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Ondansetron Given in the Acute Withdrawal from a Repeated Cocaine Sensitization Dosing Regimen Reverses the Expression of Sensitization and Inhibits Self-administration

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Male Sprague-Dawley rats were given two separate sensitizing regimens of cocaine (7 days on, 7 days off, 7 days on; 40 mg/kg/day s.c.) along with saline controls. Furthermore, animals also received the 5-HT_3 antagonist ondansetron (0.2 mg/kg s.c.) either during the second dosing regimen (3.5 h after each cocaine/saline injection) or during the first five days of the second withdrawal period. Animals were then challenged, on day 10 of withdrawal, with cocaine (7.5 mg/kg i.p.) and assessed by a behavioral rating scale and locomotor activity monitoring. The cocaine regimen induced behavioral and locomotor sensitization on day 10 of withdrawal, further, ondansetron inhibited sensitization regardless of whether given after each second cocaine regimen dose or during the second withdrawal period, although treatment 3.5 h after each cocaine injection appeared more effective. Ondansetron did not inhibit behavior in control animals. In a second experiment animals were trained to self-administer cocaine via an indwelling jugular catheter. After stable fixed-ratio responding (FR1 then FR2) they were given a progressive ratio (PR) schedule until PR each day was stable. During the first five days of withdrawal they were given either ondansetron (0.2 mg/kg s.c.) or saline injections. On day 10 of withdrawal the cocaine PR schedule was reinstated. The ondansetron treated rats showed only a non-significant decrease in break point. After day 2 of the PR session rats were again injected with either ondansetron (0.2 mg/kg s.c.) or saline, 3.5 h after each PR session for five days. Ondansetron inhibited cocaine self-administration on each of the following days. Ondansetron may be a useful treatment for cocaine addicts who have undergone previous sensitization periods.

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KEY WORDS: *Cocaine; Sensitization; Self-administration; Withdrawal; Ondansetron; Opponent processes*

The reinforcing and psychomotor stimulant effects of cocaine are thought to act mainly through the dopamine (DA) transporter and can increase the extracellular DA concentration in the nucleus accumbens which is thought to mediate cocaine's rewarding effects. However evidence from transgenic mice show that cocaine self-administration is evident in DA transporter knockout mice (Rocha et al. 1998) and these mice can also acquire conditioned place preference for cocaine (Sora et al. 1998). Further, 5-HT_{1B} receptor knockout mice show a propensity to self-administer cocaine (Rocha et al. 1997; Castanon et al. 2000). Taken together these data

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suggest that other, non-DA, mechanisms are involved in cocaine self-administration.

The case for 5-HT involvement is substantial: cocaine has a high affinity for the 5-HT transporter (Koe 1976) and the cocaine binding site on the SERT is now known (Chen et al. 1997; Rasmussen et al. 2001). Cocaine also has antagonistic properties at the 5-HT_3 receptor (Malone et al. 1991; Mair et al. 1998; Breitinger et al. 2001). The distribution of 5-HT_3 receptors within rat corticolimbic areas is relatively high (Kilpatrick et al. 1987) and 5-HT_3 receptor ligands have been shown to influence DA efflux therein. The 5-HT_3 agonists 2-methyl-5-HT and mCPBG increased basal and K^+ evoked DA efflux in rat striatal slices (King et al. 1995) and basal DA efflux in vivo (Jiang et al. 1990) and these effects were blocked by selective 5-HT_3 antagonists. Local infusion of the 5-HT_3 agonist 1-phenyl-biguanide, into the accumbens (Chen et al. 1991) increased DA efflux as measured by microdialysis, an effect again blocked by selective 5-HT_3 antagonists. Furthermore, antagonists of the 5-HT_3 receptor can inhibit the increase in striatal DA efflux caused by stimulation of the raphe nucleus (De Deurwaerdere et al. 1998) and also block increases in DA efflux induced by cocaine (McNeish et al. 1993; Kankaanpaa et al. 1996), morphine (Imperato and Angelucci 1989), nicotine (Carboni et al. 1989) and ethanol (Yoshimoto et al. 1992). Interestingly, ondansetron has been used clinically to treat alcoholism with some success (see Johnson and Ait-Daoud 2000 for a review) and has been shown to reduce withdrawal symptoms in morphine (e.g. Pinelli et al. 1997) and cocaine (e.g. Costall et al. 1990) withdrawn rats.

The ability of 5-HT_3 ligands to influence behavior may be dependent upon their time of dosing (e.g. before, during or after) in relation to the behavior being tested. Costall et al. (1987) and Reith (1990) have shown that 5-HT_3 antagonists, when given alone, have no effect on locomotor behavior but decrease stimulant-induced locomotion; however, others have found ondansetron and another 5-HT_3 antagonist, ICS 205-930, to be ineffective in inhibiting cocaine-induced locomotion (Le et al. 1996). Furthermore, 5-HT_3 antagonists have been found to both inhibit (Suzuki et al. 1992) and be ineffective (Cervo et al. 1996) in cocaine-induced conditioned place preference (CPP) and, when given 30 min *prior to* cocaine self-administration have thus far been ineffective in modifying cocaine self-administration behavior (Peltier and Schenk 1991; Lane et al. 1992; Depoortere et al. 1993; Lacosta and Roberts 1993). Interestingly, Costall et al. (1990) found that both acute and chronic ondansetron, disinhibited suppressed behavior manifested in cocaine withdrawal.

The influence of 5-HT_3 receptor ligands on animals *chronically* treated with cocaine has also been examined. Research has shown that the cocaine administration dosing regimen influences its behavioral outcome: chronic continuous cocaine (via osmotic minipumps) results in behavioral tolerance to a subsequent cocaine challenge whereas intermittent daily injections of cocaine result in behavioral sensitization (King et al. 1992). We have previously shown that ondansetron, given with intermittent or continuous cocaine, was able to block the development of both behavioral sensitization and tolerance (King et al. 1997). However, pharmacotherapy during the withdrawal period would represent a more important opportunity for treating human cocaine addicts. We have found ondansetron given during the first five days of withdrawal to block the expression of sensitization and tolerance (King et al. 1998) and this blockade was long lasting (e.g. 21 days, King et al. 2000). The hypothetical relation of sensitization in animal models to addictive behaviors has been repeatedly discussed and recently reviewed by Vanderschuren and Kalivas (2000).

