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5-HT_3 Receptor Modulation of Behavior During Withdrawal From Continuous or Intermittent Cocaine

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KING, G. R., C. M. JOYNER AND E. H. ELLINWOOD, JR. *5-HT_3 receptor modulation of behavior during withdrawal from continuous or intermittent cocaine.* PHARMACOL BIOCHEM BEHAV 47(3) 399–407, 1994.—The present experiments examined alterations in 5-HT_3 receptors during withdrawal from continuous or intermittent cocaine. Rats were pretreated with 40 mg/kg/day cocaine for 14 days by either SC injections or osmotic minipumps. The rats were then withdrawn from the pretreatment regimen for 7 days. In Experiment 1, rats received 0–16 mg/kg IP injections of ondansetron, a selective 5-HT_3 receptor antagonist. In Experiment 2, the rats received 0–16 mg/kg IP ondansetron in combination with a 15 mg/kg IP injection of cocaine. In Experiment 3, the subjects received 0–16 mg/kg IP injections of ondansetron in combination with a 7.5 mg/kg IP injection of cocaine. Following these injections, the subjects' behavior was rated using the Ellinwood and Balster (18) rating scale. The results of Experiment 1 indicated that ondansetron had no effect on the behavior of the subjects, nor was there a differential effect of pretreatment regimen on the effects of ondansetron. The results of Experiment 2 indicated that ondansetron had no effect on cocaine-induced locomotion in the saline control rats, but did have a slight, statistically significant, *suppressive* effect in the injection rats. In contrast, ondansetron had a robust *facilitative* effect on cocaine-induced locomotion in the continuous infusion rats. The results of Experiment 3 indicated that ondansetron had no effect on cocaine-induced locomotion in the saline control rats or the cocaine injection pretreatment subjects. In the continuous infusion subjects, ondansetron did have a slight, statistically significant, *facilitative* effect on cocaine-induced locomotion. The present results indicate that the continuous infusion of cocaine for 14 days may alter the ability of 5-HT_3 receptor antagonists to regulate cocaine-related behaviors.

5-HT_3 receptors Intermittent cocaine Continuous cocaine Withdrawal Rats

PREVIOUS research involving chronic cocaine administration indicates that both the dose and route of administration influence its behavioral effects: daily intermittent (IP, SC, or schedule induced) cocaine administration produces sensitization to its locomotor- and stereotypy-inducing properties, as well as its ability to alter dopamine (DA) functioning (1,19,26–30,33–36,42,43,45). In contrast, the continuous administration of cocaine via osmotic minipump results in tolerance to its behavioral and some of its neurochemical effects (29,30, 33,35,36,45). These results clearly indicate that the effects of chronic cocaine are partially dependent on the method and temporal pattern of administration.

The mechanisms underlying the differential effects of chronic cocaine as a function of administration modality are unclear. However, it is generally thought [e.g., (21)] that the psychomotor-stimulant, stereotypy-inducing, and reinforcing properties of cocaine are predominantly mediated by mesocorticolimbic DA systems [see (24) for a review of some of this literature]. Although much research indicates that the behavioral effects of cocaine are correlated with increased synaptic DA levels, several additional lines of evidence indicate that serotonin (5-hydroxytryptamine, 5-HT) systems have an inhibitory regulatory role in stimulant-induced behavior. For example, Broderick (5–8) has extensively and elegantly demonstrated some of the in vivo interrelations between 5-HT and DA release following cocaine administration [see (30,31) for a review of some of this literature].

Central 5-HT receptors can be divided into at least four general classes: 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors. The 5-HT_3 receptor is primarily distributed in corticolimbic areas (51), and apparently mediates the excitatory effects of 5-HT in the periphery and centrally (41). Behavioral research

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involving 5-HT_3 receptor antagonists indicates that these compounds have no effect on locomotor behavior when administered alone in drug-naive rats [see (17) for a review]. However, other studies indicate that 5-HT_3 receptors can modulate (drug-induced) DA-mediated behaviors under some conditions. For example, Costall et al. (13) reported that administration (either peripherally or into the accumbens) of the selective 5-HT_3 antagonist ondansetron (GR38032F) inhibited the hyperactivity induced by an intra-accumbens injection of amphetamine. Furthermore, ondansetron inhibited the hyperactivity induced by chronic perfusion of DA into the nucleus accumbens. Hagan et al. (22) demonstrated that the behavioral hyperactivity produced by increased DA release in the nucleus accumbens is blocked by the administration of 5-HT_3 antagonists (in this case, raised DA levels were induced by direct injection of the neurokinin agonist DiMe-C7 into the ventral tegmental area).

