Interactions of Cocaine with Dopamine Uptake Inhibitors or Dopamine Releasers in Rats Discriminating Cocaine

Su-Min Li, Bettye L. Campbell, and Jonathan L. Katz

Psychobiology Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health/Department of Health and Human Services, Baltimore, Maryland

Received December 23, 2005; accepted February 13, 2006

ABSTRACT

Several dopamine (DA) indirect agonists have been proposed as potential medications for treating cocaine abuse. The objective of the present study was to quantify the interactions among cocaine and DA uptake inhibitors or DA releasers to better understand how these drugs may be working when administered in combination. The DA uptake inhibitors GBR 12909 [1-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylpropyl)piperazine], WIN 35,428 [2β-carbomethoxy-3β-(4-fluorophenyl)tropane], methylphenidate, in- datarline, nomifensine, and mazindol and DA releasers methamphetamine, d-amphetamine, methcathinone, cathinone, fenfluramine, and phentermine were examined alone and in combination with cocaine in rats trained to discriminate cocaine (10 mg/kg i.p.) from saline injections. All of the DA indirect agonists dose-dependently substituted for cocaine and shifted the cocaine dose–effect curve leftward. Isobolographic analysis indicated the interactions were generally additive, although both methamphetamine and d-amphetamine were quantitatively determined to be more potent than DA uptake inhibitors in shifting the cocaine dose–effect function to the left. The potential of d-amphetamine as an effective treatment for cocaine abuse and negative clinical results with dopamine uptake inhibitors suggest that differences in shifts in dose–effect curves should be further examined with emerging clinical data as a predictive index of potential treatments for cocaine abuse.

Cocaine abuse continues to be a serious public health problem in many countries, for which there are no proven effective medications. As a result, a significant research effort has been dedicated to the discovery and evaluation of candidate pharmacotherapies (Voci, 2005). One approach has been suggested by the effective treatment of opiate abuse with opioid agonists such as methadone and of tobacco abuse with various formulations of nicotine. These medications share pharmacological mechanisms of action and many of the effects of the abused drug. Cocaine has local anesthetic effects and inhibits the uptake of dopamine (DA), serotonin, and norepinephrine through actions at their respective transporters. However, the effects of cocaine related to its abuse liability are thought to be mediated primarily by its indirect DA agonist actions mediated by the dopamine transporter (Kuhar et al., 1991), suggesting indirect agonists as potential lead compounds for discovery of treatments for cocaine abuse.

One indirect DA agonist examined rather extensively is GBR 12909, which is approximately two orders of magnitude more potent as an inhibitor of [3H]dopamine uptake than as an inhibitor of either [3H]norepinephrine or [3H]serotonin uptake, in vitro and ex vivo (Heikila and Manzino, 1984). Furthermore, in vivo GBR 12909 inhibits the uptake of DA into nerve terminals producing increases in the synaptic concentration of DA, mimicking the effects of cocaine (e.g., Tanda et al., 1997). Studies of the reinforcing effects of GBR 12909 indicated that it did not maintain rates of responding as high as those of cocaine, suggesting lower abuse liability of the compound compared with cocaine (e.g., Skjoldager et al., 1993).

Several studies of the interactions of GBR 12909 with cocaine have indicated an attenuation of the effects of cocaine by GBR 12909. For example, Baumann et al. (1994) found that treatment with selected doses of GBR 12909 attenuated the effects of cocaine on extracellular DA levels determined by in vivo microdialysis. In addition, other studies showed that pretreatment with GBR 12909 reduced subsequent rates of responding maintained by cocaine in a self-administration procedure (Glowa et al., 1995).

This work was supported by the Intramural Research Program of the National Institute on Drug Abuse.

Some of the data were reported at the Annual Meeting of the Society for Neuroscience, San Diego, CA (Oct 25, 2004) and Washington, DC (Nov 13, 2005).

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.105.100594.

ABBREVIATIONS: DA, dopamine; GBR 12909, 1-[2-[bis-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine; LED, light-emitting diode; FR, fixed ratio; CL, confidence limit; WIN 35,428, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane.
The attenuation of the effects of cocaine by GBR 12909 prompted Holtzman (2001) to further examine the interactions of GBR 12909 with either cocaine or methamphetamine. In rats trained to discriminate cocaine from saline, there was no indication of an antagonism of cocaine but rather that GBR 12909 enhanced the effects of cocaine and the DA releaser, methamphetamine. However, the interactions between GBR 12909 and cocaine quantitatively differed from those with methamphetamine. The interaction of GBR 12909 with methamphetamine was strictly additive according to an isobolographic analysis (e.g., Tallarida, 1992), whereas the interaction of GBR 12909 with cocaine was supra-additive.

