Dissociation of Cocaine-Antagonist Properties and Motoric Effects of the D1 Receptor Partial Agonists SKF 83959 and SKF 77434

DONNA M. PLATT, JAMES K. ROWLETT, and ROGER D. SPEALMAN
Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts
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ABSTRACT
Previous studies suggest that D1 receptor partial agonists may be viable candidates for development as pharmacotherapies for cocaine addiction. This study investigated the ability of the D1 receptor partial agonists SKF 83959 and SKF 77434 to modulate the behavioral effects of cocaine and compared these effects with those of the reference D1 receptor antagonist SCH 39166 and D1 receptor agonists SKF 81297 and 6-Br-APB. Squirrel monkeys were trained either to respond under a fixed-interval schedule of stimulus-shock termination or to discriminate cocaine from vehicle (procedures useful for evaluating the behavioral stimulant and subjective effects of cocaine, respectively). Additional monkeys were studied with quantitative observational techniques to evaluate the effects of the drugs on various forms of motor behavior. Like SCH 39166, but unlike SKF 81297 and 6-Br-APB, the D1 receptor partial agonists attenuated the behavioral stimulant and discriminative stimulus effects of cocaine in a dose-dependent manner, although maximum antagonism produced by SKF 77434 was not always as great as that produced by SKF 83959 or SCH 39166. In observational studies, SKF 83959 and SKF 77434 produced less severe disruptions in motor behavior than did SCH 39166 and, for SKF 83959, showed a greater separation between the dose required to antagonize the behavioral effects of cocaine and the dose that induced catalepsy (>33-fold). These results suggest that D1 receptor partial agonists can act as functional cocaine antagonists with less severe behavioral effects than D1 receptor antagonists. The prominent cocaine-antagonist properties and the low incidence of motoric side effects of SKF 83959 may reflect its unique binding profile at D1 as well as nondopaminergic receptors.

The continued abuse of cocaine has intensified efforts to develop medications for the treatment of cocaine addiction. Based on preclinical findings, dopamine (DA) agonists and antagonists have been proposed as candidate pharmacotherapies, either as maintenance medications or as cocaine antagonists (for review, see Mendelson and Mello, 1996). These drugs, however, have had limited clinical success (Kosten and McCance, 1996; Warner et al., 1997) and may be associated with debilitating side effects (Meltzer, 1993; Koller and Rueda, 1998). An alternative strategy to developing DA agonists and antagonists as pharmacotherapeutics has been to evaluate the cocaine-modulating effects of another class of DA ligand, the partial agonists (Pulvirenti and Koob, 1994; Spealman et al., 1997). Partial agonists are drugs that bind to a receptor, yet have submaximal capacity to activate its associated signal transduction mechanisms. As a result, partial agonists can exhibit either agonist-like or antagonist-like properties depending on factors such as neurotransmitter tone, receptor reserve, and the presence of exogenous ligands (Ariëns, 1983).

Cocaine, as a result of its ability to block DA transport, increases the levels of extracellular DA available for binding at DA receptors (Hurd et al., 1988; Petit and Justice, 1989). Consequently, in the presence of cocaine (a state of relatively high DA activity), DA partial agonists would be expected to function primarily as antagonists, whereas in the absence of cocaine (a state of comparatively low DA activity), DA partial agonists would be expected to act primarily as weak agonists. Partial agonists might, therefore, exhibit reduced abuse potential compared with full agonists as well as less severe motoric effects compared with antagonists.

Preclinical support for the use of DA partial agonists in the treatment of cocaine addiction comes from studies demonstrating that some of these drugs can modulate the abuse-related effects of cocaine in animals. In this regard, the D2 partial agonists terguride and SDZ 208-911 have been found

ABBREVIATIONS: DA, dopamine; DS, discriminative stimulus; FI, fixed-interval; FR, fixed-ratio; MPTP, 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine; NE, norepinephrine.
to antagonize the behavioral effects of cocaine in some studies (Callahan and Cunningham, 1993; Pulvirenti and Koob, 1994; Pulvirenti et al., 1998), although they appear to be less effective or may even exacerbate the effects of cocaine in others (Spealman, 1995; Weissenborn et al., 1996). The D1 partial agonists, in contrast, appear to have more consistent effects both across species and across procedures. For example, the D1 partial agonists SKF 38393 and SKF 75670 can attenuate the discriminative stimulus (DS) and behavioral stimulant effects of cocaine in monkeys and rodents, resulting in rightward shifts of the cocaine dose-response functions (Spealman et al., 1997; Katz et al., 1999). These same drugs also have been shown to antagonize i.v. self-administration of cocaine in both rats and monkeys (Bergman and Rosenzweig-Lipson, 1992; Katz and Witkin, 1992; Caine et al., 1999). In addition, SKF 38393 and another D1 partial agonist, SKF 83959, have been found to inhibit the reinstatement of extinguished cocaine-seeking behavior, suggesting that these compounds may be effective in attenuating relapse as well (Spealman et al., 1999). Significantly, the ability of D1 partial agonists to attenuate the effects of cocaine have been observed at doses below those that disrupt other forms of behavior (Katz and Witkin, 1992; Rosenzweig-Lipson and Bergman, 1994; Platt et al., 1998).