Human cocaine abusers typically undergo *repeated* periods of abuse and withdrawal. Therefore to more accurately model these conditions we have subjected rats to a chronic cocaine regimen and withdrawal which should establish sensitization (e.g. De La Garza and Cunningham 2000; Morrow et al. 1997), then a second regimen and withdrawal. The first question we addressed was whether a previously established sensitization (i.e. after the first regimen) could be reversed if treated during the first five days of the second regimen withdrawal. Next we questioned whether ondansetron given in acute withdrawal 3.5 h after each cocaine dose during the second dosing regimen could reverse the sensitization from the first regimen. In this paradigm, the rats receive only one regimen before treatment is started in the second regimen. In a second, related experiment we examined the effect of ondansetron on cocaine progressive-ratio (PR) self-administration. To parallel the first study ondansetron was given during the first five days of a second cocaine withdrawal period and also 3.5 h *after* each cocaine self-administration session.

METHODS

Subjects

Male Sprague-Dawley rats (Charles River Laboratories) weighing ~200 g at the start of cocaine treatment were used throughout. Animals had unlimited access to food (standard lab chow) and water and were on a 12/12 h light/dark cycle with lights on at 7 A.M. Animals were treated in accordance with the Guide for Care and Use of Laboratory Animals (NIH). Experiment 1. Cocaine Dosing and Challenge: Animals were first acclimatized to the vivarium for one week before dosing and kept two per cage throughout the experiment with animals from the same experimental group sharing a cage. Ex-

periment 2. Cocaine Self-Administration: Animals were again acclimatized to the vivarium in pairs for one week and then implanted with an indwelling jugular catheter. After surgery animals were housed separately.

Drugs

Cocaine HCl (NIDA) was dissolved (20 mg/ml) in 0.9% sterile saline. Ondansetron hydrochloride dihydrate (+/−) 1,2,3,9-tetrahydro-9-methyl-3[2-methyl-1H-imidazol-1-yl)methyl]-4Hcarbazol-4-one, monohyydrochloride dihydrate, (a gift from Glaxo Wellcome Ltd, UK) stock solution was dissolved in distilled water with further dilutions made in 0.9% saline prior to injections (0.2 mg/ml). All doses were calculated as the salt, and injection volume (∼0.3–0.6 ml) was based on body weight.

Pretreatment Dosing Regimen

There were eight groups of animals in total; four groups received cocaine for seven days were withdrawn for seven days, and were again given seven days of cocaine injections (40 mg/kg/day s.c. at 9 A.M.); four groups received an identical regimen with saline injections. Further, one cocaine group received ondansetron (0.2 mg/kg/day s.c.) during the second cocaine week 3.5 h after each cocaine injection. Another cocaine group received ondansetron during the first five days of the second withdrawal period (at 9 A.M.). The other two cocaine groups received parallel saline injections. Similarly, two of the saline groups received ondansetron either during the second dosing regimen or during the second withdrawal period. The other two groups received saline-saline injections for controls. The dosing regimens are summarized in Table 1. The 40 mg/kg s.c. cocaine dose was chosen because we have previously found this to produce a robust sensitization (King et al. 1992). We have also used this dose to be consistent with previous behavioral, neurochemical and electrophysiological studies from this lab (see Davidson et al. 2000 for review). The single 0.2 mg/kg/day ondansetron dose was chosen because our previous research had demonstrated the relative lack of a dose response effect and 0.2 mg/kg was in the middle of previously assessed doses (King et al. 1997, 1998). All pretreatment injections were given to the animals in the home cage. Acute withdrawal is defined as the withdrawal period (hours) immediately after cocaine administrations whereas chronic withdrawal is the period (days) following a cocaine dosing regimen.

Behavioral Testing

On day 10 of the second withdrawal, rats were acclimated to the test room in their home cage for 30 min under normal light conditions then placed in activity monitoring boxes for 30 min to acclimatize them to the test apparatus. The plexiglass boxes (43 × 43 × 21 cm) were located inside Opto-Varimex 'minor' activity monitors (15 photo beams, 2.5 cm apart, on each side, Columbus Instruments). For each experiment behavioral rating and locomotion was monitored for each animal for 15 min prior to cocaine challenge to determine the baseline activity levels in the absence of any drug. Animals were then given an acute cocaine challenge (7.5 mg/kg i.p.) and their behavior was monitored over the next 60 min. This represents a relatively low dose which

Table 1. Pretreatment Dosing Regimen.

Group	n	Week 1	Week 2	Week 3		Week 4	Pretreatment		Graph key	
1	12	coc	wd	coc + ond		−	coc-coc/ond		coc-coc/ond	
2	7	coc	wd	coc + sal		−	coc-coc/sal		coc-control	
3	7	coc	wd	coc		ond	coc-coc-ond		coc-coc-ond	
4	6	coc	wd	coc		sal	coc-coc-sal		coc-control	
5	7	sal	wd	sal + ond		−	sal-sal/ond		sal-sal/ond	
6	7	sal	wd	sal + sal		−	sal-sal/sal		sal-control	
7	8	sal	wd	sal		ond	sal-sal-ond		sal-sal-ond	
8	7	sal	wd	sal		sal	sal-sal-sal		sal-control	
Cocaine self-administration (SA) time-line										
ond	5	FR1	FR2	wd	PR	ond/sal	no inj	SA	5 d SA +	SA
sal	4	∼5 d	∼10-14 d	7d	stable	5 d	5 d	2 d	ond/sal	5 d

Summary of the different dosing regimens for the eight original experimental groups. coc - cocaine injection (40 mg/kg/day s.c.), sal - saline injection, ond - ondansetron injection (0.2 mg/kg/day s.c.), wd - withdrawal period. In week 3 the saline or ondansetron injection was given 3.5 h after the cocaine or saline injection. During the second withdrawal period, i.e., after the second week of cocaine (week 4), ondansetron was given for the first five days only. There were no significant differences between the coc-coc/sal and coc-coc-sal control groups or the sal-sal/sal and sal-sal-sal control groups, therefore, these were collapsed to give a single cocaine control group (coc-control) and a single saline control group (sal-control) respectively. The sequence of events for the cocaine self-administration, is also given. FR - fixed ratio, PR - progressive ratio, SA - self-administration, wd - withdrawal, d - day. During the first five days of ondansetron treatment in the SA rats, ondansetron was given during the chronic withdrawal period. During the second ondansetron administration the drug was given 3.5 h after the SA period.