Because 5-HT_3 receptors can modulate synaptic DA levels produced by drug administration, and can modulate DA-mediated behaviors, these receptors may be important in regulating the acute, chronic, and residual effects of psychomotor stimulants such as cocaine. Hence, the three experiments presented here evaluated the role of 5-HT_3 receptors in mediating the effects of continuous or intermittent cocaine administration.

GENERAL METHOD

Animals

Male Sprague–Dawley rats, initially weighing 100 to 125 g (Charles River Laboratories), were acclimated to the vivarium on a 12 L : 12 D cycle (light between 0700 and 1900) for 1 week prior to treatment. They were housed in pairs in plastic cages with continuous access to food and water.

Drugs

Cocaine HCL (received from NIDA) was dissolved in 0.9% sterile saline. Ondansetron hydrochloride dihydrate, (+/−)-1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1*H*-imidazol-1-yl)-methyl]-4*H*-carbazol-4-one, monohydrochloride dihydrate (generously supplied by Glaxo Group Research Ltd., Middlesex, UK), was dissolved in distilled water. All doses are calculated as the base, and injection volume was based on the body weight.

Minipump Preparation

Alzet osmotic pumps (model 2ML2) from Alza Corporation (Palo Alto, CA) were filled with either 2 ml of 100 mg/ml cocaine HCl, or saline (0.9%); the infusion rate was 5 μl/h resulting in an overall, average dose of 40 mg/kg/day for the cocaine pumps. The pump was primed by warming in a beaker of saline in a waterbath at 37°C for 4 h prior to surgical implantation. The minipumps were modified by adding a microdialysis fiber to the output portal to increase the surface area over which cocaine is distributed; this modification allows for the continuous infusion of high doses of cocaine without the development of necrotic skin lesions (25).

Surgery

The animals were shaved and injected locally with (0.2 cc) lidocaine (Abbott, North Chicago, IL) at the dorsal midline incision site. The animals were then anesthetized by inhalation with methoxyflurane (Metofane). A 2-cm vertical incision was made with scissors and a large SC pocket was formed with the scissors. The minipump was inserted into this pocket with the delivery portal toward the head. The opening was closed with metal surgical autoclips. On day 14, the pumps were surgically removed using the same procedure and the residual amount of cocaine measured. The amount was consistently less than 15% of the original volume, indicating that the rats approximately received the programmed daily dose.

Pretreatment

Pretreatment was for a 14-day period. On day 1 of treatment, animals were either: a) implanted with 2ML2 Alzet minipumps continuously infusing cocaine at a rate of 40 mg/kg/day (continuous infusion group), b) injected SC once daily with 40 mg/kg cocaine HCl (injection group), or c) injected SC with 0.9% saline (saline control group) once daily.

Behavioral Testing

On day 7 following pretreatment, the animals were acclimated to the test room in their home cage for 30 min under normal light conditions. The test cages were standard, clear plastic laboratory animal housing cages, 28 × 18 × 12 cm, with another cage taped, upside down, in place on top. The top cage had five air holes drilled uniformly on either side. Six of these test cages were placed in a row 12 in. apart. A modified version of the Ellinwood and Balster Rating Scale (18) was used (30–32). A rating was given to each of the animals at 5 min preinjection, and at 5-min intervals thereafter for a total of 60 min. The observation period was for 20 s with 10 s between cages.

EXPERIMENT 1: EFFECTS OF ONDANSETRON ON LOCOMOTOR BEHAVIOR

Research by Costall and colleagues (13–16) supports the hypothesis that 5-HT and 5-HT_3 receptors may be involved in withdrawal from drugs of abuse. They reported that the administration of 5-HT_3 antagonists can attenuate some of the symptoms produced by withdrawal from ethanol, nicotine, and cocaine. For example, in their studies rats were given 1 mg/kg cocaine, twice daily, for 14 days. The rats were then withdrawn from the treatment, and their social behavior examined. Withdrawal from chronic cocaine suppressed normal social interactions, and the administration of ondansetron resulted in the restoration of normal social behaviors.

In spite of the evidence that 5-HT_3 receptors can modulate some behaviors associated with drug withdrawal, the role of 5-HT_3 receptors in mediating these effects is still unclear. The studies of Costall and colleagues used a very low dose of cocaine for the chronic dosing regimen, and it is not clear if those results would generalize to a high-dose regimen that is more similar to the pattern of stimulant use exhibited by human stimulant abusers. Furthermore, all of the previous research has utilized daily intermittent injections of cocaine. It is unknown what role 5-HT_3 receptors may play in withdrawal from continuous cocaine administration.

Experiment 1 examined the role of 5-HT_3 receptors in regulating the locomotor behavior of rats withdrawn for 7 days from a 14-day pretreatment involving either continuous or intermittent cocaine administration. The rats' behavior was assessed by behavior ratings (18) over several challenge doses of the 5-HT_3 receptor antagonist ondansetron.