The purpose of this study was to investigate the potential utility of the D1 receptor partial agonists SKF 83959 and SKF 77434 as pharmacotherapies for cocaine addiction. SKF 83959 was chosen because of its low agonist efficacy as determined by its capacity to stimulate adenylyl cyclase (Arnt et al., 1999). SKF 77434 (Andersen and Jansen, 1990) was selected because it is the N-allyl derivative of the prototypical D1 partial agonist SCH 39166, and, consequently, has relatively high central nervous system bioavailability (Peiffer et al., 1982).

Monkeys were trained to respond under a fixed-interval (FI) schedule of stimulus-shock termination under conditions in which cocaine induces characteristic increases in response rate (Spealman et al., 1989). A second group of animals was trained to discriminate cocaine from vehicle, a procedure used to investigate the subjective effects of cocaine in animals (Holtzman, 1990). Both procedures engender behavior that is sensitive to the effects of cocaine, as well as to the modulation of cocaine’s effects by candidate pharmacotherapies (Spealman, 1995; Spealman et al., 1997). Because conventional DA antagonists often induce pronounced motoric side effects, it was of particular interest to determine the degree to which the cocaine-modulating effects of DA partial agonists can be dissociated from their effects on other behaviors. Therefore, partial agonists also were evaluated in quantitative observational studies. Finally, the D1 receptor agonist SCH 39166 and the D1 agonists 6-Br-APB and SKF 81297 were studied as reference compounds to determine the degree to which the effects of partial agonists resembled those of either full agonists or antagonists.

Materials and Methods

Subjects. Fourteen adult male squirrel monkeys (Saimiri sciureus), weighing 750 to 1100 g, were studied in daily experimental sessions (Monday to Friday). Between sessions, monkeys lived in individual home cages where they had unrestricted access to water. Monkeys used in observational studies and in studies of FI behavior had unrestricted access to food (Teklad Monkey Diet supplemented with fresh fruit). Monkeys used in drug discrimination studies were maintained at 85 to 90% of their free-feeding body weight by adjusting their access to food in the home cage. 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. Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Apparatus. In experiments involving FI behavior and drug discrimination, monkeys were seated in Plexiglas chairs similar to those described by Spealman et al. (1997). Either one or two response levers, depending on the experimental procedure, were mounted on the wall of the chair in front of the monkey. Each press of a lever with a minimum downward force of 0.25 N produced an audible click and was recorded as a response. Colored lights mounted above the levers could be illuminated to serve as visual stimuli. Chairs were equipped with white noise to mask external sounds. In experiments involving FI behavior, a shaved portion of the tail of the monkey was secured under brass electrodes and was coated with electrode paste to ensure low-resistance electrical contact between electrodes and tail. Brief (200 ms), low-intensity (3 mA) electric shocks could be delivered to the tail. In drug discrimination experiments, 190-mg food pellets (Formula L; P.J. Noyes Co. Inc., Lancaster, NH) could be delivered to a tray, which was accessible through an opening in the front panel of the chair.

Observational studies were conducted in a ventilated, transparent Plexiglas arena (114 × 122 × 213 cm) located in a lighted room isolated from other animals. The arena was equipped with perches, suspended plastic chains, and a wood chip foraging substrate to provide opportunities for monkeys to express a range of species-typical motor behaviors. A video camera connected to a videocassette recorder was positioned 1 m in front of the chamber and operated continuously during the observation session.

FI Behavior. Six monkeys were trained to respond under a 3-min FI schedule of stimulus-shock termination similar to the one described by Spealman et al. (1989). In the presence of a red stimulus light, electric shocks were scheduled every 3 s after a 3-min FI had elapsed. The first response after 3 min terminated the light, along with the programmed shocks, and started a 10-s timeout period. If a response was not made by the third shock, the stimulus light was extinguished, shocks were discontinued and the timeout was started. During the timeout, the chamber was dark and responses had no scheduled consequences. Each experimental session was comprised of five identical components. A component consisted of five presentations of the FI schedule and each component was preceded by a 10-min timeout during which drugs could be administered as described below.

Drug test sessions were conducted once or twice per week, with control sessions on intervening days. The effects of cocaine alone and after pretreatment with each D1 ligand were determined with a cumulative-dosing procedure similar to the one described by Spealman et al. (1989). Incremental doses of cocaine were injected i.m. at the midpoint of the timeout periods that preceded sequential components of the session. This procedure permitted determination of a five-point cumulative dose-response function in a single experimental session. In drug pretreatment experiments, selected doses of the D1 partial agonists SKF 83959 and SKF 77434, the D1 antagonist SCH 39166, and the D1 agonists SKF 81297 and 6-Br-APB were administered i.m. 5 min before the session. Cumulative doses of cocaine were then administered during the session as described above. Dose-response curves for cocaine alone and after pretreatment with each D1 agonist or antagonist typically were determined twice in each monkey. To evaluate the effect of the pretreatment...
drug alone, saline instead of cocaine was administered as the first injection of at least one test session.