Table 2. Modification of Ellinwood and Balster (1974) Rating Scale

Score	Classification	Definition
1	asleep	lying down, eyes closed
2	almost asleep	relaxed muscles, eyes partially shut
3	dystonia	abnormal posture, tense muscles
4	inactive	lying down, eyes open, infrequent sniffing
5	in-place oral behavior	vacuous oral movements, jaw tremor, yawning
6	grooming	grooming of face, body or groin
7	active movement	investigation or sniffing of cage, rearing
8	hyperactive	running characterized by rapid jerky positional changes
9	slow patterned movement	repetitive exploration of the cage at normal activity level
10	fast patterned movement	intense, rapid repetitive exploration of cage
11	stereotypy	in-place stereotypy, e.g., sniffing
12	hyper-reactive	jerky hyperactive, jumping, seizures, obstinate regression

Modified Ellinwood and Balster (1974) behavioral rating scale. Each rat is examined for 20 s at 5 min intervals and its behavior noted.

results in few intense stereotypies. Two indices of behavior were taken: (1) a behavioral rating (see Table 2) based on the Ellinwood and Balster (1974) rating scale; and (2) locomotor activity as assessed by two simultaneous beam breaks. Beam crossing was recorded in 5-min bins. A behavioral rating was given to each of the animals at 5-min intervals; the observation period was for 20 s with 10 s between boxes. It is important to measure both locomotor and behavioral activity because stimulant-induced in-place stereotypies can reduce locomotor activity (Segal and Mandell 1974).

Data Analysis for Cocaine Challenge

The behavioral rating data presented here are ordinal data; thus, differences between groups in Figure 1, panel A, which shows two groups, were analyzed by nonparametric Mann-Whitney tests. The behavioral rating data in Figure 2, panels A and B, which shows three pretreatment groups per behavior, were analyzed using 1-way ANOVA, on ranks when appropriate. Comparisons between sal-control (normal) and coc-control (sensitized) animals in Figure 2 were made by *t*-test and Mann-Whitney where appropriate. The locomotor activity data (Figure 1, panel B, and Figure 3) were analyzed using a 2-way ANOVA with post hoc Student-Newman-Keuls. The significance level is set at $p < .05$ for all comparisons. The data in all the figures are expressed as means and the standard error of the mean (SEM).

Catheterization of Jugular Vein for Self-administration

Aseptic technique was used throughout. Animals were first anesthetized with pentobarbitol (50 mg/kg i.p.) and both the dorsal and ventral neck and shoulder regions were shaved. An incision was made above the jugular vein and the vein located by blunt dissection. The skin around one side of the neck was loosened from connective tissue using blunt dissection so that the catheter could be fed from the jugular to an opening between the shoulder blades. A 0.047 inch o.d, 0.025 inch i.d. catheter (Silastic) was inserted through the vein toward the heart to ensure it would stay in place and the catheter fastened to the vein with surgical thread. The other end of the catheter was fed through the dorsal incision to a 'Covance infusion harness' (model CIH95, Instech, PA) which ensured that the animal was unable to manipulate the catheter.

Cocaine Self-administration Test Apparatus

This consisted of a 43 × 22 × 22 cm plastic cage with two identical nose-poke holes (2 cm in diameter, 9.9 cm apart) situated at one end 9 cm above floor level. Two copper plates 0.9 × 2.2 cm) were located at each side of each hole so that the nose-poke holes were located within a capacitive field and movement within these fields was monitored by computer. Power spectrum analysis (fast Fourier transform) of the movements (i.e. frequency and intensity) were measured as previously described (Ellinwood et al. 1981). Thus the animal had to sniff in the contingent nose-poke hole at between 5 and 9 Hz in order to register a response. A single response was defined by the power of the frequency (of sniffing) and an arbitrary threshold of response was set at 2000 which required approximately 10 s of sniffing. There are good reasons to use a 'sniffing' response in rats: (1) it is a prepotent rodent behavior involved in searching and acquisition (Ikemoto and Panksepp 1999); and (2) these search and acquisition behaviors become enhanced in human cocaine abusers (Rosse et al. 1993, 1994). We note that sniffing stereotypies, evident after cocaine infusion (during the post-reinforcement pause), took place away from the contingent nose-poke hole and did not contribute to the response for cocaine.

