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Cocaine Discrimination is Attenuated by Isradipine and CGS 10746B

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SCHECHTER, M. D. *Cocaine discrimination is attenuated by isradipine and CGS 10746B.* PHARMACOL BIOCHEM BEHAV 44(3) 661–664, 1993. – The discriminative stimulus properties of cocaine are thought to be mediated by dopaminergic mechanisms that may be modulated by calcium ion influx and/or interact with 5-hydroxytryptamine$_3$ (5-HT$_3$) receptors. To test these possibilities, rats were trained to discriminate between the stimulus properties of 10.0 mg/kg cocaine and its vehicle in a two-lever, food-motivated operant task. Once trained, rats showed a dose-related decrease in discriminative performance when tested with lower cocaine doses. An analysis of the dose-response curve indicated an ED_{50} value of 3.04 mg/kg. Pretreatment with the presynaptic dopamine release-inhibiting agent CGS 10746B (20–40 mg/kg) resulted in a dose-related decrease in cocaine discrimination with the highest dose significantly attenuating cocaine discrimination. Pretreatment with 10–30 mg/kg isradipine, a calcium channel blocker, also resulted in a dose-related decrease in cocaine discriminative performance. In contrast to these positive results, pretreatment with the 5-HT$_3$ receptor antagonist MDL 72222 (3.5–7.0 mg/kg), or the same doses of ibogaine, did not significantly affect cocaine discrimination. The results suggest that cocaine controls differential responding in a discriminative stimulus task by mechanisms that involve presynaptic release of dopamine, which may be regulated by neuronal calcium influx through L-type calcium channels.

Drug discrimination Cocaine Dopamine CGS 10746B Isradipine MDL 72222
Calcium channels Behavior Rat

THE ability of compounds selective for D_1 and D_2 dopamine receptors to substitute (generalize) for cocaine in a discriminative stimulus paradigm has been reported. Employing the cocaine-discriminating rat has allowed for the observation that the D_1 agonist SK&F38393 produced incomplete generalization (3,4,25), whereas the D_2 agonist quinpirole (3,4) produced complete generalization. Regarding selective dopamine antagonists, Witkin et al. (25) and Callahan et al. (4) found partial or complete antagonism, respectively, using the D_1 antagonist SCH23390, whereas the D_2-selective antagonist haloperidol did (4) or did not (25) block discrimination of 10 mg/kg cocaine. These results suggest that the dopaminergic (subreceptor) mediation of the cocaine-induced discriminative cue is complex and may necessitate stimulation of more than one receptor (10).

In addition to dopaminergically active drugs, the calcium channel blocker nimodipine (5), as well as the serotonergic antagonists ICS-205,930 and MDL 72222 (16), have been used as pretreatment in animals trained to discriminate cocaine. The purpose of the present experiment was to expand upon these cited studies by pretreating cocaine-discriminating rats with a dopaminergic antagonist that appears to act by a unique mechanism, that is, by selectively decreasing the release of presynaptic dopamine (1,2). In recent studies, this novel agent, CGS 10746B, has been shown to decrease the discrimination of the psychostimulants cathinone (18) and amphetamine (20). In addition, pretreatment with the calcium channel blocker isradipine was investigated as this compound has been shown to prevent cocaine-induced dopamine release (14), as well as antagonizing its reinforcing properties (15). The 5-hydroxytryptamine$_3$ (5-HT$_3$) antagonist MDL 72222 has been reported to block psychostimulant-induced locomotor increases (8,17), as well as cocaine-induced conditioned place preference (22), and the indolealkylamine ibogaine has been claimed to be effective in reversing cocaine addiction (12). The effect of pretreatment with these two agents was also tested in cocaine-discriminating rats.

METHOD

Subjects

Ten male Sprague-Dawley rats were purchased from Zivic-Miller (Allison Park, PA) and weighed 220–225 g upon arrival. After 1 week of isolation, animals were assigned to individual hanging wire cages and kept in a vivarium facility maintained on a 12 L : 12 D cycle (light 0600–1800 h) at a constant temperature and humidity. They were given water ad lib in their home cage, as well as daily rationing of commercial rat chow so as to maintain them at approximately 85–90% of their free-feeding weights as determined by a growth chart from the supplier. This procedure facilitated motivation of operant performance for food reward.