**Cocaine Discrimination.** Four monkeys had been trained previously to discriminate cocaine from saline with a drug discrimination procedure similar to the one described by Speelman et al. (1997). After injection of cocaine (0.3 mg/kg), 10 consecutive responses (fixed-ratio (FR) 10) on one lever produced food, whereas after injection of saline, 10 consecutive responses on the other lever produced food. Responses on the incorrect lever reset the FR response requirement. Daily 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, whichever occurred first. A 10-min timeout period, during which the lights were off and responses had no scheduled consequences, preceded each component. During most training sessions, saline was injected during timeout periods preceding the first n – 1 components, and cocaine was injected before the final component of the session. Periodically, saline was injected before each of the components of a training session to prevent an invariant association between drug and the fourth component. Injections of cocaine or saline were made in a thigh or calf muscle of either leg during the 5th minute of the 10-min timeout periods.

Drug testing began once monkeys made ≥90% of responses on the injection-appropriate lever during at least four of the last five training days. Thereafter, drug test sessions were conducted once or twice per week with training sessions scheduled on intervening days. Test sessions consisted of four components, each preceded by a 10-min timeout period. In each component, completion of 10 consecutive responses on either lever produced food. Dose-response functions were determined for cocaine with a cumulative-dosing procedure similar to the one described above for FI behavior. Incremental doses of cocaine were injected i.m. during the 5th minute of the 10-min timeout periods that preceded each FR component, permitting a 4-point cumulative dose-response function to be determined in a single session. In experiments involving drug pretreatments, different doses of SKF 83959, SKF 77434, SCH 39166, and 6-Br-APB were administered 5 min before the session, and cumulative doses of cocaine were administered during the session as described above. In general, dose-response curves for cocaine alone and after pretreatment with each D1 agonist or antagonist were determined twice in each monkey. To determine the effect of the pretreatment drug alone, saline was administered as the first injection of at least one test session.

**Observational Study.** Four monkeys initially were habituated to the observation arena and the handling and injection procedures described below for a period of ~1 month. Following habituation, 30-min observational sessions were conducted daily during which the animal’s behavior was videotaped continuously. This procedure provided an archival record of experimental sessions and permitted subsequent scoring of videotapes by independent observers. Additionally, during the 6th, 18th, and 30th minute of each 30-min session, the monkeys were removed briefly from the observation arena by a trained handler and evaluated for ataxia (defined as the inability to balance on and/or grasp a stainless steel transport pole held in the horizontal plane) and muscle rigidity (defined as greater than normal resistance to hind limb flexion and/or rigid grasping of the grid floor). During each assessment, a score of 0, 1, or 2 was assigned to these measures. For ataxia, a score of 0 indicated that the monkey was able to balance normally on the transport pole, a score of 1 indicated inability to balance effectively, and a score of 2 indicated that the monkey could neither balance nor grasp the pole. For muscle rigidity, a score of 0 indicated no abnormal resistance to hind limb flexion, a score of 1 indicated either an increased resistance to flexion or clamping to the grid floor, and a score of 2 indicated both resistance to flexion and clamping to the grid floor. Drug test sessions were conducted once or twice per week, with saline control sessions on intervening days. A full range of doses of SKF 83959, SKF 77434, SCH 39166, SKF 81297, and 6-Br-APB as well as saline controls were administered i.m. 30 min before the start of the experimental session. Videotaping and assessment of ataxia and muscle rigidity were conducted each session as described above.

Scoring of videotapes was conducted by an observer who was trained in the use of the behavioral scoring system described by Novak et al. (1992) as modified for the squirrel monkey, but who was not informed about the drugs under investigation. Four observers performed the videotape scoring for the duration of the study. To ensure reliability across observers, each individual underwent at least 20 h of training until they reached an interobserver reliability criterion of ≥90% based on percentage of agreement scores. The behavioral scoring system included 10 categories (Table 1) that were scored by recording the presence or absence of each behavior (i.e., the absolute frequency) in 15-s intervals during three 5-min observation periods across the session (0–5, 12–17, and 24–29 min). Modified frequency scores were calculated from these data as the proportion of 15-s intervals in which a particular behavior occurred. Mean modified frequency scores were determined each session for all behavioral categories in individual subjects. To facilitate data analysis, environmental manipulation and foraging were combined into the more general category exploratory behavior. Likewise, self-grooming and scratching were combined into the category self-directed behavior.

**Analysis of Drug Effects.** In studies of FI behavior, rates of responding were calculated separately in each component of the session by dividing the total number of responses in a component by the total time the component was in effect. Mean control rates of responding in each component were determined by averaging data from all noninjection control sessions that preceded drug test sessions. The effects of saline and each drug or drug combination were calculated as a percentage of the mean control rate in the corresponding component for individual subjects. The doses of cocaine (alone and after D1 ligand pretreatment) estimated to engender 50% of the maximum response rate (ED50) were determined for individual subjects by linear regression analysis in cases where the ascending limb of the log dose-response function was defined by three or more data points [if y = 50%, then ED50 = 10(Slope/ln(200)) or by linear interpolation in cases where the ascending limb was defined best by two points (cf. Speelman et al., 1989)].

In experiments involving drug discrimination, the percentage of responses on the cocaine-associated lever was calculated for individual subjects in each component of a test session by dividing the number of responses on that lever by the total number of responses on both levers and multiplying by 100. 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. Percentage of drug-lever responding was not included in the analysis if the mean response rate was ≥0.10 responses/s. The doses of cocaine estimated to engender 50% cocaine-appropriate responding were calculated as a percentage of the mean control rate in the corresponding component for individual subjects. The doses of cocaine were injected i.m. 30 min before the start of the experimental session.