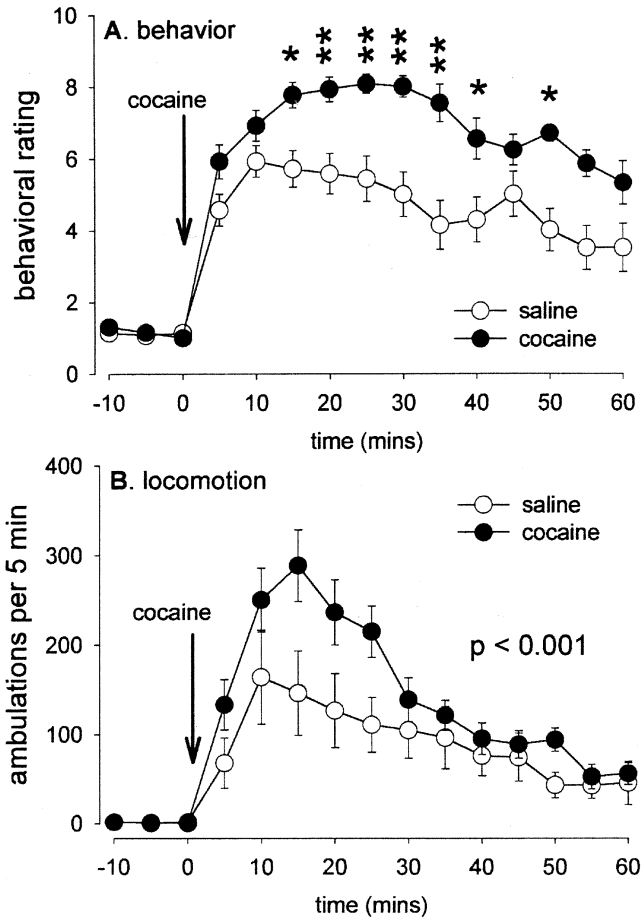

Figure 1. Repeated cocaine pretreatment causes both behavioral and locomotor sensitization. A. The cocaine pretreated groups are behaviorally sensitized versus the saline treated groups to a challenge dose of cocaine (7.5 mg/kg i.p. at time 0) on day 10 of withdrawal. B. The cocaine pretreated groups show locomotor sensitization to a challenge dose of cocaine (7.5 mg/kg i.p.) on withdrawal day 10. The temporal responses to cocaine challenge show the classical relationship of sensitized stereotypy to locomotion: locomotion increases rapidly to peak at 15 min and declines to control levels by 30 min. Stereotypy reaches a peak at 20 min and continues to be a significant factor out to at least 50 min. Values are means ± SEM. See Table 1 for details of dosing regimens.

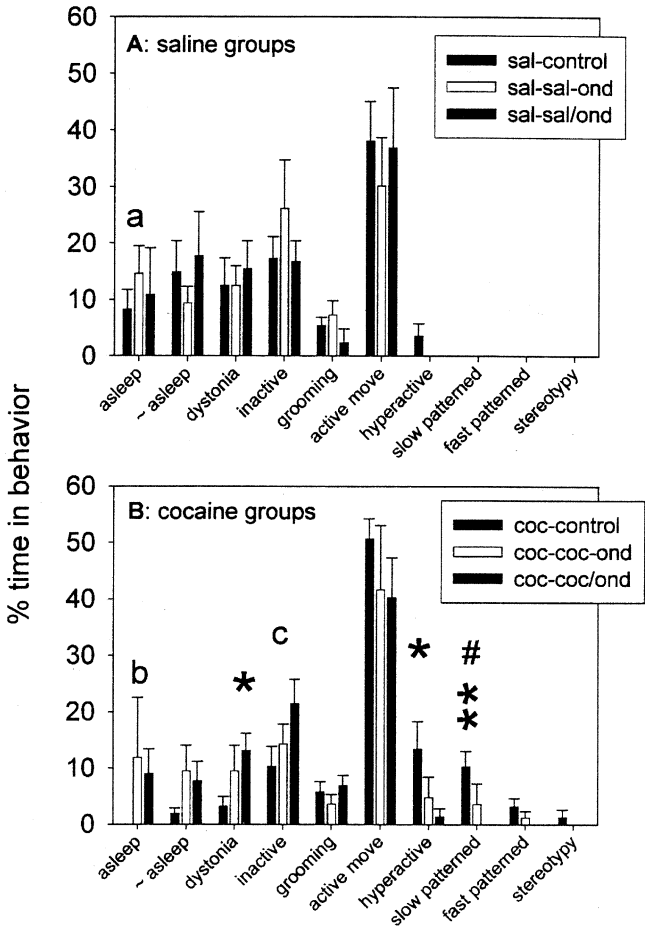

Figure 2. Ondansetron inhibits behavioral sensitization. A. Ondansetron, given either during the second week of saline injections (sal-sal/ond) or during the second withdrawal period (sal-sal-ond), has no effect on any behavior after a challenge dose of cocaine (7.5 mg/kg i.p.) given on withdrawal day 10. B. Ondansetron, given during the second week of cocaine injections (coc-coc/ond) can inhibit behavioral sensitization after a challenge dose of cocaine (7.5 mg/kg i.p.) given on withdrawal day 10. Ondansetron increased the time spent in inactive behaviors and decreased the amount of time in hyperactive behaviors. Ondansetron given in the second withdrawal period (coc-coc-ond) only tended to decrease sensitization. There was no difference between the two ondansetron treated groups either after saline pretreatment or after cocaine pretreatment. Values are means ± SEM. $^{a}p = .06$ $^{\#}p = .007$ sal-control vs. coc-control. i.e. the coc-control group shows behavioral sensitization. $^{*}p < .05$ and $^{**}p = .004$ coc-control vs. coc-coc/ond; $^{b}p = .087$; $^{c}p = .069$ for the three cocaine groups in B. See Table 1 for details of dosing regimens.

Cocaine Self-administration Procedure

Seven days after surgery the animal was introduced to the test apparatus. It was always then given a priming dose of cocaine (2 mg/kg i.v.). Criteria responses in the contingent hole resulted in a 'reinforcement' of cocaine (2 mg/kg i.v.) which was followed by a time out period of 20 s to avoid overdosing in the FR schedules. The animal was thus trained on fixed-ratio (FR1 then FR2) schedules of reinforcement (< 30 mg/kg/day to avoid overdose). Once an animal had reached a stable response rate for three consecutive days on the FR2 schedule (usually after ~10–14 days of FR training) it was withdrawn for seven days to allow for the hypothesized consolidation of sensitization. On day 8 of FR2 withdrawal they were then subjected to a PR schedule of reinforcement where the number of responses required for a reward increased on an exponential scale.