Because there is interest in the development of indirect DA agonists as treatments for cocaine abuse (e.g., Grabowski et al., 2004) and because the interactions of cocaine and GBR 12909 were different in the microdialysis and drug discrimination procedures, the present study was initiated. In this study, we compared the interactions of cocaine with both DA uptake inhibitors, including GBR 12909, and DA releasers, including methamphetamine. The interactions were examined quantitatively with an isobolographic analysis, as was done previously. In addition, we supplemented that analysis with a determination of the dose of the indirect agonist that effectively shifted the cocaine dose-effect curve 2-fold to the left. We examined the effects of several members of these two classes of DA indirect agonists to determine the generality of the parameters of the interactions.

Materials and Methods

Subjects. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 320 to 350 g served as subjects. All animals were single-housed in a temperature- and humidity-controlled colony room with a 12-h light/dark cycle (lights on at 7:00 AM). They were fed daily approximately 15 g of standard lab chow at least 1 h after testing that maintained them at their individual weights throughout the study.

Apparatus. Subjects were tested daily in two-lever operant-conditioning chambers (Med Associates, St. Albans, VT) that were housed within light- and sound-attenuating enclosures. White noise was present throughout testing to mask extraneous sounds. Ambient illumination was provided by a lamp in the top center of the front panel (house light). Levers were set 17 cm apart, with pairs of light-emitting diodes (LEDs) above each of the levers, also on the front panel. A downward force on either lever of 0.4 N through approximately 1 mm was defined as a response and produced an audible click of a relay mounted behind the front wall. Reinforced responses dispensed one 45-mg pellet (BioServe, Frenchtown, NJ) into a food tray centered between the levers on the front panel of the chamber. On-line experimental control and data collection were by computers with Med Associates interfacing equipment and operating software (Med Associates).

Discrimination Training. Subjects were initially trained to press both levers under a 20-response fixed ratio (FR 20) schedule of food reinforcement and to discriminate i.p. injections of 29.4 μmol/kg cocaine (10 mg/kg) from i.p. injections of saline. After cocaine injection, responses on only one lever were reinforced; after saline injection, responses on the other lever were reinforced. The assignment of cocaine- and saline-appropriate levers was counterbalanced across rats. Rats were placed inside the experimental chambers immediately after injections, and a 5-min time-out period followed during which the house light and LEDs were extinguished and responding had no scheduled consequences. Following the time-out period, the house light and the LEDs were illuminated, and 20 consecutive responses on the appropriate lever produced a food pellet. Responses on the inappropriate lever reset the FR response requirement. Each food presentation was followed by a 20-s time-out period. Sessions ended after 20 food presentations or 15 min, whichever occurred first. Training sessions with cocaine (D) and saline (S) injections were conducted daily and in a double alternation sequence (e.g., S, D, S, D, S, D, . . . ). Four groups of subjects were given cocaine or saline injections immediately before they were placed into chambers (5-min pretreatment time due to the 5-min time out), whereas one group of given cocaine or saline 10 min before they were placed into chambers (15-min pretreatment time).

Discrimination Testing. Testing was initiated when performances reached criteria of at least 80% appropriate responding overall and during the first FR 20 of the session over four consecutive sessions. Tests were conducted with different doses of cocaine, doses of other DA uptake inhibitors and DA releasers, or combinations of doses administered at appropriate times before sessions. After a test session, a subject was required to meet the above-mentioned performance criteria over two consecutive (cocaine and saline) training sessions to be tested again. Repeated test sessions were conducted, with at least two training sessions between tests, until entire dose effects were determined in each subject. Test sessions were identical to training sessions, with the exception that 20 consecutive responses on either lever were reinforced.

Data Analyses. For each of the rats, the overall response rate and the percentage of responses occurring on the cocaine-appropriate lever for the entire session were calculated. The mean values for groups of six subjects were calculated for each measure at each drug dose tested. For Fig. 1. and the tables, the values for cocaine were derived from 24 subjects. For the remaining figures, the data for cocaine administered alone (with vehicle) were from the group of six subjects tested with that particular drug combination. Each dose-effect curve was analyzed using standard analysis of variance and linear regression techniques, from which the ED50 values (doses producing 50% cocaine-appropriate responding) were calculated. For these analyses, points on the linear part of the ascending portions of the dose-effect curves were used (Snedecor and Cochran, 1967). To assess the degree of change in the cocaine dose-effect curve produced by coadministration of an indirect DA agonist, data were also analyzed by standard parallel-line bioassay techniques as described by Finney (1964). The relative potency value represents the dose of cocaine alone equal to 1 μmol/kg cocaine in subjects coadministered one of the indirect DA agonists (i.e., a relative potency value of 2 indicates a 2-fold shift to the left of the cocaine dose-effect curve in the presence of the indirect agonist). A significant shift in the cocaine dose-effect curve is indicated when the 95% confidence limits for the relative-potency ratio do not include the value 1.0. For the studies with a fixed dose-ratio, the ED50 (with 95% confident level) values were estimated as described above, but the ED50 (with 95% confident level) values were divided based on the proportions of the compounds in the mixture to derive values for each compound.

