Behavioral Effects of Cocaine: Interactions with D1 Dopaminergic Antagonists and Agonists in Mice and Squirrel Monkeys

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ABSTRACT

The present study compared interactions among dopamine D1-like agonists and partial agonists with cocaine on the locomotor stimulant effects of cocaine, as well as the discriminative-stimulus effects of cocaine, and effects of cocaine on rates of responding. Cocaine alone produced a dose-related stimulation of locomotor activity in Swiss-Webster mice and a dose-related increase in the proportion of responses on the cocaine-appropriate response key in squirrel monkeys (Saimiri sciureus) trained to discriminate cocaine (0.3 mg/kg i.m.) from saline. None of the D1 dopaminergic agents fully reproduced these effects, with SKF 77434 producing marginal stimulation of locomotor activity and SCH 23390, SCH 39166, and SKF 77434 producing some, although incomplete substitution for cocaine in monkeys discriminating cocaine. The D1 dopamine antagonists SCH 23390, SCH 39166, and A-69024 dose-dependently shifted the cocaine dose-effect curve for locomotor activity to the right and decreased the efficacy of cocaine. The same compounds shifted the discriminative-stimulus effects of cocaine to the right without altering efficacy of cocaine. In contrast to the effects on locomotor activity, the maximal shift to the right in the discriminative-stimulus effects of cocaine was ~3-fold, with higher doses of the antagonists producing no greater shifts in the cocaine dose-effect curve than with intermediate doses. The partial D1 agonists (±)-SKF 38393, (±)-SKF 38393, and SKF 77434 also dose-dependently shifted the dose-effect curve for locomotor stimulant effects to the right and decreased the maximal effect of cocaine. These compounds only shifted the discriminative-stimulus effects of cocaine to a 2-fold maximum. In general, cocaine effects on rates of responding in the subjects discriminating cocaine from saline were only minimally antagonized by coadministration of the D1 dopaminergic agents. Both potency for producing behavioral effects alone and in antagonizing the effects of cocaine were related to binding affinities assessed by displacement of [3H]SCH 23390 from rat striatum. These results suggest that actions mediated by D1-like receptors contribute to the behavioral effects of cocaine. However, the various limitations to the degree of antagonism accomplished indicate that D1-like dopaminergic actions appear to be more involved in the effects of cocaine on locomotor activity, relatively less involved in the discriminative-stimulus effects of cocaine, and least involved in the effects of cocaine on operant response rates. This differential involvement of D1 dopamine receptors in these various behavioral effects of cocaine suggests problems in predicting clinical efficacy of at least D1 receptor antagonists as potential treatments for cocaine abuse. Additional studies are necessary to determine whether the antagonism of cocaine can predict therapeutic efficacy at all, and, if so, which effects when antagonized are the best predictors.

The prevalence of illicit cocaine abuse has created significant public health problems in the United States over the last two decades (Chilcoat and Johanson, 1998). There have been continuing efforts to better understand the pharmacological mechanisms that underlie this abuse (Lesher, 1997), which may contribute to the identification of new leads for the discovery of medical treatments for cocaine abuse (Johnson and Vocci, 1993). Because cocaine indirectly stimulates dopaminergic receptors by blocking reuptake of dopamine (Heikkila et al., 1979), and because dopaminergic mechanisms have been implicated as mediating cocaine abuse (Wise, 1984; Kuhar et al., 1991), studies have focused on the respective roles of subtypes of dopamine receptors in mediating the pharmacological effects of cocaine.

Several studies have examined the antagonism of cocaine by D1-type dopamine receptor antagonists. For example, Woolverton and Virus (1989) found that the dopamine D1 receptor antagonist SCH 23390 decreased rates of responding maintained by cocaine in rhesus monkeys. However, these decreases in cocaine-maintained behavior were obtained only at doses of the antagonist that also decreased rates of responding maintained by food reinforcement. The

ABBREVIATIONS: FR, fixed-ratio; 5-HT, 5-hydroxytryptamine.
similarity of the potency of the antagonist in decreasing rates of responding maintained by either reinforcer suggests that this effect was not a specific action on the reinforcing effects of cocaine. Bergman et al. (1990) found that the D1 antagonist SCH 39166 as well as the D2 antagonist eticlopride shifted the dose-effect curve for cocaine to the right in squirrel monkeys (Saimiri sciureus) whose responding was maintained by cocaine. Although there were no comparisons to the effects of these antagonists on responding maintained by another reinforcer, the nature of the interaction, an antagonist-dose-dependent shift to the right in the cocaine dose-effect curve, suggested pharmacological specificity. Caine and Koob (1994) found that the dopamine D1 antagonists SCH 23390 and SCH 39166 but not A-69024 decreased rates of responding maintained by cocaine in rats at certain doses that did not alter rates of responding maintained by food reinforcement. Interestingly, the D1 antagonist A-69024 decreased rates of responding maintained by food reinforcement at doses that did not affect behavior maintained by cocaine. The specificity of the effect of the D1 antagonists in this study needs to be carefully considered because of the difference in effects obtained with A-69024.

The influential role of dopamine D1 receptors in the effects of cocaine was dramatically illustrated in studies of genetically altered mice in which the D1 receptor was targeted for removal (Xu et al., 1994). In these D1 knockout mice, cocaine was ineffective as a stimulant of locomotor activity, whereas the wild-type control mice exhibited a dose-related increase in locomotor activity. Furthermore, electrophysiological studies of cells in the nucleus accumbens showed a reduction in the inhibitory effects of cocaine on action potentials in knockout compared with wild-type subjects. These results clearly indicate a significant role of D1 dopamine receptors in the locomotor stimulant effects of cocaine and in the central effects within the nucleus accumbens presumed to mediate those effects. Nonetheless, the respective role of D1 dopamine receptors in other behavioral effects of cocaine is yet to be fully determined.

Although incomplete, there is more information on the effects of pure D1 antagonists than partial D1 agonists on the effects of cocaine (for review, see Winger, 1998). In one study, the effects of the partial agonist (±)-SKF 38393 were studied; these compounds each produce a 3-fold to the right. Similarly, Spealman et al. (1997) showed that the R-enantiomer of SKF 38393 shifted the cocaine dose-effect curve ~3-fold to the right. Similarly, Peart et al. (1997) showed that the R-enantiomer of SKF 38393 shifted the cocaine dose-effect curve ~3-fold to the right, as did the partial agonist SKF 75670 and the antagonist SCH 39166.