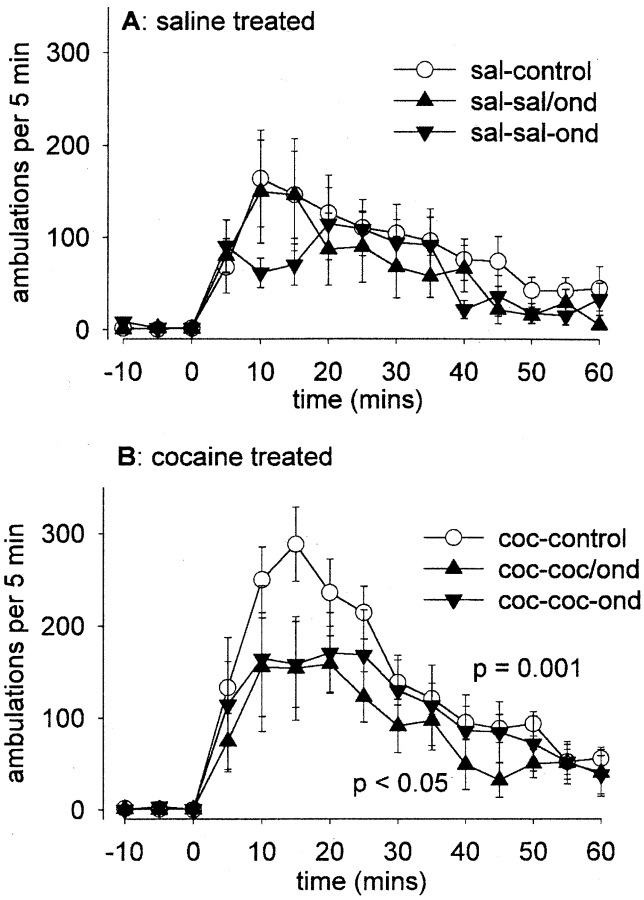

Figure 3. Ondansetron inhibits locomotor sensitization. A. Ondansetron, given either during the second week of saline injections (sal-sal/ond) or during the second withdrawal period (sal-sal-ond), has no effect on locomotor activity versus controls (sal-control) after a challenge dose of cocaine (7.5 mg/kg i.p. at time 0) given on withdrawal day 10. B. Ondansetron, given either during the second week of cocaine injections (coc-coc/ond) or during the second withdrawal period (coc-coc-ond), can inhibit locomotor sensitization after a challenge dose of cocaine (7.5 mg/kg i.p.) given on withdrawal day 10. There was no difference between the two ondansetron treated groups either after saline pretreatment or after cocaine pretreatment. Values are means ± SEM. See Table 1 for details of dosing regimens.

PR schedules are accepted to be a more accurate method of determining the rewarding effect of a reinforcer (Hodos 1961) and in particular the abuse liability and rewarding effects of cocaine (Richardson and Roberts 1996; Arnold and Roberts 1997). In the schedule used here the first reinforcer required one response, the second required two responses, and so on following this schedule 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178 etc. This series was derived from the equation:

$$\text{response ratio} = (5e^{(\text{injection number} \times 0.2)}) - 5$$

where the response ratio is rounded to the nearest integer (see Richardson and Roberts 1996).

The number of reinforcers earned, i.e. the break point (BP), was determined when the animal had stopped responding for 60 min or after 5 h, whichever came first. Typically animals reached breaking point after 3.5–4 h. The post-reinforcement pause (PRP), i.e. the time between cocaine infusion and resumption of responding at the contingent nose-poke hole, was also measured.

Ondansetron Injections in Cocaine Self-administration Experiments

Once an animal had reached a stable level of responding on the first PR schedule (typically Bp = 15, i.e. ~30 mg/kg/day cocaine) for five days of withdrawal, it was given five days of single ondansetron injection (0.2 mg/kg/day s.c.) or saline injection. On day 10 of withdrawal from cocaine self-administration it was again put on the PR schedule for two days. This was to determine whether or not the five withdrawal days of ondansetron administration were able to attenuate cocaine self-administration. On the second day of resumption of self-administration, the animals were injected with either ondansetron (0.2 mg/kg s.c.) or saline 3.5 h *after* the self-administration session for five days, and tested on the PR schedule for a further 10 days.

Data Analysis for Cocaine Self-administration. There were two groups of animals: those that received saline injections and those that received ondansetron injections. Statistical analysis was by 2-way ANOVA with the variables being Treatment group (saline vs. ondansetron) and Time (day of self-administration).

RESULTS

Throughout the ANOVA discussions 'time' refers to time after cocaine challenge and 'pretreatment' refers to the cocaine and/or ondansetron pretreatment dosing regimen.

Repeated Cocaine Dosing Regimens Produces Behavioral and Locomotor Sensitization

The two cocaine control groups (coc-coc/sal and coc-coc-sal, see Table 1) showed no statistical differences, nor did the two saline control groups (sal-sal/sal and sal-sal-sal) therefore they were collapsed to give a single cocaine and a single saline control group. Figure 1, panel A, shows that prior to cocaine challenge there was little baseline difference between the cocaine and saline groups; the animal's behavioral rating (~1) corresponds to 'lying down and eyes closed' (see Table 2). After cocaine the behavioral rating increases rapidly to

~6 for the saline treated animals and to ~8 for the cocaine treated animals. ANOVA on ranks was performed at each time point and the coc-control group was found to be significantly different (sensitized) from the sal-control group at a number of different time points starting at 15 min after the cocaine challenge. In examining each individual behavior (Figure 2) and comparing the sal-control group with the coc-control group there was a tendency for more sal-control rats to be found asleep ($p = .06$) while more of the coc-control rats were likely to be in a slow-patterned behavior ($p = .007$). Overall fewer coc-control rats were found in the less active behaviors while more of this group were found in hyperactive and stereotypy-type behaviors.