Interactions between indirect DA agonists and cocaine were further assessed using an isobolographic analysis (Tallarida, 1992). The ED50 values (and 95% CL) for cocaine in combination with the other indirect DA agonists were compared with the values predicted from a strictly additive hypothesis and considered to deviate significantly from additivity if the values of the 95% CLs did not overlap those joining the ED50 and 95% CL values for the drugs administered alone.

To further characterize the interactions, the relative potency values were fitted to the negative logarithm of the dose of the test drug. Using a linear model, the dose of the indirect agonist producing a 2-fold leftward shift (ED50) was calculated as an in vivo measure of the potency of the drug to shift the cocaine dose-effect curve.

Drugs. Phentermine hydrochloride, d-amphetamine sulfate, (+)-methamphetamine hydrochloride, (-)-cathinone hydrochloride, and (±)-N-methcathinone hydrochloride were obtained from the National Institute on Drug Abuse Drug Supply program (National...
Institute on Drug Abuse, Rockville, MD). Cocaine hydrochloride, GBR 12909 dihydrochloride, WIN 35,428, indaltraline hydrochloride, methylphenidate hydrochloride, nomifensine maleate, and mazindol were obtained from Sigma-Aldrich (St. Louis, MO). Fencamfamine (originally obtained from Merck, Darmstadt, Germany) was a gift from Dr. Roger D. Spealman (Harvard Medical School, Southborough, MA). WIN 35,428 and GBR 12909 were dissolved in water, and the other compounds were dissolved in saline (0.9% NaCl). All compounds were administered i.p. at 1 ml/kg, with doses administered on the basis of body weight. GBR 12909 at 17 mg/kg was administered at 1.7 ml/kg. Drug doses are expressed as micromoles per kilogram. Cocaine was administered immediately or 10 min before subjects were placed in chambers, resulting in 5- or 15-min pretreatment times, respectively. GBR 12909, indaltraline, and methylphenidate were administered 30 min before testing (25 min before placement in chambers); nomifensine, mazindol, and fencamfamine were administered 15 min before testing (10 min before placement in chambers); and all other drugs were administered 5 min before testing (immediately before placement in chambers).

Results

Effects of Cocaine. Cocaine (29.4 μmol/kg, 10 mg/kg), at criterion performance, produced an average of 98.7% cocaine-appropriate responses, whereas 2.64% of the responses were emitted on this lever after injection of saline. The average rates of responding after injection of cocaine (0.991 ± 0.0407 responses/s) did not significantly differ from response rates after injection of saline (0.866 ± 0.0509 responses/s) (t_{26} = 1.921, p = 0.0564), and subjects typically completed all 20 FR components following either injection. Under test conditions, increasing doses of cocaine (2.94–16.5 μmol/kg) engendered dose-related increases in the percentage of responses on the cocaine-appropriate lever (Fig. 1, filled circles). The ED_{50} value for cocaine in all subjects under these training and testing conditions was 8.83 (95% CLs, 7.64–10.2) μmol/kg (Table 1). Treatment with cocaine 15 min before testing in a group of rats trained with cocaine and saline injections 5 min before testing did not alter the cocaine dose-effect function. The ED_{50} values for cocaine administered 15 or 5 min before testing were 11.6 (95% CLs, 8.76–16.1) or 9.83 (95% CLs, 8.03–12.1) μmol/kg, respectively.

The effects of cocaine were stable over the approximate 1-year course of the study. The ED_{50} value for cocaine determined immediately after training criteria were met in a selected group of animals 9.83 (95% CLs, 8.03–12.1) μmol/kg. The value in this group of subjects determined after the completion of all tests with other drugs or drug combinations was 9.71 (95% CLs, 5.23–17.9) μmol/kg.

Effects of training with cocaine (29.4 μmol/kg) administered 15 min before testing were similar to those described above for the same dose administered 5 min before testing. At criterion, the training dose of cocaine produced an average of 99.5% responding on the cocaine-appropriate lever, and saline produced 4.16% drug-appropriate responding. The average rate of responding after injection of cocaine (0.940 ± 0.0630 responses/s) was comparable with the average response rate after injection of saline (0.850 ± 0.0700 responses/s), and subjects typically completed all 20 FR components within each session. Under test conditions, increasing doses of cocaine (2.94–16.5 μmol/kg) engendered dose-related increases in the percentage of responses on the cocaine-appropriate lever, and the ED_{50} value was 8.88 (95% CLs, 6.18–12.7) μmol/kg.

