (i.e., inhibition of DA uptake and subsequent stimulation of DA receptors) are important mechanisms underlying the abuse-related effects of cocaine. The relevance of these mechanisms for the DS effects of cocaine is supported by the findings of this study and others that other indirect DA agonists typically mimic the DS effects of cocaine. In addition, both μ and δ opioid agonists can augment release of DA in the nucleus accumbens and other brain regions implicated in the behavioral effects of abused drugs (Spanagel et al., 1990; Devine et al., 1993), providing a possible explanation for the ability of heroin, methadone, fentanyl, and SNC 80 to engender partial cocaine-like DS effects. That the selective DA receptor agonists SKF 81297

and $R(-)$ -NPA engendered at most modest substitution for cocaine further suggests that activation of either D1- or D2-like receptors individually is not sufficient for the transduction of the DS effects of cocaine. In the case of the heroin-trained monkeys, it appears likely that these subjects were discriminating primarily on the basis of a μ opioid cue. This study and others (Beardsley and Harris, 1997; Platt et al., 2001; Solecki et al., 2005) have shown that only compounds with appreciable selectivity for μ receptors mimic the DS effects of heroin.

When speedball combinations were administered to the two groups of monkeys, a distinct asymmetrical interaction profile was observed. Heroin enhanced the DS effects of cocaine in cocaine-trained subjects, but cocaine only inhibited the DS effects of heroin in heroin-trained subjects. In the case of the cocaine-trained monkeys, leftward shifts in the cocaine dose-response function also were produced by cocaine-methadone and cocaine-fentanyl, but not consistently by cocaine-SNC 80, combinations. In addition, the enhanced DS effects produced by combining cocaine and heroin could be reversed with the μ opioid antagonist naltrexone and the δ opioid antagonist naltrindole. Together, these findings suggest that μ and, based on antagonism by naltrindole, δ opioid receptor mechanisms play a role in heroin's ability to enhance the DS effects of cocaine. These findings may reflect the fact that both μ and δ opioid agonists can potentiate DA efflux induced by psychostimulants such as cocaine and amphetamine (Hemby et al., 1999; Smith et al., 2006; Bosse et al., 2008).

A role for μ and possibly δ receptor mechanisms in the ability of heroin to enhance the DS effects of cocaine is not surprising as other studies have demonstrated enhancement

of the DS effects of cocaine after pretreatment with both μ and δ opioid agonists (Spealman and Bergman, 1994; Suzuki et al., 1997; Rowlett and Spealman, 1998). The present study extends these earlier findings to another species (rhesus monkey), another route of administration (intravenous versus intramuscular, intraperitoneal, or subcutaneous), and another pattern of administration (drug combination versus pretreatment). The route and pattern of administration used in the present study more closely mimic those used by human speedball abusers (for review, see Leri et al., 2003).

When heroin-cocaine combinations were administered to heroin-trained monkeys, rightward and downward shifts in the heroin dose-response function were observed. Similar findings have been reported previously but, in general, attenuation of the DS effects of heroin by cocaine has been to a lesser degree and less consistent than we observed in the present study (Lamas et al., 1998; Platt et al., 1999). Rightward and downward shifts in the heroin dose-response function that were comparable with those produced by heroin-cocaine combinations also were observed when heroin was combined with either the D1-like agonist SKF 81297 or the D2-like agonist *R*-(−)-NPA. Moreover, the inhibitory effects of cocaine could be reversed by the D1-like antagonist SCH 39166 and the D2-like antagonist raclopride. These findings strongly implicate both D1- and D2-like receptor mechanisms in the ability of cocaine to attenuate the DS effects of heroin. Previous studies in rats have shown that D2-like agonists can attenuate the behavioral effects of heroin and other high efficacy μ opioid agonists (Cook and Picker, 1998; Cook and Beardsley, 2004). Both *R*-(−)-NPA and quinpirole, another D2-like agonist, have been shown to inhibit self-administration of heroin under a progressive ratio schedule in rhesus monkeys (Rowlett et al., 2007). Only a few studies have investigated combinations of heroin or other μ opioid agonists with D1-like agonists and none using drug discrimination procedures. Of these reports, none observed attenuation of the effects of the μ agonist by a D1-like agonist. In fact, Rowlett et al. (2007) showed that combined administration of heroin with SKF 81297 or SKF 82958 (another D1-like agonist) resulted in enhanced rather than attenuated self-administration compared with heroin alone.

Although the possibility of functional antagonism of the DS effects of heroin by cocaine and other DA ligands in our study cannot be ruled out, it seems more likely that the attenuated effects of heroin in the heroin-trained subjects reflects the perceptual masking of the heroin DS by the DA agonists (cf. Gauvin and Young, 1989). Perceptual masking has been inferred from demonstrations of attenuation of the DS effects of a drug without concomitant attenuation of its rate-altering effects. Although heroin itself did not alter response rates systematically, rate-decreasing effects were observed with the highest dose combinations at both heroin-cocaine dose ratios, the highest dose combinations at both heroin-SKF 81297 dose ratios, and all dose combinations of the highest heroin-*R*-(−)-NPA dose ratio.

In summary, the present study revealed a marked asymmetry in the generalization profile of DA and opioid agonists in monkeys trained to discriminate either cocaine or heroin from vehicle such that μ opioid agonists partially substituted for the DS effects of cocaine, but DA direct and indirect agonists did not substitute for the DS effects of heroin. An additional asymmetry was observed for the DS effects of

cocaine-heroin combinations in cocaine- compared with heroin-trained monkeys. Whereas heroin and other μ opioid agonists consistently enhanced the DS effects of cocaine, cocaine only inhibited the DS effects of heroin. Inhibition of the DS effects of heroin also was observed when heroin was combined with either a D1- or a D2-like agonist. These drug combinations also produced significant decreases in response rate, suggesting that the DA agonist-induced inhibition of the DS effects of heroin was due at least in part to masking of the heroin DS. The masking probably reflects stimulation of D1- and D2-like receptors because SCH 39166 and raclopride reversed the effects of SKF 81297 and *R*-(−)-NPA, respectively.

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Address correspondence to: Dr. Donna M. Platt, Harvard Medical School, New England Primate Research Center, One Pine Hill Dr., Box 9102, Southborough, MA 01772-9102. E-mail: donna_platt@hms.harvard.edu