**TABLE 1**

<table>
<thead>
<tr>
<th>Behavioral categories</th>
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<tr>
<td>Vocalization—any utterance including chirps, twitters, peeps, screeches, etc.</td>
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<td>Catalepsy—any static, atypical or awkward posture</td>
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<td>Locomotion—any two or more directed steps in the horizontal and/or vertical plane</td>
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<td>Exploratory behavior—combined score of the following two categories:</td>
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<tr>
<td>Environmental manipulation—any tactile or oral manipulation of features of the</td>
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<td>observation arena</td>
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<tr>
<td>Foraging—sweeping and/or picking through wood chip substrate</td>
</tr>
<tr>
<td>Self-directed behavior—combined score of the following two categories:</td>
</tr>
<tr>
<td>Self-grooming—picking, scratching, spreading, or licking of fur</td>
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<tr>
<td>Scratching—movement of digits through fur in a rhythmic, repeated motion</td>
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<tr>
<td>Visual scanning—directed eye and/or head movements, usually from a sitting position</td>
</tr>
<tr>
<td>Sleep posture—species-typical position: eyes closed, head tucked to chest, tail wrapped</td>
</tr>
<tr>
<td>Upper body</td>
</tr>
<tr>
<td>Other—any notable behavior not defined above (e.g., yawn, sneeze)</td>
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sponding (ED_{50}) were determined for individual subjects by linear interpolation or linear regression analysis as outlined above (cf. Speelman et al., 1991).

In studies of unconditioned behavior, individual mean modified frequency scores for the categories locomotion, exploratory behavior, visual scanning, and self-directed behavior were transformed to a percentage of individual saline control values and then averaged across subjects to provide group means. Statistical reliability of treatment effects were assessed with Dunnett’s q statistic, in which the effects of different doses of each drug were compared with a saline control value. Individual scores for the categories of catalepsy and sleep posture were transformed to a percentage of maximum possible score for calculation of ED_{50} values because monkeys rarely displayed these behaviors during saline control sessions. Individual scores, as well as median scores, are presented for the categories of catalepsy, sleep posture, muscle rigidity, and ataxia. To determine statistical reliability of treatment effects on each of these four categories, the effect of dose was determined for each drug by separate Friedman’s repeated-measures ANOVA on ranks. For each category, to determine whether the effects varied over the course of the study, separate Friedman’s repeated measures ANOVAs were performed on the three time periods separately for each dose. The α-level for all statistical tests was P ≤ .05.

In addition to these tests, a ratio was constructed based on the potency of each drug to antagonize the effects of cocaine and to induce catalepsy. Potencies for antagonism were based on the dose of a D1 ligand that produced a 2-fold increase in the ED_{50} for cocaine in both the FI and discrimination procedure (a measure conceptually related to an estimate of apparent affinity). Potencies for catalepsy scores were based on the lowest dose of a D1 ligand that induced a catalepsy score of ≥50% (a measure conceptually related to ED_{50}).

Drugs. (-)-Cocaine HCl, (±)-SKF 77434 HBr, (±)-SKF 81297 HBr, R(+)-6-Br-APB HBr (all purchased from Research Biochemicals, Natick, MA), SKF 83959 HBr (National Institute on Drug Abuse, Rockville, MD), and (−)-SCH 39166 HCl (Schering-Plough Research Institute, Kenilworth, NJ) were dissolved in small amounts of ethanol and 0.1 N HCl as required and diluted to the desired concentrations with sterile water or 0.9% saline solution. Compound SKF 83959 was provided by Research Biochemicals International as part of the Chemical Synthesis Program of the National Institute of Mental Health, Contract N01MH30003.

Results

FI Behavior. During control sessions that preceded drug test sessions, rates and temporal patterns of responding were characteristic for performances maintained on FI schedules of stimulus-shock termination. Responding generally occurred at low rates in the early portion of each interval and accelerated as the interval progressed. The average rate of responding ranged from 0.48 to 0.90 responses/s among individual monkeys and did not vary systematically over successive components of control sessions (±5% average deviation from the whole-session mean for each subject).

When administered alone, low-to-intermediate doses of cocaine (0.03–0.3 mg/kg) produced dose-related increases in the average rates of responding, whereas higher doses either increased responding less or decreased it (Figs. 1 and 2, filled circles). The D1 antagonist SCH 39166 produced a dose-related antagonism of the rate-altering effects of cocaine, resulting in an overall rightward shift in the cocaine doseresponse function (Fig. 1, left). The degree of antagonism depended on the dose of SCH 39166 administered before the session (Table 2). Compared with the average ED_{50} for the rate-increasing effects of cocaine alone, the ED_{50} for cocaine after pretreatment with the highest dose of SCH 39166 was increased 31-fold. Lower doses of SCH 39166 (0.03 or 0.1 mg/kg) antagonized the rate-increasing effects of cocaine to a lesser degree, resulting in a 3- to 11-fold increase in ED_{50}. In addition to antagonism of the effects of cocaine by SCH 39166, there was evidence for reciprocal antagonism of the effects of SCH 39166 by cocaine. When administered before saline, SCH 39166 produced dose-related decreases in response rate (Fig. 1, open symbols above “S”), which were overcome by administration of intermediate to high doses of cocaine during the session.

