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GR38032F, a Serotonin 5-HT_3 Antagonist, Fails to Alter Cocaine Self-Administration in Rats

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PELTIER, R. AND S. SCHENK. *GR38032F, a serotonin 5-HT_3 antagonist, fails to alter cocaine self-administration in rats.* PHARMACOL BIOCHEM BEHAV **39**(1) 133–136, 1991.—Recent data have supported a role for serotonin (5-HT) in the self-administration of cocaine by laboratory rats. More specifically, it has been suggested that 5-HT_3 receptor antagonists may be useful in the treatment of drug abuse. To assess this possibility, we compared the effects of the 5-HT_3 antagonist, GR38032F, with the dopamine D_2 receptor blocker, haloperidol, on the intravenous self-administration of cocaine (0.5 mg/kg/infusion) in rats. The serotonin antagonist (0.01, 0.1 or 1.0 mg/kg, IP) failed to alter self-administration (0.5 mg/kg/infusion). In contrast, haloperidol (0.125 mg/kg, IP) increased responding for cocaine (0.5 mg/kg/infusion), and shifted the dose-response curve for cocaine self-administration to the right. These data fail to support a role for the serotonin 5-HT_3 receptor system in the reinforcing properties of this psychostimulant. Rather, the 5-HT_1 or 5-HT_2 receptors may be the critical subtype.

Cocaine Serotonin Self-administration 5-HT_3 receptors

SEROTONIN (5-HT) receptors are currently divided into three main subtypes, including a 5-HT_1, 5-HT_2 and a 5-HT_3 site (3). The 5-HT_3 receptor corresponds to that originally described by Gaddum and Picarelli (9) as the M-receptor.

The recent development of specific 5-HT_3 agonists and antagonists has allowed for the systematic evaluation of its physiological and behavioral functions. High levels of 5-HT_3 receptor binding are associated with dopamine (DA)-containing areas (13). For example, 5-HT_3 receptors are concentrated in the nucleus accumbens, olfactory tubercle, and striatum. Based on the location of these receptors, it has been suggested that this specific 5-HT_3 system may serve to modulate central DA activity.

Converging lines of evidence support this hypothesis. For example, administration of 5-HT_3 agonists increased dopamine levels in the striatum (1,2) and nucleus accumbens (11). In addition, the release of mesolimbic DA induced by systemic injections of ethanol, nicotine, morphine, or haloperidol was reduced by pretreatment with 5-HT_3 antagonists (4).

Behavioral studies have also implied a modulatory role for the 5-HT_3 system on central DA activity. The systemic injection of 5-HT_3 antagonists inhibited the hyperactivity induced by the administration of DiMe-C7 (10), and intra-accumbens injections of dopamine or amphetamine (8). In addition, the administration of 5-HT_3 antagonists attenuated morphine and nicotine-induced place preferences (5), which have been attributed to effects on the mesolimbic DA system.

The mesolimbic dopamine system has been implicated in the rewarding properties of cocaine (19). Cocaine also blocks the reuptake of 5-HT, and recent studies have suggested its importance in cocaine's reinforcing effects. Various serotonergic manipulations including dietary L-tryptophan loading (7), administration of the nonspecific agonist, fluoxetine (6) and neurotoxic lesions to various serotonergic systems (14) have been shown to alter the reward impact of cocaine as measured by their effects on intravenous self-administration. It is possible that the effect of the various serotonergic manipulations on cocaine self-administration is mediated via the proposed modulatory role played by the 5-HT_3 system on central dopaminergic activity. If so, the suggestion that the newly developed 5-HT_3 antagonists may be effective in the treatment of drug abuse (17,18) may be warranted. As a preliminary test of this hypothesis, we examined the effects of the selective 5-HT_3 antagonist, GR38032F, on the intravenous self-administration of cocaine by laboratory rats.

METHOD

Subjects

Subjects were male Sprague-Dawley albino rats (Harlan; Houston, TX) weighing approximately 350–400 grams at the beginning of the experiment. The rats were individually housed in standard plastic rodent cages in a temperature-controlled

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(23.0 ± 1.0°C) colony room under a 12-h light/dark schedule (lights on at 0800 h). Food and water were available ad lib.

Surgery

Animals were pretreated with atropine sulfate (1.4 mg/kg; IP) and anesthetized with separate injections of ketamine (60 mg/kg; SC) and sodium pentobarbital (20 mg/kg; IP). Each rat was implanted with a chronic indwelling jugular cannula. The cannula was fixed to the right jugular vein and the silastic line, fit with a piece of 22-ga stainless steel tubing, was passed subcutaneously to the exposed skull surface. It was fixed to the rat's skull using dental acrylic anchored with stainless steel jewelers screws. Each cannula line was flushed daily with a 0.05 ml solution of saline, penicillin G sodium (250,000 units/ml) and heparin (1.25 units/ml) to verify their patency. Following a 7-day recovery period, a series of 2-hour daily self-administration tests were conducted.

