Pharmacology Biochemistry and Behavior, Vol. 46, pp. 989–993, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

BRIEF COMMUNICATION

Effects of the Dopamine Release Inhibitor, CGS 10746B, on the Locomotor Stimulant and Discriminative Stimulus Effects of Cocaine and Methamphetamine

DAWN FRENCH AND JEFFREY M. WITKIN[1]

Drug Development Group, Psychobiology Section, Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224

Received 25 March 1993

FRENCH, D. AND J. M. WITKIN. *Effects of the dopamine release inhibitor, CGS 10746B, on the locomotor stimulant and discriminative stimulus effects of cocaine and methamphetamine.* PHARMACOL BIOCHEM BEHAV 46(4) 989–993, 1993. – CGS 10746B or 5-(4-methyl-1 piperazinyl)-imadazo[2,1-b]1,3,5]benzothiadiazepine maleate is a clozapine analog that, unlike clozapine, produces decreases in neostriatal dopamine release without changing dopamine metabolism or occupying D_2 receptors. CGS 10746B also blocks neuronal impulse flow. The ability of this atypical antipsychotic candidate to alter the discriminative stimulus effects induced by cocaine or methamphetamine in rats or the stimulation of locomotor activity in mice was evaluated. A range of doses of CGS 10746B was tested against maximally effective doses of the psychomotor stimulants. Although CGS 10746B completely blocked the locomotor stimulant effects of cocaine and methamphetamine, it also decreased spontaneous activity in mice over the same dose range. Rats were trained to discriminate 10 mg/kg cocaine or 1 mg/kg methamphetamine from saline. The discriminative stimulus effects of cocaine or methamphetamine were not blocked by CGS 10746B. Thus, in contrast to other potential atypical antipsychotic compounds (e.g., D_1 receptor antagonists), CGS 10746B does not appear to produce selective blockade of these behavioral effects of psychomotor stimulant compounds.

CGS 10746B Cocaine Methamphetamine Locomotor activity Discriminative stimulus effects Mice Rats

DOPAMINE receptor antagonists have been suggested as potential treatment agents for cocaine abuse [cf. (16,24,28)]. Preclinical and clinical evaluation of these compounds, however, has suggested that they may have limited efficacy, lack behavioral selectivity, and produce side effects (16,24). For example, the dopamine D_2 receptor blocker and antipsychotic, haloperidol, does not block the locomotor stimulant effects of cocaine at doses below those that have marked sedative effects of their own (18). Haloperidol also does not consistently attenuate the discriminative stimulus effects of cocaine (3,9,15,25). Moreover, the neurological side effects of long-term treatment with haloperidol have been well documented [cf. (2)]. In contrast, certain novel potential antipsychotic drugs have been shown to block the locomotor stimulant effects and the discriminative stimulus effects of cocaine (5, 10,18,26).

CGS 10746B or 5-(4-methyl-1 piperazinyl)-imadazo[2,1-b]1,3,5]benzothiadiazepine maleate is a clozapine analog with potential antipsychotic activity (1). Unlike clozapine, behaviorally active doses of CGS 10746B produce decreases in neostriatal dopamine release without changing dopamine metabolism or occupying D_2 receptors (1). CGS 10746B, like

The facilities in which the animals were maintained are fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the studies described were conducted in accordance with the *Guide for Care and Use of Laboratory Animals* provided by the NIH and adopted by NIDA.

[1] Requests for reprints should be addressed to Jeffrey M. Witkin, Ph.D., NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224.

γ-butyrolactone, blocks neuronal impulse flow; however, the mechanisms by which these two compounds produce this effect appear to differ (1,27). CGS 10746B blocks apomorphine-induced climbing at lower doses than those that block apomorphine-induced stereotypies, a profile resembling other atypical antipsychotics (1). This finding and other pharmacological data suggest that CGS 10746B would have a lower extrapyramidal side effect profile than typical antipsychotic drugs (1). Recently, CGS 10746B has been shown to block the conditioned place preference produced by the psychomotor stimulant cathinone [cited in (6)], suggesting that this compound attenuates the reinforcing effects of psychomotor stimulant drugs.

We evaluated CGS 10746B as a potential blocker of the locomotor stimulant and discriminative stimulus effects of cocaine. Since cocaine produces dopamine release through an impulse-dependent process (4), while the dopamine-releasing effects of the amphetamines are independent of impulse flow (8), we compared the ability of CGS 10746B to block behavioral effects of cocaine and methamphetamine.