In preliminary studies of the benzazepine antagonist SCH 39166, we found a maximal 3-fold shift to the right in the discriminative-stimulus effects of cocaine, with higher doses of the antagonist ineffective in producing greater shifts to the right in the dose-effect curve. This small shift to the right in the cocaine dose-effect curve, coupled with a clearly defined limit to the degree to which the discriminative stimulus effect could be shifted, stood in marked contrast to the types of interactions obtained with other compounds, such as opioid agonists and antagonists (Bertalmio and Woods 1987), and in the dramatic elimination of locomotor stimulant effects of cocaine in D1 knockout mice (Xu et al., 1994). Thus, we initiated this study to quantitatively characterize the interaction between the indirect agonist, cocaine, and dopamine D1 receptor blockade. The effects of the antagonists on the cocaine dose-effect curves for discriminative-stimulus effects and effects on locomotor activity were quantified in terms of the degree of rightward shift, and the change in maximal efficacy of cocaine. A greater rightward shift or decrease in efficacy of cocaine is indicative of a greater degree of dopamine D1 receptor involvement in mediating that effect of cocaine. To ensure that the findings were not idiosyncratic to a particular antagonist, several D1 ligands were used, including the benzazepine antagonists SCH 23390 and SCH 39166, and the structurally distinct tetrahydroisoquinoline A-69024. In addition the partial agonists SKF 77434 and SKF 38393 were studied; these compounds each produce ~50% of maximal stimulation of cyclase (O’Boyle et al., 1989; Andersen and Jansen, 1990).

These interactions of the D1 ligands with cocaine were assessed on several behavioral effects. Locomotor activity in rodents was used as an indication of psychomotor stimulant actions of cocaine and because of the substantive effects of D1 receptor elimination on this behavior in knockout subjects. The discriminative-stimulus effects of cocaine were used as an indication of the subjective effects of cocaine, which likely play an important role in its abuse. The effects on response rates during the drug-discrimination procedure also were assessed to provide an assessment of the general behavior-disrupting effects of cocaine, with less specificity for cocaine abuse. Our results indicated differences in the degree of involvement of D1-like dopamine receptors in these behavioral effects of cocaine.

Materials and Methods

Stimulation of Locomotor Activity. Mice (male Swiss-Webster) were obtained and allowed to habituate to the animal facility for at least 1 week before use with a 12-h light/dark cycle (lights on 7:00 AM). Subjects weighed 30 to 35 g and were ~6 to 7 weeks of age at the time of the study. Locomotor activity was studied in Digiscan activity monitors (Omnitech Electronics Inc., Columbus, OH), which consisted of 40-cm2 clear acrylic chambers equipped with photoelectric detectors placed 2.56 cm apart along the walls of the chamber. One activity count was registered each instance in which the subject crossed a single photo beam. Mice were injected and placed immediately in the chamber with one subject per chamber. Activity was assessed for a 1-h session. Each dose or dose combination was studied in eight subjects, and no subject was used more than once.

Cocaine Discriminative-Stimulus Effects. Adult male squirrel monkeys weighing between 750 and 1000 g served as subjects and were housed under a 12-h light/dark cycle (lights on 7:00 AM). They were fed a daily ration of food (Purina Monkey Chow supplemented with Teklad Monkey Diet) (Ralston Purina, St. Louis, MO; Teklad Premier Laboratory Diets, Madison, WI) at least 30 min after testing that maintained their body weights at a relatively constant level throughout the course of the study. Water was always available in the individual home cages. Five or six subjects were studied for each drug or drug combination. With cocaine alone, however, eight subjects were tested because some subjects were replaced during the study, and the newly introduced subjects all received cocaine. When subjects were replaced, it was due to health reasons not related to...
the study. All monkeys had been studied previously under experimental procedures similar to those described below and had received injections of various drugs; however, drugs had not been administered for at least 1 week before the initiation of these studies.

During experimental sessions, subjects were restrained loosely about the waist in Plexiglas chairs that were placed within ventilated, sound-attenuating chambers (BRS/LVE, Laurel, MD). Continuous white noise was present in the chambers at all times to mask extraneous sounds. On the front panel of each chair (model ENV-601; MED Assoc., Inc., St. Albans, VT) were two response keys on which a downward force of at least 20 g produced an audible click and was recorded as a response. Above the response keys were three pairs of stimulus lamps (28 V d.c.); each pair was colored differently and could be independently illuminated. A food-pellet dispenser delivered 190-mg food pellets (banana flavored; Bio-Serv, Inc., Frenchtown, NJ) to the subject through an opening in the front panel of the chair. On-line experimental control and data collection were by MS-DOS computers operating MED Associates software (Med Associates, St. Albans, VT).

Subjects were initially trained to press both keys under a FR schedule of food reinforcement and subsequently trained to discriminate i.m. injections of cocaine (0.3 mg/kg) from saline. After cocaine injections, responses on only one key were reinforced; after saline injections, responses on the alternate key were reinforced. The assignment of cocaine- and saline-appropriate keys was counterbalanced across subjects. Immediately after injection, the door to the experimental chamber was closed and a 5-min time-out period was initiated, during which all stimulus lamps were extinguished and responding produced feedback clicks but had no other scheduled consequences. All lamps were then illuminated and responses on the appropriate key were reinforced. The FR value was increased to 20 (FR 20) over several training sessions. Responses on the inappropriate key reset the FR response requirement on the appropriate key. Each food presentation was followed by a 20-s time-out period during which all lamps were off, and responding had no scheduled consequences other than the feedback clicks. Sessions ended after 20 food presentations or 15 min, whichever occurred first. As the FR value increased across subjects, St. Albans, VT).

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Test sessions were initiated after criterion was met over at least four consecutive training sessions. Once initiated, subsequent test sessions were conducted between repeats of cocaine or saline sessions of the double alternation sequence. Different doses of cocaine or doses of cocaine after pretreatment with a D1 dopamine antagonist were tested only if the subject achieved criteria on both of the immediately preceding saline and cocaine training sessions. Test sessions were identical with training sessions, with the exception that 20 consecutive responses on either key were reinforced. Doses of each drug or drug combination were studied once or twice in each subject in a mixed sequence. When two determinations were made, the two values were averaged and treated as a single determination. A complete dose-effect curve was typically determined before another drug or drug combination was studied.

**Drugs and Injection Procedures.** (−)-Cocaine HCl (Sigma Chemical Co., St. Louis, MO) and the dopamine D1 receptor antagonists (−)-SCH 23390 HCl (Research Biochemicals, Inc., Natick, MA), SCH 39166 (Schering Corp., Bloomfield, NJ), and A-69024 (Abbott Laboratories, Abbott Park, IL, and National Institute on Drug Abuse, Rockville, MD) were dissolved in distilled water or water with mild acidification (0.16% tartaric acid) and heat. The partial D1 agonists (−)-SKF 38393 HCl (RB1), (−)-SKF 38393 HCl (Research Biochemicals, Inc.), and (−)-SKF 77494 HCl (Research Biochemicals, Inc.) were dissolved in distilled water. Doses for mon}

