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Behavioral effects of rimcazole analogues alone and in combination with cocaine

Jonathan L. Katz[a,*], Therissa A. Libby[a], Theresa Kopajtic[a], Stephen M. Husbands[b], Amy Hauck Newman[b]

[a] *Psychobiology Section, Medications Discovery Research Branch, NIDA Intramural Research Program, National Institutes of Health, Department of Health and Human Services, 5500 Nathan Shock Drive, Baltimore, MD 21224, USA*
[b] *Medicinal Chemistry Section, Medications Discovery Research Branch, NIDA Intramural Research Program, National Institutes of Health, Department of Health and Human Services, 5500 Nathan Shock Drive, Baltimore, MD 21224, USA*

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Abstract

Several σ receptor ligands have been reported to also have affinity for the dopamine transporter, among them rimcazole (9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)propyl]carbazole dihydrochloride). However, rimcazole lacks behavioral effects like those of other dopamine uptake inhibitors, such as cocaine and GBR 12909 (1-(2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride). Because of this profile, the interactions with cocaine of rimcazole and several of its novel analogues were assessed. The compounds studied were rimcazole, its *N*-methyl analogue, SH 1–73 (9-[3-(*cis*-3,5-dimethyl-4-methyl-1-piperazinyl)-propyl]carbazole hydrobromide), the dibrominated analogue, SH 1–76 (3,6-dibromo-9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)-propyl]carbazole hydrochloride), and the *N*-propylphenyl analogues, SH 3–24 ([3-(*cis*-3,5-dimethyl-4-[3-phenylpropyl]-1-piperazinyl)-propyl]diphenylamine hydrochloride) and SH 3–28 (9-[3-(*cis*-3,5-dimethyl-4-[3-phenylpropyl]-1-piperazinyl)-propyl]carbazole hydrobromide). The former has a diphenyl-amine group in place of the carbazole moiety of rimcazole, giving the compound additional structural similarity to GBR 12909. The rimcazole analogues produced dose-related decreases in locomotor activity, and also decreased cocaine-stimulated activity in mice. In rats trained to discriminate 10 mg/kg cocaine (i.p.) from saline injections, cocaine and GBR 12909 each produced a dose-related increase in cocaine-appropriate responding. Cocaine also increased rates of responding. SH 3–28 decreased cocaine-appropriate responding at the cocaine training dose to about 58% (SH 3–28) with two of five subjects selecting the cocaine response key. Neither rimcazole nor SH 3–24 produced a significant attenuation of the discriminative effects of cocaine. Rimcazole and its analogs all attenuated the increases in rates of responding produced by cocaine. In contrast to effects obtained with rimcazole analogs, GBR 12909 potentiated the cocaine-induced increases in locomotor activity and operant behavior, as well as the discriminative-stimulus effects of cocaine. The present results indicate that analogues of rimcazole can attenuate the behavioral effects of cocaine, and though the mechanism for these effects is not presently clear, it is possible that this attenuation maybe mediated by actions of the rimcazole analogues at the dopamine transporter and/or σ receptors.

Keywords: Rimcazole; Dopamine; GBR 12909

1. Introduction

Several σ receptor ligands have been reported to decrease the behavioral effects of cocaine. For example, Menkel et al. (1991) showed that rimcazole and BMY 14802 blocked the locomotor-stimulant effects of cocaine, which has also been reported with other σ receptor ligands (Karbon et al., 1992; McCracken et al., 1999a,b). This activity occurred more specifically than for other drugs, such as the dopamine D2 receptor antagonists which only attenuated the effects of cocaine at doses that were by themselves active (Chausmer and Katz, 2001; Menkel et al., 1991). More recently, Romieu et al. (2000, 2002) have shown that σ receptor antagonists attenuate cocaine-induced place preference and that the σ receptor is necessary for both acquisition and expression of this cocaine-induced behavior. Matsumoto et al. (2001a,b) have also shown that σ receptor ligands can

* Corresponding author. Tel.: +1-410-550-1533; fax: +1-410-550-1648.
E-mail address: jkatz@intra.nida.nih.gov (J.L. Katz).

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block some of the acute toxic effects of cocaine. These findings suggested a focus on σ receptor ligands as possible treatments for cocaine abuse.

A previous study from this laboratory indicated that rimcazole not only has affinity for σ receptors but it also has affinity for the dopamine transporter (Izenwasser et al., 1993). In that study, the affinity of rimcazole for the dopamine transporter was comparable to its reported affinity for σ receptors. In a subsequent study, rimcazole was found to have an approximately 4-fold higher affinity for the dopamine transporter ($K_i = 224$ nM) as compared to the σ receptor ($K_i = 908$ nM, Husbands et al., 1999). Notably, despite its comparable dopamine transporter affinity, the behavioral effects of rimcazole are different from those of cocaine. Rimcazole does not stimulate locomotor activity (Menkel et al., 1991), though cocaine and prototypical psychomotor stimulant drugs have this as one of their benchmark actions (Kelleher, 1977). The disparate behavioral effects of rimcazole and cocaine lead to the synthesis of novel rimcazole analogues in order to better investigate relationships between behavioral activity and pharmacological mechanisms (Husbands et al., 1999).

The present study was initiated to better define the behavioral effects of rimcazole and its analogues compared to those of cocaine. Further, because previous studies have reported some attenuation of behavioral effects of cocaine by σ receptor ligands in general (Menkel et al., 1991), and rimcazole and its analogues in particular (Matsumoto et al., 2001c), we examined interactions between rimcazole and several of its analogues with cocaine.

2. Material and methods

2.1. Animals

For studies of locomotor activity, male Swiss Webster mice (Taconic Farms) weighing 24–28 g were used. They were group housed with unrestricted access to food and water under a 12-h light/dark cycle (lights on 07:00 h). Subjects were used only once in these studies.

For studies of the discriminative-stimulus effects of cocaine, male Sprague–Dawley rats (Charles River, Wilmington MA) weighing 320–350 g were individually housed with unrestricted access to water under a 12-h light/dark cycle (lights on 07:00 h). Rats were fed daily about 15 g of standard lab chow at least 30 min after testing to maintain a constant weight within the range indicated above. A group of six subjects was typically studied with each drug in a within subjects design. Over the course of the study, two subjects were replaced due to illness with cocaine reassessed in the newly introduced subjects so that it was examined in eight subjects. Interactions of GBR 12909 with cocaine were examined in five subjects.

All subjects used in these studies were maintained in accordance with the guidelines of the NIDA Intramural Research Program, Animal Care and Use Committee, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996).

2.2. Apparatus

Locomotor activity was assessed with subjects tested individually in a clear acrylic chambers (40 × 40 cm), which were contained within a monitor (Omnitech Electronics, Columbus, OH) on which light-sensitive detectors were spaced 2.5 cm apart along two perpendicular walls. Directed at the detectors from the opposing walls were infrared light sources. One horizontal activity count was registered each time the subject interrupted a single light beam.