METHOD

Locomotor Activity

Experimentally naive, male Swiss Webster (SW) mice (Charles River, Wilmington, MA), between 10 and 12 weeks old (30–40 g), were housed five to a cage in a temperature-controlled vivarium with a 12L : 12D cycle; experiments were conducted during the light phase of the cycle (~1000–1300 h) in a dimly lit room. The mice were injected (IP) with CGS 10746B and placed back in their home cage. Thirty minutes later, the mice were injected (IP) with either cocaine or methamphetamine. After the second injection, the animals were placed in a 40 cm^3 Digiscan activity monitor (Omnitech Electronics, Columbus, OH). The activity levels for each subject were then recorded for 30 min. Each dose of CGS 10746B was evaluated simultaneously in groups (n = 5) of saline and in cocaine- or methamphetamine-treated mice using the same drug solutions. Mice were used only once.

Drug Discrimination

Sixteen adult male Sprague–Dawley rats (Charles River) were used. All were maintained at 350 g and kept in individual cages. The rats were housed using a 12L : 12D cycle, and experiments were conducted during the light phase (~1000 h). Ten rats were trained to discriminate methamphetamine, while six were trained to discriminate cocaine. All animals received other test compounds before receiving CGS 10746B, and no animal was tested with the CGS 10746B compound unless first demonstrating stable discriminative performances.

Experiments were conducted in operant-conditioning test chambers (BRS/LVE, model RTC-022) as described previously (25). Separate groups of rats (n = 5–11) were trained to discriminate 10 mg/kg cocaine or 1 mg/kg methamphetamine from saline. During drug discrimination training, the lights above both levers were illuminated. When given a drug, responses on only one lever produced food (45-mg pellets, BioServe); following saline administration, responses on the opposite lever produced food. The lever correlated with drug administration was counterbalanced across subjects in each group. A 5-min timeout occurred at the beginning of the session; during timeout the chamber was dark and responding had no scheduled consequences. After the timeout period, the house light and lights above both response levers were illuminated, and 20 consecutive responses on the appropriate lever were required for food presentation. Responses on the alternate lever reset the response requirement to 20. Timeout periods of 20 s followed each food delivery, and sessions lasted until 20 food pellets had been presented or 20 min, whichever occurred first.

Test sessions in which responding on either lever produced food were conducted following sessions in which greater than 85% of responses occurred on the injection-appropriate lever both prior to the first food presentation and during the entire training session. CGS 10746B was given 30 min prior to test sessions in which saline was given, to evaluate its effectiveness in producing responding similar to each of the training drugs. Test sessions with the training drugs were also conducted regularly during these experiments to establish control values against which to compare the effects of CGS 10746B. In addition, CGS 10746B was given 30 min prior to the training doses of cocaine or methamphetamine to assess the potential to block the discriminative stimulus effects of these drugs. Typically, injections of CGS 10746B were studied no more than twice/week and only if responding met the 85% criteria during the preceding experimental session.

Drugs

(−)-Cocaine HCl (Mallinckrodt) and (+)-methamphetamine HCl (Sigma Chemical Co.) were dissolved in 0.9% NaCl

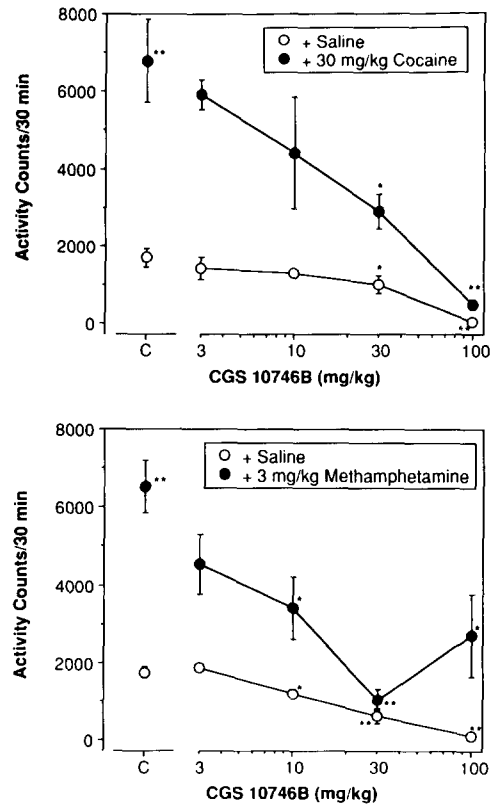

FIG. 1. Effects of CGS 10746B alone (○) or in conjunction with 30 mg/kg cocaine or 3 mg/kg methamphetamine (●) on locomotor activity of mice. Points above C represent effects of saline + saline (○) or saline + cocaine or methamphetamine (●). CGS 10746B was given 30 min prior to either saline, cocaine, or methamphetamine injections. Drug effects are mean ± SEM of at least five mice. $*p < 0.05$; $**p < 0.01$, Dunnett's test compared to respective controls (dose–effect functions compared to data point above C with the same symbol; points above C also compared with each other).