Apparatus

Twelve standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN), each containing two levers

situated 7 cm apart and 7 cm above a metal grid floor, were used as the experimental space. Equidistant between the levers was placed a food receptacle that received delivery of 45-mg Noyes food pellets. Each operant chamber was enclosed in a sound-attenuating cubicle with an exhaust fan and a 9-W houselight. Solid-state programming equipment (Med Associates, E. Fairfield, VT) was located in an adjacent room and was used to control and record discrimination sessions.

Drug Discrimination

This behavioral paradigm allowed rats to be trained to discriminate between cocaine and its (saline) vehicle; the methodology can be found in detail in a previous publication (19). Briefly, the discrimination procedure consisted of training rats to press one of two identical levers 15 min following IP administration of vehicle administered in a volume of 1 ml/kg. The initial fixed ratio 1 (FR 1) reinforcement schedule was gradually increased over 7 days until an FR 10 reinforcement schedule was attained. This procedure was then repeated with presses upon the other lever being reinforced 15 min following IP administration of a similar volume of vehicle containing 10.0 mg/ml cocaine HCl (NIDA) with the weight calculated as salt; the initial FR 1 reinforcement requirement was gradually incremented over 3 days to an FR 10. Once lever-pressing behavior was established on both levers, a biweekly repeating injection schedule was employed—V,D,D,V,V; D,V,V,D,D—where V = (saline) vehicle and D = 10.0 mg/kg cocaine. For each animal, the choice on any given day was considered correct if the first lever to accumulate 10 presses was state appropriate, that is, the cocaine lever after cocaine administration and the vehicle lever after vehicle administration. Training was continued until all rats achieved the training criterion of 8 correct first lever selections in 10 consecutive sessions.

Dose-response and Antagonist Experiments

Once the training criterion was achieved, rats were tested with doses of cocaine different from their 10-mg/kg training dose. This allowed a dose-response relationship to be observed. During this series, and in the subsequent antagonist series of experiments (below), cocaine-vehicle discrimination training was maintained by administering either the training dose of cocaine or its vehicle every second day. During these maintenance sessions, the lever pressed 10 times first was considered the selected lever and rats were allowed to continue pressing the state-appropriate lever 400 times to receive (on the FR 10 schedule) 40 additional reinforcements. In intervening test sessions, rats were placed into the experimental chamber 15 min following administration of either a lower cocaine dose (dose-response) or pretreatment with a possible antagonist and allowed to lever press until 10 presses were accumulated on either lever. Animals were then immediately removed without receiving reinforcement to preclude reinforcement/training in a drug state different than that to which they were trained. Each cocaine dose-response test was administered in a random order on two occasions with each test session preceded by one vehicle and one cocaine maintenance session, according to the following schedule: C-DR_1-V-DR_1-C-DR_2-V-DR_2-etc., where C = the training dose of cocaine, V = vehicle, and DR_1 = one other dose of cocaine and DR_2 = another cocaine dose.

Each of the putative antagonists (source), that is, CGS 10746B (Ciba-Geigy, Summit, NJ), isradipine (Sandoz Pharmaceuticals, East Hanover, NJ), MDL 72222 (Research Biochemicals, Inc., Natick, MA), and ibogaine (NIDA), was dissolved in saline and administered prior to cocaine injection at a time that, according to the scientific literature, allowed for maximum central efficacy. Thus, CGS 10746B in doses previously shown to block discrimination of amphetamine (20) was administered IP 15 min prior to injection of cocaine and animals were tested in extinction 15 min after the second injection. Similarly, isradipine was administered IP and 60 min later cocaine was injected 15 min before testing. MDL 72222 was administered 30 min prior to cocaine and, therefore, 45 min before testing, and ibogaine was injected 15 min before cocaine injection. In those test sessions in which pretreatment with the putative antagonist appeared to produce an attenuation of cocaine discrimination, the antagonist was administered in a minimum of three ascending doses until a dose (in combination with cocaine) was reached that produced behavioral disruption, that is, one or more of rats did not respond on either lever in 60 min. When disruption occurred, a second antagonist + cocaine trial at that antagonist dose was not conducted and the results do not reflect the single (disrupted) trial. In all other antagonist trials, rats (n = 10) made a lever selection within 60 min.