Apparatus

Self-administration testing took place in operant boxes (Med Associates) enclosed in sound-attenuating chambers. Rats were connected to an infusion pump (Razel Model A, with a 20 ml syringe and a 1 rpm motor), through a suspended swivel that allowed free movement. The operant chambers contained 2 levers, with a stimulus light above each lever. Depression of one lever, deemed the "active" lever, triggered a 12-second infusion of 0.1 ml of cocaine HCl and activated a lighting cue. Depression of the other lever, deemed the "inactive" lever, had no consequence. The operant boxes were interfaced with two IBM-type microcomputers. Drug delivery and data acquisition were controlled by the OPN software package [modification of (16)]. Responses were recorded at 30-min intervals across each 2-h session.

Screening

The daily test sessions consisted of 2 hours access to the operant chambers. On each day, an initial "priming" infusion was experimenter delivered. Thereafter, depression of the active lever resulted in an infusion of cocaine (0.5 mg/kg/infusion, measured as the salt). During the first 8 days of the procedure, the acquisition of self-administration was examined. Rats were included for subsequent testing if the number of reinforced responses per session exceeded 20, and did not vary by more than 10% on days 7 and 8. An additional criterion of greater than 50% active lever responding was employed.

Testing

The next phase of this experiment consisted of a series of three-day testing periods. The data from the first day were not included in any subsequent data analysis. The second day provided baseline data. On the third day, a pretreatment was administered. In one group of rats (n = 8) the specific 5-HT_3 antagonist, GR38032F (0.01, 0.1, or 1.0 mg/kg, IP; measured as the base) was administered 30 min prior to the self-administration tests. The doses of GR38032F were counterbalanced within subjects. These doses and time of administration were chosen based on previous work showing a dose-dependent suppression of intra-accumbens amphetamine (8) or intra-ventral tegmental DiMe-C7 (10) induced motor activity when GR38032F (0.01, 0.1 or 1.0 mg/kg) was administered, 30 and 40 min respectively, prior to the behavioral tests.

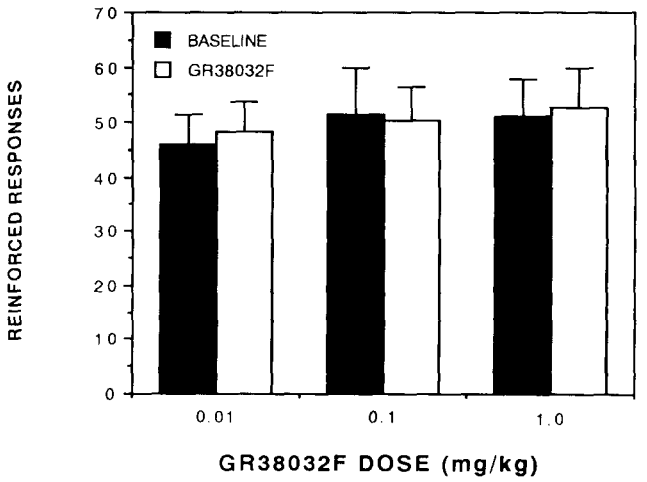

FIG. 1. Effects of various doses GR38032F on the mean number of responses (± SEM) in a 2-hour self-administration test. There were no significant differences as a function of treatment.

Before all of the GR38032F data could be collected, one of the cannulae developed a leak. Therefore, only the data from the seven rats that completed the entire testing regimen were used in subsequent analyses.

A second group of rats (n = 5) acquired self-administration of cocaine (0.5 mg/kg/infusion) using the same procedure as described above. For these rats, dose-response curves for cocaine self-administration with or without haloperidol (0.125 mg/kg, measured as the base), were then conducted. The rats were pretreated with haloperidol 2 h prior to the test session.

The first series of test days examined the effect of haloperidol on the self-administration of 0.5 mg/kg/infusion of cocaine. Since haloperidol increased the self-administration of this dose of cocaine (see the Results section), the effects on self-administration of a large range of cocaine doses was determined. The dose of cocaine in subsequent tests was repeatedly reduced by half every three days. Thus the effect of this dose of haloperidol on responding for 0.25, 0.125 and 0.0625 mg/kg/infusion of cocaine was assessed. A final dose of 0.03 mg/kg cocaine was available although the effects of haloperidol on responding for this dose of cocaine were not determined.