METHOD

For the test session, each rat received one of the following doses of ondansetron IP: 0, 1.0, 4.0, or 16.0 mg/kg. Injections were given following a 20-min adaptation period, and 5 min after receiving a baseline, no drug behavior rating. For each test session the subject types (i.e., injection, pump, saline) and ondansetron doses were randomized according to a Latin Square design. The significance level was set at $p \leq 0.05$ for all comparisons. There were 10 rats per condition.

RESULTS AND DISCUSSION

Figure 1 presents the mean behavior rating for each pretreatment group, separately for each dose of ondansetron. Panel A presents the behavior ratings of subjects receiving 0.0 mg/kg ondansetron. The results of Kruskall-Wallis analyses of variance (ANOVA) by rank indicate that there were significant differences at 40 min. The results of Mann-Whitney tests comparing the pretreatment groups at 40 min indicate that the continuous infusion subjects were significantly different than both the saline control and cocaine injection subjects.

Panel B presents the behavior ratings for subjects receiving a 1.0 mg/kg ondansetron dose. The results of Kruskall-Wallis ANOVA by ranks indicate that there were significant differences at 0 and 10 min. The results of Mann-Whitney tests comparing the pretreatment groups at 0 min indicate that the continuous infusion subjects were significantly different than the cocaine injection subjects. The results of Mann-Whitney tests comparing the pretreatment groups at 10 min indicate that the continuous infusion subjects were significantly different than both the saline control and cocaine injection subjects.

Panel C presents the behavior ratings for subjects receiving a 4.0 mg/kg ondansetron dose. The results of Kruskall-Wallis ANOVA by ranks indicate that there were significant differences at 5 min. The results of Mann-Whitney tests comparing the pretreatment groups at 5 min indicate that the continuous infusion subjects were significantly different than the cocaine injection subjects.

Panel D presents the behavior ratings for subjects receiving a 16.0 mg/kg ondansetron dose. The results of Kruskall-Wallis ANOVA by ranks indicate that there were significant differences at 10 and 15 min. The results of Mann-Whitney tests comparing the pretreatment groups at 10 min indicate that the saline control and cocaine injection subjects were significantly different. The results of Mann-Whitney tests comparing the pretreatment groups at 15 min indicate that the cocaine injec-

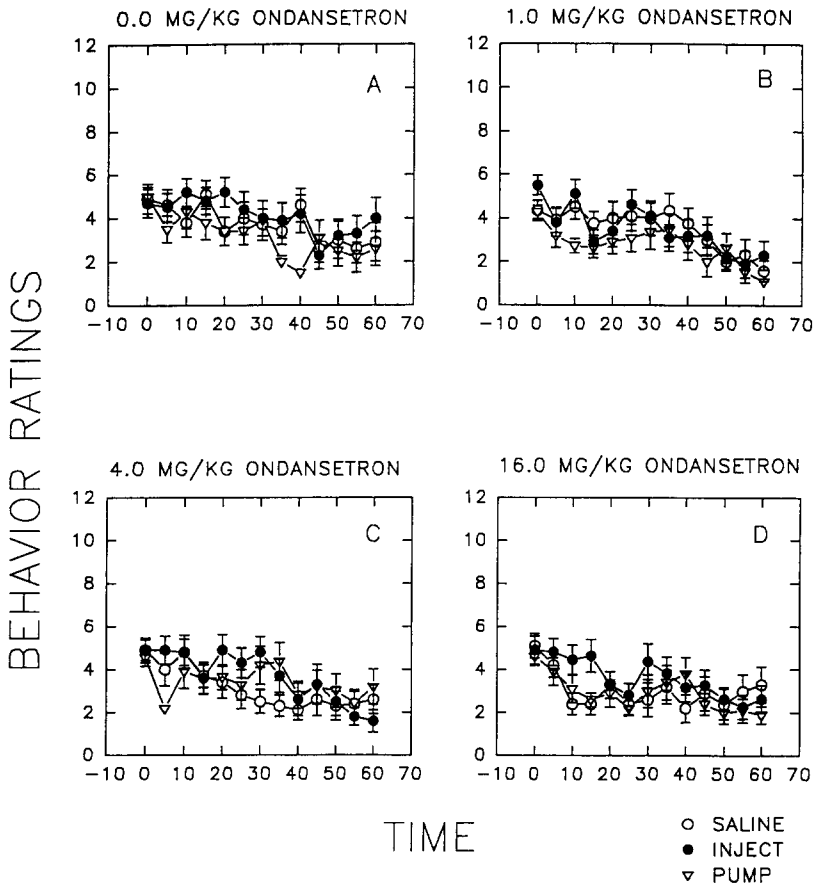

FIG. 1. The mean behavior rating for each group, separately for each dose of ondansetron. The bars represent 1 SE. The open circles (○) represent the saline pretreatment rats. The solid circles (●) represent the cocaine injection pretreatment rats. The open triangles (△) represent the continuous infusion pretreatment rats.

tion subjects were significantly different than both the saline control and continuous infusion subjects.