Substitution by Other DA Uptake Inhibitors and DA Releasers. Each of the DA uptake inhibitors examined had discriminative-stimulus effects that were qualitatively similar to those of cocaine (Fig. 1, left top). Increasing doses of each of the drugs produced dose-related increases in the percentage of cocaine-appropriate responding, with the high-
est dose occasioning full substitution in all subjects. Although the slope of the dose-effect curve for mazindol appeared to be less than that for each of the other DA uptake inhibitors, the difference was not significant (Table 1). Each of the uptake inhibitors, with the exception of GBR 12909, was more potent than cocaine (ED$_{50}$ values and potencies relative to cocaine are shown in Table 1). The order of potencies of these compounds in substituting for cocaine was WIN 35,428 > mazindol > indatraline > nomifensine > methylphenidate > cocaine $\geq$ GBR 12909. (Because the tests of some of the drugs were conducted in different groups of animals, the rank order of their potencies was determined from their potencies relative to cocaine.) In the group of rats trained to discriminate injections of cocaine and saline given 15 min before testing, increasing doses of GBR 12909 produced dose-related increases in the percentage of cocaine-appropriate responding, with the highest dose engendering full substitution in all subjects. The ED$_{50}$ value for GBR 12909 in this group of subjects 14.8 (95% CLs, 11.4–19.4) was not significantly different from that shown in Table 1. Only GBR 12909 (32.5 $\mu$/mol/kg) and mazindol substantially decreased the average rate of responding (Fig. 1, bottom left).

Each of the DA releasers also fully substituted for cocaine (Fig. 1, top right). For each compound, substitution was dose-dependent, and full substitution was obtained at the highest doses. The slopes of the dose-effect curves for each of the DA releasers were similar to each other (Table 1). Each of the DA releasers, with the exception of phentermine, was more potent than cocaine (ED$_{50}$ values and potencies relative to cocaine are shown in Table 1). The order of potencies of these compounds was methamphetamine $\approx$ d-amphetamine $\geq$ methcathinone $\approx$ cathinone $> fencamfamine$ $>>$ phentermine. Decreases in the average rate of responding were only obtained at the highest dose of d-amphetamine, and with substantial variability, there was a nonsignificant trend for increases in response rates at intermediate doses of d-amphetamine and methamphetamine (Fig. 1, right bottom).

**Effects of Combinations of DA Uptake Inhibitors and DA Releasers with Cocaine.** In general, treatment with DA uptake inhibitors dose-dependently shifted the cocaine dose-effect curve to the left (Fig. 2). Because each of the uptake inhibitors when administered alone was active (Fig. 1), some of their doses in combination with the lowest, marginally active doses of cocaine produced effects significantly greater than those obtained with cocaine alone. Table 2 shows dose-related changes in the ED$_{50}$ value for cocaine with administration of the uptake inhibitors. There was a dose-related decrease in cocaine ED$_{50}$ value as dose of the uptake inhibitor increased. These changes were evident as significant relative potency differences (reflected by 95% CLs exclusive of 1.0), typically obtained at the highest doses of the uptake inhibitors studied in combination with cocaine. There was also a trend for the slopes of the cocaine dose-effect curves to become less steep as the dose of the uptake inhibitor was increased (Fig. 2; Table 2), although these effects were not significant. Also shown in Table 2 is the calculated dose of the uptake inhibitor that shifted the cocaine dose-effect curve 2-fold to the left. This dose ranged from approximately 7.94 for GBR 12909 to 0.440 for mazindol. As might be expected, this calculated value closely followed the ED$_{50}$ value for the drug when administered alone (Table 1); however, the values were generally less than the ED$_{50}$ values, with ratios between the two that ranged from 1.30 to 3.84 (Table 2).

The DA releasers also produced dose-dependent leftward shifts in the cocaine dose-effect curve (Fig. 3), with increases in effects at the lowest doses of cocaine and a corresponding, but nonsignificant, trend for the slopes of the dose-effect curves to become less steep (Table 3). There was a dose-related decrease in cocaine ED$_{50}$ value, along with significant relative potency differences typically obtained with most doses of the DA releasers. As shown in Table 3, the calculated dose of the DA releaser that shifted the cocaine dose-effect curve 2-fold to the left ranged from approximately 1.96 for fencamfamine to 0.434 for methamphetamine. Those values also closely followed, but were less than, the ED$_{50}$ values for the drug when administered alone. The ratios of these values were the largest for methamphetamine and d-amphetamine, with the value producing a 2-fold shift in the dose-effect curve approximately 5-fold greater than the ED$_{50}$ value (Table 3).

