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Effect of Dopamine Receptor Antagonists on Renewal of Cocaine Seeking by Reexposure to Drug-associated Contextual Cues

Hans S. Crombag, Ph.D., Jeffrey W. Grimm, Ph.D., and Yavin Shaham, Ph.D.

We recently found that in rats trained to self-administer a heroin-cocaine mixture, exposure to the drug self-administration environment, after extinction of the drug-reinforced behavior in a different context, leads to renewal of drug seeking. Here we further explored the role of contextual stimuli in drug seeking by characterizing the effect of drug-associated environmental stimuli on renewal of cocaine seeking. We also investigated whether activation of dopamine receptors contributes to context-induced renewal of cocaine seeking by testing the effects of selective D1-like (SCH 23390) and D2-like (raclopride) receptor antagonists. Rats were trained for 10 days to self-administer cocaine by pressing a lever. Next, lever pressing was extinguished in the presence of the discrete cues associated with cocaine infusions for 10 days in a context that was distinctively different from the drug-taking context. On the test days, rats were pretreated with SCH 23390 (0, 5 or 10 μg/kg) or raclopride (0, 50 or 100 μg/kg) and non-reinforced lever-pressing behavior was determined either in the extinction context (Control group) or the cocaine-associated context (Renewal group). Consistent with our previous report, cocaine seeking was renewed when rats were exposed to the drug-associated context after extinction in a different context. Furthermore, pretreatment with the D1-like or the D2-like receptor antagonists attenuated context-induced renewal of cocaine seeking. These data suggest that activation of dopamine receptors is involved in reinstatement of cocaine seeking induced by exposure to the drug self-administration context.

[Neuropsychopharmacology 27:1006–1015, 2002]

KEY WORDS: *Conditioning; Occasion setting; Context; Drug self-administration; Raclopride; Reinstatement; Relapse; SCH 23390*

In humans, environmental stimuli associated with drug self-administration play an important role in relapse to drug use after prolonged abstinence (Childress et al. 1992). In laboratory animals, simple, discrete stimuli (i.e., lights or tones) that have been paired with heroin or cocaine injections (conditioned stimuli, CS) have been shown to maintain operant responding for extended periods of time in the absence of drug reward (Goldberg 1976; Everitt et al. 1999). In addition, several studies have shown that drug-paired discrete CSs reinstate drug seeking when they are re-introduced after the drug-reinforced behavior is extinguished in their absence (Schuster and Woods 1968; Davis and Smith 1976; Goldberg et al. 1981; Meil and See 1996). More recently, several investigators have used discrimination procedures (Catania 1992) to characterize the effect of drug cues on reinstatement of heroin or cocaine seek-

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Received August 3, 2001; revised April 17, 2002; accepted April 18, 2002.

Online publication: 4/24/02 at www.acnp.org/citations/Npp042402291.

Published by Elsevier Science Inc.
360 Park Avenue South, New York, NY 10010-1710

0893-133X/02/$–see front matter
PII S0893-133X(02)00356-1

ing. In these studies, discrete environmental cues (e.g., sound, smell) predict whether a drug or no drug (saline) is available during drug self-administration training (McFarland and Ettenberg 1997; Weiss et al. 2000). As in the case of drug CSs, discriminative cues that predict drug availability reinstate drug seeking when they are re-introduced after the drug-reinforced behavior is extinguished in their absence (Ciccocioppo et al. 2001). Subsequent studies using pharmacological and neurochemical methods indicate that the mesocorticolimbic dopamine (DA) system, known to be involved in the unconditioned (Wise 1996) and conditioned (Stewart 1992) behavioral effects of psychostimulant drugs, is involved in reinstatement of cocaine seeking by discrete or discriminative drug cues (Weiss et al. 2000; Alleweireldt et al. 2001; Ciccocioppo et al. 2001; See et al. 2001).

While there is now evidence that discrete drug CSs and discriminative stimuli can reinstate cocaine seeking via DA-dependent mechanisms, little is known about the role of contextual stimuli (e.g., the physical characteristics of the test environment, time of day) in reinstatement of cocaine seeking. There are many examples from studies using non-drug reinforcers demonstrating that the environmental context plays an important role in extinction and reinstatement of learned behaviors (Bouton and Swartzentruber 1991). Furthermore, contextual stimuli can modulate the behavioral and physiological effects of drugs of abuse (Siegel 1989; Robinson et al. 1998; Crombag et al. 2000).