In Figure 1, panel B, it can be seen that, prior to cocaine challenge, there is little difference between the cocaine and saline groups; the number of ambulations per 5 min is close to zero. On cocaine challenge there is a rapid increase in locomotor activity. There was a main effect of Time (cocaine challenge): $F_{14,404} = 14.685$; $p < .001$. Animals which received cocaine pretreatment were again sensitized: there was a main effect of Pretreatment: $F_{1,404} = 21.309$; $p < .001$. However, there was not a significant Time X Pretreatment interaction: $F_{14,404} = 1.525$, $p = .099$.

It should be noted that in another group of animals we found 40 mg/kg/day cocaine to cause locomotor sensitization after seven days of withdrawal (7.5 mg/kg cocaine challenge before cocaine dosing regimen, total ambulations in 60 min = 628 ± 94, after seven days cocaine and seven days withdrawal = 1085 ± 205, t = −2.283, df = 13, $p = .033$). Our dosing regimen therefore represents two sensitization periods, although the acute withdrawal treatment group received ondansetron during the second dosing regimen; thus strictly speaking it may not represent a sensitization regimen.

Ondansetron Can Reverse the Expression of Behavioral Sensitization

Figure 2, panel A, shows that ondansetron, given either with the second week of saline injections (sal-sal/ond) or during the second 'withdrawal' period (sal-sal-ond), has no effect on any of the rats' behaviors after cocaine challenge when compared with saline control rats (sal-control) $p = .196$ and .176 respectively. Conversely, Figure 2, panel B, shows that ondansetron can inhibit behavioral sensitization when given in the second week of cocaine injections (coc-coc/ond), 3.5 h after each injection, when compared with cocaine control rats (coc-control). The coc-coc/ond group were significantly different from the coc-control group with respect to dystonia (H = 7.627, df = 2, $p = .022$), hyperactivity (H = 6.155, df = 2, $p = .046$) and slow-patterned movements (H = 10.986, df = 2, $p = .004$). There was no difference between the two groups which received ondansetron.

Ondansetron Can Reverse the Expression of Locomotor Sensitization

In Figure 3 all six groups were analyzed together (both panels). ANOVA showed that cocaine challenge increased locomotor activity: there was a main effect of Time $F_{14,824} = 19.68$, $p < .001$. There was also a difference between Pretreatment groups $F_{5,824} = 11.33$, $p < .001$. There was no Time X Pretreatment interaction ($p = .889$). Student-Newman-Keuls analysis within the six pretreatment groups showed that the coc-control group was significantly different from all other groups ($p < .001$ vs. the coc-coc/ond and all three saline treated groups (sal-control, sal-sal-ond, sal-sal/ond) and $p < .05$ vs. the coc-coc-ond group). The only other differences were found between the coc-coc-ond group and the sal-sal-ond and sal-sal/ond groups (both $p < .05$). Thus ondansetron treatment given either during the acute or chronic withdrawal periods can attenuate sensitization. However, the differences between the coc-coc-ond and sal-sal-ond groups suggest that the chronic withdrawal ondansetron treatment may be less efficacious.

Ondansetron Can Inhibit Cocaine Self-administration

All rats were trained on FR1 then FR2 schedules in which they typically injected 26–30 mg/kg/day cocaine. At the end of the FR2 schedule they were withdrawn for seven days to allow for a consolidation of hypothesized sensitization to the first PR schedule. They were then subjected to a subsequent PR schedule until they reached plateau of responding over five days (typically 15 injections). They were then withdrawn for 10 days and received ondansetron or saline injections on the first five days of withdrawal.

Figure 4 shows that ondansetron, given for the first five days of withdrawal from the initial PR session did not significantly reduce BP for cocaine self-administration on day 10 or 11 of this withdrawal period. On days 11–15 ondansetron was given 3.5 h after each daily self-administration session during the second PR regimen and significantly inhibited cocaine self-administration in the following days. The effect of this ondansetron treatment was evident even three days after the last ondansetron injection. There was a significant effect of Ondansetron ($F_{1,11} = 49.357$; $p < .001$) versus Saline injections on self-administration during this second PR regimen. There was almost a significant effect of Day ($F_{1,11} = 1.864$; $p = .062$) but no Treatment X Day interaction ($F_{11,84} = 1.212$; $p = .299$). We note that a 30% reduction in BP is equal to a much larger reduction in actual responses.

Post Reinforcement Pause

In order to assess whether stereotypy had a contribution to variance in the progressive ratio, we measured

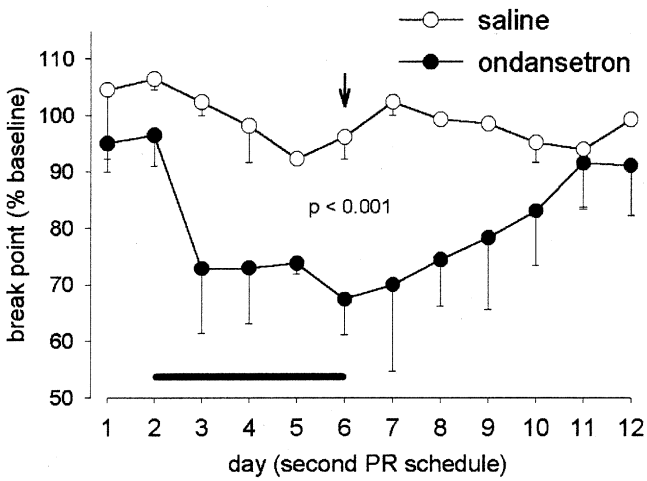

Figure 4. Ondansetron attenuates cocaine self-administration. Ondansetron (0.2 mg/kg s.c.) given for five days after stable PR responding caused only a non-significant reduction in break-point when self-administration was reintroduced after 10 days of cocaine withdrawal (day 1 and day 2 in figure). However, when ondansetron (0.2 mg/kg s.c.) was given 3.5 h after the cocaine self-administration session for five days (black bar) it attenuated cocaine self-administration the following day. This effect was evident even three days after the last ondansetron injection. Values are mean ± SEM, n = 4 for both groups. $^{*}p < .001$ saline vs. ondansetron treated groups. The arrow (↓) indicates when the PRP data in Figure 5 was taken.

the post-reinforcement pause (PRP) on several days of self-administration. In accord with Richardson and Roberts (1996), we found an average PRP of 8–10 min, before ondansetron treatment. During the 8–10 min PRP, the animals were in a stereotyped behavior away from the contingent nose-poke hole. The PRP was also assessed on day 5 of the second ondansetron treatment phase (arrow in Figure 4, Figure 5)] where it had been given 3.5 h after each self-administration. Only the first eight reinforcers were analyzed since the number of ondansetron treated rats still administering cocaine began to decrease at this point. Ondansetron treatment during the acute withdrawal tended to prolong the PRP ($F_{1,7} = 3.324$, $p = .077$).