Data analysis

Results of discriminative responding were expressed in terms of both quantal and quantitative measurements. During all dose-response and antagonist test sessions, rats were allowed to respond until 10 responses were accumulated on either of the two levers without receiving reinforcement. The lever pressed 10 times first was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for cocaine was the quantal measure of discrimination. In addition, the total number of responses on both levers made before 10 responses were accumulated on either lever constituted the quantitative measurement, that is, the number of responses on the cocaine lever divided by the total responses made on both levers times 100. The quantal data for the dose-response results, as well as the antagonist pretreatment sessions, were analyzed by the method of Litchfield and Wilcoxon (11), which employs probits vs. log-dose effects and yields both ED_{50} and ID_{50} values as generated by a computerized program (23). The utility of the quantitative measurement resides in the ability to analyze them with parametric statistics as previously discussed (21). Response rates, that is, time required to complete the FR 10 ratio, were not specifically measured.

RESULTS

The results indicate that rats can readily learn to discriminate between 10 mg/kg cocaine and its saline vehicle. The first session of 10 consecutive sessions in which at least 8 correct lever selections were made was reached in a mean of 5.6 (±3.2) sessions. Thus, all rats were capable of discriminating between cocaine and vehicle in 20 sessions (10 sessions with cocaine and 10 with vehicle). The dose-response relationship, in experiments conducted immediately after discriminative training, indicates that 10 mg/kg cocaine, as it was used in maintenance sessions, produced 95% of all first lever selections upon the cocaine lever (Fig. 1). In maintenance sessions with vehicle (0.0 mg/kg), the cocaine lever was first pressed 10 times in 10% of all sessions as the vehicle lever was selected 90% of all sessions after vehicle administration. Decreasing doses of cocaine resulted in decreasing quantal and quantita-

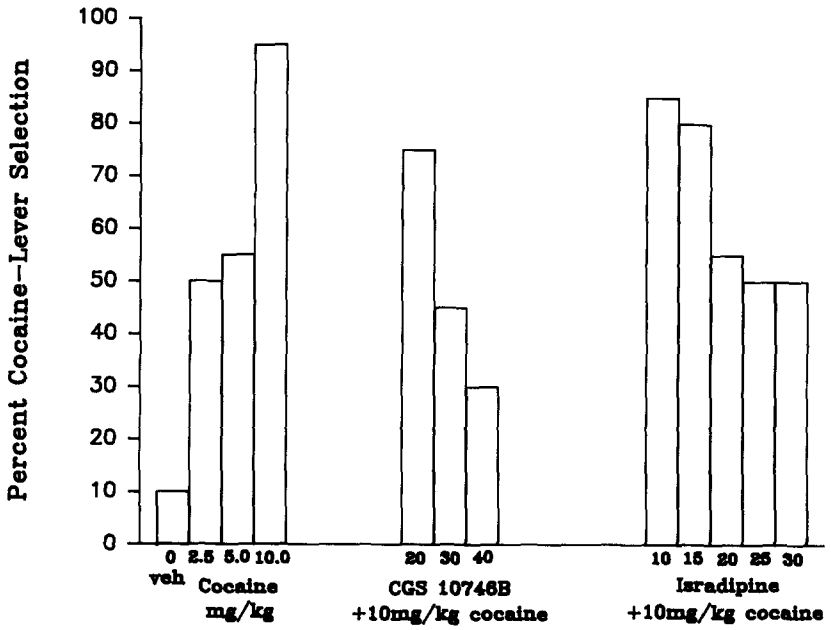

FIG. 1. Percent cocaine lever selection after IP administration of (0–10.0 mg/kg) cocaine and effect of pretreatment with (20–40 mg/kg) CGS 10746B or (10–30 mg/kg) isradipine upon discrimination of 10 mg/kg cocaine.

tive discriminative performance and the quantal ED_{50} value was calculated (11) to be 3.04 mg/kg.