Pretreatment with the D1 partial agonist SKF 83959 also produced an overall rightward shift in the cocaine dose-response function, indicative of surmountable antagonism (Fig. 1, middle). For individual subjects, the biphasic function relating dose of cocaine and response rate was retained and the maximum increases in response rate generally were comparable with those determined with cocaine alone. The degree of antagonism of the rate-altering effects of cocaine depended on the dose of SKF 83959 administered before the session (Table 2). Pretreatment with the highest dose of SKF 83959 (3.0 mg/kg) increased the average ED_{50} for the rate-increasing effects of cocaine ~43-fold and permitted testing.
Effects of cocaine alone and after pretreatment with SKF 81297 and 6-Br-APB on response rate under the FI schedule of stimulus-shock termination. The lowest dose of SKF 81297 (0.3 mg/kg) had no effect on the cocaine dose-response function and is not shown. See Fig. 1 for other details.

**TABLE 2**

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (mg/kg)</th>
<th>FI Behavior</th>
<th>Cocaine Discrimination</th>
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<tr>
<td>Cocaine alone</td>
<td>0.09 (±0.01)</td>
<td>0.13 (±0.02)</td>
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<tr>
<td>+ 0.03 SCH 39166</td>
<td>0.27 (±0.17)</td>
<td>0.28 (±0.05)</td>
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<tr>
<td>+ 0.1 SCH 39166</td>
<td>0.96 (±0.36)</td>
<td>0.53 (±0.07)</td>
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<tr>
<td>+ 0.18 SCH 39166</td>
<td>2.75 (±0.95)</td>
<td>0.46 (±0.07)</td>
<td></td>
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<tr>
<td>+ 0.1 SKF 83959</td>
<td>—&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 (±0.11)</td>
<td></td>
</tr>
<tr>
<td>+ 0.3 SKF 83959</td>
<td>0.51 (±0.09)</td>
<td>0.38 (±0.02)</td>
<td></td>
</tr>
<tr>
<td>+ 1.0 SKF 83959</td>
<td>0.63 (±0.15)</td>
<td>0.67 (±0.28)</td>
<td></td>
</tr>
<tr>
<td>+ 3.0 SKF 83959</td>
<td>3.90 (±0.89)</td>
<td>—&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>+ 1.0 SKF 77434</td>
<td>0.23 (±0.05)</td>
<td>0.32 (±0.03)</td>
<td></td>
</tr>
<tr>
<td>+ 3.0 SKF 77434</td>
<td>0.64 (±0.11)</td>
<td>0.37 (±0.07)</td>
<td></td>
</tr>
<tr>
<td>+ 5.6 SKF 77434</td>
<td>0.89 (±0.04)</td>
<td>0.43 (±0.22)</td>
<td></td>
</tr>
<tr>
<td>+ 10.0 SKF 77434</td>
<td>N.D.&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<sup>a</sup> Not tested.
<sup>b</sup> ED<sub>50</sub> value could not be determined.

Cocaine-Antagonist Properties of D1 Partial Agonists

The effects of doses of cocaine up to 10.0 mg/kg. Pretreatment with the lower doses of SKF 83959 (0.3 or 1.0 mg/kg) antagonized the effects of cocaine to a lesser extent, producing smaller (5- to 7-fold) increases in ED<sub>50</sub>. As with SCH 39166, there was evidence for reciprocal antagonism of the effects SKF 83959 by cocaine. When administered before saline, SKF 83959 produced dose-related decreases in response rate (Fig. 1, open symbols above “S”) which were reversed by administration of intermediate-to-high doses of cocaine during the session.

The effects of the D1 partial agonist SKF 77434 differed in some respects from those of SKF 83959 and SCH 39166. Doses of SKF 77434 up to 5.6 mg/kg produced modest rightward shifts in the cocaine dose-response function and increased the ED<sub>50</sub> for the rate-increasing effects of cocaine 3- to 8-fold (Fig. 1, right; Table 2). After pretreatment with an even higher dose of SKF 77434 (10.0 mg/kg), no further rightward shifts in the cocaine dose-response function were evident. Instead, this dose of SKF 77434 either suppressed or did not change response rate in combination with all doses of cocaine, resulting in a relatively flat dose-response function. As for SKF 83959 and SCH 39166, SKF 77434 dose-dependently decreased response rates in the absence of cocaine (Fig. 1, symbols above “S”), and administration of increasing doses of cocaine during the session tended to reverse these effects.

When combined with cocaine, the D1 agonists SKF 81297 and 6-Br-APB did not produce an overall rightward shift in the cocaine dose-response function (Fig. 2). Rather, pretreatment with the lowest dose of each agonist tended to suppress the rate-increasing effects of low-to-intermediate doses of cocaine and did not greatly alter the effects of higher doses. Increasing the dose of SKF 81297 and 6-Br-APB to 3.0 and 0.3 mg/kg, respectively, produced an overall downward shift and flattening of the cocaine dose-response function, reflecting suppression of the rate-increasing effects of low-to-intermediate doses of cocaine and, for SKF 81297, exacerbation of the rate-decreasing effects of higher doses of cocaine.

In the absence of cocaine, SKF 81297 and 6-Br-APB produced decreases in response rate. The rate-decreasing effects of the lower dose of SKF 81297 and both the doses of 6-Br-APB were overcome by administration of intermediate-to-high doses of cocaine during the session. However, the suppression of responding produced by the higher dose of SKF 81297 was relatively unaffected by administration of cocaine during the session.