Discriminative-stimulus effects of the drugs were assessed with subjects placed individually in operant-conditioning chambers (Med Associates, modified Model ENV 007, St. Albans, VT, USA) that were housed within light- and sound-attenuating enclosures. White noise was present throughout testing to mask extraneous sounds. Ambient illumination was by a lamp in the top center of the front panel (houselight). Two response keys (levers) were set 17 cm apart on the front panel, with pairs of light-emitting diodes (LEDs) above each. A downward force (0.4 N through about 1 mm) on either key was defined as a response, and produced an audible click. Reinforced responses dispensed one 45-mg pellet (BioServe, Frenchtown, NJ, USA) into a food tray centered between the keys. Experimental control and data collection were by PC MS-DOS computers with Med Associates interfacing equipment and operating software (Med Associates).

2.3. Procedures

Mice were injected (intraperitoneal, i.p., injections administered in volumes of 1 ml/100 g) and immediately placed in the apparatus for 1 h, with activity counts collected each 10 min. For selected studies, GBR 12909 was administered 30 min before cocaine which was administered immediately before subjects were placed in the apparatus. The subjects of these studies were not habituated to the test environment. In other studies, we have found little difference in the locomotor-stimulant effects of cocaine in habituated and non-habituated mice.

Rats were initially trained to press both keys under a 20-response fixed-ratio (FR 20) schedule of food reinforcement and to discriminate i.p. injections of cocaine (10 mg/kg) from saline (see Holtzman, 1990 for a description of the general procedures). After cocaine injection, responses on only one key were reinforced; after saline injection, responses on the other key were reinforced. The assignment of cocaine- and saline-appropriate keys was counterbalanced across rats. Rats were placed inside the experimental chambers immediately after injection; a 5-min time-out period followed, during which all stimuli were off and

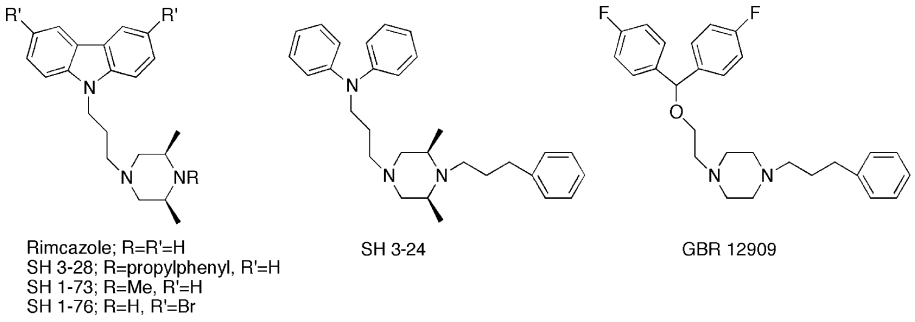

Fig. 1. Molecular structures of rimcazole and its analogues compared to that for GBR 12909.

responding had no scheduled consequences. The time out was followed by illumination of the houselight and LEDs and the beginning of the session. During the session, only responses on the appropriate key were reinforced and responses on the inappropriate key reset the FR response requirement. Each food presentation was followed by a 20-s time out during which all lamps were off, and responding had no scheduled consequences. Sessions ended after 15 min or 20 food presentations, whichever occurred first. Training sessions with cocaine and saline injections were conducted 5 days/week in a double alternation sequence [e.g. . . .saline, cocaine, cocaine, saline. . .].

Testing was initiated when performances reached criteria of at least 85% 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 the novel compounds, or combinations of doses administered prior to sessions. After a test session, a subject was required to meet the above performance criteria over two consecutive (cocaine and saline) training sessions in order 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 key were reinforced.

2.4. Drugs

The drugs tested were: (−)-cocaine HCl (MW= 340; Sigma, St. Louis, MO), GBR 12909 (1-(2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride; MW = 523.5; Research Biochemicals, Natick, MA) rimcazole (9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)propyl]carbazole dihydrochloride, MW = 394.4; RBI) and several analogues of rimcazole that were synthesized in the Medicinal Chemistry Section, NIDA Intramural Research Program (Husbands et al., 1999). Those compounds were: SH 1–73 (9-[3-(*cis*-3,5-dimethyl-4-methyl-1-piperazinyl)-propyl]carbazole hydrobromide; MW = 467.2), SH 1–76 (3,6-dibromo-9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)-propyl]-carbazole hydrochloride; MW = 549), SH 3–24 ([3-(*cis*-3,5-dimethyl-4-[3-phenylpropyl]-1-piperazinyl)-propyl]diphenylamine hydrochloride MW = 523.6), SH 3–28 (9-[3-(*cis*-3,5-dimethyl-4-[3-phenylpropyl]-1-piperazinyl)-propyl]carbazole hydrobromide MW = 520.6) and their structures with that of GBR 12909 are shown in Fig. 1. All drugs were dissolved for injection in 0.9% NaCl or sterile water. For locomotor activity studies, SH 3–28 was dissolved in 0.16% tartaric acid and for studies of discriminative-stimulus effects the compound was dissolved with mild heating in polyethylene glycol and water. The drugs were administered i.p. on the basis of body weight at 1 ml/kg (rats) or 1 ml/0.1 kg (mice).

2.5. Data analysis

Locomotor activity in mice was assessed with counts collected during each successive 10-min epoch; counts during the first and last three epochs of the 1-h assessments

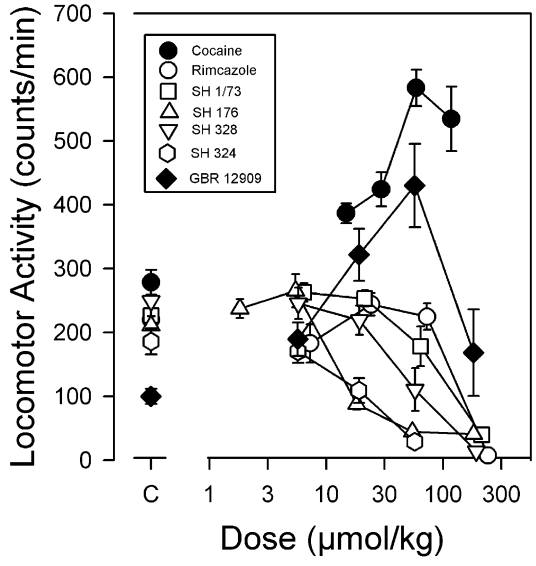

Fig. 2. Dose-dependent effects of cocaine, GBR 12909 and rimcazole and its analogues on locomotor activity in mice. Ordinates: horizontal locomotor activity (in counts/min) after drug administration. Abscissae: dose of drug in µmol/kg, log scale. Points above C represent effects obtained after control (vehicle) injections. Each point represents the average effect determined in eight mice. The effects of each drug are shown for the first 30 min after injection, except GBR 12909, for which effects are shown for the second 30 min after injection.