The results of pretreatment with 20–40 mg/kg CGS 10746B prior to the training dose of cocaine indicated that this compound attenuated cocaine discrimination in a dose-responsive manner (Fig. 1). The highest CGS 10746B dose (40 mg/kg) reduced the quantal cocaine discrimination from 90% (during interspersed maintenance trials) to 30% and the quantitative measurement in cocaine maintenance trials (82.4 ± 3.9) was significantly ($t = 13.56$, $p < 0.01$) greater than the quantitative measurement after two trials with CGS 10746B pretreatment (39.0 ± 2.5). The computer-generated (23) ID_{50} (the dose of CGS 10746B necessary to reduce cocaine discrimination to 50%) was shown to be 28.98 mg/kg. Isradipine (in 5.0-mg/kg incremental doses between 10 and 30 mg/kg) produced an increasing attenuation of cocaine discrimination, although the two highest doses (25 and 30 mg/kg) did not reduce the cocaine discriminative performance below 50% (Fig. 1). Nevertheless, a comparison of the quantitative measurement after 30 mg/kg isradipine pretreatment with cocaine was significantly lower ($t = 2.685$, $p < 0.05$) than after cocaine maintenance discriminative performance.

In contrast to the positive results obtained with both CGS 10746B and isradipine, pretreatment with two doses of MDL 72222 (3.5 and 7.0 mg/kg) allowed for 100 and 80% quantal responding, respectively, with the latter combination producing response latency (i.e., delaying FR 10 ratio lever selection) for an average of 24 min (with a range of 0–60 min). Likewise, pretreatment with 10 and 20 mg/kg ibogaine allowed for 100 and 80% cocaine quantal responding, respectively, with the highest dose of ibogaine, in combination with 10 mg/kg cocaine, eliciting an average delay of 20.5 min to complete the test session (range: 0–40 min). Thus, higher doses of both MDL 72222 and ibogaine were precluded from use as pretreatments.

DISCUSSION

Both D_1 (SCH23390) and D_2 (spiperone and haloperidol) have been employed to investigate their actions on the discriminative properties of cocaine (3,4,25). The present study used an alternative approach to postsynaptic dopaminergic blockade by employing CGS 10746B, an agent that reduces the release of dopamine yet possesses no binding affinity to postsynaptic dopaminergic receptors (1,2). The present finding that CGS 10746B is able to attenuate cocaine discriminative performance lends evidence to the dopaminergic mediation of this behavioral effect. Previous work from this laboratory has shown that 20–30 mg/kg CGS 10746B, when administered prior to either amphetamine (20) or cathinone (18), was able to inhibit discrimination of these psychostimulants. In the present study, a higher dose, viz. 40 mg/kg CGS 10746B, was necessary to produce significant attenuation of cocaine discriminative stimuli. This need for a higher dose may be caused by cocaine's more complex mechanism of action, that is, involving both dopaminergic release and reuptake inhibition (10).

The calcium channel blocker isradipine was also shown to attenuate cocaine discrimination. A recent report (15), investigating the effects of this compound (aka PN 200-110) on cocaine reinforcing properties in a conditioned place preference paradigm, indicated that in nonsedating doses (0.16–2.5 mg/kg) isradipine was able to inhibit 10 mg/kg cocaine-induced place preference. Much higher doses of isradipine were required to attenuate cocaine discrimination in the present study. The present finding would suggest that the dopamine effects of cocaine may partially be controlled by voltage-sensitive (L-type) calcium channels. Previous neurochemical studies have indicated that dopamine released in the striatum by cocaine is, in fact, mediated by calcium channel mechanisms (7,9).

5-HT_3 antagonists have previously been reported to prevent

the hyperlocomotor effects of an acute injection of cocaine (6,24), as well as block cocaine-induced conditioned place preference (22). This evidence suggests that the antagonism of 5-HT_3 receptors may, in turn, attenuate the ability of cocaine to stimulate dopamine release. In the present study, MDL 72222 did not affect cocaine discrimination. This confirms a recent report (16). Lastly, pretreatment with ibogaine at two doses did not affect cocaine discrimination. Recent claims indicate that ibogaine may have therapeutic efficacy in the treatment of human cocaine addicts (12,13).

Together, these results suggest that cocaine controls differential responding in a discriminative stimulus task by a mechanism that involves presynaptic release of dopamine and that this may, in turn, be regulated by calcium influx through L-type calcium channels. This information may help to allow a better understanding of cocaine's euphoria-producing actions in animals and suggest avenues of drug development to block cocaine's actions in human abusers.

