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Olanzapine attenuates the reinforcing effects of cocaine

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Abstract

The possibility that the atypical neuroleptic olanzapine can antagonize the ability of cocaine to produce both conditioned place preference and self-administration in rats was investigated. Pre-treatment with olanzapine (3.0, 4.5 mg/kg, but not 1.5 mg/kg) significantly attenuated conditioned place preference produced by cocaine (10 mg/kg). However, the higher dose of olanzapine administered alone resulted in conditioned place aversion. Pre-treatment with olanzapine also produced a dose-dependent decrease in cocaine self-administration (0.33 mg/infusion) under a fixed-ratio 2 schedule of reinforcement. Olanzapine produced a similar dose-responsive attenuation in operant responding for food (fixed-ratio 10) suggesting that olanzapine produces a nonspecific decrease in operant behavior. Pre-treatment with 4.5 mg/kg olanzapine significantly attenuated cocaine-induced hyperactivity, whereas lower olanzapine doses had little effect upon cocaine-induced hyperactivity. These results suggest that pre-treatment with olanzapine is capable of blocking the reinforcing effects of cocaine and illustrates the value of using multiple tests of reinforcement when evaluating the pharmacological effects of newer psychotherapeutic agents. © 1997 Elsevier Science B.V.

Keywords: Olanzapine; Cocaine; Conditioned place preference; Self-administration; Locomotor activity; (Rat)

1. Introduction

Several converging lines of evidence support the hypothesis that the reinforcing properties of cocaine reside in its ability to potentiate mesolimbic dopaminergic transmission by blocking dopamine reuptake (Roberts et al., 1980; Ritz et al., 1987; Pettit and Justice, 1991). Some of this research is based upon studies showing that dopamine receptor antagonists attenuate cocaine's reinforcing effects; however, these results have proven highly sensitive to experimental variables. For example, pre-treatment with dopamine receptor antagonists increases the rate of cocaine self-administration under 'rich' reinforcement schedules, in which the unit dose of cocaine is on the descending limb of cocaine's dose–response function (Roberts and Vickers, 1984; Koob et al., 1987; Britton et al., 1991; Hubner and Moreton, 1991; Caine and Koob, 1994; Glowa and Wojnicki, 1996). In contrast, pre-treatment with dopamine receptor antagonists decreases the rate of cocaine self-administration under 'lean' reinforcement schedules, where the unit dose of cocaine is on the ascending limb of the dose–response function (Woolverton and Virus, 1989; Kleven and Woolverton, 1990; Caine and Koob, 1994). Interpretation of self-administration studies employing fixed-ratio schedules of reinforcement, like those cited, is further complicated since the rate of responding represents an ambiguous measure of reinforcement. Antagonist-induced augmentation of responding is typically interpreted as a compensatory response to a partial blockade of reinforcement (Yokel and Wise, 1976), whereas it has been suggested that dopamine receptor antagonists may actually increase response rates independent of reinforcement by antagonizing cocaine-induced inhibition of operant response rates (Wilson and Schuster, 1972; Glowa and Wojnicki, 1996). Conversely, decreases in the rate of cocaine self-administration may represent a disruption of reinforcement (Roberts et al., 1977) or a global suppression of behavior (Woolverton and Virus, 1989), the latter of which may be particularly important given the effects of dopamine receptor antagonists upon the extrapyramidal motor system (Meil and See, 1994; Ebadi and Srinivasan, 1995). This difficulty in interpreting the effects of dopamine receptor antagonism as seen in cocaine self-administration experimentation illustrates the importance of parametric examination of experimental

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variables and suggests the use of other behavioral paradigms which may provide alternative measures of reinforcement.

The conditioned place preference paradigm avoids many of the interpretive difficulties observed in drug self-administration, since reinforcement is assessed in the non-drugged state (Schechter and Calcagnetti, 1993). Although several reports have failed to demonstrate blockade of cocaine-induced conditioned place preference by dopamine receptor antagonists (Spyraki et al., 1982; Morency and Beninger, 1986), other studies have found conditioned place preference to cocaine was attenuated by dopamine receptor antagonists, including haloperidol (Kosten et al., 1996), 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 23390) (Cervo and Samanin, 1995; Pruitt et al., 1995), sulpiride and eticlopride (Pruitt et al., 1995), pimozide (Morency and Beninger, 1986) and α-flupenthixol (Aulisi and Hoebel, 1983). Employing the progressive ratio schedule of reinforcement in self-administration studies, which measure reinforcement in a manner less dependent on response rate than fixed-ratio schedules, also suggests that dopamine receptor antagonists block cocaine's reinforcing effects. Thus, haloperidol (Roberts et al., 1989), spiperone (Hubner and Moreton, 1991) and SCH 23390 (Hubner and Moreton, 1991) have all been shown to produce a dose-dependent decrease in the 'breaking point' on progressive ratio schedules; this is indicative of decreased reinforcer efficacy. Taken together, studies employing several paradigms are consistent with the hypothesis that dopamine receptor antagonists attenuate the reinforcing effects of cocaine.