Based on these previous findings, we recently examined the effect of contextual stimuli on reinstatement of speedball (a heroin-cocaine combination) seeking using a *renewal* procedure (Bouton and Bolles 1979). In this procedure, conditioned responses to discrete CSs are recovered when they are reintroduced in the original conditioning context (where they were paired with the primary reinforcer) after extinction in a different context. We found that when rats were returned to the context associated with speedball self-administration, after lever-pressing behavior had been extinguished in a different context in the presence of the discrete CSs (cue light, sound of pumps) previously associated with speedball infusions, drug seeking was renewed to pre-extinction levels (Crombag and Shaham 2002). These findings demonstrate an important modulatory role of contextual stimuli in reinstatement of drug seeking.

Here we further characterized the role of contextual cues in drug relapse by studying the effects of drug-associated contextual stimuli on renewal of drug seeking in rats with a history of cocaine self-administration. Furthermore, we tested whether activation of DA receptors is involved in renewal of cocaine seeking induced by contextual stimuli by determining the effects of the D1-like antagonist, SCH 23390 (Hyttel 1983), and the D2-like receptor antagonist, raclopride (Kohler et al. 1985).

MATERIALS AND METHODS

Subjects

Male Long-Evans (Charles River, Raleigh, NC) rats (325–375 g) were housed individually in a climate-controlled animal colony (lights on from 8 P.M. to 8 A.M.) with food and water freely available. All training and testing took place during the dark phase of the light/dark cycle. All procedures used followed the Principles of Laboratory Animal Care (NIH publication No. 86-23, 1996) and were approved by the Animal Care and Use Committee of NIDA/IRP.

Surgery

Intravenous (IV) catheters were implanted using procedures described previously (Shalev et al. 2000). Briefly, rats were pretreated with the analgesic buprenorphine (0.01 mg/kg, SC) and then anesthetized with a ketamine+xylazine mixture (100+10 mg/kg, IP). The catheter was inserted into the jugular vein and was then passed subcutaneously to the top of the skull where it was attached to a modified 22-gauge cannula (Plastics One, Roanoke, VA) mounted to the skull with jeweler's screws and dental cement. After catheter implantation, rats were allowed to recover for at least five days. Throughout the experiment the catheters were flushed daily with sterile saline containing gentamicin (0.08 mg/ml).

Drugs

Cocaine HCl (NIDA/USA) was dissolved in sterile saline. SCH 23390 HCl and raclopride tartrate (Sigma, St. Louis, MO) were dissolved in saline and injected SC. The doses used have minimal impact on operant responding maintained by non-drug reinforcers (Nakajima 1989; Caine and Koob 1994; Weissenborn et al. 1996) and also have no effect on footshock stress-induced reinstatement of heroin seeking (Shaham and Stewart 1996).

Apparatus

Rats were trained and tested in standard operant chambers (25 × 27 × 30 cm) located inside sound-attenuating cabinets (Med Associates, Georgia, VT). The ceiling, back wall and hinged front door of the operant chambers were constructed of clear acrylic plastic and the sidewalls were made of aluminum. Each chamber was equipped with two levers located 9 cm above the grid floor. Responding on one lever, the active retractable lever, resulted in the illumination of a white cue light located above the lever and activation of the infusion pump. Responses on the other lever, the inactive (non-retractable) lever, located on the opposite wall of the

chamber, were recorded but had no programmed consequences. A fan was located in the sound-attenuating cabinets for air circulation.

Two different contexts were provided by two sets of operant chambers located in nearby rooms. In one context, the floor consisted of 19 stainless steel rods (4.8 mm in diameter) spaced 10.8 mm apart. A white house light provided low level illumination and the waste trays underneath the grid floor were filled with water containing a small amount of Vicks Vaporub®. In the second context, the floor consisted of 23 stainless steel rods (3.2 mm in diameter) spaced 9.4 mm apart. Illumination was provided by a red houselight and a white noise generator provided low-level background noise (~68 dB). The waste tray underneath the grid floor contained a 1% acetic acid solution. Finally, the two contexts differed temporally: sessions in one context occurred in the morning, whereas sessions in the second context occurred in the afternoon.

Procedures

Experiment 1. Effect of SCH 23390 on Renewal of Cocaine Seeking. In Experiment 1 we tested the effect of the D1-like receptor antagonist, SCH 23390, on renewal of cocaine seeking. The experiment was conducted over 27 days and consisted of three phases: acquisition of cocaine self-administration (10 days), extinction training (10 days), and testing for renewal (7 days). Rats were assigned to either the Renewal (n = 9) or the Control (n = 7) group, which were tested in the following manner. The contexts are referred to as A and B, where A is the context where drug self-administration occurred and B is the alternative context. The physical environments that provided contexts A and B were counterbalanced within each group. Rats assigned to the Renewal group were trained to self-administer cocaine in context A; responding was then extinguished in context B and on the test days these rats were returned to context A (ABA condition). Rats assigned to the Control group were trained to self-administer cocaine in context A; responding was extinguished in context B and on the test days these rats remained in context B (ABB condition).

