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RAPID COMMUNICATION

Facilitating Role of *m*-Chlorophenylbiguanide in a Cocaine Discrimination

LEE KOETZNER, JOHN MASTROPAOLO AND STEPHEN I. DEUTSCH[1]

Psychiatry Service 116A, Department of Veterans Affairs Medical Center, 50 Irving Street NW, Washington, DC 20422

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KOETZNER, L., J. MASTROPAOLO AND S. I. DEUTSCH. *Facilitating role of* m-*chlorophenylbiguanide in a cocaine discrimination.* PHARMACOL BIOCHEM BEHAV **52**(4) 815–818, 1995. – To examine the interaction between serotonergic systems and the discriminative stimulus effects of cocaine, the effects of the selective 5-HT_3 agonist *m*CPBG were examined in 46 Sprague–Dawley rats trained to discriminate 10 mg/kg cocaine from saline. *m*CPBG substitution tests showed partial substitution for the cocaine stimulus. In interaction tests, the combination of *m*CPBG and low doses of cocaine resulted in increased cocaine lever selection. *m*CPBG treatment reduced response rates, but not below a 50% criterion. These results point toward a limited modulatory role of serotonergic systems in the cocaine discriminative stimulus.

mCPBG Cocaine Drug discrimination Rats

SEVERAL studies have reported a link between 5-HT_3 receptors and cocaine's behavioral effects. In one study, the 5-HT_3 antagonists zacopride, MDL 72,222, and tropisetron all antagonized cocaine-induced hyperactivity; the effect of zacopride was virtually abolished by treatment with the serotonin depleter *p*-chlorophenylalanine (9). Using an in vivo microdialysis procedure, it was found that a dose of zacopride, which virtually abolished cocaine-induced hyperactivity, produced a substantial decrease in the amount of dopamine recovered after cocaine administration (6). There is also specific evidence that serotonin interacts with cocaine's discriminative stimulus. For example, a low dose of the serotonin uptake inhibitor fluoxetine shifts the cocaine dose-response function to the left in rats trained to discriminate cocaine from saline (2). In addition, 5-HT_3 antagonists can partially antagonize the ethanol discriminative stimulus in pigeons trained to discriminate ethanol from water. Zacopride partially blocked and tropisetron and MDL 72,222 completely blocked the ethanol cue (3).

Unlike cocaine-induced hyperactivity, however, there is a reported failure of 5-HT_3 antagonists to block the discriminative stimulus effects of cocaine. In one study, rats were trained to discriminate 10 mg/kg cocaine from saline; since a dose of 5 mg/kg engendered 100% drug-appropriate responding, it was used for antagonism tests. Neither tropisetron nor MDL 72,222 significantly attenuated drug-appropriate responding at any dose tested (7). Using similar training conditions, another study found ondansetron unable to antagonize a 10 mg/kg dose of cocaine (5). These results may indicate a phenomenon generally applicable to dopamine uptake inhibitors, as MDL 72,222 has failed to modify the cathinone discriminative stimulus (8).

There are several possible reasons why the interaction between 5-HT_3 receptors and cocaine did not extend to these drug discrimination studies. For example, it is possible that cocaine discrimination is mediated by a brain region that is not affected by 5-HT_3 receptors (1); however, the nucleus accumbens, which appears to mediate the cocaine cue (10) does respond to 5-HT_3 receptor manipulations (11). Another possibility is that the administration of 5-HT_3 antagonists reduces dopamine concentrations in the accumbens, but not below the amount necessary to create an identifiable cocaine stimulus. For example, a dose of zacopride that abolished cocaine-induced hyperactivity and that had no effect on its own re-

[1] To whom requests for reprints should be addressed.

duced the cocaine-induced dopamine overflow from 338% to 246% of baseline (6). This overflow of 246% may still present a cocaine stimulus.