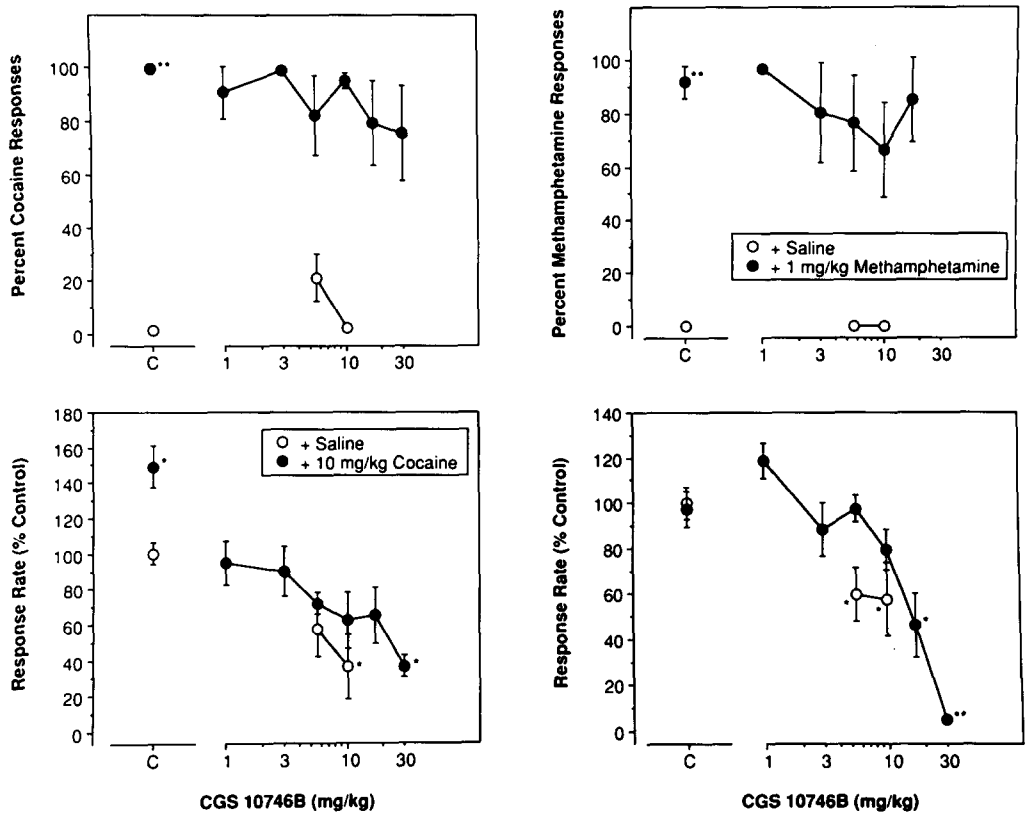

FIG. 2. Effects of CGS 10746B alone (○) or in conjunction 10 mg/kg cocaine or 1 mg/kg methamphetamine (●) in rats discriminating 10 mg/kg cocaine from saline. Top panel: effects on the percentage of cocaine-lever responses; bottom panel: response rates as a percent of control values. Points above C represent effects of saline (○), 10 mg/kg cocaine, or 1 mg/kg methamphetamine alone (●). CGS 10746B was given 30 min prior to either saline, cocaine, or methamphetamine injections. Drug effects were evaluated in at least six rats and are shown as mean ± SEM. In cases where response rates were reduced to 85% of control values or greater, data on the percent cocaine-lever responses were derived from values in at least three rats. $*p < 0.05$; $**p < 0.01$, Dunnett's test compared to respective controls.

(saline). CGS 10746B (Ciby Geigy Corp.) was dissolved in distilled water. Drugs were injected IP in a volume of 1 ml/kg (rats) or 10 ml/kg (mice). Doses are expressed as the drug forms noted above.