**Isobolographic Analysis of the Effects of DA Uptake Inhibitors and DA Releasers on Cocaine Discrimination.** In general, the ED$_{50}$ values for cocaine in the presence of different doses of the various DA uptake inhibitors (Fig. 4, top row) or DA releasers (Fig. 4, bottom row) were within the limits of variability of the ED$_{50}$ values for the drugs when administered alone. There were occasional instances in which trends toward supra-additivity were evident. This was most notable with mazindol, d-amphet-

### Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ED$_{50}$</th>
<th>Slope</th>
<th>Relative Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>8.83 (7.64–10.2)</td>
<td>85.0 (72.1–97.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Cocaine 15 min prior</td>
<td>11.6 (8.76–16.1)</td>
<td>99.8 (70.1–129)</td>
<td>7.53 (4.97–11.7)</td>
</tr>
<tr>
<td>WIN 35,428</td>
<td>1.09 (0.753–1.45)</td>
<td>88.1 (58.2–118)</td>
<td>6.07 (3.50–10.6)</td>
</tr>
<tr>
<td>Mazindol</td>
<td>1.68 (1.15–2.53)</td>
<td>60.0 (41.7–78.3)</td>
<td>3.62 (2.37–5.54)</td>
</tr>
<tr>
<td>Indatraline</td>
<td>2.29 (1.68–3.04)</td>
<td>99.9 (67.3–132)</td>
<td>2.22 (1.42–3.37)</td>
</tr>
<tr>
<td>Nomifensine</td>
<td>2.05 (2.40–3.78)</td>
<td>112 (77.3–146)</td>
<td>1.99 (1.09–3.63)</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>7.26 (6.30–8.60)</td>
<td>195 (134–257)</td>
<td>0.749 (0.571–0.992)</td>
</tr>
<tr>
<td>GBR 12909</td>
<td>13.2 (11.0–15.5)</td>
<td>134 (99.7–168)</td>
<td>5.91 (4.19–8.33)</td>
</tr>
<tr>
<td>Methcathinone</td>
<td>1.67 (1.21–2.33)</td>
<td>93.1 (62.7–123)</td>
<td>5.24 (3.65–7.51)</td>
</tr>
<tr>
<td>Cathinone</td>
<td>1.87 (1.28–2.80)</td>
<td>98.9 (61.5–136)</td>
<td>6.25 (4.29–9.18)</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>2.18 (1.49–3.08)</td>
<td>78.3 (53.9–103)</td>
<td>6.18 (4.58–8.36)</td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>2.23 (1.86–2.74)</td>
<td>87.0 (70.8–103)</td>
<td>1.96 (1.18–3.10)</td>
</tr>
<tr>
<td>Fencamfamine</td>
<td>3.26 (2.45–4.37)</td>
<td>86.8 (58.8–115)</td>
<td>1.13 (0.740–1.73)</td>
</tr>
</tbody>
</table>

$^{a}$ Cocaine ED$_{50}$ and slope are the overall calculation of data from 24 animals (four groups).

$^{b}$ The value is an estimate because there was a significant deviation from parallel.

$^{c}$ The value is an estimate because there was a significant deviation from linearly.
amine, and methamphetamine. However, the majority of the interactions were indicative of a strictly additive interaction.

Because the additive effects of GBR 12909 and cocaine differed from those previously reported (Holtzman, 2001), we examined this interaction further. In the study by Holtzman, the combinations were studied with cocaine administered 15 min before testing, as opposed to the 5 min employed in the present study. In addition, that study was conducted using fixed ratios of doses, rather than the fixed doses as reported above. We also examined the effects of the combination of fixed ratios of doses of cocaine and GBR 12909 when subjects were tested with cocaine administered 5 min prior to sessions. The isobolographic analysis of those data (dose-effect curves not shown) indicates simple additive interaction between these two compounds (Fig. 5A). In these same subjects, dose ratios were examined with subjects tested at 15 min after cocaine injection, with little evidence that the interaction was anything more than strictly additive (Fig. 5B). We then trained another group of animals in which cocaine was given 15 min before training and testing sessions. The results showed that treatment with GBR 12909 dose-dependently shifted the cocaine dose-effect curve to the left; the ED50 value for cocaine was changed from 8.88 (95% CLs, 6.18–12.7) to 5.65 (95% CLs, 2.57–9.03) by the respective administration of 3.0 and 5.6 mg/kg GBR 12909, 30 min before sessions (data not shown). As in the above study, this combination of drugs did not differ from predictions of a strictly additive interaction (Fig. 5C).

**TABLE 2**

Interactions of different dopamine uptake inhibitors with cocaine on discriminative stimulus in rats