Acquisition of Cocaine Self-administration. For the first seven days of this phase rats were trained during 2-h sessions on a fixed-ratio-1 schedule of reinforcement (FR-1; each lever press is reinforced), to self-administer 0.75 mg/kg/injection cocaine. Each day, rats were transported from the animal colony to the testing room, where they were placed in the operant chambers. Each session commenced when the house light was illuminated and the active lever was inserted into the chamber. Responding on the active lever resulted in an injection of cocaine, delivered in a volume of 0.13 ml over 4.5 s. At the same time, the cue light located above the active lever was activated for 5 s and lever presses during this time were recorded but were not reinforced (a timeout period). At the end of each session, the house light turned off, the active lever retracted and the rats were returned to the animal colony. During the last three days of the acquisition phase, the dose of cocaine was reduced to 50% of the original training dose (i.e., 0.375 mg/kg/injection). This was done to verify that rats acquired robust cocaine-taking behavior as indicated by an increase in responding to compensate for the decrease in the cocaine dose.

Extinction Training. During the extinction phase all procedures were identical to those used during the self-administration phase, except that the drug syringes were removed from the infusion pumps. Thus, presses on the formerly active lever activated the cue light and the syringe pump, but did not result in drug delivery. Both groups underwent extinction training in a context that was distinctively different from the drug-taking context.

Testing for Renewal. The testing phase began 24 h following the final extinction session. During three 1-h sessions rats were tested for cocaine seeking after exposure to the context previously paired with drug self-administration (Renewal group) or the context previously paired with extinction training (Control group). Fifteen minutes prior to each test session, rats were pretreated with vehicle (0 dose) or SCH 23390 (5 or 10 μg/kg, SC). Doses of SCH 23390 were given in a counterbalanced order. The test sessions were conducted every third day and on the intervening days the rats were exposed to the extinction conditions (see above, and Figure 1).

Experiment 2. Effect of Raclopride on Renewal of Cocaine Seeking. In Experiment 2 we tested the effects of the D2-like receptor antagonist, raclopride, on renewal of cocaine seeking. Rats were assigned to either the Renewal (n = 11) or the Control (n = 10) group. The procedures were identical to those of Experiment 1, except that vehicle (0 dose) or raclopride (50 or 100 μg/kg, SC) was given, in a counterbalanced order, prior to the tests for renewal of cocaine seeking.

Statistical Analyses

The data were analyzed separately for the self-administration, extinction, and test phases. For the training phase, the dependent measures were the total number of cocaine infusions, number of active lever responses (infusions + responses during the 5-s timeout period), and the number of inactive lever responses. For the extinction and test phases, the dependent measures were the total (non-reinforced) responses on the active and inactive levers. For the tests for renewal, initial analyses indicated that there were significant effects on inactive-