If there is any interaction between 5-HT_3 receptor activity and the cocaine discriminative stimulus, it may be more readily apparent with a 5-HT_3 agonist. The present experiment used rats trained to discriminate 10 mg/kg cocaine from saline. The selective 5-HT_3 agonist meta-chlorophenylbiguanide [*m*CPBG; (5)] was administered alone and in combination with varying doses of cocaine during substitution tests.

METHOD

Subjects

Forty-six adult male Sprague-Dawley rats (Zivic Miller, Zelinople, PA), experimentally naive and not exposed to psychotropic drugs outside of this study, served as subjects. All animals were maintained at 85% of their free-feeding weight by food earned during sessions and measured postsession feeding; estimated free-feeding weight was adjusted for age. Water was available continuously in the home cages. The colony room was maintained at 22 ± 2°C; lights were on from 0700–1900 h. Not all subjects completed every test session; however, doses were tested twice whenever possible, and most data points were determined twice for each subject.

Apparatus

Training and test sessions were conducted in standard operant test chambers (Med Associates, East Fairfield, VT); responses were recorded, and contingencies were programmed using Med-PC state notation software (Med Associates). Each chamber was fitted with response levers located on either side of a food pellet delivery cup. A stimulus light with a 2.54 cm diffuser lens was located above each lever; these lights signaled the availability of a schedule of reinforcement. Lever lights were extinguished and a house light was illuminated for 0.5 s whenever food was delivered. Reinforcers were 45 mg machined food pellets (Noyes, Lancaster, PA).

Procedure

After magazine training, subjects were exposed to fixed ratio (FR) schedules of food reinforcement. At all times during the study, the schedules for the two levers were independent: presses on one lever had no effect on the schedule set for the other lever. The ratio requirement was increased to a terminal schedule of FR10 (i.e., 10 appropriate responses produced a food pellet, regardless of inappropriate responding ocurring during the ratio).

After subjects reliably completed the FR requirement on each lever, drug discrimination training began. Each subject was administered 10 mg/kg cocaine or 0.9% saline and placed in the test chamber. For each animal, one lever (right or left) was designated the drug-appropriate lever; the choice of drug lever was balanced across subjects to control for bias. After 10 min, the stimulus lights were illuminated; food presentations were available and the lights remained illuminated until 200 injection-appropriate responses were emitted or 20 min passed, whichever occurred first. Responses on the other lever (i.e., injection-inappropriate) were recorded but had no programmed consequence. Drug and vehicle training sessions were scheduled in a double alternation sequence.

The criterion for discriminative control was set at 85% (i.e., two or less injection-inappropriate responses during the first ratio component). When this criterion was met for 4 days, substitution tests began. These tests were scheduled between drug training sessions and saline training sessions, so that 6 days would include two of each type of session (for example, cocaine-saline-test-saline-cocaine-test). If a subject did not meet the 85% criterion for the four training sessions preceding the substitution test, no session was conducted for that subject on the test day. Test sessions were terminated when the first ratio was completed.

Drugs

Cocaine (MW 339.82; Research Biochemicals Incorporated) was dissolved in saline; meta-chlorophenylbiguanide (*m*CPBG; MW 248.12; Research Biochemicals Incorporated) was dissolved in warm distilled water. All doses were calcu-