Given the effects of dopamine receptor antagonists upon cocaine's reinforcing effects in laboratory animals, some investigators have suggested dopamine receptor antagonists may serve as pharmacotherapies for cocaine dependence in humans. While these studies have yielded mixed results (Gawin, 1986; Gawin et al., 1989, Sherer et al., 1989), the fact that most dopamine receptor antagonists produce extrapyramidal side-effects (Ebadi and Srinivasan, 1995) limits their potential long-term usefulness. In contrast, 'atypical' neuroleptics, such as clozapine and olanzapine, produce fewer, if any, motor side-effects (Bruhwyler et al., 1990; See and Ellison, 1990; Moore et al., 1992) and, therefore, may represent more acceptable pharmacotherapy for cocaine dependence. Atypical neuroleptics, which possess high affinity for dopamine ($D_1/D_2/D_4$), 5-hydroxytryptamine (5-HT), and muscarinic acetylcholine receptors (Meltzer et al., 1989; Van Tol et al., 1991) may produce an enhanced efficacy in treating cocaine dependence since they appear to have a preferential action upon mesolimbic/mesocortical dopamine pathways (Batholoni, 1977; Hand et al., 1987), which have been implicated in cocaine's reinforcing effects (Roberts et al., 1980; Roberts and Koob, 1982). The 5-HT receptor antagonism produced by atypical neuroleptics may also be involved in cocaine's subjective effects since neither dopamine D_1 nor D_2 receptor antagonists alone, or together, completely antagonize the discriminative stimulus effects of cocaine (Witkin, 1994).

The purpose of the present study was to investigate the ability of olanzapine [LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-B][1,5]benzodiazepine], a clozapine-like atypical neuroleptic (Moore et al., 1992; Bymaster et al., 1996), to block the reinforcing effects of cocaine. In this study, the ability of olanzapine to block cocaine-induced conditioned place preference, as well as its ability to produce conditioned place aversion, was examined. In addition, the effects of olanzapine on cocaine self-administration under a fixed-ratio schedule of reinforcement was investigated. Since dopamine receptor antagonists often produce non-specific inhibition of behavior, the effects of olanzapine upon spontaneous locomotor activity, cocaine-induced hyperlocomotion and operant responding for food were also examined.

2. Materials and methods

2.1. Subjects

Sixty-five male, Sprague–Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) weighing 250–300 g at the start of the experiment were individually housed and maintained on a 12:12 h reverse light–dark cycle with lights on at 06.00 h. Rats had free access to food and water except those in the food self-administration experiment and those being trained to lever press for food in the cocaine self-administration experiment. These animals were maintained at 85–90% of their free-feeding weights. Behavioral testing was conducted in laboratories separate from the animal colony during the first half of the dark cycle.

2.2. Conditioned place preference and aversion

Conditioning/testing was conducted using a 3-chambered place conditioning apparatus (Lafayette Instrument Company, Lafayette, IN). The units included a gray center section and two end-chambers, each $20.5 \times 30.5 \times 20$ cm. The end chambers were made distinctive as to sight, texture and smell. The 'dark' chamber was illuminated with a 6 W, 30 V red light bulb and had a smooth, black Plexiglas floor. The 'light' chamber was illuminated with a 6 W, 30 V white light bulb and had a steel-bar grid floor with pine shavings in the drop pan below. The amount of time spent in each end-chamber was measured by weight sensitive microswitches and recorded as the time (s) by computer. Pine shavings were changed after each subject's session and the chambers were washed with warm water and dried. Application of 70% isopropyl alcohol to wash the chambers at the end of each day helped to minimize olfactory cues.

The 'biased' place conditioning protocol was used in the present study. Training and test sessions were performed Monday–Friday. On day 1 of the training procedure, rats were handled for 10 min in the testing room. On days 2 and 3, rats were allowed free access to the 3-chambered apparatus for 30 min in order to habituate them to the test apparatus. On day 4, the amount of time spent in each chamber was recorded over 30 min. The chamber in which rats spent most of their time was defined as its 'preferred' side. Animals who spent more than 80% of their time in one chamber were removed from the study. On four of the next eight training days, each rat received an intraperitoneal (i.p.) injection of cocaine (10 mg/kg) or saline and were placed back into their home cage for 30 min. Rats were then given an i.p. injection of olanzapine (1.5, 3.0, 4.5 mg/kg) or saline and confined, by insertion of metal dividers, to their 'non-preferred' end chamber for 30 min. Subjects were randomly assigned to the treatment conditions. Cocaine–HCl (National Institute on Drug Abuse) was dissolved in saline; olanzapine (Eli Lilly, Indianapolis, IN) was dissolved in a mixture of Tween 80 (10%) and saline. On alternate days, each subject received an i.p. saline injection; 30 min later, rats were given an i.p. olanzapine vehicle (Tween 80) injection and were confined to their preferred end-chamber for 30 min. All injections were delivered in a 1 mg/kg volume. On day 13 of testing, animals were given a post-conditioning preference test in which they were placed in the center chamber and given free access to all chambers for 30 min. The time spent in each chamber was recorded and the amount of time spent in the non-preferred chamber was later compared to the time measured on day 4, the baseline test day.