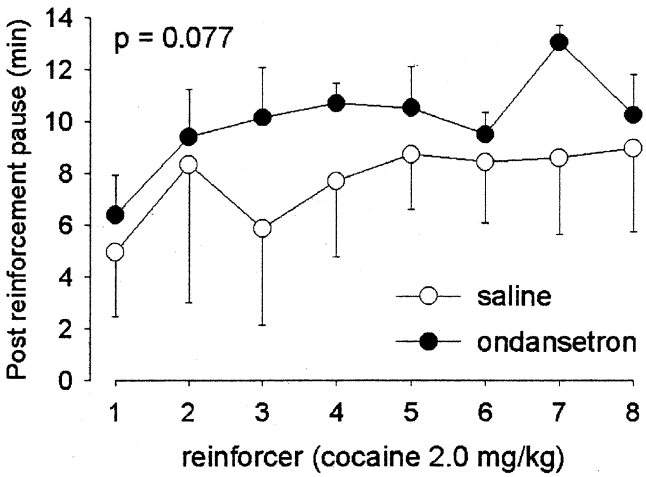

Figure 5. The effect of ondansetron on post reinforcement pause (PRP). The PRP is approximately 8–10 min as previously shown (Richardson and Roberts 1996). Ondansetron tends to increase PRP gradually over the first eight reinforcement schedules (only eight are shown because many of the ondansetron treated rats gave up (reached break point). These data are taken from day 5 in Figure 4.

DISCUSSION

As expected, the two cocaine dosing regimens induce behavioral and locomotor sensitization 10 days after the second withdrawal. The multiple dosing regimen also resulted in significantly greater sensitization (Davidson et al. 2002). Furthermore, ondansetron, when given either with the second cocaine dosing regimen (3.5 h after cocaine) or during the first five days of the second withdrawal period, can inhibit the expression of sensitization and normalize behavior when tested several days later. Ondansetron given to the control groups did not reduce cocaine-induced locomotor behavior or behavioral rating relative to the saline control group. Thus, ondansetron, when given during acute or chronic withdrawal, acts specifically to inhibit the sensitized response and not locomotion or behavior per se. This is in contrast to 5-HT_3 antagonists given as a treatment 30 min before cocaine challenge (Reith 1990; Le et al. 1996) where locomotion was reduced in control (non-sensitized) animals. However, there was a difference in blockade of behavioral sensitization between animals that received ondansetron during cocaine acute withdrawal and those that received ondansetron during chronic withdrawal. As can be seen from Figure 2, panel B, the coc-coc/ond treatment was more efficacious. The locomotor data show no difference between the coc-coc/ond and coc-coc-ond groups. However the coc-coc-ond group exhibits a greater response to cocaine challenge than the sal-sal-ond group suggesting that this chronic withdrawal ondansetron treatment may be less efficacious. The acute withdrawal ondansetron treatment, however, *completely blocks* the expression of locomotor sensitization.

To our knowledge, this is the only report of a treatment given during the withdrawal period of a second dosing regimen that inhibits the expression of cocaine sensitization *previously established by a prior dosing regimen* and only one other report has found effective treatments when given during a second dosing regimen (Li et al. 2000). Given that cocaine abusers usually have many sensitization and withdrawal episodes in their progression from use to abuse, various forms of the multi-regimen paradigms may provide the most appro-

priate animal model to explore medication development.

This coc-coc-ond study is an extension of our previous data in which ondansetron permanently blocked sensitization when given during the first five days of withdrawal from a single chronic cocaine regimen (King et al. 2000). The coc-coc-ond study differs from previous work in that we directly compare the treatment effects after *two* sensitization treatment regimens to test the hypothesis that a previously established sensitization could be inhibited. In the coc-coc/ond study we gave ondansetron 3.5 h *after* the cocaine injections in the second regimen whereas in previous studies ondansetron was given before or at the same time as cocaine (King et al. 1997, 1998). These studies provide insight into potential treatment of cocaine abusers: (1) treatment during the first five days of the chronic withdrawal period inhibits previously established sensitization yet probably will not prevent reestablishment of sensitization with subsequent abuse; (2) treatment every day with ondansetron, whether before cocaine (King et al. 1997, 1998) or after cocaine (present study) may well attenuate subsequent reestablishment of sensitization. The use of sustained release formulations of ondansetron have not been tested but hold additional promise.

In addition, we tested the relevance of these behavioral results to a model of cocaine self-administration. Ondansetron, given 3.5 h *after* cocaine self-administration, substantially inhibited self-administration the following day but was much less effective when it had previously been given during the first five days of the chronic withdrawal period and tested before the acute withdrawal treatments. Further, the reluctance to resume responding (PRP) tended to increase after the second ondansetron treatment. The PRP has been thought to be a measure of the cost of reward (Felton and Lyon 1966; Neill and Justice 1981; Brown et al. 1996), or may simply reflect the animal's attempts at titrating cocaine levels in the blood or brain (e.g. Richardson and Roberts 1996). The former explanation is consistent with cocaine showing less reinforcement value after ondansetron treatment. The latter explanation would suggest the unlikely scenario that ondansetron increased the level of cocaine in the blood/brain or potentiated its reinforcing effects. These results contrast to previous studies which have found ondansetron (Peltier and Schenk 1991; Lane et al. 1992; Depoortere et al. 1993) and other 5-HT_3 antagonists (Lacosta and Roberts 1993) to be ineffective in cocaine self-administration models, when given 30 min *before* the cocaine self-administration session. Finally, Cervo et al. (1996) using the CPP paradigm showed that none of the 5-HT_3 antagonists tested (given 30–60 min prior to testing) affected acquisition of cocaine CPP. Taken together, and given the rapid absorption and short half-life of ondansetron in rats ($t_{1/2}$ ~12 min, Saynor and Dixon 1989), we suggest that *the time of ondansetron dosing is critically important*. Given the $t_{1/2}$ in humans ($t_{1/2}$ ~3–4 h, Saynor and Dixon 1989; Roila and Del Favero 1995), the time of dosing may also be a clinical concern.