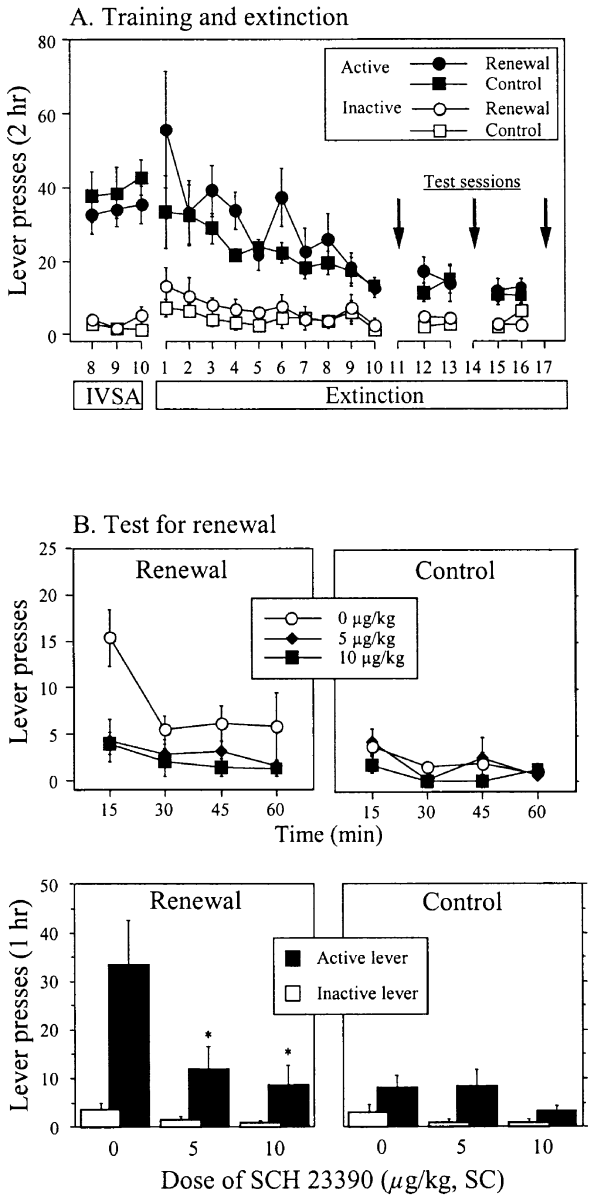

Figure 1. Effect of SCH 23390 on drug context-induced renewal of cocaine seeking. A. The left side shows the mean ± SEM number of responses on the active and the inactive levers during the final three sessions of cocaine self administration. The middle and right sides show the mean number of responses on the previously active and the inactive lever during 10 consecutive extinction sessions, and during the extinction session that separated the three test sessions for renewal of cocaine seeking. B. The time course (upper panels) and the total number of responses (lower panels) on the active and inactive levers (mean ± SEM) during the 1-h test for reinstatement sessions for the Renewal and Control groups following pretreatment with SCH 23390. * Different from the saline condition, $p < .05$.

lever responding, a measure of non-specific activity and/or response generalization. Therefore, the data from the tests for renewal were analyzed in a mixed-regression procedure (SAS PROC MIXED, V. 8.0), which enables inactive-lever presses for the three dose-conditions (vehicle, low, high) to be treated as covariates for active-lever presses at these doses. This analysis was done separately for Experiments 1 and 2 and it included one between-subjects factor (Group) and one repeated factor (Dose), while controlling for inactive-lever presses at each dose. Follow-up post-hoc comparisons between different doses of SCH 23390 or raclopride were conducted using the Fisher PLSD test. Significant differences are reported for p values of less than .05.

RESULTS

Experiment 1. Effect of SCH 23390 on Renewal of Cocaine Seeking

The results from the acquisition, extinction and test phases are shown in Figure 1.

Acquisition of Cocaine Self-administration. Rats rapidly acquired cocaine self-administration on a training dose of 0.75 mg/kg/inf. As expected, when the dose of cocaine was reduced to 0.375 mg/kg/inf during the last three cocaine self-administration sessions, active lever responding increased (mean ± SEM per 2 h of 19 ± 9.6 for the 0.75-mg/kg-unit-dose versus 36.4 ± 15.4 per 2 h during the last three days for the 0.375-mg/kg-unit-dose). There were no group differences for the number of cocaine infusions during the last three days of training ($p > .05$ for Group, Session and Group X Session effects). Responding on the inactive lever was low in both groups and no group differences were observed ($p > .05$; Figure 1, panel A).

Extinction Training. Following stable cocaine self-administration, both groups underwent extinction training in a context distinctively different from the drug self-administration context. Figure 1, panel A, shows that responding on the active lever progressively decreased over consecutive extinction sessions (Session, $F_{9,126} = 4.7$, $p < .01$), and that there were no differences between the Renewal and Control groups ($p > .05$ for Group and Session X Group effects). Analysis of inactive lever responses similarly revealed a significant decrease over extinction sessions (Session, $F_{9,126} = 2.2$, $p < .05$), but no group differences ($p > .05$ for Group and Session X Group effects).

Test for Renewal. Figure 1, panel B, shows the time course (top panels) and the total number of responses (bottom panels) on the previously active lever and on the inactive lever for the Renewal and Control groups after pretreatment with vehicle or SCH 23390. The mixed-regression analysis on the total number of responses on the previously active lever (using inactive-lever responses as a covariate) revealed significant effects of Group ($F_{1,14} = 8.0$, $p < .05$), Dose ($F_{2,27} = 4.1$, $p <$