Data Analysis

Rates of responding and the percentage of responses on the cocaine- or methamphetamine-appropriate lever were obtained for individual animals. The percentage of responses on the drug-appropriate lever was not considered reliable if the overall rate of responding was reduced by 85% or greater, compared to saline controls, in individual animals; therefore, these data were not used in the calculation of the dose–effect curves for discriminative stimulus effects. Dose–effect functions were analyzed using data from the linear portion of the curves using standard bioassay analysis of variance (ANOVA) techniques (14,22). Individual contrasts were evaluated with two-tailed Dunnett's test.

RESULTS

CGS 10746B did not decrease spontaneous locomotor activity in mice (i.e., in saline-pretreated mice) until doses of 30 mg/kg and higher. At 100 mg/kg, activity was profoundly suppressed (Fig. 1, open circles). Administration of 30 mg/kg cocaine produced fourfold increases in locomotor activity (Fig. 1; compare unconnected circles above C). CGS 10746B attenuated the stimulatory effects of cocaine (Fig. 1, filled circles) but only at doses that also decreased spontaneous activity. ED_{50} values for the suppressant effects of CGS 10746B on spontaneous- and cocaine-stimulated locomotor activity were 24.67 (95% confidence limits: 16.29–37.38) and 17.63 (9.1–34.13), respectively.

Locomotor activity experiments with methamphetamine revealed qualitatively similar effects of CGS 10746B. In these experiments, 3 mg/kg methamphetamine increased locomotor activity to about the same extent as 30 mg/kg cocaine (Fig. 1, filled circle above C). CGS 10746B decreased both spontaneous (Fig. 1, open circles) and methamphetamine-stimulated (Fig. 1, filled circles) locomotor activity at comparable doses. ED_{50} values for the suppressant effects of CGS 10746B on spontaneous and methamphetamine-stimulated locomotor activity were 19.57 (95% confidence limits: 14.30–26.79) and 10.32 (2.04–52.16), respectively.

Group data indicated that CGS 10746B did not significantly block the discriminative stimulus effects of cocaine (Fig. 2, top panel, filled circles). However, CGS 10746B did completely block this behavioral effect of cocaine in one rat at 10, 17, and 30 mg/kg; no blockade was observed in the other animals. When given alone, CGS 10746B did not substi-

tute for cocaine (Fig. 2, unfilled circles). Response rates were reduced somewhat more when CGS 10746B was given alone than when it was given in combination with cocaine (Fig. 2, bottom panel).

CGS 10746B also did not significantly block the discriminative stimulus effects of methamphetamine when evaluated in groups of rats (Fig. 2, top panel, filled circles). However, at doses of 3–17 mg/kg, CGS 10746B partially or completely blocked the discriminative stimulus effects of methamphetamine in one or two rats out of the eight tested. CGS 10746B did not produce methamphetamine-appropriate responses (Fig. 2, unfilled circles). Response rates were decreased somewhat more by CGS 10746B alone than when CGS 10746B was given in conjunction with methamphetamine (Fig. 2, bottom panel).

DISCUSSION

In the present study, CGS 10746B attenuated the increases in locomotor activity produced by cocaine or methamphetamine only at doses that also decreased spontaneous locomotor activity in mice. This nonselective blockade has also been reported with typical antipsychotic agents as well as with other atypical antipsychotic drugs like clozapine (5,18). In contrast, other putative antipsychotic compounds have been shown to block the locomotor stimulant effects of cocaine or amphetamines at doses that do not significantly suppress spontaneous locomotion. These include the dopamine D_1 antagonist SCH 23390, but not the D_2 antagonist (−)-sulpiride (5), and several sigma ligands including BMY 14802, rimcazole, and NPC 16377 (18,26). A cogent explanation for the lack of selectivity of CGS 10746B is not readily apparent. Haloperidol is a more selective inhibitor of apomorphine-induced climbing than CGS 10746B; it blocks climbing at doses at least 83 times lower than those exhibiting sedation (as measured by the traction response), whereas the selectivity of CGS 10746B is only on the order of 2.5 by the same route (1).