The results from Experiment 1 indicate that ondansetron had no *consistent* effect on the locomotor behavior of the rats withdrawn from either continuous or intermittent cocaine, although there were some effects at isolated time points. However, the work of Costall and colleagues (12-17) indicated that ondansetron was effective in attenuating some of the aversive consequences of withdrawal from intermittent cocaine. The results from Experiment 1 are not entirely consistent with Costall's work. The differences between the present and previous results are probably due to the different behavioral assays used in the different experiments. We measured general exploratory and locomotor behaviors, while the previous experiments examined social behavior. Hence, an effect of ondansetron on behavior may have been detected had different behavioral assays such as social behavior, an elevated plus maze, or light/dark maze, been used. Further research should examine these possibilities.

EXPERIMENT 2: EFFECTS OF ONDANSETRON ON COCAINE-INDUCED LOCOMOTION

Behavioral studies indicate that 5-HT_3 receptors can modulate DA-mediated behaviors. For example, Hagan et al. (22) demonstrated that the behavioral hyperactivity produced by increased nucleus accumbens DA release is blocked by the administration of 5-HT_3 antagonists. Given these results, the administration of 5-HT_3 antagonists should be able to attenuate some of the behavioral effects of cocaine administration. Indeed, a variety of 5-HT_3 antagonists (e.g., ICS 205-930, zacopride, ondansetron, MDL 72222) have been shown to block the locomotor-stimulating (44,46,50), but not the discriminative stimulus [e.g, (39)] or reinforcing [e.g., (40)], effects of acute cocaine administration. In spite of this research, it is unknown to what extent chronic cocaine administration results in alterations in 5-HT_3 receptor regulation of cocaine-induced behaviors.

Experiment 2 examined the role of 5-HT_3 receptor antagonists in regulating cocaine-induced hyperactivity of rats withdrawn for 7 days from either continuous or intermittent cocaine administration. The rats' behavior was assessed by behavior ratings over several challenge doses of the 5-HT_3 receptor antagonist ondansetron in conjunction with a 15 mg/kg IP cocaine injection.

METHOD

Behavioral Testing

The subjects received on of the following doses of ondansetron IP, 30 min prior to the session: 0.0, 1.0, 2.0, 4.0, 8.0, or 16.0 mg/kg. The cocaine injections were also IP, and were given immediately prior to the session. For each test session in Experiment 2, the subject types and ondansetron doses were randomized according to a Latin Square design. The significance level was set at $p \leq 0.05$ for all comparisons. There were 10 rats per condition.

RESULTS AND DISCUSSION

Figure 2 presents the mean behavior rating for each pretreatment group, separately for each condition. Panel A presents the behavior ratings for the 0.0 mg/kg ondansetron plus 15.0 mg/kg cocaine combination. The results of Kruskall-Wallis ANOVA by rank indicate that there were significant differences between the pretreatment groups from 5-40 min. Mann-Whitney tests comparing the saline control and cocaine injection groups indicate that the injection group had significantly higher behavior ratings than the saline control group from 10-35 min. Mann-Whitney tests comparing the saline control and continuous infusion groups indicate that the saline control group had significantly higher behavior ratings than the continuous infusion group from 5-15 and at 35 min. Mann-Whitney tests comparing the cocaine injection and continuous infusion groups indicate that the injection group had significantly higher behavior ratings than the continuous infusion group from 5-40 min. These results indicate that a history of daily cocaine injections results in sensitization to, while the history of the continuous of cocaine results in tolerance to, the behavioral effects of cocaine challenges. These results are consistent with previous research using this pretreatment regimen (29,30,45).

Panel B presents the behavior ratings for the 1.0 mg/kg ondansetron plus 15.0 mg/kg cocaine combination. The results of Kruskall-Wallis ANOVA by ranks indicate that there were significant differences between the pretreatment groups from 35 to 55 min. Mann-Whitney tests comparing the saline control and cocaine injection groups indicate that the injection group had significantly higher behavior ratings than the saline control group from 35 to 55 min. Mann-Whitney tests comparing the saline control and continuous infusion groups indicate that there were no significant differences at any time point. Mann-Whitney tests comparing the cocaine injection and continuous infusion groups indicate that the injection group had significantly higher behavior ratings than the continuous infusion group from 35 to 45 min.