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Dose-effect functions were analyzed with ANOVA and linear regression techniques (Snedecor and Cochran, 1967), and effects were considered to be significant at the p < 0.05 level. Because each mouse was used only once, locomotor activity data were always analyzed with a one-way ANOVA. Effects of individual doses or dose combinations in mice were considered to be significantly different from vehicle if so indicated by subsequent planned comparisons (Stevens, 1990). If all squirrel monkeys had received all doses of the drugs, a repeated measures ANOVA was used to increase power by analyzing between-subjects variability. The ED50 values and their 95% CL were derived from data from the linear ascending portions of the dose-effect curves for locomotor stimulation and drug discrimination, and the descending portions for response rates. ED50 values were not calculated if the linear regression was not significant. To assess the significance and magnitude of change in the cocaine dose-effect curve produced by coadministration of the D1 dopaminergic agents, data also were analyzed by standard parallel-line bioassay techniques as described by Finney (1964). This analysis determined whether the slopes of the two dose-effect curves were significantly different from parallel, and fit a common slope to the two dose-effect curves. The ratio of doses for equivalent effects is derived from the difference between x-intercepts to provide a value for relative potency as a measure of the degree of shift in the cocaine dose-effect curve. The relative potency value is a unitless measure that indicates the multiple of a the dose of cocaine alone that produces an equivalent effect in subjects coadministered one of the dopaminergic agents (i.e., a
relative potency value of 3 indicates that in the presence of the dopaminergic agent it takes a 3-fold higher dose of cocaine to produce the effects obtained with cocaine alone. A significant shift in the cocaine dose-effect curve is indicated when the 95% CL for the relative potency ratio do not include the value 1.0. Because the antagonists often had pronounced effects of their own on locomotor activity and response rates, the range of effects (y-axis values) was often significantly different when cocaine was given with the antagonist compared with when it was given alone (an effect of preparations, Finney, 1964). Thus, a significant effect of preparations can reflect a significant decrease in the maximal effect of cocaine due to antagonist administration when there is an overlap of y-values across all but the highest doses.

To assess the potency of the D1 dopaminergic agents in shifting the cocaine dose-effect curve, the calculated ED_{50} values were used to estimate an apparent affinity constant (apparent K_{B} value) for the antagonist (Kenakin, 1993). These values were only used from interactions in which there was a significant shift in the cocaine dose-effect curve at some dose of the antagonist. In addition, calculated relative potency estimates (Finney, 1964) for these same interactions also were used (in place of dose ratios) to provide a second estimate of the apparent affinity of the antagonist. The two estimates of apparent antagonist affinity at each antagonist dose were averaged to provide a single estimated apparent affinity constant. In the studies of drug discrimination, there was a limit to the degree of rightward shift produced by the antagonists both for discriminative-stimulus effects and for response rates; thus, apparent antagonist affinity at each antagonist dose were averaged of the apparent affinity of the antagonist. The two estimates of the cocaine dose-effect curve, the calculated ED_{50} values were only computed for dose ratios up to those that produced the maximum shift in the cocaine dose-effect curve.

**D1 Receptor Binding.** Brains from male Sprague-Dawley rats weighing 200 to 225 g (Taconic Farms Inc., Germantown, NY) were removed and striatum was dissected and rapidly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of 50 mM Tris, pH 7.4 at 25°C (buffer A), with a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, NY) (setting 6 for 20 s), and centrifuged at 50,000g for 10 min at 4°C. The resulting pellet was resuspended in buffer and centrifuged again at the same parameters. The supernatant was discarded, and the final pellet was resuspended in cold Tris-HCl containing 120 mM NaCl, 5 mM CaCl_{2}, and 1 mM MgCl_{2}, pH 7.4 at 25°C (buffer B) to a concentration of 3.3 mg/ml (original wet weight). Ligand-binding experiments were conducted in assay tubes containing 1.0 ml of buffer B for 30 min at 37°C. Each tube contained 0.3 nM [³H]SCH 23390 (New England Nuclear, Boston, MA), 1 μM mianserin (Research Biochemicals Inc.), and 2.5 mg of striatal tissue. Nonspecific binding was determined with 1 μM fluphenazine. Incubations were terminated by rapid filtration through Whatman GF/B filters (Whatman Specialty Products, Inc., Fairfield, NJ) with a Brandel cell harvester (Brandel Laboratories, Gaithersburg, MD). The filters were washed twice with 5 ml of cold buffer B and transferred to scintillation vials. Beckman Ready Safe (3.0 ml) was added, and the vials were counted the next day with a Beckman 6000 liquid scintillation counter (Beckman Instruments, Fullerton, CA). Triplicate samples were used in each assay and assays were replicated at least three times. Data were analyzed with GraphPad Prism software (San Diego, CA).

**Results**

**Direct Effects of Cocaine and Dopamine D1 Receptor Ligands.** Cocaine produced a dose-related stimulation of locomotor activity in Swiss-Webster mice. Maximal stimulation to 700 counts/min was produced by a dose of 59 μmol/kg, with both higher and lower doses producing less stimulation (Fig. 1). The other compounds produced only dose-related decreases in locomotor activity across the range of active doses in the first 30 min following injection (Fig. 1, top panel). The dopamine D1 antagonist SCH 23390 was ~5-fold more potent than SCH 39166 in producing decreases in locomotor activity (Fig. 1, top panel, squares and upward pointing triangles, respectively), followed by A-69024 (open circles), exhibiting an ~34-fold lower potency than SCH 23390. The other compounds were appreciably less potent than these three compounds (Table 1, column A). Similar results were obtained in the second 30 min after injection; however, the partial agonist SKF 77434 exhibited a significant stimulation in locomotor activity at the 30-μmol/kg (10-mg/kg) dose.

Subjects trained to discriminate cocaine (0.3 mg/kg) showed a dose-related increase in the percentage of responses emitted on the cocaine-appropriate response key with increasing cocaine dose, with virtually exclusive cocaine-appropriate responding at the training dose (Fig 2, top panel, filled circles). This effect of cocaine was reliable with several replications conducted at various times throughout the course of the study. Table 1 shows ED_{50} values and 95% CL for cocaine determined on four occasions during the course of the study demonstrating no appreciable change in cocaine sensitivity over a period of 58 months.

None of the D1 dopaminergic ligands produced full substitution at any of the doses tested, although the antagonists SCH 39166 and SCH 23390 and the partial agonist SKF 77434 produced a level of drug-appropriate responding significantly greater than vehicle levels (Fig. 2, top panel). Each of the D1 dopaminergic ligands produced a dose-related decrease in rates of responding (Fig. 2, bottom panel). The
significant differences from saline in the percentage of cocaine-appropriate responses emitted were obtained with SCH 39166 doses that produced large decreases in the ongo-
ing rates of responding. The two benzazepine antagonists SCH 23390 and SCH 39166 were the most potent of the D1 ligands in decreasing response rates (Table 1, column C). The tetrahydroisoquinoline antagonist A-69024 was about 10-fold less potent (Table 1) than the benzazepine antagonists. The partial agonists (±)-SKF 38393 and (+)-SKF 38393 were the least potent of the D1 ligands. The order of potency for producing these decreases was similar to that for the decreases in locomotor activity, with the exception of SKF 77434. This compound, which was the only one that produced a significant increase in locomotor activity, was relatively more potent in decreasing response rates than in decreasing locomotor activity.