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ED50 Value</th>
<th>Relative Potency</th>
<th>Slope</th>
<th>ED2x</th>
<th>ED50/ED2x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>8.83 (7.64–10.2)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.73 GBR 12909 and cocaine</td>
<td>6.92 (4.18–10.2)</td>
<td>1.40 (0.934–2.15)</td>
<td>78.8 (46.3–111)</td>
<td>7.94</td>
<td>1.66</td>
</tr>
<tr>
<td>10.7 GBR 12909 and cocaine</td>
<td>2.80</td>
<td>2.47 (1.29–5.97)</td>
<td>48.8 (1.04–98.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.30 WIN 35,428 and cocaine</td>
<td>5.47 (1.87–9.46)</td>
<td>1.39 (0.722–2.95)</td>
<td>69.5 (29.1–110)</td>
<td>0.445</td>
<td>2.45</td>
</tr>
<tr>
<td>0.53 WIN 35,428 and cocaine</td>
<td>4.16 (1.39–6.94)</td>
<td>1.68 (0.943–3.32)</td>
<td>60.7 (29.1–92.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.99 WIN 35,428 and cocaine</td>
<td>1.05 (0.0715–2.10)</td>
<td>5.63 (2.72–14.2)</td>
<td>47.1 (13.8–80.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.91 Indatraline and cocaine</td>
<td>7.02 (4.07–10.7)</td>
<td>1.16 (0.675–2.06)</td>
<td>86.1 (48.4–124)</td>
<td>1.42</td>
<td>1.61</td>
</tr>
<tr>
<td>1.70 Indatraline and cocaine</td>
<td>3.50 (0.912–5.28)</td>
<td>2.60 (1.53–4.81)</td>
<td>117 (29.9–205)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.71 Methylphenidate and cocaine</td>
<td>6.93 (3.14–11.9)</td>
<td>1.40 (0.824–2.51)</td>
<td>81.3 (36.2–127)</td>
<td>6.92</td>
<td>1.30</td>
</tr>
<tr>
<td>6.30 Methylphenidate and cocaine</td>
<td>4.85 (1.03–8.96)</td>
<td>1.84 (0.722–2.95)</td>
<td>66.1 (23.0–109)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.58 Nomifensine and cocaine</td>
<td>5.56 (1.46–10.3)</td>
<td>1.06 (0.582–1.97)</td>
<td>69.2 (24.3–114)</td>
<td>2.33</td>
<td>1.31</td>
</tr>
<tr>
<td>2.28 Nomifensine and cocaine (1.3 mg only)</td>
<td>2.26 (0.00–4.02)</td>
<td>2.61 (1.51–4.96)</td>
<td>73.8 (2.61–145)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.35 Mazindol and cocaine</td>
<td>6.24 (4.01–10.2)</td>
<td>1.65 (0.993–2.71)</td>
<td>64.9 (43.2–86.6)</td>
<td>0.440</td>
<td>3.84</td>
</tr>
<tr>
<td>0.60 Mazindol and cocaine</td>
<td>3.58 (1.96–5.81)</td>
<td>2.69 (1.58–4.79)</td>
<td>60.9 (38.2–83.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 The value is an estimate because there was a significant effect of preparations.
2 The value is an estimate because there was a significant deviation from linearity.
3 The value is an estimate because there was a significant deviation from parallel.
To characterize the interactions of indirect dopaminergic agonists, we examined both uptake inhibitors and releasers of dopamine in rats trained to discriminate cocaine from saline injections. We replicated previous findings that the present DA uptake inhibitors (e.g., Broadbent et al., 1991; Baker et al., 1993; Kleven et al., 1999; Katz et al., 2000) and DA releasers substitute completely for cocaine (e.g., D'Mello and Stolerman, 1977; Risner et al., 1985; Wood and Emmett-Oglesby, 1988; Ukai et al., 1993; Young and Glennon, 1993; Koetzner et al., 1996). When DA uptake inhibitors and DA releasers were given before or concurrently with cocaine, the cocaine dose-effect function was shifted to the left, with a trend toward a change in the slope of the dose-effect curve. Isobolographic analysis revealed that the interactions were generally additive. However, there were instances in which trends toward supra-additivity were evident with mazindol, d-amphetamine, and methamphetamine, and these trends were reflected in relatively greater ratios of ED50 to ED2x values.

The rank order of potency among the dopamine uptake inhibitors for substituting for cocaine and for shifting the cocaine dose-effect curve leftward was WIN 35,428 > indatraline > nomifensine > cocaine = methylphenidate > GBR 12909. This potency relation is similar to potency relationships observed for these drugs in previous studies of self-administration (Risner and Silcox, 1981; Bergman et al., 1989; Roberts, 1993; Lile et al., 2003), increases in rates of schedule-controlled operant behavior (Spealman et al., 1989), and discriminative-stimulus effects (Broadbent et