Panel C presents the behavior ratings for the 2.0 mg/kg ondansetron plus 15.0 mg/kg cocaine combination. The results of Kruskall-Wallis ANOVA by ranks indicate that there were significant differences between the pretreatment groups at 15 and 30 min. Mann-Whitney tests comparing the saline control and cocaine injection groups indicate that the injection group had significantly higher behavior ratings than the saline control group at 15 and 30 min. Mann-Whitney tests comparing the saline control and continuous infusion groups indicate that the saline control group had significantly higher behavior ratings than the continuous infusion group only at 15 min. Mann-Whitney tests comparing the cocaine injection and continuous infusion groups indicate that the injection group had significantly higher behavior ratings than the continuous infusion group at 15 and 30 min.

Panel D presents the behavior ratings for the 4.0 mg/kg ondansetron plus 15.0 mg/kg cocaine combination. The results of Kruskall-Wallis ANOVA by ranks indicate that there were significant differences between the pretreatment groups at 30 and 60 min. Mann-Whitney tests comparing the saline control and cocaine injection groups indicate that the injection group had significantly higher behavior ratings than the saline control group at 30 min. Mann-Whitney tests comparing the saline control and continuous infusion groups indicate that the saline control group was not significantly different. Mann-Whitney tests comparing the cocaine injection and continuous infusion groups indicate that the injection group had significantly higher behavior ratings than the continuous infusion group at 30 and 60 min.

Panel E presents the behavior ratings for the 8.0 mg/kg ondansetron plus 15.0 mg/kg cocaine combination. The results of Kruskall-Wallis ANOVA by ranks indicate that there were significant differences between the pretreatment groups at 25, 30, and 40 min. Mann-Whitney tests comparing the

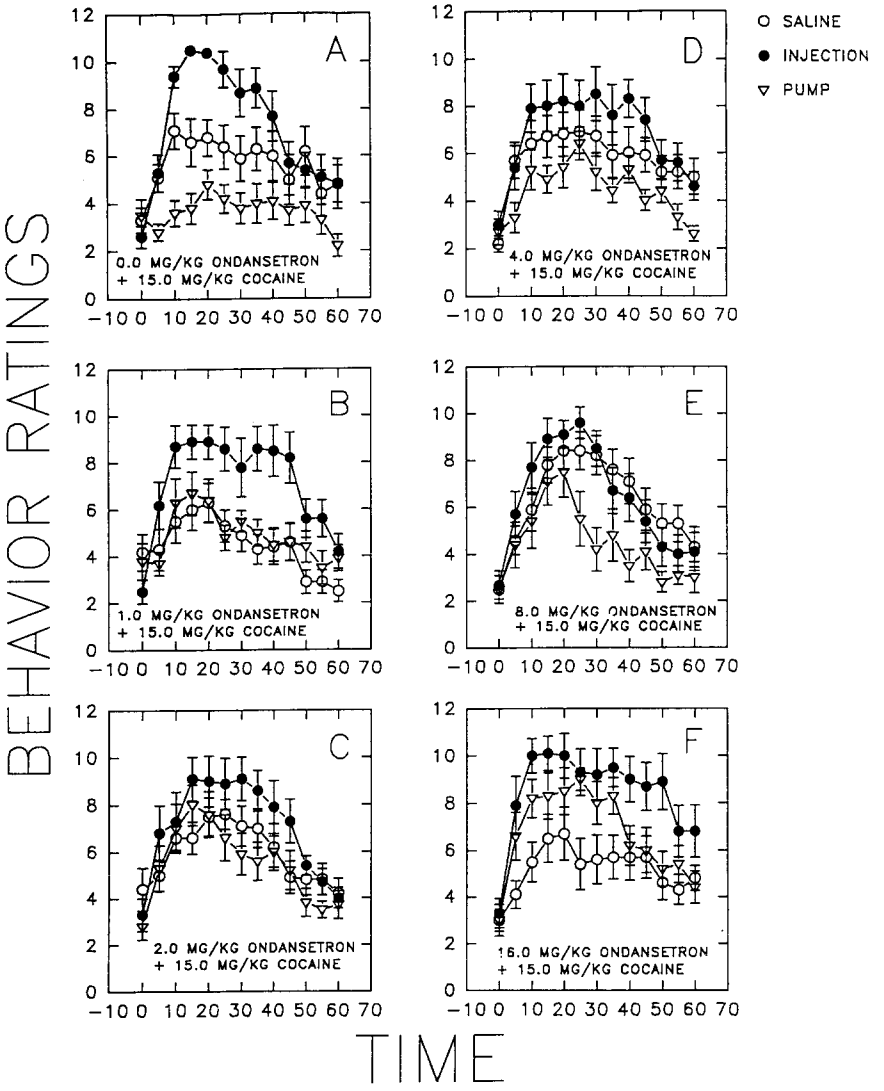

FIG. 2. The mean behavior rating for each group, separately for each combination of cocaine and ondansetron. The bars represent 1 SE. The open circles (○) represent the saline pretreatment rats. The solid circles (●) represent the cocaine injection pretreatment rats. The open triangles (△) represent the continuous infusion pretreatment rats.