**Table 1**

ED$_{50}$ values for drug effects on locomotor activity, discriminative stimulus, and response-rate-decreasing effects

<table>
<thead>
<tr>
<th>Drug</th>
<th>Locomotor Activity$^a$</th>
<th>Discriminative Stimulus Effects</th>
<th>Response-Rate-Decreasing Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$mol/kg</td>
<td>$\mu$mol/kg</td>
<td>$\mu$mol/kg</td>
</tr>
<tr>
<td>Cocaine (all)</td>
<td>19.09 (15.26–23.85)$^b$</td>
<td>0.50 (0.47–0.53)</td>
<td>1.82 (1.50–2.21)</td>
</tr>
<tr>
<td>Cocaine 1</td>
<td>0.44 (0.41–0.50)</td>
<td>2.47 (1.71–3.62)</td>
<td></td>
</tr>
<tr>
<td>Cocaine 2</td>
<td>0.50 (0.50–0.53)</td>
<td>1.65 (1.26–2.18)</td>
<td></td>
</tr>
<tr>
<td>Cocaine 3</td>
<td>0.62$^c$ (0.41–0.97)</td>
<td>2.00 (0.62–6.29)</td>
<td></td>
</tr>
<tr>
<td>Cocaine 4</td>
<td>0.50 (0.50–0.53)</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>SCH 23390</td>
<td>0.28 (0.19–0.40)</td>
<td>0.09 (0.06–0.19)</td>
<td>0.06 (0.03–0.09)</td>
</tr>
<tr>
<td>SCH 39166</td>
<td>1.31 (0.03–6.20)</td>
<td>0.11$^e$ (0.06–0.20)</td>
<td>0.06 (0.03–0.11)</td>
</tr>
<tr>
<td>A-69024</td>
<td>6.23 (4.50–8.63)</td>
<td>NS$^d$ (0.29–0.55)</td>
<td>0.41</td>
</tr>
<tr>
<td>SKF 77434</td>
<td>338.74 (284.22–403.74)</td>
<td>4.82 (3.25–7.11)</td>
<td>1.96 (0.99–3.89)</td>
</tr>
<tr>
<td>(±)-SKF 38393</td>
<td>163.40 (85.78–311.24)</td>
<td>NS</td>
<td>17.44</td>
</tr>
<tr>
<td>(+)-SKF 38393</td>
<td>436.87 (135.13–1412.44)</td>
<td>NS</td>
<td>(11.14–27.31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(9.42–20.63)</td>
</tr>
</tbody>
</table>

$^a$ Values shown in italics represent those for locomotor stimulant effects; all others in this column represent locomotor-depressant effects.
$^b$ ED$_{50}$ value and 95% CL are estimates due to a significant deviation from linearity.
$^c$ ED$_{50}$ value.
$^d$ NS, nonsignificant substitution for cocaine.

**Interactions of Cocaine with Dopamine D1 Receptor Antagonists.** At the lowest dose studied (0.01 mg/kg), SCH 23390 only marginally changed the potency of cocaine for stimulation of locomotor activity (Fig. 3, left panel, triangles). The ED$_{50}$ value for cocaine in the presence of SCH 23390 was not appreciably different from that for cocaine alone (Table 2, column A). However, the relative potency analysis indicated an ~1.5-fold shift to the right in the dose effect curve (Table 2, column B), which although small, was significant (95% CL of that value excluded the value 1.0). The maximal stimulation produced by cocaine was decreased by this dose of SCH 23390, as reflected by a significant effect of preparations in the relative potency analysis. This dose of SCH 23390 was inactive when administered alone (Fig. 3, left panel). Higher doses of SCH 23390 (0.03 mg/kg, 0.1 mg/kg) produced further dose-related decreases in the maximal effect of cocaine; these doses, respectively, produced marginal or substantial decreases in activity when administered alone (Fig. 3). Only the highest dose of SCH 23390 appreciably altered the ED$_{50}$ value for cocaine relative to cocaine alone (Table 2, column A), although this and the intermediate dose significantly decreased the potency of cocaine as indicated by the relative potency analysis (Table 2, column B).

The other antagonists, SCH 39166 and A-69024, produced similar patterns of attenuation of the locomotor stimulatory effects of cocaine, characterized by a dose-related decrease in the maximal effect and potency of cocaine (Fig. 3, middle and right panels). For SCH 39166, the attenuation of the maximal effect of cocaine was not significant at doses of 0.01 to 0.03 mg/kg, although it was at 0.056 mg/kg (Fig. 3, middle panel; Table 2, column B, significant effect of preparations only at 0.056 mg/kg). At the highest dose (0.1 mg/kg), there was an appreciable attenuation of the effects of cocaine that appeared to be at least partially surmountable but only at a relatively high (80 mg/kg) dose of cocaine (Fig. 3, middle panel). This dose of SCH 39166 also significantly decreased locomotor activity when administered alone. The ED$_{50}$ values for cocaine with SCH 39166 doses of 0.01 to 0.056 mg/kg were not appreciably changed from those for cocaine alone (Table 2, column A), although relative potency analyses indicated a significant decrease in potency of cocaine in the presence of SCH 39166 at all but the 0.01-mg/kg dose (Table 2, column B).

The tetrahydroisoquinoline antagonist A-69024 produced dose-related decreases in the maximal locomotor stimulant effects of cocaine (Fig. 3, right panel; Table 2, column B, significant effects of preparations), with the attenuation appearing at least in part surmountable at A-69024 doses of 0.3 and 1.0 mg/kg (Fig. 3, right panel). Only the lowest dose of A-69024 was without effects on locomotor activity when administered alone. The ED$_{50}$ value for cocaine was increased by 1.0 mg/kg A-69024 (Table 2, column A), and at this dose there was a significant shift to the right in the cocaine dose-effect curve (Table 2, column B). At the highest doses of
A-69024, an ED50 value for cocaine and relative potency were not calculated because there were no significant stimulant effects (Fig. 3, right panel; Table 2, column A). At these doses, the effects of A-69024 were not surmounted by cocaine doses up to 235 μmol/kg.

The discriminative-stimulus effects of cocaine were shifted marginally to higher doses (Fig. 4A, top panel, triangles) at the lowest dose (0.01 mg/kg) of SCH 23390 studied. This shift was significant (95% CL of the relative potency value did not include 1.0; Table 2, column D). The highest dose of SCH 23390 (0.1 mg/kg; Fig. 4A, diamonds) also shifted the cocaine dose-effect curve to the right. The shift in the cocaine dose-effect curve was ~4-fold (Table 2, column D), although this change should be considered an estimate only because the relative potency analysis indicated significant deviations from parallel dose-effect curves.

The dose-related decreases in response rates produced by cocaine also were altered by SCH 23390 (Fig. 4A, bottom panel), although these changes were obscured by the pronounced effects of the antagonist on response rates (Fig. 2, bottom panel). However, the decreases in response rates produced by SCH 23390 were progressively antagonized by low-to-intermediate doses of cocaine. As a result, the relative potency analyses of the antagonist with cocaine were conducted with the descending linear portion of the cocaine-alone dose-effect curve (at doses of 2.9 to 8.8 μmol/kg). These analyses indicated significant rightward shifts of the cocaine dose-effect curve to a maximum of ~2-fold (Table 2, column F).