**Cocaine Discrimination.** Cocaine maintained consistent stimulus control of behavior over the course of the study. Averaged across all training sessions that preceded drug test sessions, individual monkeys made 98 ± 1% (mean ± S.E.) of responses on the cocaine lever after injection of cocaine and 2 ± 1% of responses on the cocaine lever after injection of saline. The average rate of responding after injection of cocaine (1.7 ± 0.1 responses/s) was consistently greater than the average response rate after injection of saline (0.7 ± 0.04 responses/s). During test sessions, cocaine engendered dose-related increases in the percentage of responses on the cocaine lever (Fig. 3, top, filled circles). The average rate of responding increased after administration of low-to-intermediate doses of cocaine (0.03–0.3 mg/kg) and decreased to ~50% of the saline control rate after administration of the highest dose of cocaine (Fig. 3, bottom, filled circles).

Pretreatment with SCH 39166 resulted in a dose-dependent rightward shift in the dose-response function for the DS effects of cocaine (Fig. 3, top) accompanied by an increase in the average ED<sub>50</sub> of up to 4-fold (Table 2). In the absence of cocaine, SCH 39166 did not engender any cocaine-appropriate responding (open circles above “S”). Pretreatment with SKF 83959 and SKF 77434 also attenuated the DS effects of cocaine in a dose-related manner and produced overall rightward shifts in the cocaine dose-response function. Compared with the average ED<sub>50</sub> for cocaine alone, the average ED<sub>50</sub> for cocaine was increased 4- to 5-fold after pretreatment with the highest dose of either SKF 83959 or SKF 77434 (Table 2). Lower doses of SKF 83959 and SKF 77434 produced less pronounced antagonism of the DS effects of cocaine and smaller increases in ED<sub>50</sub>. When saline was administered in the first component after pretreatment with either SKF 83959 or SKF 77434, neither drug engendered any cocaine lever responding (open circles above “S”).

In addition to antagonism of the DS effects of cocaine, SKF 83959, SKF 77434, and SCH 39166 often attenuated the
effects of cocaine on response rate (Fig. 3, bottom). Consistent with their effects on FI behavior, pretreatment with increasing doses of SKF 83959, SKF 77434, and SCH 39166 produced dose-dependent rightward shifts in the ascending limb of the cocaine dose-response function, reflecting the antagonism of the rate-increasing effects of low-to-intermediate doses of cocaine (0.1–0.3 mg/kg). Higher doses of SCH 39166, SKF 83959, and SKF 77434 also attenuated the rate-decreasing effects of 1.0 mg/kg cocaine. In addition, the highest doses of SCH 39166 produced a clear rightward shift in the descending limb of the cocaine dose-response function. In the absence of cocaine, at least one dose of SKF 83959, SKF 77434, and SCH 39166 produced substantial (∼50%) decreases in response rate (open symbols above “S”), which could be overcome to varying degrees by cocaine administered cumulatively during the session.

Pretreatment with 6-Br-APB did not antagonize the DS effects of cocaine (Fig. 3, right, top). Instead, this drug produced a leftward shift in the cocaine dose-response function such that low doses of cocaine engendered a greater proportion of cocaine-lever responses in the presence than in the absence of 6-Br-APB (Fig. 3) and reduced the average ED$_{50}$ for cocaine by as much as 5-fold. In general, the effects of cocaine combined with 6-Br-APB were similar to those that would be expected by simply adding the percentage of cocaine-lever responses engendered separately by the two drugs because 6-Br-APB itself engendered 18 to 27% cocaine-lever responding when combined with saline (open symbols above “S”). Pretreatment with 6-Br-APB produced an overall suppression of response rate, which was not overcome by administration of cocaine during the session (Fig. 3, right, bottom).

**Observed Behavior.** SKF 83959 and SKF 77434, as well as SCH 39166, eliminated locomotion, exploration, and self-directed behaviors at one or more doses in all monkeys (Table 3). SKF 83959 and SKF 77434 also reduced visual scanning maximally to ∼50% of the saline control value. The highest dose of SCH 39166 eliminated visual scanning. In contrast to the effects of the D1 antagonist and partial agonists, SKF 81297 and 6-Br-APB had less pronounced effects on all of these behaviors, although SKF 81297 did significantly decrease self-directed behavior and significantly increase visual scanning.

For catalepsy, sleep posture, ataxia, and muscle rigidity, no statistically reliable effects across the three time periods were observed; therefore, only effects of dose collapsed across time are discussed. SCH 39166 and, to a lesser extent, SKF 83959 and SKF 77434 induced catalepsy at high doses (Fig. 4). A maximum median catalepsy score of 20.0 was observed after the highest dose of SCH 39166 (0.3 mg/kg), indicating that all animals displayed catalepsy throughout the entire session ($\chi^2[4] = 11.84, P < .05$). SKF 83959 and SKF 77434, however, produced maximum median catalepsy scores of only 10.0 and 16.3 (50.0 and 81.3% of maximum), respectively, indicating that not all animals displayed catalepsy at a given dose and/or that the animals were not cataleptic for the entire session. For SKF 83959, the largest catalepsy scores were observed at the highest doses tested (10 and 17.8 mg/kg), and no reliable effect of dose was observed (Friedman’s ANOVA, $P > .05$). For SKF 77434, the
The largest score was observed at an intermediate dose, and smaller effects were observed when the dose was >10 mg/kg; similar to SKF 83959, no reliable effect of dose was observed (Friedman's ANOVA, P >.05). Catalepsy was not observed after administration of any dose of SKF 81297 or 6-Br-APB (data not shown). Higher doses of these drugs were not tested because seizures were observed in one animal after administration of 3.0 mg/kg SKF 81297.