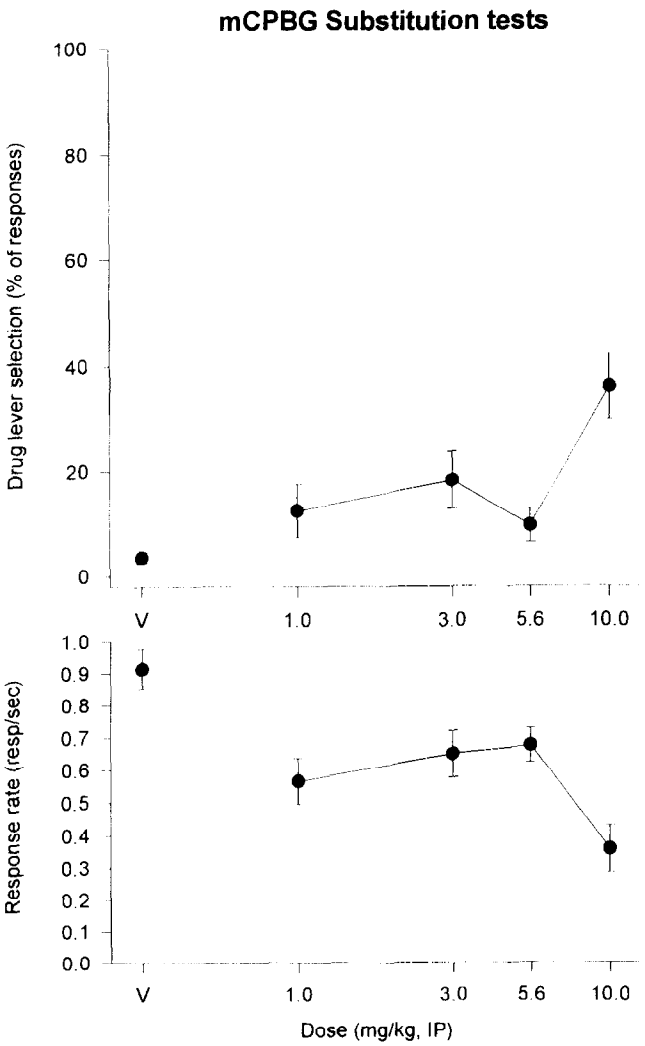

FIG. 1. Upper panel: proportion of drug-appropriate responding following varying doses of *m*CPBG (administered IP, 15 min before the beginning of the test session). Lower panel: rate of responding during test sessions. Bars represent standard errors of the mean in both panels.

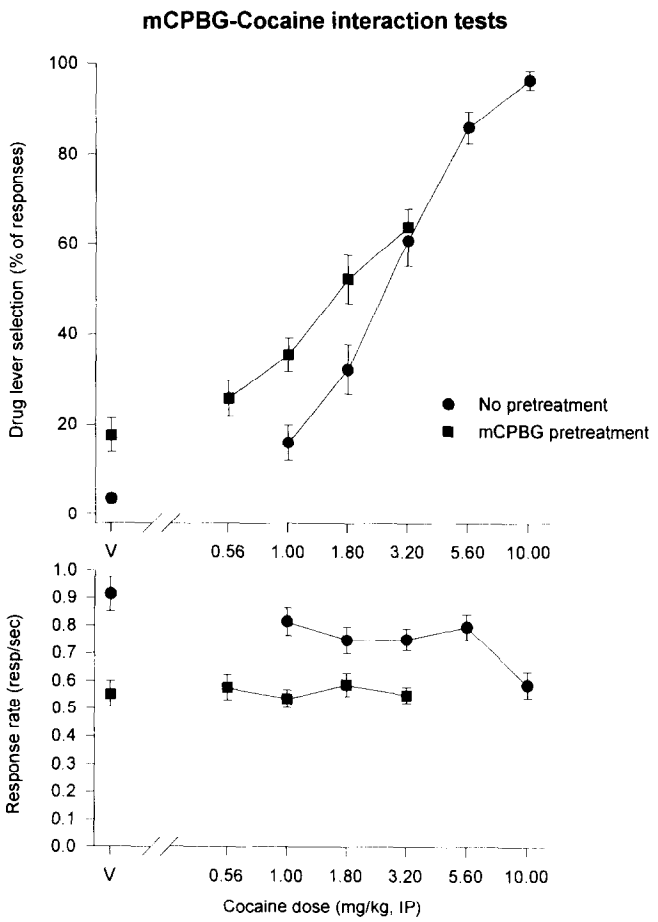

FIG. 2. Upper panel: proportion of drug-appropriate responding following varying doses of cocaine (IP, administered 15 min before the beginning of the test session) with and without a pretreatment of *m*CPBG (5.6 mg/kg, administered IP, 8 min before cocaine injections). Lower panel: rate of responding during test sessions. Bars represent standard errors of the mean in both panels.

lated from the hydrochloride salt weights and administered intraperitoneally in a volume of 1 ml/kg body weight. Drug solutions were prepared fresh daily. Different doses of a drug treatment were given in random order.