Table 1
Comparisons of potencies (ED_{50} values with 95% confidence limits) of cocaine, GBR 12909 with rimcazole and its analogues on the various behavioral effects

Drug	Locomotor activity (μmol/kg)	Discriminative stimulus effects (μmol/kg)
Cocaine	*19.1*[a] *(15.3–23.8)*	12.0 (9.35–15.7)
GBR 12909	*11.6*[b] *(4.26–19.9)*	20.6 (17.2–25.5)
Rimcazole	137 (110–172)	No substitution
SH 1–73	106 (79.9–142)	NT[c]
SH 1–76	12.8 (18.9–28.6)	NT
SH 3–24	21.6 (15.9–30.4)	No substitution
SH 3–28	46.2 (32.5–69.1)	No substitution

[a] Values shown in italics represent those for locomotor stimulant effects; all others in this column represent locomotor depressant effects.
[b] This value is for effects obtained during 30–60 min after injection; all others in this column represent effects during 0–30 min after injection.
[c] Not tested.

were cumulated for separate analyses of the first and last 30 min. Except where noted, statistical analyses of effects in the first and last 30 min were similar and results of these analyses are presented for the data from the first 30 min.

Each dose–effect curve was subjected to an analysis of variance (ANOVA) and subsequent planned comparisons (Stevens, 1990). In addition, linear regression techniques (Snedecor and Cochran, 1967) were used to determine ED_{50} values and their 95% confidence limits from the ascending (cocaine, GBR 12909) linear portions of the dose–effect curves, or the descending portions for the drugs that did not increase activity. In order to assess the degree of change in the cocaine dose–effect curve produced by treatments (co-administration of the rimcazole, its analogues, or GBR 12909), data were also analyzed by standard parallel-line bioassay techniques (Finney, 1964). This analysis consisted of a one-way ANOVA, which determined whether the ascending slopes of the linear portions of the two dose–response curves were significantly different from parallel, and fitted a common slope to the two dose–response curves. The relative potency, a ratio of doses for a 50% effect, was derived from the linear portions of the dose–effect curves providing a measure of the degree of shift in the cocaine dose–effect curve. The relative potency value represents the dose of cocaine, in subjects co-administered one of the treatments, equal to 1 μmol/kg of cocaine alone (i.e. a relative potency value of 2.0 indicates a 2-fold shift to the right in the cocaine dose–effect curve due to the treatment). A significant shift in the cocaine dose–effect curve is indicated when the 95% confidence limits for the relative

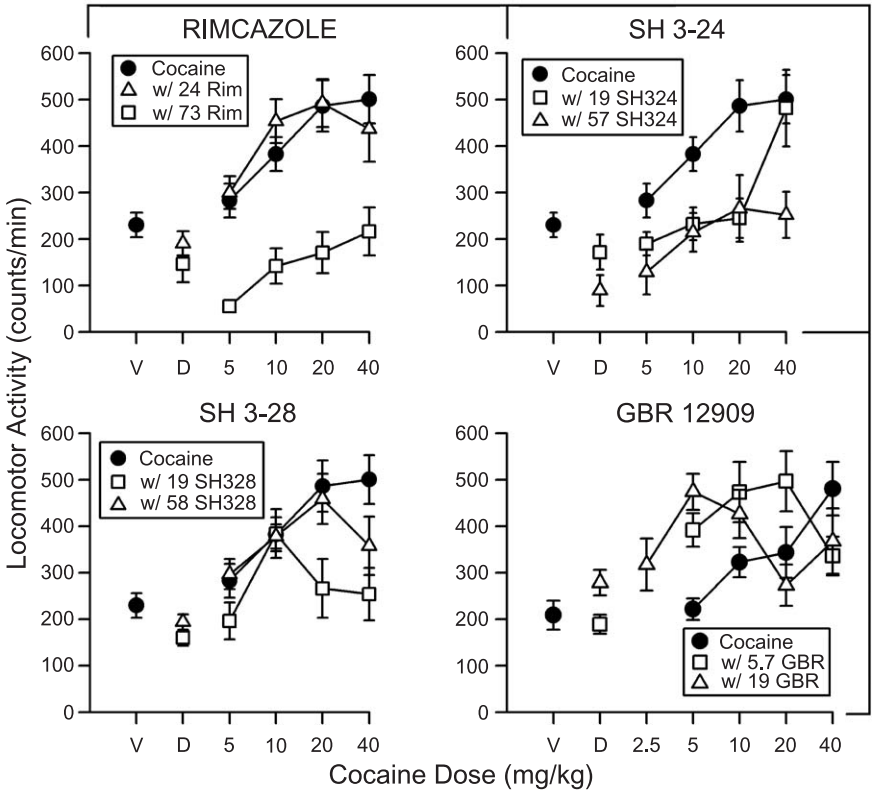

Fig. 3. Effects of combinations of cocaine and rimcazole and its analogues on locomotor activity in mice. Ordinates: horizontal locomotor activity (in counts/min) after drug administration. Abscissae: cocaine dose in mg/kg, log scale. Points above V represent effects obtained after control (two vehicle) injections. Points above D represent effects obtained after control injections of rimcazole, SH 3–24, SH 3–28, or GBR 12909 with cocaine vehicle. The doses of rimcazole, SH 3–24, SH 3–28, and GBR 12909 provided in the keys are in μmol/kg. Each point represents the average effect determined in eight mice.

Table 2
Effects on locomotor activity of interactions of rimcazole and its analogues, and GBR 12909 with cocaine

Treatment	Potency relative to cocaine (95% confidence limits)	Maximal effect (% of cocaine alone)
Cocaine with 24 μmol/kg rimcazole	0.696 (0.364–1.32)	98.3 at 58.8 μmol/kg
Cocaine with 73 μmol/kg rimcazole	18.6 (6.66–181)	43.1 at 118 μmol/kg
Cocaine with 19 μmol/kg SH 3–24	2.89 (1.56–7.66)	96.2 at 118 μmol/kg
Cocaine with 57 μmol/kg SH 3–24	5.12 (2.07–51.1)	53.1 at 58.8 μmol/kg
Cocaine with 5.8 μmol/kg SH 3–28	0.969 (0.533–2.01)	91.6 at 58.8 μmol/kg
Cocaine with 19 μmol/kg SH 3–28	1.35 (0.70–4.35)	76.7 at 29.4 μmol/kg
Cocaine with 5.7 μmol/kg GBR 12909 (0 min prior)	0.24 (0.05–0.51)	103.4 at 58.8 μmol/kg
Cocaine with 19 μmol/kg GBR 12909 (0 min prior)	0.163 (0.068–0.310)	98.7 at 14.7 μmol/kg
Cocaine with 5.7 μmol/kg GBR 12909 (30 min prior)	0.254 (0.105–0.441)	131 at 58.8 μmol/kg
Cocaine with 19 μmol/kg GBR 12909 (30 min prior)	0.063 (0.026–0.115)	100.5 at 7.4 μmol/kg

potency ratio do not include the value 1.0. Effects of cocaine were determined on three separate occasions: when administered alone and compared with other compounds, when administered with vehicle and administered with rimcazole, SH 3–24, or SH 3–28, and when administered with vehicle and GBR 12909. Because the effects of these drugs were determined with different shipments of subjects, the effects of cocaine were redetermined on several occasions. All analyses of interactions between drugs with cocaine were conducted with the cocaine data obtained contemporaneously with the effects of the other drug.