CGS 10746B was ineffective in significantly altering the discriminative stimulus of either cocaine or methamphetamine. Nonetheless, behaviorally active doses were studied as revealed by the decreases in rates of responding. Although higher doses of CGS 107846B did block the discriminative stimulus effects of these psychomotor stimulants in individual rats, the effect was not dose dependent. However, there was a tendency for cocaine and methamphetamine to block the rate-suppressant effects of CGS 10746B, an effect of cocaine reported in experiments on schedule-controlled behavior [cf. (23)]. As noted in the Introduction, typical antipsychotics have also not been shown to markedly or consistently block the discriminative stimulus effects of cocaine, but have generally shown good efficacy as blockers of the discriminative stimulus effects of amphetamines [cf. (9,19,20)]. Atypical antipsychotics such as molindone and clozapine have been shown to block the discriminative stimulus effects of *d*-amphetamine (20). In contrast, the sigma ligand NPC 16377, which blocks the locomotor stimulant effects of cocaine, did not block the discriminative stimulus effects of cocaine or methamphetamine (26). Other potential antipsychotic drugs like SCH 23390 (3,5,7,25) or the sigma ligand Dup 734 (10) have been shown to block both behavioral effects in rodents.

While the lack of selectivity of CGS 10746B in the locomotor activity experiments may be partly related to the small separation in the dopamine-blocking vs. the sedative effects of CGS 10746B (1), this does not likely account for the lack of efficacy of CGS 10746B in the drug discrimination experiments where sedative-like effects can be measured separately from the control of behavior by drug injections. Opposing behavioral effects of CGS 10746B also do not appear to be responsible for the blockade by CGS 10746B of the conditioned place preference induced by the psychomotor stimulant cathinone [cited in (6)]. In that experiment, conditioned place preference was blocked by doses of CGS 10746B that did not produce the opposing conditioned place aversion (M. D. Schechter, personal communication).

γ-Butyrolactone has been shown to decrease the outflow of dopamine induced by cocaine, but not *d*-amphetamine, in the nucleus accumbens of freely moving rats (8). This terminal area of the mesolimbic dopamine system has been thought to be involved in the reinforcing effects of stimulant compounds (13) as well the locomotor stimulant (11) and discriminative stimulus effects of these drugs (12). Although CGS 10746B, like γ-butyrolactone, also blocks neuronal impulse flow, CGS 10746B did not selectively block the behavioral effects of cocaine or methamphetamine in the present study. Regardless of the ultimate explanation for these findings, the dopamine release inhibitor CGS 10746B does not, in our hands, appear to be a viable candidate for further consideration as a treatment agent for cocaine or amphetamine abuse.

(While the present article was under review, Schechter et al. [Pharmacol. Biochem. Behav. 44:661–664; 1993] reported that CGS 10746B [20–40 mg/kg] dose-dependently blocked the discriminative stimulus effects of 10 mg/kg cocaine in rats. These results and those reporting blockade of the discriminative stimulus effects of *d*-amphetamine by CGS 10746B (21) are at odds with the present data; we have, at present, no definitive explanation for this discrepancy.)

ACKNOWLEDGEMENTS

We thank Ms. Pam Ranberg and Ms. Stephanie Cornish for secretarial support and Dr. Lovell for help in obtaining donations of CGS 10746B.

REFERENCES

1. Altar, C. A.; Wasley, A. M.; Liebman, J.; Gerhardt, K. H.; Welch, J. J.; Wood, P. L. CGS 10746B: An atypical antipsychotic candidate that selectively decreases dopamine release at behaviorally effective doses. Life Sci. 39:699–705; 1986.
2. Baldessarini, R. J. Drugs and the treatment of psychiatric disorders. In: Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P., eds. Goodman and Gilman's the pharmacological basis of therapeutics. New York: Pergamon Press; 1990:383–435.
3. Barrett, R. L.; Appel, J. B. Effects of stimulation and blockade of dopamine receptor subtypes on the discriminative stimulus properties of cocaine. Psychopharmacology (Berlin) 99:13–16; 1989.
4. Broderick, P. A. In vivo voltammetric studies on release mechanisms for cocaine with γ-butyrolactone. Pharmacol. Biochem. Behav. 40:969–975; 1991.
5. Cabib, S.; Castellano, C.; Cestari, V.; Filibeck, U.; Puglisi-Allegra, S. D1 and D2 receptor antagonists differently affect cocaine-induced locomotor hyperactivity in the mouse. Psychopharmacology (Berlin) 105:335–339; 1991.
6. Calcagnetti, D. J.; Schechter, M. D. Conditioned place aversion following the central administration of a novel dopamine release inhibitor CGS 10746B. Pharmacol. Biochem. Behav. 40:255–295; 1991.
7. Callahan, P. M.; Appel, J. B.; Cunningham, K. A. Dopamine