SCH 39166 also dose-dependently shifted the discriminative-stimulus effects of cocaine to the right (Fig. 4B, top panel), with corresponding increases in the cocaine ED50 values (Table 2, column C). The relative potency analysis indicated an ~2.4- to 2.6-fold shift to the right in the cocaine dose-effect curve produced by the highest doses of SCH 39166 (Table 2, column D). However, at these doses there were significant deviations from parallel, precluding a precise estimate of the degree of shift (Table 2, column D).

The dose-related decreases in response rates produced by cocaine were affected by pretreatment with SCH 39166 in a manner similar to that obtained with SCH 23390 (Fig. 4B, bottom panel). None of the doses of SCH 39166 tested appreciably increased the ED50 value of cocaine compared with that for cocaine alone (Table 2, column E), and the relative potency analyses indicated a significant rightward shift in the cocaine dose-effect curve to a maximum of ~2 fold at the highest dose studied (Table 2, column F).

As with the other antagonists, A-69024 antagonized the discriminative-stimulus effects of cocaine in a dose-related manner. At the lowest dose (0.03 mg/kg) studied, there were small if any changes in the effects of cocaine (Fig. 4C, top panel; Table 2, columns C and D); higher doses shifted the cocaine dose-effect curve to the right (Fig. 4C, top panel). At 0.1 mg/kg A-69024, the ED50 value for cocaine was greater than that for cocaine alone (Table 2, column C), and the relative potency analysis indicated a maximum 3-fold shift to the right in the cocaine dose-effect curve relative to cocaine alone (Table 2, column D).

In contrast to the other antagonists, A-69024 failed to significantly alter the dose-related decreases in response rates produced by cocaine, by even a small factor (Fig. 4C, bottom panel). The ED50 values for effects of cocaine alone and cocaine in the presence of A-69024 were not altered by administration of A-69024 (Table 2, column E), nor did the relative potency analysis indicate a significant shift to the right in the cocaine dose effects on rates of responding (Table 2, column F).

Interactions of Cocaine with Dopamine D1 Receptor Partial Agonists. Each of the doses of SKF 77434 examined decreased the maximal effects of cocaine (Fig. 5, left panel). The two lowest doses studied (3.0 and 10.0 mg/kg) had comparable effects, and only at these doses were some significant stimulant effects of cocaine preserved. There was a trend toward an increase in cocaine ED50 values at these doses of
Antagonism of the effects of cocaine on locomotor activity in Swiss-Webster mice by SCH 23390, SCH 39166, and A-69024. Top panel, locomotor activity counts during the first 30 min of a 1-h observation period as a function of cocaine dose. Bottom panel, locomotor activity counts during the second 30 min of a 1-h observation period as a function of cocaine dose. Ordinates, average locomotor activity counts (counts/min); abscissae, dose (mg/kg) of drug, log scale. Each point is the average of eight subjects, with vertical bars representing ±1 S.E. Points above C represent effects of the antagonists with vehicle injections (△, □, ○, △), or two vehicle injections (○).

TABLE 2
Quantitative assessments of interaction data for D1-like antagonists

<table>
<thead>
<tr>
<th>Drug</th>
<th>Locomotor Activity</th>
<th>Cocaine Discrimination</th>
<th>Response Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED₅₀ value (95% CL)</td>
<td>Relative potencyᵃ</td>
<td>ED₅₀ value (95% CL)</td>
</tr>
<tr>
<td></td>
<td>µmol/kg</td>
<td></td>
<td>µmol/kg</td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>19.09</td>
<td>0.50</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>(15.26–23.85)</td>
<td>(0.47–0.53)</td>
<td></td>
</tr>
<tr>
<td>with 0.01 mg/kg SCH 23390</td>
<td>20.00</td>
<td>1.49ᵇ</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>(14.44–27.68)</td>
<td>(1.10–2.15)</td>
<td></td>
</tr>
<tr>
<td>with 0.03 mg/kg SCH 23390</td>
<td>NS</td>
<td>0.38</td>
<td>1.91ᵇ</td>
</tr>
<tr>
<td></td>
<td>(2.24–11.29)</td>
<td>(0.12–1.35)</td>
<td></td>
</tr>
<tr>
<td>with 0.1 mg/kg SCH 23390</td>
<td>34.65</td>
<td>3.36ᵇ</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>(29.00–41.44)</td>
<td>(2.48–5.13)</td>
<td></td>
</tr>
<tr>
<td>with 0.01 mg/kg SCH 39166</td>
<td>23.88</td>
<td>1.29</td>
<td>1.84ᵇ</td>
</tr>
<tr>
<td></td>
<td>(19.24–29.68)</td>
<td>(0.99–1.73)</td>
<td></td>
</tr>
<tr>
<td>with 0.03 mg/kg SCH 39166</td>
<td>22.82</td>
<td>1.92</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>(14.26–36.44)</td>
<td>(1.28–3.06)</td>
<td></td>
</tr>
<tr>
<td>with 0.056 mg/kg SCH 39166</td>
<td>25.03</td>
<td>1.54ᵇ</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>(20.88–30.03)</td>
<td>(1.20–2.05)</td>
<td></td>
</tr>
<tr>
<td>with 0.1 mg/kg SCH 39166</td>
<td>No Max</td>
<td>96.8</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.74–2.38)</td>
</tr>
<tr>
<td>with 0.03 mg/kg A-69024</td>
<td>NT</td>
<td>NT</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.47–0.59)</td>
</tr>
<tr>
<td>with 0.1 mg/kg A-69024</td>
<td>NT</td>
<td>NT</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.29–2.59)</td>
</tr>
<tr>
<td>with 0.3 mg/kg A-69024</td>
<td>18.82</td>
<td>1.32ᵇ</td>
<td>1.71ᵇ</td>
</tr>
<tr>
<td></td>
<td>(14.76–24.00)</td>
<td>(0.95–2.08)</td>
<td></td>
</tr>
<tr>
<td>with 1.0 mg/kg A-69024</td>
<td>57.50</td>
<td>10.24ᵇ</td>
<td>2.59ᵇ</td>
</tr>
<tr>
<td></td>
<td>(30.68–107.79)</td>
<td>(4.93–43.08)</td>
<td></td>
</tr>
<tr>
<td>with 3.0 mg/kg A-69024</td>
<td>NSS</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>with 10.0 mg/kg A-69024</td>
<td>NSS</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

ᵃ Relative potency values with 95% CL exclusive of 1.0 are significant (see Materials and Methods).
ᵇ Significant effect of preparations.
ᶜ NS, nonsignificant linear regression; NT, not tested; NSS, ED₅₀ value not calculated because there was a nonsignificant stimulation of locomotor activity; NC, analysis not conducted because antagonism was not surmounted; No Max, the ED₅₀ value was not calculated and the relative potency value is an estimate because a clear maximum to the dose-effect curve was not obtained.
ᵈ The results of the relative-potency analysis should be considered an estimate because the two regression lines significantly deviated from parallel.
SKF 77434 (Table 3, column A), and the relative potency analysis indicated that both 3.0 and 10.0 mg/kg SKF 77434 significantly shifted the cocaine dose-effect curve to the right (Table 3, column B). These changes in the effects of cocaine were obtained with doses that were inactive when administered alone (Fig. 5, left panel, disconnected points at left). The partial agonist (±)-SKF 38393 and its active (+)-enantiomer produced similar effects. Both of these compounds...
produced dose-related decreases in the potency of cocaine (Fig. 5, middle and right panels). Both \((\pm)\)- and \((+)\)-SKF 38393 increased the cocaine ED\(_{50}\) values (Table 3, column A), and the relative potency analyses (Table 3, column B) indicated, respectively, significant 3- or 10-fold decreases in the potency of cocaine when administered with either \((\pm)\)- or \((+)\)-SKF 38393. These compounds also produced a dose-related decrease in the maximal effect of cocaine (Fig. 5, middle and right panels; Table 3, column F). At the highest doses of either compound, there was a significant leftward shift in the response rate-decreasing effects of cocaine (Table 3, column F).