---

**Table 3**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ED50 Value</th>
<th>Relative Potency</th>
<th>Slope</th>
<th>ED2x ED50/ED2x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>8.83 (7.64–10.2)</td>
<td>1</td>
<td>85.0 (72.1–97.8)</td>
<td>4.48</td>
</tr>
<tr>
<td>0.54 d-Amphetamine and cocaine</td>
<td>6.06 (2.99–9.56)</td>
<td>2.23 (1.46–3.54)</td>
<td>75.8 (39.1–113)</td>
<td>0.448</td>
</tr>
<tr>
<td>0.54 Methamphetamine and Cocaine</td>
<td>5.18 (1.92–10.9)</td>
<td>2.42 (1.29–4.69)</td>
<td>47.8 (24.1–71.4)</td>
<td>0.434</td>
</tr>
<tr>
<td>0.54 Methamphetamine and Cocaine</td>
<td>0.713 (0.00435–2.18)</td>
<td>9.57 (4.89–21.2)</td>
<td>33.4 (9.38–57.5)</td>
<td>0.54</td>
</tr>
<tr>
<td>0.54 Cathinone and cocaine</td>
<td>5.66 (2.72–8.88)</td>
<td>1.69 (1.05–2.93)</td>
<td>81.7 (42.7–121)</td>
<td>0.626</td>
</tr>
<tr>
<td>0.54 Cathinone and cocaine</td>
<td>1.61 (0.0066–3.93)</td>
<td>5.41 (2.75–12.4)</td>
<td>56.7 (5.91–107)</td>
<td>0.925</td>
</tr>
<tr>
<td>0.50 Methcathinone and cocaine</td>
<td>7.88 (4.31–13.0)</td>
<td>1.24 (0.73–2.16)</td>
<td>75.0 (38.9–117)</td>
<td>0.925</td>
</tr>
<tr>
<td>0.50 Methcathinone and cocaine</td>
<td>2.64 (0.0429–5.82)</td>
<td>2.60 (1.51–5.25)</td>
<td>49.7 (12.1–87.3)</td>
<td>0.925</td>
</tr>
<tr>
<td>1.02 Phenetermine and cocaine</td>
<td>4.49 (2.95–6.65)</td>
<td>1.77 (1.04–3.01)</td>
<td>67.9 (47.7–88.0)</td>
<td>1.86</td>
</tr>
<tr>
<td>5.39 Phenetermine and cocaine</td>
<td>1.62 (0.406–2.95)</td>
<td>4.55 (2.41–9.36)</td>
<td>63.3 (22.8–104)</td>
<td>1.86</td>
</tr>
<tr>
<td>1.19 Fencamfamine and cocaine</td>
<td>6.55 (3.88–9.93)</td>
<td>0.985 (0.576–1.68)</td>
<td>85.7 (47.9–124)</td>
<td>1.96</td>
</tr>
<tr>
<td>2.22 Fencamfamine and cocaine</td>
<td>2.15 (0.678–4.05)</td>
<td>2.21 (1.11–4.54)</td>
<td>48.3 (25.1–71.6)</td>
<td>2.22</td>
</tr>
</tbody>
</table>

*a* The value is an estimate because there was a significant deviation from linearity.

*b* The value is an estimate because there was a significant effect of preparations.

*c* The value is an estimate because there was a significant deviation from parallel.

---

**Discussion**

To characterize the interactions of indirect dopaminergic agonists, we examined both uptake inhibitors and releasers of dopamine in rats trained to discriminate cocaine from saline injections. We replicated previous findings that the present DA uptake inhibitors (e.g., Broadbent et al., 1991; Baker et al., 1993; Kleven et al., 1999; Katz et al., 2000) and DA releasers substitute completely for cocaine (e.g., D’Mello and Stolerman, 1977; Risner et al., 1985; Wood and Emmett-Oglesby 1988; Ukai et al., 1993; Young and Glennon, 1993; Koetzner et al., 1996). When DA uptake inhibitors and DA releasers were given before or concurrently with cocaine, the cocaine dose-effect function was shifted to the left, with a trend toward a change in the slope of the dose-effect curve. Isobolographic analysis revealed that the interactions were generally additive. However, there were instances in which trends toward supra-additivity were evident with mazindol, d-amphetamine, and methamphetamine, and these trends were reflected in relatively greater ratios of ED50 to ED2x values.

The rank order of potency among the dopamine uptake inhibitors for substituting for cocaine and for shifting the cocaine dose-effect curve leftward was WIN 35,428 > indatraline > nomifensine > cocaine = methylphenidate > GBR 12909. This potency relation is similar to potency relationships observed for these drugs in previous studies of self-administration (Risner and Silcox, 1981; Bergman et al., 1989; Roberts, 1993; Lile et al., 2003), increases in rates of schedule-controlled operant behavior (Spealman et al., 1989), and discriminative-stimulus effects (Broadbent et
In addition, the observed relative potencies of these compounds were consistent with their relative binding affinities for the DA transporter (Schoemaker et al., 1985; Andersen, 1989; Madras et al., 1989; Valchar and Hanbauer, 1993). These results suggest that the effects observed in the present study were due to the known actions of the drugs in blocking the uptake of DA.

Among the DA releasers, the rank order of potency relative to cocaine was methamphetamine, methcathinone, cathinone, fencamfamine, and phentermine. This potency relation is similar to those in reports in i.v. drug self-administration (Risner and Cone, 1986; Stafford et al., 2001), discriminative-stimulus effects (D’Mello and Stolerman, 1977; Risner et al., 1985; Wood and Emmett-Oglesby, 1988; Ukai et al., 1993; Young and Glennon, 1993; Koetzner et al., 1996), and increases in rates of schedule-controlled operant behavior (Risner et al., 1985). In addition, the observed relative potency of these compounds is consistent with their in vitro potencies in producing DA release (Seyfried, 1983; Rothman et al., 2001). These results suggest that the effects observed in the present study were due to the known actions of the drugs in releasing DA.