For each of the rats studied in the cocaine-discrimination procedure, the overall response rate and the percentage of responses occurring on the cocaine-appropriate key were calculated. The mean values were calculated for each measure at each drug dose tested. If less than half of the rats responded at a particular dose, no mean value was calculated for percentage of cocaine-appropriate responding at that dose. At least 15% cocaine-appropriate responding was adopted as a conservative criterion for a significant difference from saline; 85% or higher cocaine-appropriate responding was taken as similar to the training dose of cocaine, and intermediate levels of cocaine-appropriate responding were considered partial substitution.

Each dose–effect curve was analyzed using standard two-way (cocaine dose and treatment) ANOVA and linear regression techniques. ED_{50} values and their 95% confidence limits were derived from data using the ascending linear portions of the dose–effect curves (Snedecor and Cochran, 1967). The degree of change in the cocaine dose–effect curve produced by treatments (co-administration of the rimcazole, its analogues, or GBR 12909), was also analyzed by standard parallel-line bioassay techniques (Finney, 1964) as described above.

One of the analogs of rimcazole appeared to reduce the number of subjects showing a full substitution at the highest dose of cocaine. In order to determine if this was a significant effect, the number of subjects showing a full substitution for cocaine (greater than 85% drug-appropriate responding was considered full substitution) was determined

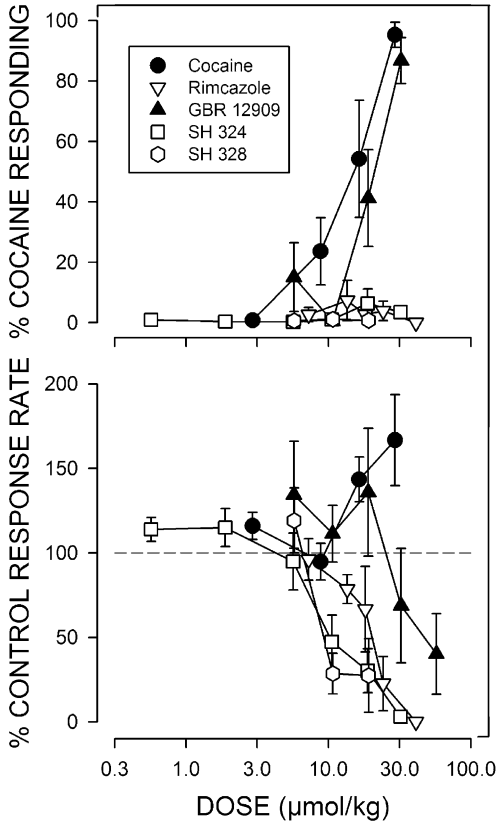

Fig. 4. Effects of cocaine, GBR 12909, and rimcazole and its analogues in rats trained to discriminate injections of cocaine (10 mg/kg) from saline. Ordinates for top panels: percentage of responses on the cocaine-appropriate key. Ordinates for the bottom panels: rates at which responses were emitted (as a percentage of response rate after saline administration). Abscissae: drug dose in μmol/kg (log scale). Each point represents the effect in at least six rats. Polyethylene glycol and water injections administered as a vehicle control produced an average of 1.68% cocaine-appropriate responding and was not different from rates during saline training sessions (data not shown).

for each dose of cocaine alone and in combination with the various rimcazole analogs. A Fisher's Exact test was applied to these data to determine if the number of subjects showing a full substitution for cocaine was altered.

3. Results

3.1. Locomotor activity

Cocaine produced dose-related increases in ambulatory activity with a maximum of approximately 580 counts/min at 59 μmol/kg (Fig. 2, filled circles). GBR 12909 (Fig. 2, filled diamonds) also increased locomotor activity, although this effect was greater in the second 30 min after injection (Fig. 2, diamonds) and remained less than that obtained with cocaine in the first 30 min after injection. Rimcazole and its analogues generally produced dose-related decreases in locomotor activity (Fig. 2, open symbols). Among the rimcazole analogues, SH 3–24 and SH 1–76 were the most potent, with the parent compound, rimcazole, least potent (ED_{50} values are provided in Table 1). Effects of the rimcazole analogues in the second 30 min after injection (data not shown) were generally comparable to those obtained in the first 30 min after injection, however, a significant but small increase in activity was obtained with rimcazole, at a dose of 72.7 μmol/kg, in the second 30 min after injection.

Rimcazole attenuated the locomotor stimulant effects of cocaine (Fig. 3) in a dose-related manner ($F(2,105) = 47.138$, $P < 0.001$). The 24-μmol/kg dose of rimcazole was inactive, however, a dose of 73 μmol/kg decreased the stimulant effects of all doses of cocaine. This higher dose of rimcazole was marginally active when administered alone (Fig. 3, points above D). Table 2 shows the co-administration of rimcazole did not appreciably affect the potency of cocaine; the 95% confidence limits of the relative potency estimates include 1.0 (Table 2). The decreases in the maximal effect of cocaine on locomotor activity produced by rimcazole are also shown in Table 2.

The rimcazole analogues, SH 3–24 ($F(2,105) = 13.163$, $P < 0.001$) and SH 3–28 ($F(2,105) = 10.087$, $P < 0.001$), also attenuated the locomotor stimulant effects of cocaine. The 19-μmol/kg dose of SH 3–24 shifted the cocaine dose–effect curve to the right without an appreciable change in maximal effect of cocaine (Fig. 3 and Table 2). The higher dose primarily decreased the maximal effects of cocaine without changes in potency of cocaine (Fig. 3; Table 2).

In contrast to the effects of rimcazole and its analogues, GBR 12909 potentiated the effects of cocaine. These effects (Fig. 3) were obtained when GBR 12909 was administered