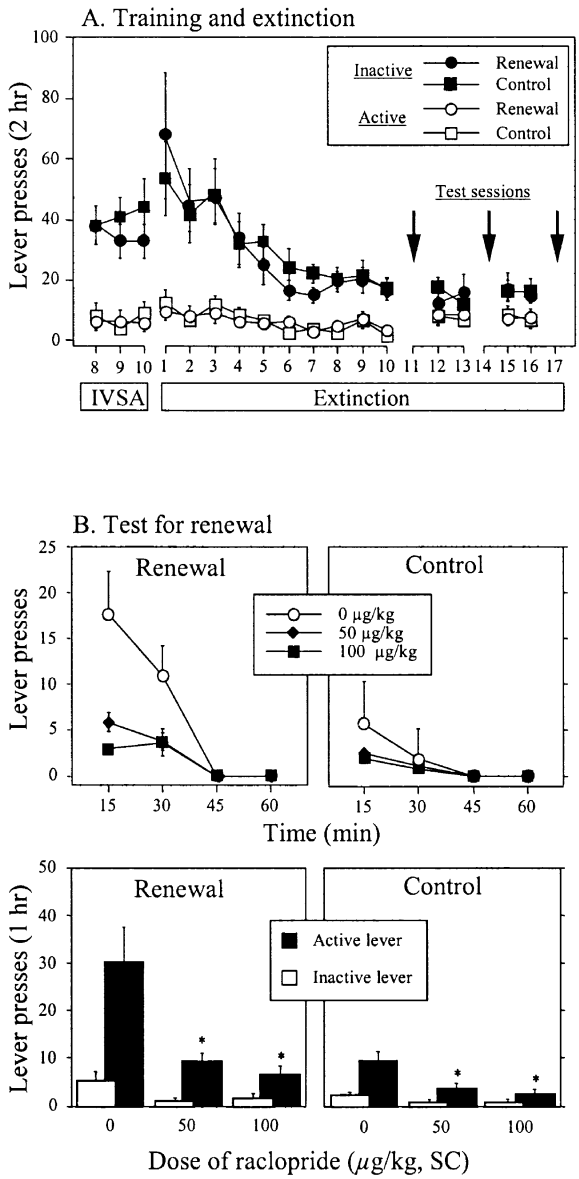

Figure 2. Effect of raclopride on drug context-induced renewal of cocaine seeking. A. The left side shows the mean ± SEM number of responses on the active and the inactive levers during the final three sessions of cocaine self administration. The middle and right sides show the mean number of responses on the previously active and the inactive lever during 10 consecutive extinction sessions, and during the extinction session that separated the three test sessions for renewal of cocaine seeking. B. The time course (upper panels) and the total number of responses (lower panels) on the active and inactive levers (mean ± SEM) during the 1-h test for reinstatement sessions for the Renewal and Control groups following pretreatment with raclopride. * Different from the saline condition, $p < .05$.

.05) and Group X Dose ($F_{2,27} = 4.3$, $p < .05$). Subsequent post-hoc tests revealed a reduction in responding on the previously active lever following both 5 and 10 µg/kg of SCH 23390 in the Renewal group ($p < .05$). In contrast, SCH 23390 did not significantly alter active-lever responding in the Control group ($p > .05$; Figure 1, panel B).

Taken together, the results indicate that, after taking into account potential non-specific effects of SCH 23390 (as indicated by responses on the inactive lever), the D1-like receptor antagonist selectively blocked renewal of cocaine seeking induced by re-exposure to the drug-associated context.

Experiment 2. Effect of Raclopride on Renewal of Cocaine Seeking

The results of the acquisition, extinction and test phases are shown in Figure 2.

Acquisition of Cocaine Self-administration. Rats rapidly acquired cocaine self-administration on a training dose of 0.75 mg/kg/inf and when the dose of cocaine was reduced to 0.375 mg/kg/inf during the last three cocaine self-administration sessions, active lever responding increased (mean ± SEM per 2 h of 26.5 ± 15.1 for the 0.75-mg/kg-unit-dose versus 39.8 ± 14.6 per 2 h during the last three days for the 0.375-mg/kg-unit-dose). There were no differences between groups in the number of cocaine infusions during the last three days of training ($p > .05$ for Group, Session and Group X Session effects). Responding on the inactive lever was low in both groups and no group differences were observed ($p > .05$; Figure 2, panel A).

Extinction Training. Following stable cocaine self-administration, both groups underwent extinction training in a context distinctively different from the drug self-administration context. Figure 2, panel A, shows that responding on the previously active lever progressively decreased over consecutive extinction sessions (Session, $F_{9,126} = 4.7$, $p < .01$), and there were no differences between the Renewal and Control group ($p > .05$ for Group and Group X Session effects). Analysis of inactive lever responding similarly revealed a significant decrease over extinction sessions (Session, $F_{9,126} = 2.23$, $p < .05$), and no group differences ($p > .05$ for Group and Group X Session effects).