Because of the actions of cocaine as an indirect DA agonist, there has been a focus on drugs with a similar mechanism as potential treatments for cocaine abuse (Gorelick et al., 2004). For example, d-amphetamine, methamphetamine, and methylphenidate have been tested as treatments for cocaine dependence, whereas others have been tested in rodent and nonhuman primates (Grabowski et al., 2004). Perhaps the compound for which there has been the most preclinical attention is GBR 12909. Studies of in vivo microdialysis indicated that pretreatment with GBR 12909 attenuated the effectiveness of cocaine in elevating extracellular DA levels (Baumann et al., 1994). In addition, GBR 12909 has been reported to decrease rates of cocaine-maintained responding (Glowa et al., 1995; see also Skjoldager et al., 1993). In contrast, GBR 12909 enhanced the discriminative-stimulus effects of cocaine (present study; Spealman, 1993; Holtzman 2001; Katz et al., 2003), as well as its effects on locomotor activity (Katz et al., 2003) and schedule-controlled behavior (Spealman, 1993). Because self-administration of drugs is a complex procedure, there can be many interpretations and
potential mechanisms for the reported decreases. Although decreases in response rates suggest decreases in the reinforcing effects of cocaine, this conclusion is not always justified. Because the dose-effect curve for self-administration often has a bell shape, a rightward or leftward shift in the dose-effect curve could be reflected in a decrease at a single dose of cocaine. Parsimony with the present findings suggests that the decrease in cocaine self-administration obtained with pretreatment of indirect DA agonists represents an additive enhancement of the effects of cocaine. However, at present, there is no way to reasonably reconcile the additive behavioral effects of GBR 12909 and cocaine in the present and previous study and biochemical studies showing that phen- termine (Rothman et al., 1996) and GBR 12909 (Baumann et al., 1994) attenuate the rise in extracellular DA in the nu- cleus accumbens produced by cocaine.

To characterize the interactions of cocaine and DA uptake inhibitors, isobolographic analyses were conducted. In gen- eral, the effects of none of the compounds substantially dif- fered from a simple additive model. Holtzman (2001) showed that the combination of GBR 12909 with cocaine produced a supra-additive discriminative-stimulus effect. To reconcile our findings with those of Holtzman, we examined the effects of the different pretreatment times for cocaine, and the dosing procedures. Neither of these differences appeared to be responsible for the differences between the present results and those previously reported. Remaining potential reasons for the discrepancy between the findings might at least in- clude that we used food reinforcement; whereas Holtzman used avoidance of electric shock to maintain responding, we used food-deprived animals, and Holtzman used free-feeding animals.

Although isobolographic analysis revealed that the inter- actions were generally additive, methamphetamine and d- amphetamine were generally more potent in shifting the cocaine dose-effect curve to the left than were the other indirect DA agonists based on the calculated ED_{50}/ED_{25} ra- tios. The results showed that DA uptake inhibitors engen- dered a ratio normally smaller than 2, whereas those for the DA releasers engendered a ratio normally bigger than 2, indicating that DA releasers are more potent than DA uptake inhibitors in potentiating the cocaine discriminative stimu- lus in the present study.

Although acute administration of the indirect DA agonists did not block the behavioral effects of cocaine, it should be noted that an agonist maintenance (or replacement) medical treatment for drug abuse typically shares pharmacological effects with the abused drug. For example, methadone, an effective treatment for opioid abuse, shifts the morphine dose-effect curve leftward (Li et al., 2005), as the present indirect DA agonists shifted the cocaine dose-effect curve. There were some differences among the indirect DA agonists with regard to the degree to which they shifted the effects of cocaine. Whether those differences predict efficacy in treatment of cocaine abuse is not currently clear. However, the reported negative clinical findings with several uptake inhib- itors (e.g., Silva de Lima et al., 2002; Gorelick, 2004) and the potential of d-amphetamine as an effective treatment (Grabowski et al., 2004) suggest that these differences in shifts in dose-effect curves should be further examined with emerging clinical data.

Acknowledgments

We thank Ronald J. Tallarida and Steven S. Negus for expert advice on the data analysis and Roger D. Spearman for generously supplying fencamfamine. We also thank Patty Bailerstadt for administrative assistance.

References


Address correspondence to: Dr. Jonathan L. Katz, Psychobiology Section, National Institute on Drug Abuse, Intramural Research Program, 5500 Nathan Shock Drive, Baltimore, MD 21224. E-mail: jkatz@intra.nida.nih.gov