Data Analysis

Drug lever selection was calculated as the mean ratio of drug lever responses to total responses (both counted up to the time that the first reinforcer would have been delivered) and averaged across subjects. Response rate was calculated as the total number of responses divided by the time elapsed between the beginning of the lights on period and the time that the first reinforcer would have been delivered; if the animal did not emit 10 responses on one lever or the other, the data were discarded. A reduction in the response rate to below 50% of the rate calculated for the vehicle control was taken as evidence for disruption of the discrimination during test sessions. Differences between conditions were assessed using one-way or two-way (with interaction) analysis of variance; post hoc analyses used Scheffé tests. The alpha criterion was set at 0.05 for hypothesis tests.

RESULTS

As can be seen in Fig. 1, responding during test sessions following the training injections (i.e., saline and 10 mg/kg cocaine) reflected clear stimulus control. As expected, varying doses of cocaine substituted for the training dose in a dose-dependent manner, $F(6, 515) = 43.67$, $p < 0.001$. Response rates only decreased after the highest dose of cocaine.

*m*CPBG administration occasioned drug-appropriate responding, with the greatest proportion of drug-appropriate responding after the highest dose, $F(3, 135) = 4.74$, $p < 0.004$. However, the greatest proportion of drug lever selection was only 36% (see Fig. 2). *m*CPBG treatment reduced response rate, $F(3, 135) = 4.73$, $p < 0.004$, but post hoc analysis revealed that rates differed only after the 10.0 mg/kg treatment. A dose of 5.6 mg/kg *m*CPBG was chosen for interaction tests as a compromise between stimulus properties and rate-decreasing effects.

Interaction tests with both *m*CPBG and cocaine administered before the session resulted in a slight potentiation of the cocaine discriminative stimulus (two-way ANOVA for replicated doses; cocaine dose term $F(2, 456) = 39.01$, $p < 0.001$; *m*CPBG dose term $F(1, 456) = 15.02$, $p < 0.001$; interaction term $F(2, 456) = 2.59$, $p < 0.09$). Drug lever selection following doses of 0.56, 1.0 and 1.8 mg/kg cocaine was increased (see Fig. 1). At a dose of 3.2 mg/kg cocaine, however, this effect was diminished. Response rates did not differ from those obtained after *m*CPBG/vehicle administration. mCPBG tended to reduce response rate, but never below the 50% criterion; Scheffé analysis showed that rates only differed between vehicle and *m*CPBG/vehicle treatments.

DISCUSSION

Administration of the 5-HT_3 agonist *m*CPBG resulted in 36% drug-appropriate responding at the highest dose tested. After 10 mg/kg, response rates were decreased in those subjects completing a ratio requirement; many subjects did not fulfill the requirement. Accordingly, a lower dose (5.6 mg/kg) was tested in combination with varying doses of cocaine. At low doses, subjects selected the drug lever more often after the *m*CPBG pretreatment. However, this potentiation of the cocaine stimulus dissipated as the dose of cocaine increased, pointing to a limited modulatory role of 5-HT_3 agonists.

In light of the present findings, the previously reported failure of 5-HT_3 antagonists to attenuate cocaine's stimulus properties does not represent a lack of interaction between these two receptor systems with regard to stimulus properties. Instead, it suggests that 5-HT_3 can play a limited modulatory role. As such, 5-HT_3 receptor antagonists may still have utility as adjuvants, which in combination with other drugs, can attenuate cocaine's stimulus properties, although alone they will not be therapeutic for cocaine abuse. In addition, a 5-HT_3 agonist could provide a weak agonist for the cocaine cue, with potential therapeutic value. Regardless of the clinical effect, however, research with these drugs highlights the specificity of drug discrimination as a tool for investigating the effects of drugs like cocaine.

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