The procedure used to test conditioned place aversion was identical to that used in testing conditioned place preference except that rats received olanzapine (3.0 or 4.5 mg/kg) on four of the eight training days 30 min prior to being restricted to their preferred end chamber. On alternate training days, rats received an i.p. injection of saline 30 min before being restricted to their non-preferred side. The time spent on the preferred side on days 4 and 13 was then compared.

2.3. Drug self-administration

Operant chambers consisted of Plexiglas enclosures (30 × 20 × 20 cm) with a houselight and single lever which extended 2.5 cm into the chamber (Med Associates, St. Albans, VT). A food pellet dispenser from which 45-mg food pellets (P.J. Noyes, Lancaster, NH) were dispensed was located to the left of the lever. Each chamber was enclosed in a separate sound-attenuating enclosure and was equipped with a small fan to decrease external noise. An infusion pump (Med Associates, St. Albans, VT) delivered cocaine through a polyethylene tube attached to a weighted swivel apparatus located above each chamber. From the swivel, a polyethylene tube, encased in a wire coil, was screwed into the external mount of the animal's guide cannulae. Experimental events and data collection were controlled by a computer running OPN software (Emmett-Oglesby et al., 1982).

Prior to surgery, animals were trained to lever press for food pellets on a fixed-ratio 2 schedule. Each animal received a minimum of three 3-h food training sessions in which at least 100 food pellets were earned. Rats were then anesthetized with ketamine (100 mg/kg) and (2 mg/kg) xylazine. Chronic polyethylene guide cannulae were implanted in the right jugular vein for delivery of cocaine based on previously described procedures (Caine et al., 1993). Briefly, catheters were constructed from two thicknesses of polyethylene tubing (PE-10, PE-50) fused together at their intersection and coated with silastic. The smaller tubing was inserted into the vein and the catheter secured to tissue near the entry point of the vein. The catheter was then run subcutaneously to an exit point on the rat's back where it was attached to an external guide cannula. This junction was glued and surrounded by a 2.5 cm square of Marlex mesh using cranioplastic cement. Intramuscular penicillin (10,000 units) was administered following surgery. Catheters were flushed twice daily with 0.1 ml heparinized saline (10 units/ml) for the 5 days following surgery and then after each self-administration session to prevent clotting. Catheters were also flushed with 0.1 ml saline prior to each self-administration session. A 'dummy' stylet was inserted into the guide cannula when the rat was not connected to the infusion system.

Five days post-surgery, rats were placed into the self-administration chambers for a 3 h daily session of cocaine self-administration. During each session, animals had the opportunity to lever press for cocaine–HCl (0.33 mg/0.05 ml bolus over 3 s) on a fixed-ratio 2 schedule of reinforcement. Following at least 3 consecutive days of stable responding (±5%), animals received an i.p. injection of one of 3 doses of olanzapine (1.5, 3.0, 6.0 mg/kg) or saline 30 min prior to the self-administration session. The doses of olanzapine (administered in random order) were tested with at least two days of stable responding between each drug challenge. No drug challenge data were used from rats whose responding did not recover within 2 days. The procedure used to assess the effects of olanzapine on operant responding for food was the same as that used in cocaine self-administration animals except rats responded for food pellets on a fixed-ratio 10 schedule and they were not catheterized.

2.4. Activity testing

Rats used in the conditioned place preference procedure were also used for activity testing. Two days after the completion of conditioned place preference tests, rats were placed in activity boxes (Columbus Instruments Corp., Columbus, OH) for 1 h to habituate to the environment. Four transparent Plexiglas activity boxes (43 × 43 × 20

cm) with 16 evenly-spaced photocell beams going across both the width and length of the boxes approximately 4.0 cm above the chamber floor were used. Horizontal activity was measured as the distance traveled, in cm, by counting interruptions of the photocell beams that were, in turn, interfaced to a computer-controlled system (Columbus Instruments International Corporation, Columbus, OH). The following day, rats received an i.p. injection of cocaine (10 mg/kg) or saline (1 ml/kg injection volume) and were placed back into their home cage. Thirty min later, the rats were given a second i.p. injection of olanzapine (1.5, 3.0, 4.5 mg/kg) or saline and were placed into the activity chamber for 60 min. Rats previously tested for conditioned place aversion received an i.p. injection of olanzapine (3.0 or 4.5 mg/kg) 30 min prior to being placed into the activity chamber for 60 min. Each animal was administered the same experimental treatment that they had received during conditioned place preference-testing.

