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BRIEF COMMUNICATION

Clozapine Increases Breaking Points on a Progressive-Ratio Schedule Reinforced by Intravenous Cocaine

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LOH, E. A., T. FITCH, G. VICKERS AND D. C. S. ROBERTS. *Clozapine increases breaking points on a progressive-ratio schedule reinforced by intravenous cocaine.* PHARMACOL BIOCHEM BEHAV **42**(3) 559–562, 1992.—The effect of the atypical neuroleptic clozapine on cocaine self-administration reinforced on a progressive-ratio schedule in rats was examined. The rat's first response on a lever each day produced an IV infusion of cocaine (0.6 mg/injection) after which the requirements of the schedule escalated with each infusion until the frequency of responding on the lever fell below a criterion level. The final ratio completed was defined as the breaking point. Doses of 5 and 20 mg/kg clozapine produced either no effect or a nonspecific disruption in responding. Rats pretreated with 10 mg/kg clozapine responded to significantly higher breaking points, indicating an increased motivation to self-administer cocaine.

Self-administration Cocaine Clozapine Dopamine Serotonin Progressive ratio Atypical neuroleptic

SELF-ADMINISTRATION of psychomotor stimulant drugs is highly sensitive to manipulations of central dopamine (DA) systems. 6-Hydroxydopamine lesions of the mesolimbic DA system have been shown to attenuate the reinforcing effects of cocaine and amphetamine (10,15,17). Similarly, DA antagonists such as chlorpromazine, trifluoperazine, haloperidol, perphenazine, α-flupenthixol, sulpiride, metaclopramide, thioridazine, and SCH 23390 have all been found to increase the rate of cocaine or amphetamine self-administration (7,20). Typically, such increases in rate of drug intake have been interpreted to reflect a compensatory response to the blockade of psychostimulant reinforcement (25). Clozapine, an atypical neuroleptic, has been shown to decrease the rate of cocaine self-administration in rats on a fixed-ratio (FR) 1 schedule of reinforcement, in contrast to all other DA antagonists tested to date (20). These data are difficult to interpret, however, since a decrease in the rate of drug intake has been thought to indicate either an increase or a decrease in the drug's reinforcing efficacy (23,25).

In the present experiment, we reexamined the effect of clozapine on cocaine self-administration through the use of a progressive-ratio (PR) schedule. On a PR schedule, the ratio requirement for successive reinforcements is increased until responding falls below a criterion level. Rather than using rate of responding as a dependent variable, the final ratio (or breaking point) is used as an indicator of the animal's motivation to self-administer cocaine (9,18).

METHOD

Subjects and Training

Male Wistar rats (Charles River Farms, Quebec) weighing 275–300 g at the start of the experiment served as subjects. One week following their arrival from the supplier, rats were food deprived for 24 h, then trained to press a lever for food reinforcement on an FR 1 schedule. Thereafter, food was available ad lib. Once trained to press a lever for food reinforcement, each rat was implanted with a chronically indwelling silastic jugular cannula that exited from the animal's back at the mid scapular region (16). Following cannulation, rats were singly housed in 50 × 50 × 40 cm (h) operant testing chambers. The cannula was mounted on a counterbalanced swivel apparatus that allowed the animal free movement in the operant chamber.

Rats were allowed access to a response lever for a 5-h period each day on an FR 1 schedule. Each lever response acti-

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vated a syringe driver delivering 0.12 ml saline solution containing 5.0 mg/ml cocaine HCL over a 5-s period, resulting in a dose of 0.6 mg/ml (or approximately 1.8 mg/kg). Previous studies have demonstrated that this dose falls in the middle of the dose-response curve so that both increases and decreases in breaking points may be observed (18). Concurrent with the start of the injection, a stimulus light was activated that signalled a 20-s postinfusion time-out period, during which time responses had no programmed consequence. Rats were first trained on an FR 1 schedule until they developed a consistent pattern of cocaine intake characterized by interinfusion intervals of 7–10 min. After 3 days of consistent cocaine intake, the PR reinforcement schedule was imposed.

On the PR schedule, after each cocaine infusion the number of responses required to obtain the next infusion was increased following the exponential progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, and 603. At the start of each daily session, the PR schedule was reset to 1.