D_1 and D_2 mediation of the discriminative stimulus properties of d-amphetamine and cocaine. Psychopharmacology (Berlin) 103: 50–55; 1991.
8. Carboni, E.; Imperato, A.; Perezzani, L.; Di Chiara, G. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28:653–661; 1989.
9. Colpaert, F. C.; Niemegeers, C. J. E.; Janssen, P. A. J. Discriminative stimulus properties of cocaine and d-amphetamine, and antagonism by haloperidol: A comparative study. Neuropharmacology 17:937–942; 1978.
10. Cook, L.; McElroy, J. F. and Rohrbach, K. W. DUP 734 differentially antagonizes the locomotor stimulant effects of cocaine, D-amphetamine and apomorphine in mice and rats. Soc. Neurosci. Abstr. 18:548; 1992.
11. Cools, A. R. Basic considerations on the role of concertedly working dopaminergic, GABA-ergic, cholinergic and serotonergic mechanisms within the neostriatum and nucleus accumbens in locomotor activity, stereotyped gnawing, turning and dyskinetic activities. In: Ellinwood, E. H., Jr.; Kilbey, M. M., eds. Cocaine and other stimulants. New York: Plenum Press; 1977:97–141.
12. Cunningham, K. A.; Dworkin, S. I.; Smith, J. E. Neurobiology of cocaine: Reinforcing and stimulus effects. In: Lakoski, J. M.; Galloway, M. P.; White, F. J., eds. Cocaine: Pharmacology, physiology, and clinical strategies. Boca Raton, FL: CRC Press; 1991:91–113.
13. Di Chiari, G.; Imperato, A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85: 5274–5278; 1988.
14. Finney, D. J. Statistical methods in biological assay, 2nd ed. New York: Hafner; 1964.
15. Jarbe, T. U. C. Cocaine as a discriminative cue in rats: Interaction with neuroleptics and other drugs. Psychopharmacology (Berlin) 59:183–187; 1978.
16. Johanson, C-E.; Fischman, M. M. The pharmacology of cocaine related to its abuse. Pharmacol. Rev. 41:3–52; 1989.
17. Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The dopamine hypothesis of the reinforcing effects of cocaine. Trends Neurosci. 14:299–302; 1991.
18. Menkel, M.; Terry, P.; Pontecorvo, M.; Katz, J. L.; Witkin, J. M. Selective sigma ligands block stimulant effects of cocaine. Eur. J. Pharmacol. 201:251–252; 1991.
19. Nielsen, E. B.; Randrup, K.; Andersen, P. H. Amphetamine discrimination: Effects of dopamine receptor agonists. Eur. J. Pharmacol. 160:253–262; 1989.
20. Nielsen, E. B.; Jepsen, S. A. Antagonism of the amphetamine cue by both classical and atypical antipsychotic drugs. Eur. J. Pharmacol. 11:167–176; 1985.
21. Schechter, M. D.; Boja, J. W. CGS 10746B is able to attenuate the effects of amphetamine: Further evidence for dopaminergic mediation. Pharmacol. Biochem. Behav. 36:189–192; 1988.
22. Snedecor, G. W.; Cochran, W. G. Statistical methods, 6th ed. Ames, IA: Iowa State University Press; 1967:135–171.
23. Spealman, R. D. Antagonism of behavioral effects of cocaine by selective dopamine receptor blockers. Psychopharmacology (Berlin) 101:142–145; 1990.
24. Witkin, J. M. Pharmacotherapy of cocaine abuse: Preclinical development. Neurosci. Biobehav. Rev. (in press).
25. Witkin, J. M.; Nichols, D. E.; Terry, P.; Katz, J. L. Behavioral effects of selective dopaminergic compounds in rats discriminating cocaine injections. J. Pharmacol. Exp. Ther. 257:706–713; 1991.
26. Witkin, J. M.; Terry, P.; Menkel, M.; Hickey, J.; Pontecorvo, M.; Ferkany, J.; Katz, J. L. Effects of the selective sigma ligand, NPC 16377, on behavioral and toxic effects of cocaine. J. Pharmacol. Exp. Ther. (in press).
27. Wood, P. L.; Altar, C. A.; Kim, H. S. Presynaptic inhibition of nigrostriatal dopamine release in the mouse: Lack of cross tolerance between apomorphine, GBL, and CGS 10746B. Life Sci. 42: 1503–1506; 1988.
28. Woolverton, W. L.; Johnson, K. M. Neurobiology of cocaine abuse. Trends Pharmacol. Sci. 13:193–200; 1992.