The lowest dose of SKF 77434 (0.3 mg/kg) did not affect the cocaine-induced decreases in response rates as shown in Fig. 6A, bottom panel, squares, and as reflected in the cocaine ED\(_{50}\) and relative potency values (Table 3, columns E and F, respectively). As with the antagonists, SKF 77434 alone had effects on response rates (Fig. 2, bottom panel), and these effects were antagonized in a dose-related manner by cocaine (Fig. 6A, bottom panel). At the higher doses of cocaine in combination with SKF 77434, there was little evidence of a shift to the right in the cocaine dose-effect curve (Fig. 6A, bottom panel). Accordingly, the ED\(_{50}\) values for effects of cocaine on response rates and the relative potency estimates indicated no effect at doses of 0.3 and 1.0 mg/kg SKF 77434; at the highest dose, there was a significant leftward shift in the response rate-decreasing effects of cocaine (Table 3, column F).

The effects of \((\pm)\)-SKF 38393 (Fig. 6B, top panel) and \((+)\)-SKF 38393 (Fig. 6C, top panel) on the discriminative stimulus effects of cocaine were uniformly more modest than those obtained with the other compounds. The cocaine ED\(_{50}\) values generally were not changed by administration of either of these dopamine D1 partial agonists (Table 3, column C), and with the exception of 5.6 mg/kg \((\pm)\)-SKF 38393, the 95% CL of the relative potencies were inclusive of 1.0 (Table 3, column D). Similarly, neither \((\pm)\)-nor \((+)\)-SKF 38393
produced a shift to the right in the cocaine dose-effect curve for decreasing response rates (Fig. 6, B and C; bottom panel). The relative potency analyses indicate, that if anything, the coadministration of \((\pm)-SKF\) 38393 or its \((+)-enantiomer\) shifted the cocaine dose-effect curves to the left (Table 3, column \(F\); 95% CL are below and exclude 1.0).

The effects of \((\pm)-\) and \((+)-SKF\) 38393 on the cocaine dose-effect curve were even less pronounced when these drugs were administered concurrently with cocaine 5 min before testing. The cocaine ED\(_{50}\) values with 3.0 mg/kg \((\pm)-SKF\) 38393 were 0.15 mg/kg (95% CL, 0.13–0.18) and 0.47 mg/kg (95% CL, 0.17–1.27) for percentage of cocaine responding and response rate, respectively. At 10.0 mg/kg \((\pm)-SKF\) 38393, the respective values were 0.17 mg/kg (95% CL, 0.17–0.18) and 0.45 mg/kg (95% CL, 0.29–0.70). The cocaine ED\(_{50}\) values with 3.0 and 10 mg/kg \((+)-SKF\) 38393 for percentage of cocaine responding were 0.23 mg/kg (95% CL, 0.14–0.36) and 0.35 mg/kg (95% CL, 0.09–1.33), respectively. ED\(_{50}\) values for the effects of cocaine on response rates with these doses of \((+)-SKF\) 38393 could not be determined because the linear regression was not significant.

All of the compounds displaced \[^{3}H\]SCH 23390 from rat striatum (Table 4), with affinities \((K_i\) values) that ranged from 0.61 to 21.31 nM. The highest affinity was obtained with SCH 39166, which had an affinity that was \(~12\)-fold greater than that for \((\pm)-SKF\) 38393. Table 4 shows significant correlations between binding affinities and in vivo behavioral potencies when acting on behavior alone (columns B and F). In addition, there was a high correlation among binding affinities and apparent \(K_i\) values computed from shifts in the cocaine dose-effect curve (columns C and E).

There were insufficient numbers of values to compute meaningful correlation coefficients for the other behavioral effects (columns D and G).

### Discussion

In the present study, the effects of several compounds acting at dopamine D1 receptors were examined alone and in combination with cocaine. The effects examined were the cocainelike discriminative-stimulus effects and the concurrently assessed effects on rates of lever pressing, as well as the effects on locomotor activity. Locomotor activity in rodents is used as an indication of psychomotor stimulant actions, and the discriminative-stimulus effect of cocaine is used as an indication of the subjective effects of these drugs, which likely play an important role in cocaine abuse. The effects on response rates during the drug-discrimination procedure provide an index of the disruption in behavior produced by cocaine, which is not an effect that is specifically related to cocaine abuse.

Cocaine and the D1 partial agonist SKF 77434 produced increases in locomotor activity, but only cocaine produced substantial stimulation. Results similar to those of our study have been reported by others. For example, stimulation of locomotor activity in rats was reported for \((\pm)-SKF\) 38393 (Murray and Waddington, 1989; Meyer and Shults, 1993; although see Tirelli and Terry, 1993). Similarly, decreases in locomotor activity with the remaining compounds, or schedule-controlled behavior with all of the dopaminergic D1 agents, have been reported (Bergman et al., 1991, 1995, 1996; Criswell et al., 1992; Katz et al., 1995).
The ED$_{50}$ values for producing each of the various effects examined in the present study and their apparent affinities computed from the antagonism experiments were related to their binding affinities determined from displacement of $[^{1}H]$SCH 23390. The benzazepine antagonists SCH 23390 and SCH 39166 were most potent in decreasing locomotor activity in mice and in decreasing response rates in monkeys discriminating cocaine injections. These two compounds were equally potent in decreasing response rates and had the highest affinity in the present study of displacement of $[^{1}H]$SCH 23390. Our affinity values are consistent with those previously reported in rats (Chipkin et al., 1988) and primates (Madras et al., 1988; Bergman et al., 1991), and with previous assessments of potencies in vivo (Bergman et al., 1991, 1995; Criswell et al., 1992). The relatively lower potency of A-69024 in decreasing locomotor behavior or response rates is consistent with its lower affinity (present study; Kerkman et al., 1989; Kassiou et al., 1995). The ~30-fold difference in potency between SKF 77434 and SCH 23390 in decreasing response rates is consistent with its ~12-fold lower affinity for D1 dopamine receptors (Table 4; Andersen et al., 1985; Andersen and Jansen, 1990). However, in decreasing locomotor activity, the potency of SKF 77434 was disproportionately lower than SCH 23390.