saline control and cocaine injection groups indicate that the injection group had significantly higher behavior ratings than the saline control group from 25–30 min. Mann–Whitney tests comparing the saline control and continuous infusion groups indicate that the groups were not significantly different. Mann–Whitney tests comparing the cocaine injection and continuous infusion groups indicate that the injection group had significantly higher behavior ratings than the continuous infusion group at 25, 30, and 40 min.

Panel F presents the behavior ratings for the 16.0 mg/kg ondansetron plus 15.0 mg/kg cocaine combination. The results of Kruskall–Wallis ANOVA by ranks indicate that there were significant differences between the pretreatment groups from 5–50 min. Mann–Whitney tests comparing the saline control and cocaine injection groups indicate that the injection group had significantly higher behavior ratings than the saline control group from 5–50 min. Mann–Whitney tests comparing the saline control and continuous infusion groups indicate that the groups were significantly different at 10, 25, and 35 min. Mann–Whitney tests comparing the cocaine injection and continuous infusion groups indicate that the injection group had significantly higher behavior ratings than the continuous infusion group at 10–20 and 30–50 min.

Overall, the results of Experiment 2 would seem to suggest that 5-HT$_3$ antagonists have no effect on cocaine-induced locomotion and stereotypies in drug-naive rats. However, in rats that have a history of daily intermittent cocaine injections, 5-HT$_3$ antagonists seem to have a slight, inconsistent, but statistically significant, suppressive effect on cocaine-induced locomotion. In contrast, in rats that have a history of the continuous infusion of cocaine, 5-HT$_3$ antagonists have a pronounced faciliatative effect on cocaine-induced locomo-

tion. Indeed, examination of the results for the continuous infusion rats in 16.0 mg/kg ondansetron plus 15.0 mg/kg cocaine condition indicates that these rats strongly resemble sensitized rats (i.e., rats with a history of daily cocaine injections). These results are interesting because they would seem to indicate that the differential drug histories may have a substantial effect on the subsequent actions of some 5-HT_3 antagonists in modulating cocaine-induced behaviors.

EXPERIMENT 3: EFFECTS OF ONDANSETRON ON LOCOMOTION INDUCED BY LOW-DOSE COCAINE

In spite of the interesting and counterintuitive nature of the results from Experiment 2, those results could be the result of a rate dependency (i.e., suppression of high-rate behaviors and enhancement of low-rate behaviors). As stated in the Introduction, intermittent cocaine injections induce sensitization, while the continuous infusion of cocaine results in tolerance to the behavioral effects of cocaine challenges. Indeed, in this experiment condition 1 resulted in significantly greater locomotion in the injection group, compared to the saline control and continuous infusion groups, while the continuous infusion subjects exhibited significantly less locomotion in condition 1, compared to both the saline control and daily injection subjects.

Given these considerations, Experiment 3 partially replicates Experiment 2, except that the cocaine challenge dose was 7.5 mg/kg instead of 15.0 mg/kg. This change in the cocaine challenge dose should eliminate or attenuate the behavioral differences engendered by the pretreatment regimen (i.e., the degree of sensitization and tolerance should be attenuated). If the results of Experiment 2 are the result of rate dependency, then 5-HT_3 antagonists should have no (differential) effects on cocaine-induced locomotion, between the pretreatment groups, but should result in a dose-dependent facilitation of cocaine-induced locomotion.

METHOD

Behavioral Testing

For the test session, each rat received one of the following doses of ondansetron IP: 0, 1.0, 4.0, or 16.0 mg/kg 30 min prior to the session, and then a 7.5 mg/kg IP cocaine injection immediately prior to the session. For each test session the subject types and ondansetron doses were randomized according to a Latin Square design. The significance level was set at $p \leq 0.05$ for all comparisons. There were 10 rats per condition.