Test for Renewal. Figure 2, panel B, shows the time course (top panels) and the total number of responses (bottom panels) on the previously active lever and on the inactive lever for the Renewal and Control groups after pretreatment with vehicle or raclopride. The mixed-regression analysis for active-lever responding (using inactive-lever responding as a covariate) revealed significant effects of Group ($F_{1,19} = 7.3$, $p < .05$), Dose ($F_{2,37} = 9.1$, $p < .01$) and Group X Dose ($F_{2,37} = 3.8$, $p < .05$). Subsequent post-hoc tests revealed a reduction in responding on the (previously) active lever following both 50 and 100 µg/kg of raclopride in the Renewal group ($p < .05$).

However, these post-hoc tests also revealed that both doses of raclopride significantly decreased active lever responses in the Control group ($p < .05$).

In summary, as with SCH 23390, raclopride decreased responding on the previously active lever in rats re-exposed to the drug-associated context (Renewal group), after its effects on inactive lever responding were statistically controlled. However, unlike SCH 23390, raclopride also decreased active lever responding under extinction conditions in the Control group. Thus, the selectivity of the effect of raclopride on renewal of cocaine seeking by reexposure to the cocaine self-administration context has not been clearly established (see Discussion).

DISCUSSION

Using a *renewal* procedure (Bouton and Bolles 1979) we report that rats with a history of cocaine self-administration renew drug seeking when exposed to the drug-associated environment following extinction training in a different environment. This observation extends our finding with speedball-trained rats (Crombag and Shaham 2002) and data from studies using non-drug reinforcers on context-induced renewal of learned behaviors after extinction (Bouton and Swartzentruber 1991). More important, we found that D1- or D2-like receptor antagonists attenuate context-induced renewal of cocaine seeking. These data suggest that activation of DA receptors is involved in context-induced renewal of cocaine seeking and extend previous findings on the role of DA in reinstatement of cocaine seeking induced by discrete CSs or discriminative cues (see below). In the sections below we address in detail the role of D1- and D2-like receptors in reinstatement by conditioned drug cues and discuss methodological issues associated with the use of DA receptor antagonists. We also briefly discuss theoretical and clinical implications of the present data.

Role of Dopamine Receptors in Reinstatement of Cocaine Seeking by Conditioned Drug Cues

DA receptor mechanisms have been implicated in the behavioral activating and incentive motivational effects of psychostimulant drugs (Wise and Bozarth 1987; Robinson and Berridge 1993), including reinstatement of cocaine seeking following periods of abstinence (Stewart 2000). Thus, both D1- and D2-like receptors are involved in cocaine priming-induced reinstatement of drug seeking, although they play fundamentally different roles (Self and Nestler 1998). For example, D1- and D2-like receptor antagonists attenuate cocaine-induced reinstatement (Khroyan et al. 2000) but whereas D2-like receptor agonists reinstate cocaine seeking, D1-like agonists not only fail to do so but they also attenuate cocaine priming-induced reinstatement (Wise et al. 1990; Self et al. 1996; De Vries et al. 1999; Khroyan et al. 2000).

Conditioned drug cues are thought to reinstate drug seeking by inducing a "drug-like" state similar to that induced by the drug itself, which is mediated in part by enhanced DA neurotransmission (Stewart et al. 1984). In agreement with this idea, exposure to discrete or discriminative cocaine-associated cues increases dopamine utilization (microdialysis) or signal (chronoamperometry) in terminal regions of the mesocorticolimbic DA system, including the amygdala (Weiss et al. 2000) and the nucleus accumbens (Gratton and Wise 1994; Kiyatkin and Stein 1996; Weiss et al. 2000), but see Neisewander et al. (1996) and Bradberry et al. (2000). Furthermore, D1-like receptor antagonists (SCH 23390 or SCH 39166) attenuate reinstatement of cocaine seeking provoked by discriminative cues or discrete CSs (Alleweireldt et al. 2001; Ciccocioppo et al. 2001; Weiss et al. 2001). See et al. (2001) also reported that SCH 23390, injected directly into the basolateral amygdala (BLA), attenuates discrete cue-induced reinstatement of cocaine seeking. These data and our findings on the effect of SCH 23390 on context-induced renewal of cocaine seeking indicate that D1-like receptors play a critical role in reinstatement of cocaine seeking induced by environmental cues. This conclusion is in agreement with data from studies using non-drug reinforcers on the role of D1-like receptors in the behavioral effects of conditioned cues (Beninger and Miller 1998; Sutton and Beninger 1999).