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REFERENCES

1. Altar, C. A.; Wesley, A. M.; Boyar, W. C.; Liebman, J.; Gerhardt, S.; Wood, P. L. Dopamine neurochemical profile of atypical antipsychotics resembles that of D_1 antagonists. Naunyn Schmiedbergs Arch. Pharmacol. 338:162–168; 1988.
2. Altar, C. A.; Wesley, A. M.; Liebman, J.; Gerhardt, S.; Kim, 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.
3. Barrett, R. L.; Appel, J. B. Effects of stimulation and blockade of dopamine receptor subtypes on the discriminative stimulus properties of cocaine. Psychopharmacology (Berl.) 99:13–16; 1989.
4. 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 (Berl.) 103: 50–55; 1991.
5. Callahan, P. M.; Cunningham, K. A. The discriminative stimulus properties of cocaine: Effects of BAY K 8644 and nimodipine. Eur. J. Pharmacol. 186:143–147; 1990.
6. Costall, B.; Domeney, A. M.; Naylor, R. J.; Tyers, M. D. Effects of the 5-HT_3 receptor antagonist, GR38032F, on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain. Br. J. Pharmacol. 92:881–894; 1987.
7. Di Chiara, 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.
8. Hamon, M. 5-HT_3 receptors are critically involved in 5-HT/DA interactions. Biol. Psychiatry 29:107S; 1991.
9. Hurd, Y.; Ungerstedt, U. Cocaine: An in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum. Synapse 3:48–54; 1989.
10. Kleven, M. S.; Anthony, E. W.; Woolverton, W. L. Pharmacological characterization of the discriminative stimulus effects of cocaine in rhesus monkeys. J. Pharmacol. Exp. Ther. 254:312–317; 1990.
11. Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 69:99–106; 1949.
12. Lotsof, H. Rapid method for interrupting the cocaine and amphetamine abuse syndrome. U.S. Patent 4,587,243, 1986.
13. Maisonneuve, I. M.; Keller, R. W., Jr.; Glick, S. D. Interactions between ibogaine, a potential antiaddictive agent, and morphine: An in vivo microdialysis study. Eur. J. Pharmacol. 199:35–42; 1991.
14. Pani, L.; Kuzmin, A.; Diana, M.; De Montis, G.; Gessa, G. L.; Rossetti, Z. L. Calcium receptor antagonists modify cocaine effects in the central nervous system differently. Eur. J. Pharmacol. 190:217–221; 1990.
15. Pani, L.; Kuzman, A.; Martellotta, M. C.; Gessa, G. L.; Fratta, W. The calcium antagonist PN 200-110 inhibits the reinforcing properties of cocaine. Brain. Res. Bull. 26:445–447; 1991.
16. Paris, J. M.; Cunningham, K. A. Serotonin 5-HT_3 antagonists do not alter the discriminative stimulus properties of cocaine. Psychopharmacology (Berl.) 104:475–478; 1991.
17. Reith, M. E. A. 5-HT_3 receptor antagonists attenuate cocaine-induced locomotion in mice. Eur. J. Pharmacol. 186:327–330; 1990.
18. Schechter, M. D. Effect of altering dopamine or serotonin neurotransmitters upon cathinone discrimination. Pharmacol. Biochem. Behav. 41:37–41; 1991.
19. Schechter, M. D. Rats bred for differences in preference to cocaine: Other behavioral measurements. Pharmacol. Biochem. Behav. 43:1015–1021; 1992.
20. Schechter, M. D.; Boja, J. W. CGS 10746B is able to attenuate the effects of amphetamine: Further evidence for dopaminergic mediation. Pharmacol. Biochem. Behav. 30:189–192; 1988.
21. Stolerman, I. P.; D'Mello, G. D. Role of training conditions in discrimination of central nervous system stimulants by rats. Psychopharmacology (Berl.) 73:295–303; 1981.
22. Suzuki, T.; Shiozaki, Y.; Masukawa, Y.; Misawa, M. 5-HT_3 receptor antagonists block cocaine- and methamphetamine-induced placed preference. Jpn. J. Pharmacol. (in press).
23. Tallarida, R. J.; Murray, R. B. Manual of pharmacologic calculations with computer programs. 2nd ed. New York: Springer-Verlag; 1986.
24. Van der Hoek, G. A.; Cooper, S. J. Evidence that ondansetron, a selective 5-HT_3 antagonist, reduces cocaine's psychomotor stimulant effects in the rat. Psychopharmacology (Berl.) 101:S59; 1990.
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.