RESULTS AND DISCUSSION

Figure 3 presents the mean behavior rating for each pretreatment group, separately for each combination of ondansetron and 7.5 mg/kg cocaine. Panel A presents the behavior ratings of subjects receiving 0.0 mg/kg ondansetron plus 7.5 mg/kg cocaine. The results of Kruskall-Wallis ANOVA by rank indicate that there were significant differences between the pretreatment groups at 10 and 15 min. The results of Mann-Whitney tests comparing the pretreatment groups at 10 min indicate that the behavior ratings for the continuous infusion subjects were significantly smaller than those for both the saline control and cocaine injection subjects. The results of Mann-Whitney tests comparing the pretreatment groups at 15 min indicate that the behavior ratings for the continuous infusion subjects were significantly smaller than those for the cocaine injection subjects. No other comparison was significant. Thus, the continuous infusion subjects exhibited tolerance to the behavioral activating effects of a 7.5 mg/kg cocaine challenge on day 7 of withdrawal from the pretreatment regimen. However, the cocaine injection subjects did not apparently demonstrate sensitization to the behavioral effects of a 7.5 mg/kg cocaine challenge.

Panel B presents the behavior ratings for subjects receiving a 1.0 mg/kg ondansetron and 7.5 mg/kg cocaine combination dose. The results of Kruskall-Wallis ANOVA by rank indicate that there were significant differences between the pretreatment groups at 10 and 15 min. The results of Mann-Whitney tests comparing the pretreatment groups at 10 min indicate that the behavior ratings for the continuous infusion subjects were significantly less than those for both the saline control and cocaine injection subjects. Furthermore, the behavior ratings for the saline control group were significantly less than the those for the cocaine injection subjects. The results of Mann-Whitney tests comparing the pretreatment groups at 15 min indicate that the behavior ratings for the continuous infusion subjects were significantly less than those for both the saline control and the cocaine injection subjects. No other comparison was significant.

Panel C presents the behavior ratings for subjects receiving a 4.0 mg/kg ondansetron and 7.5 mg/kg cocaine combination dose. The results of Kruskall-Wallis ANOVA by rank indicate that there were significant differences between the pretreatment groups at 5 and 15 min. The results of Mann-Whitney tests comparing the pretreatment groups at 5 min indicate that the behavior ratings for the continuous infusion subjects were significantly less than those for both the saline control and cocaine injection subjects. The results of Mann-Whitney tests comparing the pretreatment groups at 15 min indicate that the behavior ratings for the continuous infusion subjects were significantly less than those for the cocaine injection subjects. No other comparison was significant.

Panel D presents the behavior ratings for subjects receiving a 16.0 mg/kg ondansetron and 7.5 mg/kg cocaine combination dose. The results Kruskall-Wallis ANOVA by ranks indicate that there were no significant differences between the pretreatment groups at any time point.

The results of Experiment 3 are in partial agreement the results from Experiment 2 for the cocaine pump subject: a) These subjects demonstrated tolerance to the behavioral effects of a 7.5 mg/kg cocaine challenge, and b) the 16.0 mg/kg ondansetron injection produced a slight facilitative effect on cocaine-induced hyperactivity, which attenuated the tolerance exhibited with the 7.5 mg/kg cocaine injections. However, the effects in the cocaine pump subjects were not nearly as robust as those from Experiment 2. Furthermore, the cocaine injection subjects did not demonstrate sensitization to the behavioral effects of a 7.5 mg/kg cocaine injection, nor did any dose of ondansetron have any effect on the behavior of these subjects induced by a 7.5 mg/kg cocaine challenge.

GENERAL DISCUSSION

The present results support and extend previous findings, which indicate that the effects of chronic cocaine depend on the route and temporal pattern of administration. Chronic, daily SC injections of cocaine produce sensitization to a subsequent cocaine challenge; this result is consistent with previous research using daily injections of cocaine (29,30,34,43,45). In contrast, the continuous infusion of an overall, equivalent