2.5. Data analysis

Conditioned place preference was analyzed using a mixed model repeated measures analyses of variance (ANOVA) to compare the amount of time (s) spent in the non-preferred side, pre- (day 4) and post- (day 13) conditioning between treatment conditions. A similar ANOVA was conducted to assess conditioned place aversion by comparing the time spent on the preferred side, pre- and post-conditioning, between treatment conditions. A one-way repeated measures ANOVA was performed on the total number of cocaine infusions per 3 h daily session for animals pre-treated with olanzapine or saline. A one-way repeated measures ANOVA was applied to the number of food pellets earned per 1 h daily session for animals pre-treated with olanzapine versus saline. Locomotor activity (distance traveled in cm) between treatment groups was compared using a between subjects ANOVA. Post-hoc comparisons for individual groups were conducted using the Student–Newman–Keuls method for pairwise comparisons. The criterion for statistically significant differences was set at $P < 0.05$.

3. Results

3.1. The effects of olanzapine on conditioned place preference for cocaine

Fig. 1 represents the increase in the amount of time (s) spent on the non-preferred side prior to and after conditioning with drugs and between treatment conditions. There was no significant difference in the amount of time spent on the non-preferred side pre- and post-conditioning in the saline-saline treated animals (30.74% increase). Thus, the effect of multiple injections and time did not appear to alter the rats' baseline non-preference for a particular side.

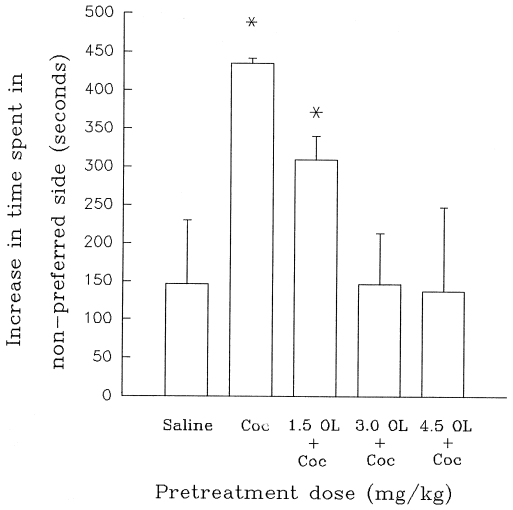

Fig. 1. The effect of olanzapine (OL) pre-treatment on conditioned place preference for 10 mg/kg cocaine. Rats given 10 mg/kg of cocaine showed a significant increase in the amount of time spent on the non-preferred (cocaine-paired) side pre- versus post-conditioning ($n = 7$). Pre-treatment with 1.5 mg/kg of olanzapine and cocaine also resulted in a significant increase in the amount of time spent on the non-preferred side ($n = 8$). Pre-treatment with 3.0 ($n = 7$) and 4.5 ($n = 8$) mg/kg of olanzapine did not significantly alter the amount of time spent on the non-preferred side. There was no change in the amount of time spent on the non-preferred side in rats pre-treated with saline in both chambers ($n = 5$). Asterisks indicate a significant difference in the amount of time spent on the non-preferred side pre- versus post-conditioning ($P < 0.05$).

There was a significant overall difference in the amount of time spent on the non-preferred side pre- versus post-conditioning ($F(1, 29) = 26.86$; $P < 0.0001$). Conditioned place preference was observed in rats pretreated with cocaine and 1.5 mg/kg olanzapine in combination with cocaine. Rats administered 10 mg/kg of cocaine and confined to their non-preferred side, showed a 104% increase in the amount of time spent on the non-preferred side post-conditioning ($P < 0.05$). Likewise, rats pretreated with 1.5 mg/kg olanzapine and then given 10.0 mg/kg cocaine showed a 72.5% increase in the amount of time spent on the non-preferred side post-conditioning ($P < 0.05$). In contrast, pre-treatment with the two higher doses of olanzapine (3.0 and 4.5 mg/kg) blocked conditioned place preference for cocaine as indicated by time spent in their non-preferred side post-conditioning, 29.1 and 28.7%, respectively.

3.2. Conditioned place aversion to olanzapine

Fig. 2 represents data indicating the decrease in the amount of time (s) spent in the baseline-determined preferred side pre- versus post-conditioning in rats pre-treated only with one of two doses of olanzapine. There was a significant difference in the amount of time spent on the preferred side pre- versus post-conditioning ($F(1, 23) = 7.64$; $P < 0.05$) in the rats receiving 4.5 mg/kg of olanzapine in that they showed a 50.4% decrease in the amount

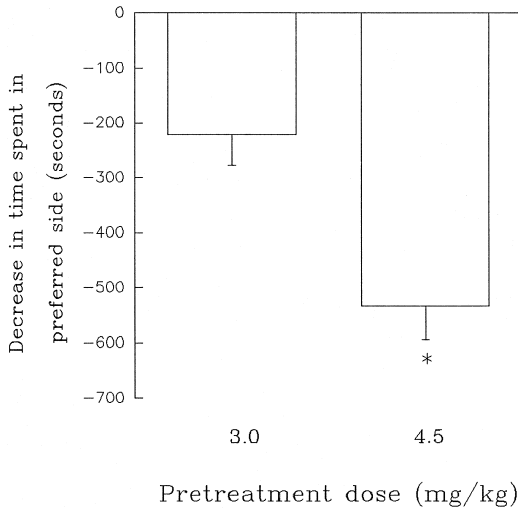

Fig. 2. Conditioned place aversion following pre-treatment with 3.0 or 4.5 mg/kg olanzapine. Pre-treatment with 4.5 mg/kg of olanzapine produced a significant decrease in the amount of time spent on the preferred (drug-paired) side pre- versus post-conditioning (n = 6). Pre-treatment with 3.0 mg/kg of olanzapine did not significantly alter the amount of time spent on the preferred side ($n = 6$). The asterisk indicates a significant difference in the amount of time spent on the preferred side pre- versus post-conditioning ($P < 0.05$).