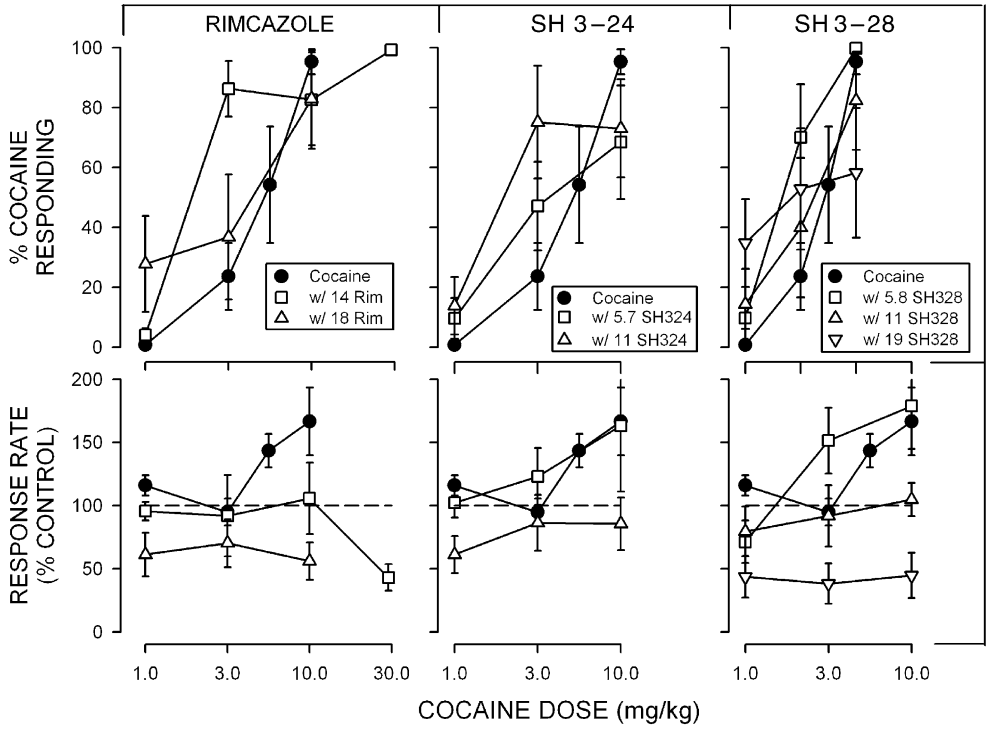

Fig. 5. Changes in the cocaine dose–effect curve for discriminative-stimulus effects produced by pretreatments with rimcazole or its analogues. Ordinates for top panels: percentage of responses on the cocaine-appropriate key. Ordinates for the bottom panels: rates at which responses were emitted (as a percentage of response rate after saline administration). Abscissae: cocaine dose in mg/kg (log scale). The doses of rimcazole, SH 3–24, and SH 3–28 provided in the keys are in μmol/kg. Each point represents the effect in at least five rats.

with cocaine immediately before testing ($F_{2,88}=11.211$, $P<0.001$), or when administered 30 min before cocaine and testing (data not shown; $F_{2,86}=15.484$, $P<0.001$). The changes in the dose effects of cocaine are shown as dose-dependent shifts to the left in the cocaine dose–effect curve (Fig. 3, Table 2). These shifts to the left in the cocaine dose–effect curve were obtained at doses of GBR 12909 that were inactive or minimally active when administered alone.

3.2. Cocaine discrimination

As in previous studies, all subjects readily acquired the cocaine discrimination. After cocaine injection during the baseline conditions, subjects responded almost exclusively on the cocaine-appropriate key, with 99.7 ± 0.13% of the responses on the cocaine-appropriate key. After vehicle injection only 0.98 ± 1.08% of the responses were emitted on the cocaine-appropriate key. During testing with different doses of cocaine, there was a dose-related increase in the percentage of cocaine-appropriate responses as dose was increased from 3 to 29 μmol/kg (Fig. 4, top panel, filled circles). Cocaine produced a significant effect of dose, with an ED_{50} value for its discriminative-stimulus effects of 12.0 μmol/kg (Table 1). Cocaine also produced a significant effect of dose on rates of responding at the 29-μg/kg dose (Fig. 4, bottom panel, filled circles). The dopamine uptake inhibitor, GBR 12909, produced a dose-related increase in cocaine-appropriate responding as dose was increased from 11 to 33 μmol/kg (Fig. 5, top panel, triangles). GBR 12909 was approximately half as potent as cocaine, with an ED_{50} value of 20.6 μmol/kg (Table 1).

Neither rimcazole nor its analogues produced a level of drug-appropriate responding that significantly exceeded the level obtained after vehicle injections at any of the doses tested (Fig. 4, open symbols, top panel). The lack of substitution of the rimcazole and its analogues was obtained over a range of doses from the low doses, that had no effects on response rates, to those that virtually eliminated responding (Fig. 4, open symbols, bottom panel).

Coadministration of rimcazole produced complex changes in the discriminative-stimulus effects of cocaine (Fig. 5). At 14 μmol/kg, there was a leftward shift in the cocaine dose–effect curve, with the cocaine ED_{50} value decreased from 12.0 (cocaine with vehicle) to 5.44 (cocaine with rimcazole). However, the relative potency assessment (RP = 0.67) indicated that this approximate 1.5-fold leftward shift was not significant (Table 3). At the 18-μmol/kg dose of rimcazole, the effects of cocaine were again not significantly altered. The ED_{50} value at this dose was 9.21 μmol/kg, and the relative potency (RP = 0.72) indicated an approximate 1.4-fold leftward shift that was not significant.

As can be seen in Fig. 5 (lower left panel), rimcazole attenuated the stimulant effects of cocaine on response rates. A two-way ANOVA indicated a significant effect of rimcazole pretreatment on the effects of cocaine ($F(2,59)=8.046$, $P<0.001$). The dose of cocaine that increased response rates when administered alone did not do so when administered with rimcazole.

Table 3
Effects of interactions of rimcazole and its analogues, and GBR 12909 on the discriminative-stimulus effects of cocaine

Treatment	ED_{50} value in μmol/kg (95% CL)	Relative potency (95% CL)	% Subjects with full substitution for cocaine
Cocaine alone	12.0 (9.35–15.7)	–	100
Cocaine with 14 μmol/kg rimcazole	5.44 (4.38–6.74)	0.672 (0.376–1.10)	83.3 ($P=1.0$)
Cocaine with 18 μmol/kg rimcazole	9.21 (3.80–22.3)	0.720 (0.369–1.35)	83.3 ($P=1.0$)
Cocaine with 5.7 μmol/kg SH 3–24	12.6 (5.80–27.3)	0.926 (0.516–1.67)	66.7 ($P=0.45$)
Cocaine with 11 μmol/kg SH 3–24	8.22 (3.92–17.2)	0.664 (0.358–1.17)	66.7 ($P=0.45$)
Cocaine with 5.8 μmol/kg SH 3–28	7.09 (5.06–9.94)	0.574 (0.369–0.859)	100 ($P=1.0$)
Cocaine with 11 μmol/kg SH 3–28	10.5 (4.23–36.8)	0.816 (0.448–1.47)	83.3 ($P=1.0$)
Cocaine with 19 μmol/kg SH 3–28	NS[a]	0.741[b]	40.0 ($P=0.035$)
Cocaine with 5.7 μmol/kg GBR 12909	4.82 (2.94–7.88)	0.875 (0.462–1.61)	100 ($P=1.0$)
Cocaine with 11 μmol/kg GBR 12909	3.58 (2.20–5.84)	0.655 (0.343–1.15)	100 ($P=1.0$)

[a] Nonsignificant linear regression.
[b] Relative potency value is an estimate due to a significant deviation from parallel.

Neither dose of SH 3–24 significantly altered the discriminative-stimulus effects of cocaine (Fig. 5). The ED_{50} values of 12.6 and 8.22 μmol/kg obtained with combinations of cocaine and 5.7 and 11 μmol/kg SH 3–24, respectively, did not differ significantly from that for cocaine alone (Table 3). The corresponding relative potency estimates of 0.93 and 0.66, respectively, were also not significant (Table 3). As with rimcazole, SH 3–24 attenuated the cocaine-induced stimulation of response rates (Fig. 6, bottom middle panel). A two-way ANOVA indicated a significant effect of pretreatment with SH 3–24 on the stimulation of response rates produced by cocaine ($F(2,57)=4.409$, $P=0.017$).