The role of D2-like receptors in reinstatement of drug seeking by environmental cues is less clear. See et al. (2001) found that intra-BLA infusions of a single dose of raclopride (5 μg/side) had no effect on discrete cue-induced reinstatement of cocaine seeking. Also, using an operant runway procedure, McFarland and Ettenberg (1997) reported that the preferentially D2-like receptor antagonist haloperidol had no effect on reinstatement of heroin seeking by discriminative cues. Weiss et al. (2001), however, found that a single dose of the D2-like antagonist, nafadotride (1 mg/kg) attenuated discriminative cues-induced reinstatement of cocaine seeking. Consistent with the latter finding, we found that the D2-like receptor antagonist raclopride attenuates context-induced renewal of cocaine seeking. Thus, taken together with Weiss et al. (2001) our data provide some support for the idea that D2-like receptors are involved in reinstatement of cocaine seeking by contextual and discriminative cues. However, as discussed next, it cannot be ruled out that some non-specific behavioral effects of the drug were involved in this effect.

Methodological Considerations

We used DA receptor antagonists to study the role of DA neurotransmission in context-induced renewal of lever-pressing behavior previously reinforced by co-

caine. A critical issue in the interpretation of the present data is the degree to which the observed behavioral effects were due to the effect of the DA receptor antagonists on the motivation versus their effect on motor performance (see Fouriezos and Wise 1976; Ettenberg et al. 1981; Salamone 1994). In the case of the D1-like receptor antagonist, we argue that it is unlikely that the effect of SCH 23390 is due to its effect on motor performance. While the drug had some effect on the very low rate of responding on the inactive lever, it had no significant effect on lever-pressing behavior in the Control group. In this regard, it should be pointed out that while inactive lever responding may have some value in detecting non-specific motor activation, it is not the optimal measure for detecting non-specific motor depression in reinstatement studies (Shalev et al. 2002). The main reason for this is that responding on this lever is typically very low (about 4–5 responses per hour in the present study). Also, it is not clear whether changes in inactive lever responding during reinstatement testing reflect response generalization, which is often observed during extinction (see Figs. 1-2, and Catania 1992), or non-specific activity (Shalev et al. 2002). For these reasons, we have suggested that reinstatement studies should incorporate complementary procedures in which rats perform an operant task at a high rate to determine the potential effect of the pharmacological manipulations on performance (Shalev et al. 2002). Thus, in a pilot study we found that SCH 23390 did not alter higher rates of lever presses for a sucrose reinforcer (mean ± SEM vehicle, 50.6 ± 7.2/60 min; 5 μg/kg, 50.8 ± 5.6/60 min; 10 μg/kg, 42.8 ± 5.4/60 min, $p > .05$; n = 8). The latter data are in agreement with previous studies that found that at the dose range used here SCH 23390 had minimal impact on high rates of responding for food or brain stimulation reward (Nakajima 1989; Goudie and Smith 1999). In addition, previous studies using similar doses of SCH 23390 found that it does not alter operant responding for food in cocaine-experienced rats (Caine and Koob 1994; Weissenborn et al. 1996). These findings are particularly important since prior experience with cocaine, as was the case in Experiments 1 and 2, may produce sensitization to the cataleptic effects of SCH 23390 (Ushijima et al. 1995).

The selective D2-like receptor antagonist, raclopride, also attenuated context-induced renewal of cocaine seeking. However, in contrast to SCH 23390, the present results do not allow us to rule out that the effect of raclopride on behavior in the Renewal group was not, in part, due to an effect on motor behavior. First, in a pilot study we found that the high dose of raclopride modestly reduced high rates of lever presses for a sucrose reinforcer (mean ± SEM vehicle, 71.7 ± 10.4/60 min; 50 μg/kg, 68.7 ± 8.8/60 min; 100 μg/kg, 52.2 ± 11.9/60 min, $p < .05$, n = 8). Second, raclopride also had some effect on baseline extinction responding on the active lever in the Control group. Third, a detailed analysis of the behavioral effects of dopamine receptor antagonists led Fowler and Liou (1994) to conclude that, in contrast to SCH 23390, raclopride has a tendency to produce micro-catalepsy even at low doses. Similarly, Beninger and Miller (1998) concluded that while the effect of D1-like receptor antagonists on behaviors controlled by unconditioned and conditioned rewards can be dissociated from their effect of performance, a similar dissociation is more difficult to achieve with D2-like receptor antagonists.

It is important to note, however, that other investigators found that in cocaine-experienced rats, raclopride (given at doses similar or higher than the ones used here) does not alter operant responding for food (Weissenborn et al. 1996). In addition, raclopride (at the dose range used here) has minimal effect on high rates of responding for food or brain stimulation reward (Nakajima 1989; Goudie and Smith 1999). Therefore, an alternative explanation for the effect of raclopride on extinction responding in the Control group could be that extinction training was incomplete and thus raclopride may have decreased the residual cocaine seeking maintained by the "incompletely" extinguished CSs. Such an interpretation would be consistent with a notion that D2-like receptors, like D1-like receptors, are involved in reinstatement of cocaine seeking by environmental cues by affecting motivation-related processes (Weiss et al. 2001).