The contrast of ondansetron inhibiting the establishment of sensitization in behavior models when given prior to cocaine (King et al. 1997, 1998) versus having no effect on self-administration requires further study of the differences in these two models. Similarly the relative lack of effect of 5-day withdrawal ondansetron treatment in the progressive ratio model versus sensitization model requires further study. However, in both the sensitization and self-administration models the ondansetron treatment during the acute withdrawal period appears to be more effective.

Here the multiple cocaine dosing regimen, used to establish repeated consolidation of sensitization, not only better models the human condition but parallels the self-administration study. Direct establishment of sensitization in the self-administration rats would be difficult since the baseline measure would occur during training. We chose not to pretreat with experimenter or yoked dosing with cocaine to establish sensitization to avoid the reported differences (Wilson et al. 1994; Dworkin et al. 1995; Hemby et al. 1997; Mutschler and Miczek 1998; Mark et al. 1999; Galici et al. 2000; Mutschler et al. 2000). However it is generally acknowledged that such a cocaine self-administration schedule does result in sensitization (Hooks et al. 1994; Phillips and Di Ciano 1996; Schenk and Partridge 1997; De Vries et al. 1998).

Opponent processes have been postulated to be involved in psychostimulant addiction (Koob et al. 1997). Koob and Bloom (1988) suggest that the adaptive opponent processes are initiated to counter the effects of the drug (acute tolerance) and these processes persist *after the drug has been cleared from the brain,* thereby leaving the opponent processes unopposed. Solomon and Corbit (1974) hypothesized that pleasant affect is automatically opposed by centrally mediated mechanisms that reduce the intensity of these affective states. They define opponent-processes as counter adaptive and can be further defined as: (1) a process (positive hedonic effects) occurring shortly after presentation of the reinforcer but showing acute tolerance; and (2) other processes (negative anhedonic effects) which appear after the initial reinforcing process has been terminated, are slow to the decay and can get larger with repeated exposure.

Thus one possibility is that ondansetron is inhibiting a negative effect in withdrawal. Serotonin-3 receptors have the potential to mediate negative opponent processes since they are related to a number of aversive conditions. Ondansetron is an antiemetic and effective in a spectrum of aversive conditions such as nociception (Greenshaw and Silverstone 1997), the Bezold-

Jarisch reflex (Malinowska et al. 1996) and gut hypermotility (Scarpignato and Pelosini 1999). Cocaine can be aversive and cocaine self-administering rats have been shown to emit increased stress-related ultrasonic vocalizations (USV) and increased startle response to tactile startle stimuli (Barros and Miczek 1996; Mutschler and Miczek 1998) at least as early as 6 h after withdrawal. These increased responses may be correlates of the increased anxiety seen in human binge abusers during the 'crash' phase of withdrawal (Ellinwood and Petrie 1977; Gawin and Ellinwood 1988). We hypothesize ondansetron to act upon these aversive effects when given after the cocaine self-administration. Indeed, ondansetron has previously been found to inhibit some of the behavioral consequences (light-dark exploration and social interaction deficits) of cocaine withdrawal (Costall et al. 1990). By decreasing such withdrawal symptoms we suggest that the need to self-medicate for these effects is diminished and the animal is less likely to self-administer the next day and for several days later. It is also possible that ondansetron could be reducing the reinforcing value of cocaine, as the PRP data suggests. As a caveat we also note that ondansetron may simply lead to a generalized inhibition of operant responding. However, as ondansetron treatment 30 min prior to self-administration does not lead to a decrease in break point (Peltier and Schenk 1991; Lane et al. 1992; Depoortere et al. 1993) we feel this explanation is unlikely.

In conclusion, this study shows that repeated cocaine dosing regimens produce both behavioral and locomotor sensitization. This sensitization is inhibited by ondansetron when given in the acute withdrawal periods in the second cocaine dosing regimen and, perhaps to a lesser extent, when given during the early chronic withdrawal period. Furthermore, ondansetron given at the same dose during the acute withdrawal period was also shown to decrease cocaine self-administration the following day. Importantly ondansetron was given, not before, as previously reported, but 3.5 h *after* the self-administration session. This suggests that the acute withdrawal period represents a window for the action of putative therapeutic agents as it potentially allows for interaction of the agent with mechanisms underlying hypothesized ongoing withdrawal opponent processes caused by cocaine. This acute withdrawal study extends previous findings that treatments in the chronic withdrawal period, e.g. ondansetron (King et al. 2000), MK801 + quinpirole or SKF81297 (however, given prior to cocaine injections during withdrawal; Li et al. 2000) reversed sensitization. The coc-coc/ond as well as the coc-coc-ond study are consistent with the potential for a means of treating cocaine abusers who have already established a sensitized profile of self-administration. It is well known that abusers in treatment programs will frequently challenge medication; thus medicine on board during the acute withdrawal period could be effective. The evidence we have of daily post-cocaine ondansetron dosing reversing sensitization and attenuating self-administration may represent a novel way of thinking about disassociation of post-cocaine processes effects from cocaine's initial effects and its potential relationship in reversing sensitization mechanisms. If these findings can be confirmed, it presents a potential novel means of treating cocaine abusers.

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