In contrast to the other compounds, SH 3–28 significantly attenuated the discriminative-stimulus effects of cocaine. This alteration was evident at the 19 μmol/kg dose, and was characterized by a significant change in the slope of the cocaine dose–effect curve ($F(1,36)=7.23$, $P=0.011$). In addition, there was a decrease in the effectiveness of the highest dose of cocaine (Fig. 5, right top panel). This

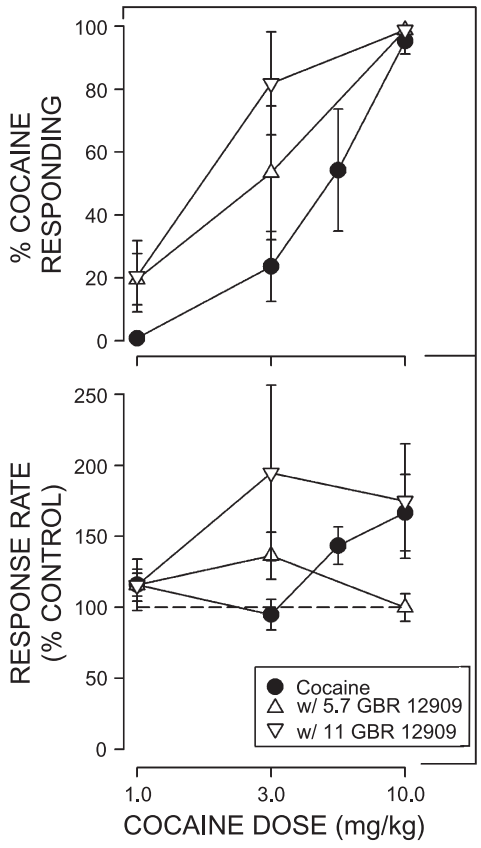

Fig. 6. Changes in the cocaine dose–effect curve for discriminative-stimulus effects produced by pretreatments with GBR 12909. Ordinates for top panels: percentage of responses on the cocaine-appropriate key. Ordinates for the bottom panels: rates at which responses were emitted (as a percentage of response rate after saline administration). Abscissae: cocaine dose in mg/kg (log scale). The doses of GBR 12909 provided in the key are in μmol/kg. Each point represents the effect in at least six rats.

reduction in effect was due to differences in the number of subjects showing a full substitution for cocaine at the highest dose. Table 3 also shows the percentage of subjects for which the training dose of cocaine fully substituted either given alone or with one of the rimcazole analogues, and the results of the Fisher's Exact test. This analysis indicated that 19 μmol/kg SH 3–28 produced a significant reduction in the number of subjects in which 10 mg/kg cocaine fully substituted ($P = 0.035$).

As with the other drugs, SH 3–28 significantly attenuated the effects of cocaine on response rates (Fig. 5, lower right panel). A two-way ANOVA indicated that pretreatment with SH 3–28 significantly attenuated the stimulation in rates of responding produced by cocaine ($F(3,79) = 10.738$, $P < 0.001$).

At doses of 5.7 and 11 μmol/kg, GBR 12909 produced a trend towards a shift to the left in the cocaine dose–effect curve (Fig. 6, top panel). The 12.0-μmol/kg cocaine ED_{50} value was dose-dependently changed to 4.82 and 3.58 when administered with the 5.7 or 11 μmol/kg of GBR 12909, respectively. These ED_{50} values significantly differed from those of cocaine (95% confidence limits did not overlap), however, those shifts were not significant as the 95% confidence limits for the relative potency values were inclusive of 1.0 (Table 3). Higher doses of GBR 12909 were not tested with cocaine because those doses produced significant cocaine-appropriate responding when administered alone (Fig. 4).

4. Discussion

The present study investigated the pharmacology of rimcazole and several of its analogues and compared those actions to those of cocaine and GBR 12909, drugs that have behavioral effects mediated primarily by actions at the dopamine transporter (e.g. Heikkila and Manzino, 1984; Heikkila et al., 1979). Previous studies had shown that rimcazole and its analogues had affinities for the dopamine transporter that were comparable to that of cocaine (Izenwasser et al., 1993; Husbands et al., 1999). Despite their dopamine transporter affinities, the behavioral effects of these drugs were distinct from those of cocaine and GBR 12909. None of the compounds produced a robust stimulation of locomotor activity, indeed only rimcazole significantly increased activity, and this effect was marginal compared to that produced by cocaine and the dopamine uptake inhibitor, GBR 12909. Further, neither rimcazole nor its analogues substituted for cocaine in rats trained to discriminate cocaine from saline whereas GBR 12909 did substitute for cocaine and, as has been reported previously (Holtzman, 2001) potentiated the effects of cocaine.

Menkel et al. (1991) showed that rimcazole blocked the locomotor stimulant effects of cocaine, and Ujike et al. (1996) reported that several σ receptor antagonists blocked the development of sensitization to the locomotor stimulant effects of cocaine. In the study by Menkel et al. (1991), the stimulant effects of cocaine were blocked by rimcazole and another σ receptor antagonist, BMY 14802. These effects were obtained at relatively low doses that did not have pronounced effects of their own on locomotor activity.

Recently, Matsumoto et al. (2001b), extended the study of interactions of cocaine and σ receptor antagonists to rimcazole and several of its analogues, including SH 3–24. A number of other σ receptor ligands have been reported to block the locomotor stimulant and acute toxic (convulsions and lethality) effects of cocaine (McCracken et al., 1999a,b; Matsumoto et al., 2001a,c). In the present study, the attenuation by rimcazole of the effects of cocaine on locomotor activity were replicated, and extended. Effects similar to those described by Menkel et al. (1991) were obtained for locomotor stimulation in mice. In addition, each of the analogues of rimcazole produced a similar attenuation of the stimulant effects of cocaine on locomotor behavior in mice, and the stimulant effects of cocaine on rates of responding by rats in the drug discrimination procedure. For each of these effects, the predominate alter-

ation of the cocaine dose–effect curve was a decrease in the maximal stimulation produced by cocaine. These results were in contrast to those obtained with the dopamine uptake inhibitor GBR 12909, which produced locomotor stimulation when administered alone, and shifted to the left the locomotor stimulant dose effects of cocaine. However, the present changes in the cocaine dose–effect curve produced by rimcazole and its analogs are similar to those described for dopamine D2-like receptor antagonists (Chausmer and Katz, 2001). In that study, the D2-like dopamine antagonists altered the effects of cocaine on locomotor activity predominately by decreasing maximal effects of cocaine. In contrast to the present effects, effective D2-like antagonist doses were those that significantly decreased locomotor activity when administered alone.