Finally, the effects of SCH 23390 and raclopride on context-induced renewal of cocaine seeking were not clearly dose-dependent: both the low and the high doses of the DA receptor antagonists blocked renewal drug seeking, and lower doses were not tested. It could be argued, therefore, that the lack of dose-dependency was due to non-specific effects, potentially on non-DA receptors. For example, SCH 23390 also binds at high affinity to 5-HT2c (previously 5-HT1c) receptors (Bischoff et al. 1986). It is possible, therefore, that the effect SCH 23390 on context–induced renewal of cocaine seeking is due, in part, to an action on 5-HT receptors. Although we cannot completely rule out this possibility, this seems unlikely because the effect of SCH 23390 on reinstatement provoked by discriminative cocaine cues is mimicked by the highly selective D1-like receptors, SCH 39166 (Ciccocioppo et al. 2001), which has low affinity for 5-HT receptors (McQuade et al. 1991). In addition, there is little evidence that 5-HT2 agents mimic the effect of D1-like receptor antagonists on conditioned responses (Fletcher and Korth 1999; Sutton and Beninger 1999).

Theoretical and Clinical Implications

The present data suggest that activation of DA receptors is involved in context-induced renewal of cocaine seeking. An important issue arising from these data is con-

cerned with the learning mechanisms involved in context-controlled behavior. Based on previous studies in the learning field, we suggested (Crombag and Shaham 2002) that drug-associated contextual stimuli reinstate drug seeking because of their occasion setter (Holland 1992) or modulator (Rescorla et al. 1985) properties. Specifically, contexts can function as retrieval cues in cases where the meaning of the discrete CSs is ambiguous because they have been paired with both reinforcement (training conditions) and non-reinforcement (extinction conditions) (Bouton 1993). In the present experiments, the presence/absence of cocaine was reliably signaled by different contextual stimuli, and thus lever presses in response to the discrete CSs could have been determined by whether the contextual stimuli in the background retrieved the training or the extinction experience. An interesting question that emerges, therefore, is whether DA receptor antagonists block context-induced renewal of cocaine seeking by interfering with the putative occasion setting properties of the context or by acting on neuronal systems that underlie conditioned behaviors elicited by discrete CSs (which are modulated by the contextual drug cues). This distinction is important because different neuronal circuits underlie the behavioral effects of discrete CSs versus contextual stimuli (Phillips and LeDoux 1992; Holland and Bouton 1999).

Finally, present data may have some clinical implications. Relapse to drug use after periods of abstinence is common in cocaine and heroin users (Mendelson and Mello 1996; O'Brien 1997). The renewal model used here is a variation of the well-established reinstatement procedure wherein the effect of exposure to drugs or non-drug stimuli on reinstatement of drug seeking is determined following extinction of drug-reinforced behavior (Stewart and de Wit 1987). The reinstatement procedure appears to have good predictive validity because conditions thought to provoke relapse and craving in humans also reinstate drug seeking in laboratory animals. Specifically, exposure to drugs, drug-related cues, or stressors was reported to increase drug craving and relapse to drug use in humans (Childress et al. 1992; de Wit 1996; Sinha et al. 1999) and these also reinstate drug seeking in rats, even following prolonged withdrawal periods (De Vries et al. 1998; Tran-Nguyen et al. 1998; Ciccocioppo et al. 2001; Grimm et al. 2001; Shalev et al. 2001).

To the degree that our preclinical model is of relevance to relapse to drug use in humans our findings are in agreement with clinical reports demonstrating high rates of relapse when drug users return to their home environment after successful extinction of the physiological and psychological responses to drug-associated discrete cues in the clinic (Childress et al. 1993). Furthermore, many drug addicts resume drug taking following successful inpatient detoxification upon returning to the home environment (Meyer and Mirin 1979). Taken together, these findings clearly suggest that for drug abuse interventions to succeed it is critical that contextual stimuli are considered and that treatment strategies aimed at extinguishing the complex association between contextual cues, discrete cues and drug-taking behavior may improve long-term drug abstinence.

ACKNOWLEDGMENTS

This study was supported by NIDA/IRP. We thank Dr. David Epstein for expert-advise on the statistical analyses of the data and Jasmine Yap for her technical assistance.

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