Ratios representing the differential between cocaine-modulating doses and cataleptogenic doses were determined for SCH 39166, SKF 77434, and SKF 83959. For both SCH 39166 and SKF 77434, the lowest dose that induced catalepsy scores of ≥50% was 10 times higher than the lowest dose that antagonized the effects of cocaine (i.e., increase the ED50 for cocaine at least 2-fold) in both the FI and cocaine discrimination procedures. For SKF 83959, the lowest dose that produced a catalepsy score of ≥50% was 10.0 mg/kg; which was 33 times higher than the lowest dose that antagonized the behavioral stimulant effects of cocaine, and 100 times higher than the lowest dose that antagonized the DS effects of cocaine.

SCH 39166, SKF 83959, and SKF 77434 also increased the incidence of sleep posture (Fig. 4). However, no reliable effect of dose was observed for SCH 39166, SKF 83959, or SKF 77434 (Friedman’s ANOVA, P >.05). SKF 81297 and 6-Br-APB had little or no effect on the incidence of sleep posture regardless of dose (data not shown).

One or more doses of SCH 39166, SKF 83959, and SKF 77434 induced ataxia (Fig. 5, top) and muscle rigidity (Fig. 5, bottom) in most subjects. The maximum median scores for muscle rigidity (3.0) and ataxia (3.5) produced by SKF 77434 were comparable with those produced by SCH 39166 (4.5 and 3.0, respectively), whereas the maximum median scores for muscle rigidity and ataxia produced by SKF 83959 were considerably less (both 1.5). The effect of dose on ataxia for both SCH 39166 and SKF 77434 was statistically reliable (Friedman’s ANOVA, χ²[4] = 14.44, and χ²[6] = 13.61, P < .05), whereas the effect of dose for SKF 83959 was not reli-

### Table 3

Maximum effects of D1 ligands on observed behavior

Data are mean ± S.E. expressed as percentage of saline control values.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose Range</th>
<th>% Saline Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Visual Scan</td>
</tr>
<tr>
<td>SKF 83959</td>
<td>0.1–17.8</td>
<td>51.27 ± 18.66</td>
</tr>
<tr>
<td>SKF 77434</td>
<td>1.0–30.0</td>
<td>54.02 ± 45.72</td>
</tr>
<tr>
<td>SCH 39166</td>
<td>0.01–0.3</td>
<td>134.80 ± 15.81b</td>
</tr>
<tr>
<td>SKF 81297</td>
<td>0.3–3.0</td>
<td>121.64 ± 13.66</td>
</tr>
<tr>
<td>R(-)+6-Br-APB</td>
<td>0.1–0.3</td>
<td>81.68 ± 15.85</td>
</tr>
</tbody>
</table>

* Saline control values (mean modified frequency values): visual scan = 15.86 ± 0.20; locomotion = 11.14 ± 0.29; exploratory = 6.99 ± 0.27; self-directed = 1.42 ± 0.08.

b Means differ from control values as determined by Dunnett's q statistic (α = 0.05)

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**Fig. 4.** Effects of SCH 39166, SKF 83959, and SKF 77434 on catalepsy and sleep posture. Data points represent individual scores. The solid line indicates the median score for each dose; ●, S-365; △, S-89; ▼, S-430; ◊, S-450.
able \( (P > .05) \). For muscle rigidity, however, a reliable effect of dose was observed for SKF 77434 only (Friedman’s ANOVA, \( \chi^2[6] = 12.61, P < .05 \)), although the effect for SCH 39166 approached statistical reliability (Friedman’s ANOVA, \( \chi^2[4] = 9.33, P = .053 \)). No dose of SKF 81297 or 6-Br-APB induced ataxia or muscle rigidity (data not shown).

**Discussion**

To date, no broadly effective DA-based pharmacotherapy has been identified for the treatment of cocaine addiction. An alternative strategy to developing DA agonists and antagonists as pharmacotherapies has been to evaluate the cocaine-modulating effects of the DA partial agonists. In the absence of cocaine, DA partial agonists would be expected to act as weak agonists and perhaps serve as cocaine maintenance medications, whereas in the presence of cocaine, DA partial agonists would be expected to function primarily as cocaine antagonists. In this study, no evidence of cocaine-like effects of the D1 partial agonists was observed. Rather, SKF 83959 and SKF 77434 functioned exclusively as cocaine antagonists. In this respect, the cocaine-modulating effects of the partial agonists were qualitatively similar to those of the D1 antagonist SCH 39166 and unlike those of the D1 agonists SKF 81297 and 6-Br-APB. However, in contrast to SCH 39166, SKF 77434 and especially SKF 83959 had less debilitating motor effects in our observational study. From the perspective of medication development, a desirable characteristic of an effective cocaine antagonist would be a maximal separation between doses that modulate the effects of cocaine and doses that disrupt other behaviors. In this study, the partial agonist SKF 77434 blocked the DS and behavioral stimulant effects of cocaine at doses ~10 times lower than those that induced catalepsy and ataxia. A similar separation of doses was evident for the antagonist SCH 39166. SKF 83959, however, displayed a more impressive separation (33-to 100-fold) between doses that antagonized cocaine and doses that induced catalepsy.