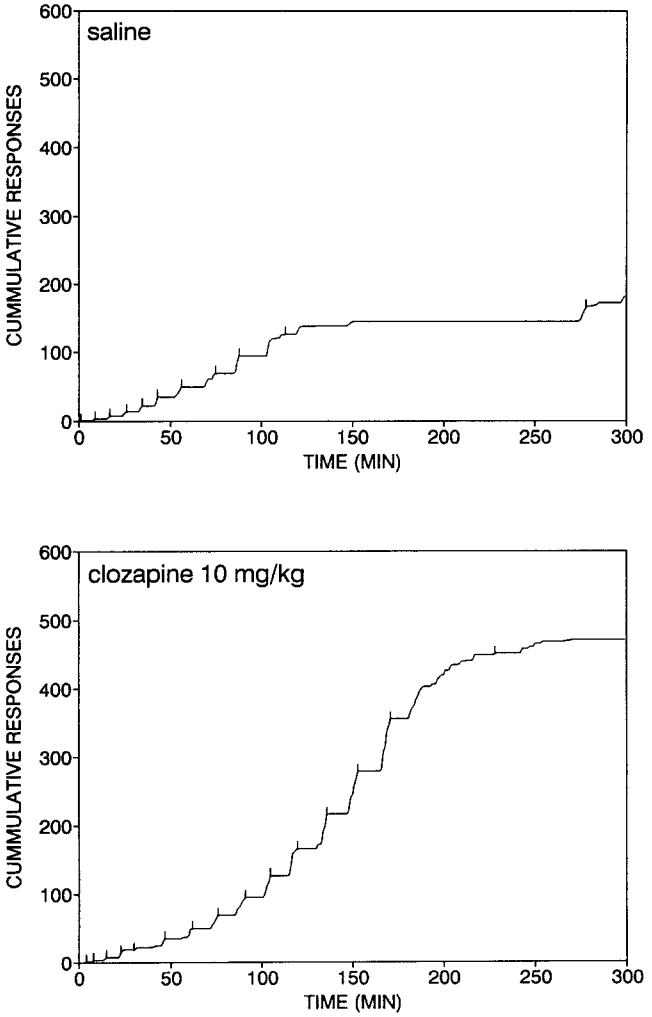

FIG. 1. Example of the effect of clozapine on cocaine self-administration behavior reinforced on a PR schedule. Lines represent cumulative records from consecutive daily 5-h test sessions. The time of each cocaine injection is indicated by an upward deflection of the pen. The effect of saline (top) and 10 mg/kg clozapine (bottom) pretreatment is illustrated.

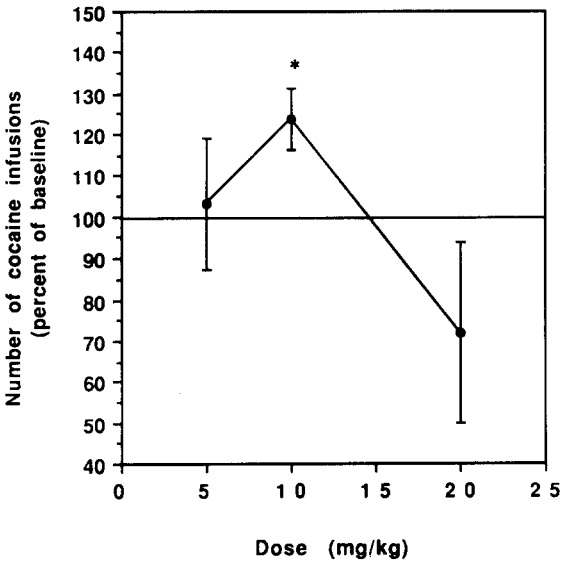

FIG. 2. Effect of clozapine on cocaine self-administration behavior reinforced under a PR schedule. Each point represents the mean breaking point (±SEM) expressed as a percentage of the preceding baseline test days. $*p < 0.01$.

The ordinal value of the final ratio completed was used as the dependent measure and was called the breaking point. If a rat did not obtain an infusion for a 1-h period, it was considered that it had reached its breaking point. For example, if a rat obtained its fifteenth infusion of cocaine by completing a ratio of 95 responses, but failed to make 118 responses for the next infusion (in the succeeding 1-h period), the final ratio would be 95 and the breaking point 15. See Roberts and Richardson (19) for a complete description of the implementation of the PR schedule.

Drug Testing

Once a rat had developed a consistent baseline level of responding (3 consecutive days of testing with a range of ≤3 breaking points), it was given a saline injection 1 h prior to testing. On the following day, the rat was given an injection of one of three doses of clozapine (5, 10, or 20 mg/kg, IP) 1 h prior to testing. Baseline responding was reestablished in each animal before the next dose of clozapine was tested. The order of dose presentation was varied. Cannula blockage or mechanical problems with the swivel apparatus prevented all animals from receiving all dosages.

Drugs

Clozapine (Sandoz, Quebec) was first dissolved in a small amount of 0.1 N HCl and distilled water; the pH was then increased to 6.0 by adding NaOH. For the control injections, the pH of the saline solution was reduced to 6.0 by adding a small amount of HCl.

RESULTS

Results are based on 14 animals. Six rats received two doses of clozapine while the remaining eight were tested at only one dose. Figure 1 (top) illustrates a typical baseline response

profile of a rat self-administering cocaine on a PR schedule. Note that the rat obtained cocaine at regularly spaced intervals.