of time spent in the previously preferred chamber. In contrast, pre-treatment with 3.0 mg/kg of olanzapine did not produce a significant conditioned place aversion. Rats pretreated with 3.0 mg/kg olanzapine showed a 24.4% decrease in the amount of time spent in the previously preferred chamber.

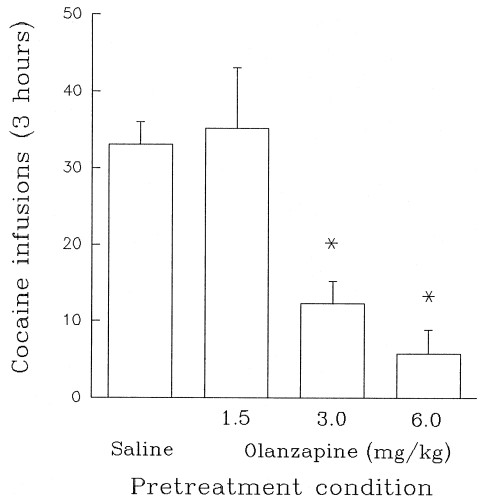

Fig. 3. The effect of olanzapine pre-treatment on the number of cocaine infusions (0.33 mg/infusion) earned during 3 h daily limited access sessions on a fixed-ratio 2 schedule of reinforcement. The number of cocaine infusions earned during cocaine self-administration did not significantly differ between rats pre-treated with saline ($n = 6$) and those pre-treated with 1.5 mg/kg of olanzapine ($n = 7$). Rats pre-treated with 3.0 ($n = 9$) and 6.0 ($n = 8$) mg/kg of olanzapine received significantly fewer cocaine infusions than those pre-treated with saline. Asterisks indicate a significant differences in the number of cocaine infusions compared to saline pre-treated rats ($P < 0.05$).

3.3. The effects of olanzapine on cocaine self-administration

The mean (±S.E.M.) number of cocaine infusions received during the 3 h baseline cocaine self-administration sessions was 31.18 ± 3.44. The mean number of infusions earned during baseline sessions of cocaine self-administration did not differ between treatment groups prior to the drug challenge test days. Fig. 3 shows the effects of saline or olanzapine pre-treatment on the number of cocaine infusions received during drug self-administration. Olanzapine produced a significant decrease in the number of drug infusions ($F(3, 15) = 8.53$; $P < 0.001$). Post-hoc comparisons revealed rats pre-treated with either 3.0 or 6.0 mg/kg earned significantly fewer infusions than rats pre-treated with saline or 1.5 mg/kg of olanzapine.

3.4. Effects of olanzapine on operant responding for food

Pre-treatment with olanzapine produced a significant dose-dependent decrease in operant responding for food ($F(3, 15) = 5.64$, $p < 0.01$). Fig. 4 shows the number of reinforcers earned on a fixed-ratio 10 schedule of reinforcement following pre-treatment with either saline or one of three doses of olanzapine. Rats pre-treated with 4.5 mg/kg earned significantly fewer food pellets than rats

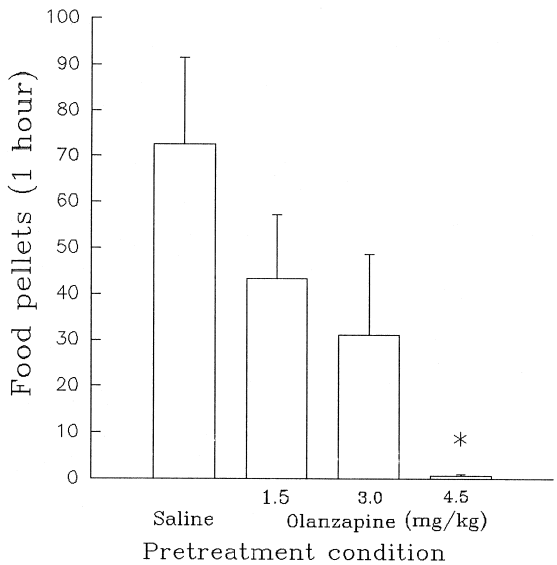

Fig. 4. The effect of olanzapine pre-treatment on the number of food pellets earned by rats responding on a fixed-ratio 10 schedule of reinforcement during daily 3 h sessions. The number of food pellets earned by rats pre-treated with saline did not significantly differ from those pre-treated with 1.5 and 3.0 mg/kg of olanzapine. Rats pre-treated with 4.5 mg/kg of olanzapine received significantly fewer food pellets than rats pre-treated with saline or 1.5 or 3.0 mg/kg of olanzapine ($n = 6$). Asterisk indicates a significant difference in the number of food pellets earned compared to saline pre-treated rats ($P < 0.05$).

pre-treated with either saline, 1.5 or 3.0 mg/kg of olanzapine. In addition, rats pre-treated with 3.0 mg/kg of olanzapine earned significantly fewer food pellets than rats pre-treated with saline ($P < 0.05$).