SH 3–28 was the only analogue that also blocked the discriminative-stimulus effects of cocaine. However, the blockade of the discriminative effect of cocaine that was obtained with SH 3–28 was not a rightward shift in the cocaine dose–effect curve. Rather, the maximal substitution of cocaine was reduced by co-administration of SH 3–28, and this was a reflection of a decrease in the proportion of subjects that exhibited a full substitution. In addition, there were nonsignificant trends toward leftward shifts in the cocaine dose–effect curve with rimcazole, and indications of increased effects of the lower doses of cocaine when administered in combination with SH 3–28. Matsumoto et al. (2001c) also noted unique features of the interactions of cocaine and σ receptor antagonists. For example, the dose–effect curves for blockade of the effects of cocaine by several σ receptor ligands (in this particular study, conformation-restricted analogues of BD 1008) were apparently very steep with full effects often occurring at the lowest active dose of the σ receptor ligand. Those findings were obtained along with a general absence of parallel shifts to the right in the dose–effect curves for other activities of cocaine blocked by rimcazole analogues (e.g. Matsumoto et al., 2001a). Taken together with the present data, the studies of interactions of sigma receptor ligands and cocaine suggest a complex interaction between these drugs that is, as would be expected, appreciably different from a classic antagonism.

The mechanism by which rimcazole and its analogues blocked the effects of cocaine has not as yet been determined. A number of studies have suggested interactions between σ receptor systems and the behavioral effects of cocaine. At a molecular level, several studies have suggested cross-recognition between σ receptor ligands and cocaine analogues. For example, the photoaffinity label [^{125}I]iodoazidococaine recognizes cocaine binding sites, however when photoactivated, it selectively derivatized a 26-kDa polypeptide with the pharmacology of a σ receptor, rather than the dopamine transporter (Kahoun and Ruoho, 1992). Moreover, low-affinity binding (K_i = 6.7 μM) of cocaine to σ receptors labeled with [^{3}H]haloperidol has been demonstrated (Sharkey et al., 1988). Thus, Matsumoto et al. (2001a) suggested that the interaction between cocaine and σ receptor ligands is a competitive antagonism of effects of cocaine mediated by the σ receptor. However as noted above, the changes in dose–effect curves are not indicative of a competitive antagonism.

Izenwasser et al. (1993) found that several σ receptor ligands inhibited dopamine uptake and had micromolar affinity for the dopamine transporter. Thus, an alternative mechanism for the interaction between σ receptor ligands and cocaine is competition between the drugs at the dopamine transporter. Consistent with this interpretation is a finding by Husbands et al. (1997). In that study, an isothiocyananate analogue of rimcazole had affinity for, and as expected, bound in an irreversible manner to the dopamine transporter. Analysis of the binding of [^{3}H]WIN 35,428 after incubation with this compound suggested a selectivity of the rimcazole analogue for the dopamine transporter in the low-affinity state, suggesting that rimcazole and its analogues bind to the dopamine transporter in a manner that is different from that for cocaine and its analogues. A different mode of interaction with the dopamine transporter may account for the lack of stimulant activity of rimcazole analogues that bind to the dopamine transporter. Further, complex interactions between the dopamine transporter in the high- and low-affinity state may account for the interactions between rimcazole analogues and cocaine. However, these suggestions are currently only speculative.

There are several reports of the modulation of dopaminergic actions by the actions of σ receptor ligands. For example, Wachtel and White (1988) showed that BMY 14802 reversed the suppressant effects of apomorphine on the firing of both A9 and A10 dopamine neurons. Similar effects were not obtained with the established dopamine receptor antagonists, haloperidol and clozapine, suggesting dopamine receptors were not the mechanism underlying the interaction, and that other mechanisms (σ receptors) were necessary. More recently, Gonzalez-Alvear and Werling (1994) established a modulatory effect on dopamine release mediated by σ receptors. Hayashi et al. (2000) showed that σ receptors are linked to intracellular calcium signalling mechanisms. Any of these mechanisms may be responsible for the alteration in the behavioral effects of cocaine.

Most drugs with affinity for the dopamine-transporter mimic the behavioral effects of cocaine (e.g. Witkin et al., 1991). Rimcazole and its analogues may not exhibit a cocaine-like activity because those effects are suppressed by the action of these ligands via σ receptors. Certainly, σ receptor ligands lacking affinity for the dopamine transporter can block at least some of the effects of cocaine (e.g. McCracken et al., 1999b; Romieu et al., 2000, 2002). However, rimcazole, SH 3–24 and SH 3–28 each show comparable affinities at the dopamine transporter and at σ receptors (Husbands et al., 1999), which may explain their unique behavioral profiles. The relative contribution of both dopamine transporter and σ receptor actions might govern

both the degree to which the compounds have cocaine-like actions and the degree to which the compounds can attenuate cocaine-like activity. Depending on the relative binding affinities, different behavioral outcomes may be mediated and potentially predicted. Further investigation into structure–activity relationships at both dopamine transporter and σ receptors has provided additional tools with which to investigate the mechanistic role of these targets in the behavioral actions of this class of rimcazole analogues (Cao et al., 2001; Newman and Kulkarni, 2002). Behavioral evaluation of ligands with varying affinities for the dopamine transporter and σ receptors will provide further evidence regarding the effects of σ receptor antagonists on dopamine transporter-mediated actions. Furthermore, ligands that are structurally related to rimcazole and the analogues described herein that are selective for the dopamine transporter over σ receptors may provide further insight into the role of a low-affinity site/state of the dopamine transporter in the actions of these agents.

The present study extends previous ones indicating an attenuation of the effects of cocaine by rimcazole and its analogues. This effect may be of practical importance in efforts to identify lead compounds for the discovery of pharmacological treatments for cocaine abuse. Despite significant efforts, novel drugs that antagonize the behavioral effects of cocaine in laboratory animal models of drug abuse have not been discovered. In fact, the compounds RTI 112 and GBR 12909 that have been developed preclinically and are currently being evaluated in clinical trials for treatment of cocaine abuse are potent dopamine uptake inhibitors, with known cocaine-like behavioral profiles (Carroll et al., 1999; Rothman and Glowa, 1995). An alternative strategy which involves both the dopamine transporter and the σ receptor, might prove advantageous, as these compounds do not have profound cocaine-like actions nor do they strongly potentiate cocaine's effects. If the present results prove reliable, and can be extended to human subjects, it would appear that rimcazole analogues may have promise in further drug discovery efforts toward the treatment of cocaine-abuse.

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References

Cao, J., Husbands, S.M., Kopajtic, T., Katz, J.L., Newman, A.H., 2001. [3-*cis*-3,5-Dimethyl-1-piperazinyl)alkyl]-bis-(4′-fluorophenyl)amine analogs as novel probes for the dopamine transporter. Bioorg. Med. Chem. Lett. 11, 3169–3173.

Carroll, F.I., Howell, L.L., Kuhar, M.J., 1999. Pharmacotherapies for treatment of cocaine abuse: preclinical aspects. J. Med. Chem. 42, 2721–2736.

Chausmer, A.L., Katz, J.L., 2001. The role of D_2-like dopamine receptors in the locomotor stimulant effects of cocaine in mice. Psychopharmacology 155, 69–77.

Finney, D.J., 1964. Statistical Methods in Biological Assay, 2nd ed. Hafner, New York.