Although both partial agonists acted as cocaine antagonists in the FI and discrimination procedures, in the FI procedure SKF 77434 had effects that differed from those of SCH 39166 and SCH 39166. In this procedure, low-to-intermediate doses of SKF 77434 produced rightward shifts in the cocaine dose-response function, an effect shared with SKF 83959 and SCH 39166; the highest dose of SKF 77434 flattened the cocaine dose-response function, an effect shared with SKF 81297 and 6-Br-APB. In vitro, SKF 77434, although characterized as a partial agonist, is capable of stimulating cAMP to a greater degree than SKF 83959 (Arnt et al., 1992; Weed et al., 1997). SKF 77434 also has been shown to produce effects in vivo similar to those of D1 full agonists. For example, SKF 77434, in common with the full agonists SKF 81297 and SKF 82958, but not the partial agonist SKF 38393, induces hyperactivity in rodents (Katz et al., 1999). Thus, the more agonist-like appearance of the highest dose of SKF 77434 in the FI procedure may be due to its comparatively high D1 efficacy.

Although SKF 77434 binds with high affinity at the D1 receptor, it shows significant binding at D2 receptors as well (Andersen and Jansen, 1990; Weed et al., 1998). In addition, there is evidence suggesting that SKF 77434 can, under some circumstances, act as a D2 as well as a D1 receptor agonist in vivo. For example, SKF 77434 is the only D1 partial agonist found to maintain i.v. self-administration (Self and Stein, 1992) and has been shown to increase rearing and thigmotaxis (putative D2 receptor-mediated effects; Meyer and Shults, 1993) at doses that also induced intense grooming (a
putative D1 receptor-mediated effect; Murray and Waddington, 1989). D2 agonist-like effects, which would be expected to emerge at high doses of SKF 77434, also may account for the biphasic effect of SKF 77434 on catalepsy, because D2 receptor agonists have been shown to ameliorate the catalepsy induced by D1 receptor antagonists (Morelli and Di Chiara, 1985). Collectively, these results raise the possibility that the D2 agonist-like effects of SKF 77434, alone or in conjunction with D1 agonist-like effects, may contribute to its unique profile of effects.

In this study, SKF 83959 attenuated both the DS and behavioral stimulant effects of cocaine in a manner similar to that of the antagonist SCH 39166. In other procedures, however, the effects of SKF 83959 more closely resembled those of D1 agonists. In a nonhuman primates model of Parkinson’s disease, for example, SKF 83959, like prototype D1 receptor full agonists, reversed motor deficits induced by the neurotoxin 1-methyl-4-(2’-methylphenyl)-1,2,3,6-tetrahydropyridine (MPTP), whereas other D1 partial agonists typically are ineffective in this model (Gnanalingham et al., 1995). The ability of SKF 83959 to ameliorate MPTP-induced motor deficits has been attributed to differences in its ability to stimulate D1 receptors coupled to transduction mechanisms other than or in addition to adenyl cyclase (e.g., phosphoinositide hydrolysis; Clifford et al., 1999). Although the capacity of SKF 83959 to stimulate inositol phosphate accumulation has not been reported, efficacy values are available for other D1 partial agonists (Undie et al., 1994). D1 agonists with high efficacy in this assay, such as SKF 38393 and SKF 75670, actually induce catalepsy to antagonist-like levels (Rosenzweig-Lipson and Bergman, 1994), suggesting that the reduced cataleptogenic effects of SKF 83959 are not related to high efficacy in stimulating phosphoinositide hydrolysis.

In addition to its partial agonist effects at D1 receptors, SKF 83959 acts as both an α₂-adrenoceptor antagonist and an inhibitor of norepinephrine (NE) transport (Andringa et al., 1999a). When combined with DA antagonists, α₂-receptors have been shown to inhibit the expression of catalepsy (Kalkman et al., 1998). This observation suggests that blockade of α₂-receptors by SKF 83959 may limit its liability to engender catalepsy. The NE-modulating effects of SKF 83959 also may contribute to its ability to alleviate motor deficits induced by MPTP. Deficient NE mechanisms have been postulated to govern the progressive degeneration of nigrostriatal DA neurons and the expression of Parkinsonian symptoms (Colpaert, 1994). It remains to be determined whether the NE-modulating effects of SKF 83959 can account for the wide separation between cataleptogenic and cocaine antagonistic doses of this drug.

In summary, efficacy (at least as determined by stimulation of adenyl cyclase) appeared to play an important, although perhaps not exclusive, role in the interaction between cocaine and the D1 partial agonist SKF 77434 and SKF 83959. Consistent with receptor theory (Ariens, 1983), relatively low intrinsic efficacy may confer antagonist-like properties to SKF 77434 and SKF 83959 in the presence of cocaine at doses that do not produce catalepsy. In addition, interactions with neurotransmitter systems other than DA (e.g., the NE system) may play a role in minimizing the disruptive motor effects of SKF 83959, implying that drugs with both D1 partial agonist and NE agonist-like properties warrant further consideration as candidate medications for cocaine addiction.

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References

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