3.5. Effects of olanzapine on cocaine-induced hyperactivity and spontaneous locomotor activity

Pre-treatment with olanzapine 30 min prior to locomotor testing significantly altered spontaneous activity, as well as cocaine-induced hyperactivity in 1 h test sessions ($F(6, 40) = 7.24$; $P < 0.0001$) (Fig. 5). Cocaine pre-treatment (10 mg/kg) produced a 2.5 fold increase in locomotor activity compared to saline ($P < 0.05$). The highest dose of olanzapine tested completely blocked cocaine's ability to produce locomotor hyperactivity. Pre-treatment with 4.5 mg/kg of olanzapine prior to cocaine administration attenuated locomotor activity compared to rats receiving cocaine alone and those treated with 1.5 and 3.0 mg/kg of olanzapine in combination with cocaine ($P < 0.05$). Locomotor activity in animals pre-treated with 1.5 and 3.0 mg/kg of olanzapine and cocaine did not significantly differ from those pre-treated with cocaine alone. Locomotor activity in olanzapine (3.0 and 4.5 mg/kg) treated animals was attenuated, but was not significantly different from activity in saline-treated animals habituated to the testing environment.

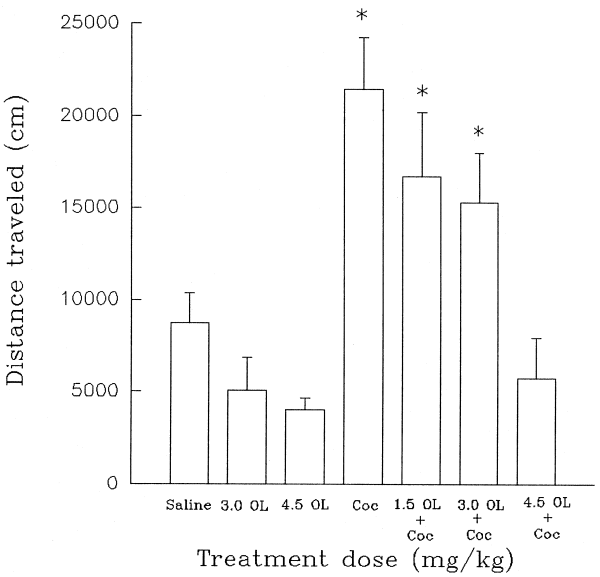

Fig. 5. The effect of olanzapine (OL) on spontaneous and cocaine-induced hyperactivity during 1 h locomotor activity sessions. Rats pre-treated with cocaine (10 mg/kg) ($n = 7$) showed increased locomotor activity, as measured by the distance traveled (cm), than rats pre-treated with saline ($n = 5$). Locomotor activity in rats pre-treated with 1.5 ($n = 8$) and 3.5 ($n = 7$) mg/kg of olanzapine in combination with cocaine also were observed to have a significantly greater activity level than rats pre-treated with saline. Locomotor activity in rats ($n = 8$) pre-treated with 4.5 mg/kg olanzapine and cocaine was significantly attenuated compared to rats pre-treated with cocaine or 1.5 and 3.0 mg/kg of olanzapine and cocaine ($P < 0.05$), but was not significantly different from rats pre-treated with saline. Pre-treatment with 3.0 ($n = 6$) and 4.5 ($n = 6$) mg/kg of olanzapine alone did not significantly alter locomotor activity when compared to saline treated rats. Asterisks indicate significantly different locomotor activity compared to saline-treated rats ($P < 0.05$).

4. Discussion

The results of the present study indicate that pre-treatment with olanzapine can attenuate the ability of cocaine to produce a conditioned place preference in rats; this suggests that olanzapine can effectively block the reinforcing properties of cocaine. However, the blockade of conditioned place preference by the highest dose of olanzapine (4.5 mg/kg) cannot be exclusively attributed to inhibition of cocaine's reinforcing properties since, at this dose, olanzapine alone produced a conditioned place aversion. Therefore, this dose may be decreasing cocaine's reinforcing activity by a simple oppositional action. This mechanism of antagonism does not serve to explain the effects of 3.0 mg/kg olanzapine pre-treatment, which was shown, by itself, not to be aversive (Fig. 2) but to significantly attenuate cocaine's reinforcing effects in the conditioned place preference-task (Fig. 1). Olanzapine's antagonism of conditioned place preference to cocaine is consistent with that previously reported for another 'atypical' neuroleptic clozapine (Kosten and Nestler, 1994). Olanzapine was 3–4 times more potent than clozapine at blocking conditioned place preference to cocaine, which is consistent with the differences in potency for blocking cocaine's discriminative stimulus properties (Meil et al., 1997). Taken together, these results suggest that the mixed dopamine and 5-HT receptor antagonism may be effective in attenuating cocaine's reinforcing effects.