The partial agonist SKF 77434 stimulated locomotor activity at lower doses than those that decreased locomotor activity (albeit during the second 30 min after injection). If its potency as a locomotor stimulant is considered along with the potencies of the other compounds, the agreement among in vivo potencies and affinities for D1 receptor binding among all of the compounds is relatively high. This agreement is consistent with an interpretation that the stimulation of activity produced by the partial agonist SKF 77434 is mediated by agonist actions at D1 dopamine receptors, whereas the decreases in locomotor activity produced by the remaining ligands is the result of antagonist effects at D1 dopamine receptors. The differences in activity of SKF 77434 and the other D1 ligands might be explained on the basis of the greater intrinsic efficacy of SKF 77434 compared with the antagonists; however, remaining without explanation is the lack of stimulant effects of (±)- or (+)-SKF 38393. The racemic form of this compound has been shown to maximally stimulate locomotor activity in C57BL mice between 50 and 90 min after injection (Tirelli and Terry, 1993). Thus, it is possible that the stimulant effects of either form of SKF 38393 would have been obtained with our Swiss-Webster mice, although at an even later time point. Alternatively, SKF 38393 may have decreased locomotor activity by a mechanism other than D1 dopamine receptor activation (cf. Terry and Katz, 1992).

In monkeys trained to discriminate cocaine from saline injections, cocaine produced dose-related increases in responding on the cocaine-appropriate lever, with virtually exclusive responding on that lever at the training dose. None of the D1 dopaminergic agents fully substituted for cocaine, although with SCH 39166, SCH 23390, and SKF 77434 there was a substitution that was significantly greater than saline levels. Similar results have been reported in rodents and primates (Kleven et al., 1990; Callahan et al., 1991; Witkin et al., 1991; Terry et al., 1994; Spealman et al., 1997). The partial substitution for cocaine by the compounds with some agonist activity has been interpreted as consistent with the notion that activation of D1 dopamine receptors contributes to, but does not fully reproduce, the discriminative effects of cocaine.

The partial substitution of D1 dopamine receptor antagonists is not currently understood. Several studies have found paradoxical agonist actions of dopamine D1 receptor antagonists. For example, Starr and Starr (1986) first reported substantial grooming behavior induced by relatively low doses of SCH 23390, below those that antagonized the effects of SKF 38393 (see also G orell et al., 1986; Wachtel et al., 1992). Similarly, Wachtel and White (1995) found a D1 agonist-like effect of both SCH 23390 and SCH 39166 on glutamate-stimulated firing in neurons of the nucleus accumbens of rats. These effects also were seen at low doses, and fur-
The D1 antagonists A-69024, SCH 23390, and SCH 39166 and the partial agonists SKF 77434, (±)-SKF 38393, and (+)-SKF 38393 each produced a rightward shift in the cocaine dose-effect curve for stimulation of locomotor activity (ascending limb of the dose-effect curve). The antagonism of the effects of cocaine on locomotor activity by the D1 antagonist SCH 23390 has been reported (Cabib et al., 1991); however, characterization of the cocaine dose-effect curve as a result of that antagonism has not been previously reported. One interesting result of our study is that the antagonists not only shifted the cocaine dose-effect curve to the right but also decreased maximal efficacy for stimulation of locomotor activity, such that the antagonism was not fully surmountable.

The shift to the right in the dose-effect curve concomitant with a decrease in maximal effect is consistent with what is expected with the antagonism of an indirectly acting agonist by a competitive antagonist (Kenakin, 1993). In addition, the characteristics of the antagonism reported herein are consistent with a differential antagonism of the ascending and descending portions of the cocaine dose-effect curve. If these two limbs of the dose-effect curve represent differently mediated and offsetting effects, then a preferential shift to the right in the ascending limb of the curve would result in a decrease in maximal effect. Finally, the antagonist-induced decreases in maximal effects of cocaine could represent a functional antagonism resulting from the opposing effects of cocaine (stimulation of activity) and the D1 ligands (decreases in activity) observed when the drugs are administered alone. However, the decreases in maximal effect of cocaine often occurred at doses of the antagonists and partial agonists that alone had no substantial effects on locomotor activity, as well as at doses that alone decreased activity. Therefore, the decrease in maximal cocaine effect in the presence of the D1 ligands is at least not uniformly due to a functional or physiological antagonism. Thus, the shift to the right in the cocaine dose-effect curve concomitant with a decrease in maximal effect in the studies of locomotor activity appears to be either characteristic of antagonism of the effects of an indirect-acting agonist, or differential antagonism of the differently mediated limbs of the bitonic dose-effect curve.

One major difference in the antagonism of the behavioral effects examined herein was that in the cocaine discrimination, there were generally no changes in the efficacy of cocaine in the presence of increasing doses of the D1 antagonists. At all of the doses of the D1 ligands, the antagonism was surmounted by appropriate doses of cocaine, a result that contrasts with that obtained for the effects of cocaine on locomotor activity. If the antagonism of the effects of cocaine on locomotor activity is characteristic of indirect agonist-antagonist interactions (as described above), the question remains as to why the antagonism of the discriminative-stimulus effects was fully surmountable. These differences in antagonism may be related to the differences in species used to assess discriminative effects and locomotor stimulation (however, see below), or to differences in exposure to cocaine as the discrimination procedure necessitates repeated administration of the training drug. Alternatively, it is possible that there are differences in dopamine receptor occupancy necessary for the two effects. Kenakin (1993) notes that even with interactions between indirect-acting agonists and competitive antagonists, when small amounts of pharmacological stimulus are necessary for an effect, the amount of parallel shift in dose-effect curves will be extended before there are decreases in maximal effect. In the opioid system for example, there is compelling evidence that discriminative-stimulus effects are “sensitive” based on their appearance at doses lower than those producing other opioid effects (Woods et al., 1988). It follows from these observations that the discriminative effects of opioids would require a small receptor occupancy for the expression of a full effect compared with other effects. Our results suggest a similar relationship for the discriminative-stimulus effects of cocaine.

Several studies have demonstrated an antagonism of the discriminative-stimulus effects of cocaine by D1 dopamine antagonists. For example, Kleven et al. (1990) showed an ~3-fold shift to the right in the discriminative effects of cocaine in rhesus monkeys by SCH 23390. Spealman et al. (1991) showed similar degrees of antagonism by SCH 39166. In our study, the antagonists dose-dependently shifted the cocaine dose-effect curve for effects on locomotor activity, often reaching doses that completely prevented stimulation at any dose of cocaine studied. In contrast, in the cocaine discrimination studies the antagonists exhibited a maximal degree to which the cocaine dose-effect curve could be shifted (typically 3-fold), with higher doses ineffective in producing greater shifts to the right in the discriminative-stimulus effects of cocaine. Interestingly, among the published studies of antagonism of cocaine by D1 antagonists, ~3-fold shifts to the right in the dose-effect curve are generally the rule (Kleven et al., 1990; Spealman, 1990; Bergman et al., 1990; Spealman et al., 1991; Vanover et al., 1991; Spealman et al., 1997).