Drugs

Atropine (Sigma Chemical Co.; St. Louis, MO) and GR38032F (Glaxo, UK) were dissolved in water. Cocaine HCl (Sigma Chemical Co.; St. Louis, MO) was dissolved in 0.9% saline. Haloperidol (Sigma Chemical Co.; St. Louis, MO) was dissolved in a 1% lactic acid solution, and adjusted to pH 5.0–5.5 with [2M] NaOH. Sodium pentobarbitol (Sigma Chemical Co.; St. Louis, MO) was dissolved in 50% H_2O, 40% propylene glycol and 10% EtOH.

RESULTS

Figure 1 shows the effects of each dose of GR38032F on the total number of reinforced lever responses emitted during the 2-h cocaine self-administration tests. Baseline data were derived on day 2 of the 3-day test period, whereas GR38032F data were derived on day 3. The number of reinforced responses was markedly similar on the baseline and GR38032F days. A 2-way

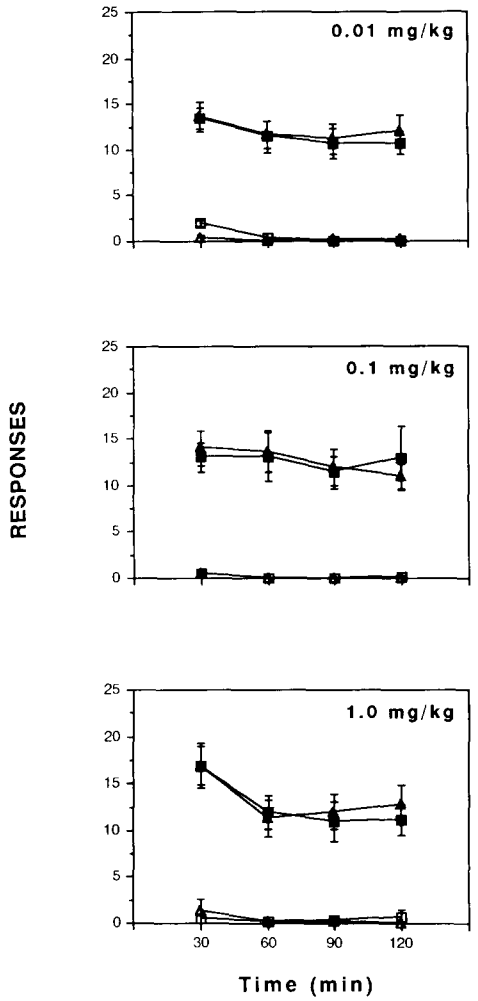

FIG. 2. Effects of GR38032F on the number of cocaine-reinforced (active) and nonreinforced (inactive) responses during 30-min blocks of a 2-hour self-administration test. Symbols represent the mean and vertical lines represent teh SEM. ■: Baseline-Active; ▲: GR38032F-Active; □: Baseline-Inactive; △: GR38032F-Inactive.

(treatment × dose) Analysis of Variance (ANOVA) was performed, with no significant differences in the number of reinforced responses as a function of treatment, $F(1,6) = 1.306$, NS.

To determine whether the analysis of total number of responses obscured the effects of a possible short duration of drug action, the effect of GR38032F on the number of reinforced and nonreinforced responses during the two-hour test session was broken down into thirty-minute blocks (Fig. 2). Across all time periods, the number of reinforced responses was markedly similar, regardless of the treatment condition. A 3-way ANOVA (Treatment × Dose × Time) failed to reveal any significant differences in the number of reinforced responses as a function of treatment, $F(1,6) = 1.57$, NS. Baseline data are remarkably consistent across the three 3-day test periods, indicating the stability of responding across days.

Figure 3 shows the effects of haloperidol (0.125 mg/kg) on cocaine self-administration. In contrast to the data from Fig. 1, haloperidol increased responding for 0.5 mg/kg cocaine, $t(4) = 3.17$, $p = 0.034$. A shift to the right in the dose-response curve