The present study also found that olanzapine significantly attenuated cocaine self-administration on a fixed-ratio schedule of reinforcement. These results are consistent with other studies showing self-administration of cocaine at low and intermediate unit injection doses of cocaine is attenuated by pre-treatment with dopamine receptor antagonists (Woolverton, 1986; Corrigall and Coen, 1991; Glowa and Wojnicki, 1996; Hemby et al., 1996). This decrease is often interpreted to reflect an antagonism of cocaine's reinforcing effects. However, olanzapine, like other dopamine receptor antagonists that decrease cocaine self-administration (Glowa and Wojnicki, 1996; Hemby et al., 1996) also produced a dose-dependent decrease in operant responding for food. This suggests that the decrease in cocaine self-administration produced by olanzapine may, in fact, be due to non-selective behavioral effects. In support of this hypothesis, the potency of olanzapine for producing deficits in responding for cocaine and food was similar. Thus, the lowest dose of olanzapine (1.5 mg/kg) did not significantly alter cocaine or food intake; the

middle dose of olanzapine (3.0 mg/kg) attenuated responding by 35 and 57% for cocaine and food, respectively, and the highest dose of olanzapine (4.5 mg/kg) produced profound and near complete suppression of responding for both reinforcers. Although olanzapine is markedly less potent than typical neuroleptics for producing extrapyramidal side-effects, such as catalepsy (ED_{50} = 34.9 mg/kg) (Moore et al., 1992), animals treated with olanzapine (3.0, 4.5 mg/kg) show little spontaneous locomotor activity and an attenuation of cocaine-induced behavioral hyperactivity at a dose of 4.5 mg/kg olanzapine. The effects of olanzapine on cocaine-induced hyperactivity and spontaneous locomotor activity are consistent with those reported by Arnt (1995) who found that olanzapine attenuated D-amphetamine hypermotility and inhibited spontaneous locomotor activity at doses similar to those that inhibited activity following a high dose of D-amphetamine. Because olanzapine produces non-specific behavioral deficits which may interfere with operant behavior, the ability of olanzapine to antagonize the reinforcing effects of self-administered cocaine are difficult to assess using only the drug self-administration paradigm.

The present results illustrate the importance of employing multiple measures of reinforcement and appropriate controls when evaluating the effects of pharmacological antagonists upon drug-induced reinforcement. The drug self-administration paradigm involves an operant task and provides a measure of the response reinforcing properties of a drug. Because antagonists can interfere with operant behavior independent of a drug's reinforcing effects, tests evaluating the ability of an antagonist to produce non-specific inhibition of behavior, or changes in operant response rates, are necessary to rule out alternate explanations for alterations in drug self-administration. In the present study, olanzapine produced a dose-dependent decrease in cocaine self-administration, operant responding for food, and spontaneous locomotor activity; all tests suggesting that attenuation of cocaine self-administration by olanzapine may result from non-specific effects on behavior. Therefore, the self-administration paradigm does not provide an adequate test of olanzapine's effects on cocaine-induced reinforcement. The conditioned place preference paradigm, a Pavlovian-type task, measures the ability of a drug to increase the extent to which an animal is attracted to a drug-associated environment when the animal is tested in a non-drugged state (Schechter and Calcagnetti, 1993). This advantage is apparent in its preclusion of drug effects upon locomotor activity at the time of testing. While Skinnerian (operant) and Pavlovian reinforcement represent processes that may be fundamentally different in theory and procedure (Wise and Hoffman, 1992), the fact that self-administration and conditioned place preference studies generally yield consistent results (Carr et al., 1989) suggests that these two types of reinforcement may share a common neural substrate.

Recently, it has been suggested that the aversive properties of an antagonist represent a further confound in studies investigating drug reinforcement. For example, Pizzi and Cook (1996) have shown that isradipine and carbamazepine produce conditioned taste aversion at the same doses at which they block place preference and self-administration of morphine and cocaine. Therefore, the ability of an antagonist to block drug consumption or place preference may be explained by its untoward or toxic effects rather than its apparent blockade of the neural substrates for reinforcement. This confound may be particularly important when evaluating the effects of dopamine receptor antagonists since these compounds are often dysphorigenic (Rech, 1982). The importance of a test for aversion is underscored in the present study since olanzapine produced conditioned place aversion at the highest of two doses of olanzapine that blocked conditioned place preference for cocaine.