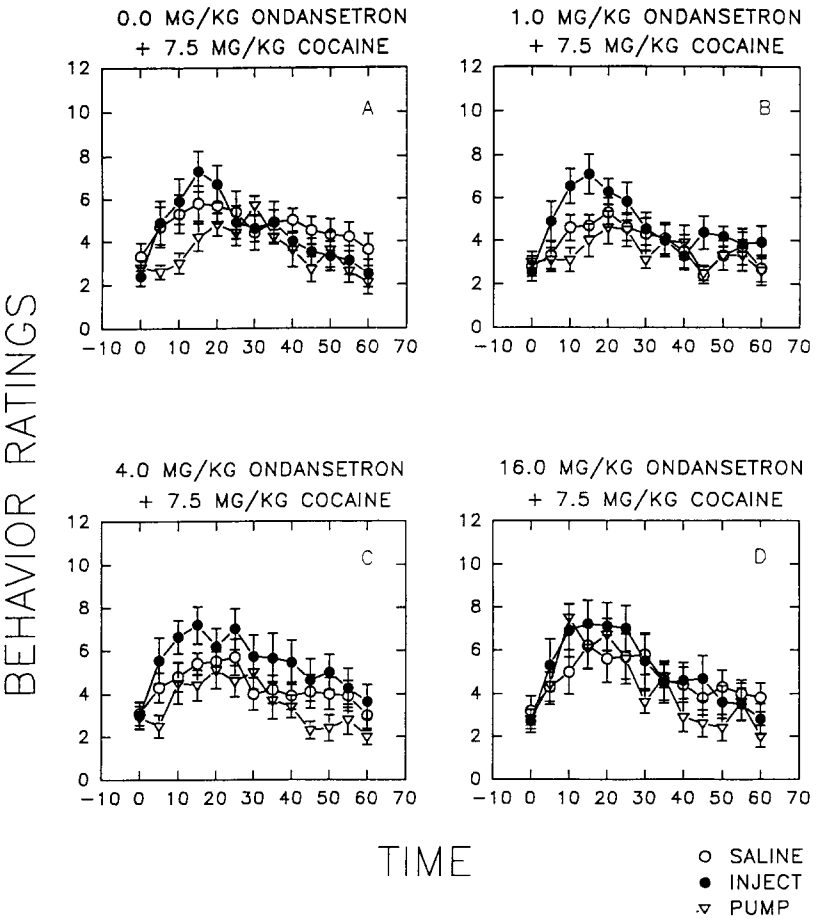

FIG. 3. The mean behavior rating for each group, separately for each combination of cocaine and ondansetron. The bars represent 1 SE. The open circles (○) represent the saline pretreatment rats. The solid circles (●) represent the cocaine injection pretreatment rats. The open triangles (△) represent the continuous infusion pretreatment rats.

daily dose of cocaine produces tolerance to a subsequent cocaine challenge. These results are also consistent with previous studies (29,30,45).

The present experiments were based on the hypothesis that withdrawal from continuous or intermittent cocaine alters the abilities of 5-HT$_3$ receptors to regulate behavior:

a) Cocaine is, amongst other things, a 5-HT$_3$ receptor antagonist (20,47), and therefore its chronic administration should have some effect on the functional capacities of 5-HT$_3$ receptors,
b) 5-HT$_3$ receptors are important in the regulation of neurotransmitter release; 5-HT$_3$ receptor agonists induce DA release in mesocorticolimbic areas (2–4,9–11,23), and
c) 5-HT$_3$ receptors are important in the regulation of some (44,46,50), but not all (39,40), aspects of stimulant-induced, DA-mediated behavior.

Experiment 1 examined the ability of ondansetron to regulate behavior during withdrawal. The results indicated that ondansetron had no consistent effects on the behavior of the subjects. In contrast, Costall and colleagues (12–17) have elegantly demonstrated that ondansetron was effective in attenuating some of the consequences of withdrawal from intermittent cocaine. The differences between the present and previous results are probably due to the different behavioral assays used in the different experiments. We measured general exploratory and locomotor behaviors, while the previous experiments examined social behavior. Hence, an effect of ondansetron on behavior may have been detected had different behavioral assays, such as social behavior, an elevated plus maze, or the light/dark maze, been used. Further research should examine these possibilities.

Experiments 2 and 3 examined whether chronic daily cocaine injections, or the continuous infusion of cocaine, altered the ability of 5-HT$_3$ receptor antagonists to regulate cocaine-induced behaviors. As stated above, acutely these compounds suppress cocaine-induced hyperactivity (44,46,50). The results of Experiments 2, and to a lesser extent Experiment 3, indicate that withdrawal from continuous cocaine reverses the effects of ondansetron: ondansetron *enhances* the behavior induced by a 15.0 mg/kg, and to a lesser extent 7.5 mg/kg, cocaine challenge. The results from Experiment 2, while not dose dependent, were fairly robust. In contrast, the results from Experiment 3 were less robust. The highest dose of ondansetron did enhance the locomotor behavior induced by a 7.5 mg/kg cocaine challenge in the continuous infusion subjects; how-

ever, this effect was rather weak and somewhat transient. The overall pattern of results from Experiments 2 and 3 indicates that the continuous infusion of cocaine alters the abilities of 5-HT_3 receptor antagonists to regulate DA-mediated behaviors. However, the nature of this alteration is unclear.

In summary, the present results indicate that the continuous infusion of cocaine results in tolerance to subsequent challenge doses of cocaine. Furthermore, the continuous infusion of cocaine results in alterations in the ability of 5-HT_3 receptor antagonists to regulate cocaine-induced locomotion. Daily intermittent injections of cocaine do not alter the ability of 5-HT_3 receptor antagonists to regulate cocaine-induced locomotion. In contrast to the effects of ondansetron on cocaine-induced locomotion, ondansetron, when administered alone, had no effect on behavior.

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