A relatively greater antagonism of the discriminative-stimulus effects compared with the response-rate-decreasing effects of cocaine could explain the ~3-fold limit to the antagonism of the discriminative-stimulus effects if pronounced decreases in response rates precluded an assessment of further antagonism of the discriminative effects. However, even at doses that decreased response rates, there was usually a
rate of responding sufficient to adequately assess the discriminative-stimulus effects.

The differences in the sensitivity of the discriminative-stimulus effects and the effects on response rates to antagonism by the D1 antagonists suggest differing mechanisms underlying these effects of cocaine. The degree of D1 receptor involvement in the effects of cocaine on response rate appears to be substantially less than that for the discriminative-stimulus effects of cocaine. Similarly, the degree of D1 receptor involvement in the effects of cocaine on locomotor activity appears to be greater than that for the other effects. This latter conclusion, however, is tempered by the species differences in assessing these effects. However, a study on D1 dopamine receptor knockout mice (Miner et al., 1995) lends support to the conclusions of differential D1 receptor involvement in different behavioral effects of cocaine. In that study, conditioned place preference induced by cocaine was retained in the D1 knockout mice. As mentioned in the Introduction, Xu et al. (1994) found D1 knockout mice to be insensitive to the locomotor stimulant effects of cocaine. These results along with our findings clearly indicate a significant role of D1 dopamine receptors in the locomotor stimulant effects of cocaine. Moreover, these findings together suggest a more complex and possibly more limited involvement of D1 dopamine receptors in the effects mediating the covert stimulus effects underlying discriminative effects and possibly conditioned place preference.

The maximal shift in the dose-effect curve for the discriminative-stimulus effects of cocaine is not entirely surprising when considered in light of the multiplicity of the actions of cocaine. Cocaine not only inhibits the uptake of dopamine but also that of serotonin and norepinephrine. With its indirect dopaminergic agonist actions, each of the dopamine receptor subtypes should be stimulated. Selective antagonism of any one of these dopaminergic sites should remove part, but not all of the interoceptive stimulus effects of cocaine. How these various actions of cocaine contribute to its discriminative-stimulus effects is not clear. However, the current study suggests that there is a limit to the potential blockade of the discriminative effects of cocaine through antagonist actions at dopaminergic D1 receptors. It is possible that the limited antagonism of the discriminative effects of cocaine by selective antagonists occurs because only the D1 component of the stimulus is eliminated. As such, the discriminative-stimulus effects of cocaine may resemble those of a compound stimulus, with individual components combining in some manner to produce the discriminative effect. The elimination of any one component may not be sufficient to preclude a discriminative effect produced by the remaining components (Stolerman et al., 1991). The results of substitution studies with direct agonists, however, are not consistent with this interpretation. For example, many of the selective D2-like and D1-like agonists do not fully substitute for cocaine when administered alone, regardless of dose (Callahan et al., 1991; Witkin et al., 1991). However, full substitution has been on occasion reported following administration of D2-like agonists (Barrett and Appel, 1989; Ukai et al., 1993). Thus, there may be differences in the relative contributions of D1-like and D2-like dopamine receptors to the discriminative stimulus complex produced by cocaine.

For the discriminative effects of cocaine, there were differences in the interactions with cocaine of antagonists SCH 23390 and SCH 39166, and the partial agonists SKF 77434, (±)-SKF 38393, and (±)-SKF 38393. The antagonists each produced 3-fold shifts, whereas the partial agonists produced less of a shift, if at all. The present results with (±)-SKF 38393 somewhat differ from a previous finding; in that study (Spealman et al., 1997), the dose-effect curve for cocaine was shifted ~3-fold to the right by (±)-SKF 38393. In that study, the effects of cocaine were determined with a cumulative-dosing procedure, whereas in the present study single doses were administered. Although there have been reports that the cumulative-dosing procedure can interject effects specific to its use (Schindler et al., 1990; Walker and Branch, 1998), it is not clear that those effects were involved, or how they would have contributed to the differences between our study and the one by Spealman et al. (1997).

Interestingly, SKF 77434 is similar to SKF 38393 in terms of its stimulation of adenylyl cyclase activity in rodent tissue (O’Boyle et al., 1989), and SKF 77434 also antagonized the effects of cocaine in a manner that like that of the D1 antagonists. Because SKF 77434 is also a partial agonist, it is clear that in the present study the antagonism of cocaine could be obtained with drugs having some intrinsic efficacy. The lack of a robust antagonism of cocaine by SKF 38393 may have been due to its actions mediated by other systems. For example, Zarrindast et al. (1991) found behavioral effects of SKF 38393 to be antagonized by the 5-HT antagonist metergolide, rather than by the dopamine D1 antagonist SCH 23390. Those results suggest that the actions of this drug that are mediated by 5-HT receptors (Bischoff et al., 1986) are more potent than its actions at D1 receptors. These 5-HT-mediated effects could have interfered with its ability to antagonize the effects of cocaine via a D1 partial agonist action.

The shifts in the cocaine dose-effect curves for discriminative-stimulus effects produced by the D1 antagonists in the present study were relatively modest, ~3-fold shifts. Greater shifts were obtained in the effects of cocaine on locomotor activity. These findings indicate differences in the involvement of D1 dopamine receptors in mediating these effects of cocaine. It is currently not clear which of these effects, if either, will be predictive of the therapeutic effects of the compounds in treating cocaine abuse. The discriminative-stimulus effects of cocaine are thought to be indicative of subjective effects that are presumably involved in the abuse of cocaine. If so, the current 3-fold shifts in the cocaine dose-effect curve may be, at least on first blush, disappointing. However, a 3-fold shift in the cocaine dose-effect curve may be adequate for a therapeutic agent. Surmounting even a minimal 3-fold antagonism of the effects of cocaine may very well be beyond the economic means of at least a subset of cocaine abusers.

In summary, several dopaminergic agents were examined alone and in combination with cocaine to better understand the role of D1 dopamine receptors in certain behavioral effects of cocaine. There was a clear limit to the degree to which the D1 ligands could shift the dose-effect curve for the effects of cocaine on response rates, with a greater shift in its discriminative-stimulus effects. There also were defined limits to the shifts in cocaine discriminative-stimulus effects, with a maximal 3-fold shift in the dose-effect curve. Furthermore, there was evidence that the activity of D1 dopaminergic receptors may be redundant with other mechanisms mediat-
ing the discriminative effects of cocaine. The locomotor-stimulant effects of cocaine were shifted to the right to an extent greater than that for the other behavioral effects examined and the maximal effects of cocaine were decreased by the antagonists. The antagonism was progressively increased with increasing doses of the D1 antagonists up to doses that produced an insurmountable antagonism. These results indicate a contribution of D1-receptor agonist actions in each of these effects of cocaine, however, with appreciable differences in the respective roles of D1 dopamine receptors in mediating each of these behavioral effects of cocaine.

Acknowledgments

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