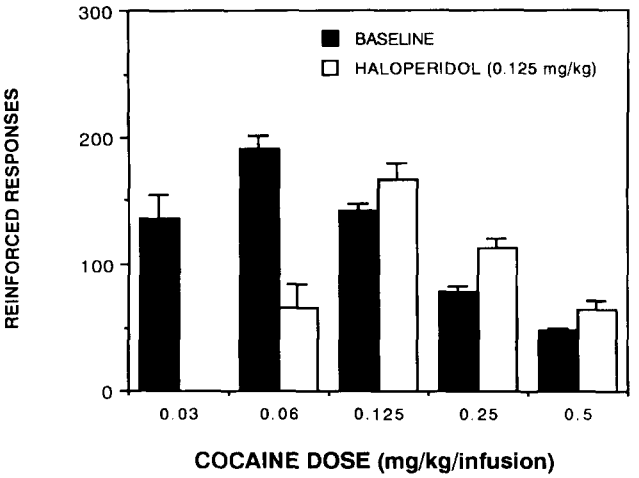

FIG. 3. Effects of haloperidol (0.125 mg/kg) on the mean number (±SEM) of active responses for various doses of cocaine during a 2-hour self-administration test.

for self-administration was also apparent.

Figure 4 shows individual data for each of the 5 rats treated with haloperidol. Haloperidol produced a shift to the right of the dose-response curve for cocaine self-administration in all 5 subjects.

DISCUSSION

The selective serotonin 5-HT$_3$ antagonist, GR38032F, failed to alter the self-administration of cocaine. Although it is possi-

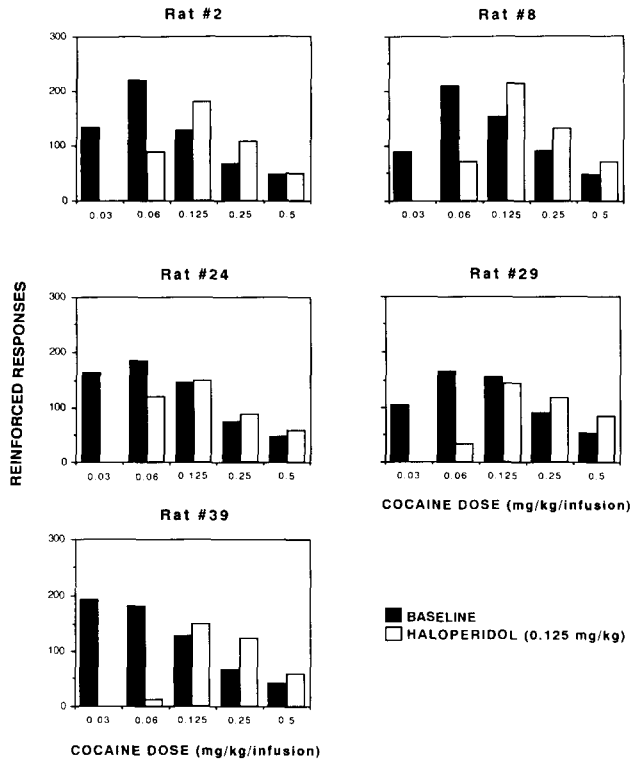

FIG. 4. Individual dose-response curve data for the 5 rats tested with haloperidol (0.125 mg/kg IP). In each case, haloperidol shifted the dose-response curve for cocaine self-administration to the right.

ble that the doses employed in the present study were subthreshold, this is unlikely since these doses have been employed in other behavioral studies and have been shown to be effective in inhibiting the locomotor hyperactivity induced by central administration of amphetamine, an indirect DA agonist (8), or DiMe-C7 (10), a peptide that has been suggested to produce hyperactivity associated with mesolimbic DA activation.

The ability for a low dose (0.125 mg/kg) of the dopamine receptor blocker, haloperidol, to increase responding for 0.5 mg/kg/infusion cocaine and to shift the dose-response curve for self-administration to the right supports the hypothesis that the integrity of central dopaminergic receptors is critical for cocaine reward (15,19). The data suggest that a blockade of these receptors reduced the reward effectiveness of cocaine. The failure to observe a similar effect of GR38032F suggests that the drug does not decrease the effectiveness of dopamine in a similar manner.

It has been suggested that 5-HT_3 antagonists may serve as effective pharmacotherapies for drug abuse (17,18). Insofar as the use of rate of responding in the animal model of self-administration is a valid model for human substance abuse, the negative data obtained in the present study would argue that these drugs would have little, if any, applicability in the treatment of cocaine abuse.

There is a growing literature base that is describing the interaction between central serotonergic systems and the reinforcing properties of cocaine. The effects of general manipulations of serotonin, including the administration of precursors (7), reuptake blockers (6) and local administration of serotonergic neurotoxins (14), have suggested that serotonin may interact with dopamine to modulate the effectiveness of cocaine as a reinforcer. If so, the present data would suggest that the 5-HT_1 or 5-HT_2 receptor subtype may be the critical ones to concentrate on in evaluating the role of serotonin in cocaine self-administration.

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