Olanzapine and clozapine possess similar neurochemical and behavioral profiles which differ from those associated with 'typical' neuroleptics. Both olanzapine and clozapine exhibit high affinity for dopamine D_1, D_2, D_4, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, muscarinic, α_1-adrenergic and histamine H_1 receptors (Bymaster et al., 1996). In contrast, haloperidol, the prototypical 'typical' neuroleptic has high affinity for dopamine D_2 and D_4 receptors, moderate affinity for dopamine D_1 and α_1-adrenoceptors and relatively low affinity for 5-HT, muscarinic and histamine receptors (Bymaster et al., 1996). Olanzapine has been shown to elicit behavioral effects similar to clozapine and different from 'typical' neuroleptics on several measures, including blockade of phencyclidine-induced hyperlocomotion (Gleason and Shannon, 1997), increased rates of punished responding (Moore et al., 1994; Bevenga and Leander, 1995), inhibition of *d*-amphetamine-induced hyperactivity (Arnt, 1995), antagonism of 5-hydroxytryptophan-induced head twitching (Moore et al., 1992) and oxotremorine-induced tremor (Moore et al., 1994). Olanzapine also appears to antagonize the behavioral effects of cocaine in a manner similar to that produced by clozapine and some evidence suggests that these effects may differ from those observed following administration of typical neuroleptics. For example, both olanzapine (present results) and clozapine (Kosten and Nestler, 1994) block conditioned place preference for cocaine, whereas typical neuroleptics have been shown to block conditioned place preference for cocaine in some studies (Cervo and Samanin, 1995; Pruitt et al., 1995; Kosten et al., 1996), but not in others (Spyraki et al., 1982; Morency and Beninger, 1986). However, studies failing to demonstrate that typical neuroleptics block the development of cocaine-induced conditioned place preference have been conducted using the same species and experimental protocol (biased conditioned place preference procedure, number of drug and compartment pairings, and confinement time) as were used in the present study. Olanzapine and clozapine also block the discriminative stimulus effects of cocaine in rats (Meil

et al., 1997), although in monkeys clozapine produces only a partial blockade of cocaine discrimination (Vanover et al., 1993). Much evidence in rats suggests that blockade of cocaine discrimination by typical neuroleptics appears to be less consistent and complete (e.g. Witkin et al., 1991; Geter-Douglass and Riley, 1996).

As with the present findings with olanzapine, Roberts and Vickers (1984) reported that rats pre-treated with clozapine show a dose-dependent decrease in cocaine self-administration on a fixed-ratio schedule (Roberts and Vickers, 1984). However, their study also reported that, under the same experimental conditions, several 'typical' neuroleptics produced a dose-dependent augmentation of cocaine self-administration. Differences in the effects of 'typical' and 'atypical' neuroleptics may also be observed on progressive ratio schedules of cocaine self-administration. Although it remains difficult to explain, clozapine has been shown to increase the motivation to self-administer cocaine on a progressive ratio schedule of reinforcement (Loh et al., 1992). In contrast, haloperidol has been shown to attenuate cocaine self-administration on this schedule (Roberts et al., 1989). Taken together with the present results, these studies suggest that the 'atypical' neuroleptics may be capable of antagonism of cocaine's reinforcing effects in a manner that is different from that seen with 'typical' neuroleptics.

Olanzapine's ability to block the reinforcing properties of cocaine could result from one, or a combination of several, pharmacological effect(s). One likely candidate is the dopamine D_1 receptor, in that specific antagonists of this receptor have been shown to block the reinforcing effects of cocaine using drug self-administration (Koob et al., 1987; Corrigall and Coen, 1991; Caine and Koob, 1994) and conditioned place preference (Cervo and Samanin, 1995; Pruitt et al., 1995). Nevertheless, the dopamine D_2 receptor has also been implicated in the reinforcing effects of cocaine (Roberts and Vickers, 1984; Britton et al., 1991) yet olanzapine has a relatively low affinity at this site (Bymaster et al., 1996). In addition, several studies suggest that dopamine D_1 receptors may be more critical in cocaine-induced reward than dopamine D_2 receptors (Koob et al., 1987; Cervo and Samanin, 1995). While olanzapine has high affinity for dopamine D_4 receptors, little is known regarding the contribution of these receptors to reinforcement. Olanzapine also has high affinity for several 5-HT receptor subtypes (Bymaster et al., 1996). However, research implicating 5-HT neurotransmission in the reinforcing effects of cocaine has been limited such that neither 5-HT_2 (Porrino et al., 1989) nor 5-HT_3 (Peltier and Schenk, 1991) receptor antagonists alter cocaine self-administration in rats. Although 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT), a 5-HT_{1A} receptor antagonist, has been found to attenuate cocaine self-administration (Peltier and Schenk, 1993), olanazapine does not possess high affinity for this receptor (Bymaster et al., 1996). Forebrain serotonergic lesions have also been found to increase cocaine self-administration in rats responding under a progressive-ratio schedule (Loh and Roberts, 1990). However, pre-treatment with a mixed 5-HT_1/5-HT_2 receptor antagonist, as well as a 5-HT_2 and a 5-HT_3 receptor antagonists failed to alter breaking points maintained by cocaine on progressive ratio schedules (Lacosta and Roberts, 1993).

The present results indicate that a 3.0 mg/kg dose of olanzapine can attenuate reinforcing effects of cocaine. At this dose olanzapine blocked cocaine conditioned place preference without producing its own conditioned aversive effect, as well as altering cocaine-induced hyperactivity, although this dose did attenuate operant responding for food. Therefore, olanzapine may show promise as a potential pharmacotherapy for cocaine dependence.

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