Individual animals demonstrated stable daily breaking points within 5–7 days of testing on the PR schedule. There was a considerable degree of variability in breaking point between animals. Breaking points during baseline testing ranged from 5–23 (overall mean = 13.0, SEM ± 4.26). Consequently, changes in the number of infusions obtained on drug and postdrug days were calculated as percentages of the mean breaking point attained on the preceding 3 baseline days.

Pairwise *t*-tests indicated that the 10-mg/kg dose of clozapine significantly increased breaking points, ($t = 4.42$, $p < 0.01$) (Fig. 2). The mean breaking point at this dose during baseline testing days was 12.1, which represents an average of approximately 50 responses for the final infusion. The mean breaking point increased to 14.9 (95 responses for the last cocaine infusion) when rats were pretreated with 10 mg/kg clozapine. This elevation in breaking point represented an average increase of 90% in the number of lever responses performed to receive the final infusion of drug.

The 5- and 20-mg/kg doses of clozapine had no significant effect. However, the 20 mg/kg dose produced a large decrease in the breaking point in some animals that appeared to be a nonspecific disruption of responding as the response profile indicated that these rats exhibited long pauses in responding between cocaine infusions or behavioral stereotypy.

DISCUSSION

We have previously shown that clozapine produces a dose-dependent decline in the rate at which rats self-administer cocaine on an FR 1 reinforcement schedule (20). Since changes in rate of drug self-administration are difficult to interpret, the present experiment reexamined the effects of clozapine pretreatment on cocaine self-administration using a PR reinforcement schedule. The present results demonstrate that pretreatment with clozapine (10 mg/kg) produces a significant increase in breaking points on a PR schedule reinforced by intravenous cocaine and potentiates cocaine reinforcement. It would appear that animals have an increased motivation to self-administer cocaine following pretreatment with this particular dose of clozapine.

These results are somewhat surprising. Previous experiments have shown that dopamine antagonists decrease the reinforcing effects of cocaine on the PR schedule (18). Moreover, there is a substantial literature that indicates that DA receptor stimulation is essential for cocaine self-administration. Since clozapine is known to be a DA antagonist, we expected that it would diminish, rather than augment, the reinforcing efficacy of cocaine in the PR paradigm.

It is not clear why clozapine differs in many respects from typical neuroleptics. Clozapine does not produce extrapyramidal side effects typical of most neuroleptics and potentiates rather than antagonizes amphetamine-induced stereotypy (21). Other studies have indicated that clozapine exhibits a selective affinity for limbic structures (1,2), which may explain its unique effects. Clozapine has a greater affinity for the D_1 relative to the D_2 dopamine receptor site (3,4); however, this would not seem to account for the present results since both D_1 and D_2 dopamine antagonists attenuate the reinforcing effects of cocaine (7,24). Recently, a novel D_4 receptor that is expressed in both humans and rats has been cloned (22). Clozapine has been shown to have an extremely high affinity for this novel receptor. Further research is required to determine the role of the D_4 receptor in the mediation of cocaine reinforcement.

Beyond its effects as a DA receptor blocker, clozapine has been found to be a potent 5-hydroxytryptamine$_2$ (5-HT$_2$) antagonist (11). While most neuroleptics have some effects at this receptor site (6,13), clozapine appears to affect the 5-HT system differentially. Injections of clozapine will produce a substantial reduction in 5-HT$_2$ receptor binding, which is not the case for the classical neuroleptic haloperidol (8,12). This may play a role in the mediation of its unique profile in cocaine self-administration. Previous studies have indicated that, following neurotoxic depletions of forebrain 5-HT, rats will respond to significantly higher breaking points on a PR schedule for cocaine reinforcement (9). Conversely, the administration of the indirect 5-HT agonist fluoxetine reduces breaking points (14). Thus, it would appear that 5-HT systems exert a regulatory influence on cocaine reinforcement and it may be the 5-HT antagonistic properties of clozapine that contribute to the increased motivation to self-administer cocaine.

Clozapine also has anticholinergic properties (5). Wilson and Schuster (23) demonstrated that atropine will increase the rate of cocaine self-administration in rhesus monkeys; however, atropine does not appear to alter the rate of cocaine self-administration in rats. Further, it is also possible that clozapine might have a peripheral effect such as inhibiting the metabolism and clearance of cocaine from the blood.

Regardless of the explanation, these results indicate that clozapine does not block the reinforcing effects of cocaine and a neuroleptic profile is not a sufficient condition for a drug to attenuate cocaine reinforcement. It is possible that clozapine possesses nonneuroleptic properties that eclipse the antireinforcement effects it shares with other antipsychotics.

It is unlikely that clozapine would be an effective treatment for cocaine abuse. On the contrary, clozapine may actually exacerbate the addictive potential of cocaine and caution should be exercised when administering clozapine to individuals at risk for psychomotor stimulant abuse.

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