Gonzalez-Alvear, G.M., Werling, L.L., 1994. Regulation of [^{3}H]dopamine release from rat striatal slices by sigma receptor ligands. J. Pharmacol. Exp. Ther. 271, 212–219.

Hayashi, T., Maurice, T., Su, T.-P., 2000. Ca^{2+} signaling via σ_1-receptors: novel regulatory mechanism affecting intracellular Ca^{2+} concentration. J. Pharmacol. Exp. Ther. 293, 788–798.

Heikkila, R.E., Manzino, L., 1984. Behavioral properties of GBR 12909, GBR 13069 and GBR 13098: specific inhibitors of dopamine uptake. Eur. J. Pharmacol. 103, 241–248.

Heikkila, R.E., Cabbat, F.S., Manzino, L., Duvosin, R.C., 1979. Rotational behavior induced by cocaine analogs in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra: dependence upon dopamine uptake inhibition. J. Pharmacol. Exp. Ther. 211, 189–194.

Holtzman, S.G., 1990. Discriminative stimulus effects of drugs: relationship to potential for abuse. In: Adler, M.W., Cowan, A. (Eds.), Modern Methods in Pharmacology. Testing and Evaluation of Drugs: Relationship to Potential for Abuse, vol. 6. Wiley-Liss, New York, pp. 193–210.

Holtzman, S.G., 2001. Differential interaction of GBR 12909, a dopamine uptake inhibitor, with cocaine and methamphetamine in rats discriminating cocaine. Psychopharmacology 155, 180–186.

Husbands, S.M., Izenwasser, S., Loeloff, R.J., Katz, J.L., Bowen, W.D., Vilner, B.J., Newman, A.H., 1997. Irreversible analogs of rimcazole: novel probes for the dopamine transporter. J. Med. Chem. 40, 4340–4346.

Husbands, S.H., Kopajtic, T., Izenwasser, S., Bowen, W.D., Vilner, B.J., Katz, J.L., Newman, A.H., 1999. Structure–activity relationships at the monoamine transporters and σ receptors for a novel series of 9-[3-*cis*-3,5-dimethyl-1-piperazinyl)propyl] carbazole (rimcazole) analogs. J. Med. Chem. 42, 4446–4455.

Izenwasser, S., Newman, A.H., Katz, J.L., 1993. Cocaine and several σ receptor ligands inhibit dopamine uptake in rat caudate–putamen. Eur. J. Pharmacol. 243, 201–205.

Kahoun, J.R., Ruoho, A.E., 1992. (^{125}I) Iodoazidococaine, a photoaffinity label for the haloperidol-sensitive sigma receptor. Proc. Natl. Acad. Sci. U. S. A. 89, 1393–1397.

Karbon, E.W., Abreu, M.E., Erickson, R., Clissold, D., Pontecorvo, M.J., Witkin, J.M., Katz, J.L., Ferkany, J.W., 1992. Biochemical, neurochemical and behavioral profile of aminoalkoxychromones, potent and selective ligands for sigma sites. In: Kamenka, J.-M., Domino, E.F. (Eds.), Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Protection. NPP Books, Ann Arbor, MI, pp. 75–97.

Kelleher, R.T., 1977. Psychomotor stimulants. In: Pradhan, S.N., Dutta, S.N. (Eds.), Drug Abuse: The Clinical and Basic Aspects. C.V. Mosby, St. Louis, pp. 116–147.

Matsumoto, R.R., McCracken, K.A., Friedman, M.J., Pouw, B., de Costa, B.R., 2001a. Conformationally restricted analogs of BD1008 and an antisense oligodeoxynucleotide targeting sigma1 receptors produce anti-cocaine effects in mice. Eur. J. Pharmacol. 419, 163–174.

Matsumoto, R.R., Hewett, K.L., Pouw, B., Husbands, S.M., Cao, J.J., Newman, A.H., 2001b. Rimcazole analogs attenuate the convulsive effects of cocaine: correlation with binding to sigma receptors rather than dopamine transporters. Neuropharmacology 41, 878–886.

Matsumoto, R.R., McCracken, K.A., Pouw, B., Miller, J., Bowen, W.D., Williams, W., de Costa, B.R., 2001c. *N*-alkyl substituted analogs of the

sigma receptor ligand BD1008 and traditional sigma receptor ligands affect cocaine-induced convulsions and lethality in mice. Eur. J. Pharmacol. 411, 261–273.

McCracken, K.A., Bowen, W.D., Matsumoto, R.R., 1999a. Novel sigma receptor ligands attenuate the locomotor stimulatory effects of cocaine. Eur. J. Pharmacol. 365, 35–38.

McCracken, K.A., Bowen, W.D., de Costa, B.R., Matsumoto, R.R., 1999b. Two novel sigma receptor ligands, BD1047 and LR172, attenuate cocaine-induced toxicity and locomotor activity. Eur. J. Pharmacol. 370, 225–232.

Menkel, M., Terry, P., Pontecorvo, M., Katz, J.L., Witkin, J.M., 1991. Selective sigma ligands block stimulant effects of cocaine. Eur. J. Pharmacol. 201, 251–252.

Newman, A.H., Kulkarni, S.S., 2002. Probes for the dopamine transporter: new leads toward a cocaine-abuse therapeutic—a focus on analogues of benztropine and rimcazole. Med. Res. Rev. 22, 1–36.

Romieu, P., Martin-Fardon, R., Maurice, T., 2000. Involvement of the sigma1 receptor in the cocaine-induced conditioned place preference. NeuroReport 11, 2885–2888.

Romieu, P., Phan, V.L., Martin-Fardon, R., Maurice, T., 2002. Involvement of the sigma(1) receptor in cocaine-induced conditioned place preference: possible dependence on dopamine uptake blockade. Neuropsychopharmacology 26, 444–455.

Rothman, R.B., Glowa, J.R., 1995. A review of the effects of dopaminergic agents on human, animals and drug-seeking behavior, and its implications for medication development. Mol. Neurobiol. 11, 1–19.

Sharkey, J., Glen, K.A., Wolfe, S., Kuhar, M.J., 1988. Cocaine binding at sigma receptors. Eur. J. Pharmacol. 149, 171–174.

Snedecor, G.W., Cochran, W.G., 1967. Statistical Methods, sixth ed. University Press, Iowa State University Press, Ames, IO, pp. 135–171.

Stevens, J.P., 1990. Intermediate Statistics. Lawrence Erlbaum Associates, Hillsdale, NJ.

Ujike, H., Kuroda, S., Otsuki, S., 1996. Sigma receptor antagonists block the development of sensitization to cocaine. Eur. J. Pharmacol. 296, 123–128.

Wachtel, S.R., White, F.J., 1988. Electrophysiological effects of BMY 14802, a new potential antipsychotic drug, on midbrain dopamine neurons in the rat: acute and chronic studies. J. Pharmacol. Exp. Ther. 244, 410–416.

Witkin, J.M., Nichols, D.E., Terry, P., Katz, J.L., 1991. Behavioral effects of selective dopaminergic compounds in rats discriminating cocaine injections. J. Pharmacol. Exp. Ther. 257, 706–713.