A dopamine D3 receptor partial agonist blocks the expression of conditioned activity

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The partial dopamine D3 receptor agonist BP 897 attenuates cocaine seeking suggesting that BP 897 will attenuate conditioned activity to environmental stimuli paired with amphetamine. During conditioning, amphetamine (2.0 mg/kg) stimulated activity and co-treatment with BP 897 (1.0 mg/kg) had no effect. In the saline test, groups conditioned with amphetamine or amphetamine plus BP 897 showed conditioned activity. Treatment with BP 897 in the test following conditioning with saline produced no significant effect but following conditioning with amphetamine BP 897 blocked conditioned activity. Results extend previous findings that BP 897 attenuates responding for cocaine-paired stimuli to amphetamine-paired stimuli in a different paradigm and support the potential of BP 897 as a therapeutic agent for the prevention of drug seeking. *NeuroReport* 13:1–4 © 2002 Lippincott Williams & Wilkins.

Key words: Amphetamine; BP 897; Conditioned activity; D3 receptors; Dopamine; Drug seeking; Relapse

INTRODUCTION

Environmental stimuli that predict the effects of abused drugs can cause craving and drug seeking in abstinent drug users, leading to relapse of drug taking [1,2]. The successful treatment of relapse depends on understanding the mechanisms underlying this phenomenon and identifying potential therapeutics.

One candidate treatment for lessening drug seeking is the partial dopamine D3 receptor agonist, 1-(4-(2-naphthoyl-amino)butyl)-4-(2-methoxyphenyl)-1A-piperazine HCl (BP 897). This compound significantly and dose-dependently reduced responding for conditioned stimuli associated with cocaine self-administration. BP 897 did not affect responding for cocaine itself nor did it support self-administration in rats [3] or monkeys [4]. Thus, BP 897 selectively inhibited cocaine seeking.

Another paradigm involving the control of behaviour by drug-associated stimuli is conditioned activity. Conditioned activity occurs when a history of administration of a drug, such as cocaine or amphetamine, in a particular environment results in the ability of that environment to elicit stimulant properties in the absence of the previously administered substance [5,6]. The development of conditioned activity depends on intact dopaminergic neurotransmission [7] and it has been argued that conditioned activity, like drug self-administration and a number of related phenomena, is an example of dopamine-mediated incentive learning [6].

Di Chiara [8] has argued that drug abuse can be understood as resulting from excessive control over behaviour by drug-related stimuli produced by dopamine-mediated incentive learning. From this point of view, the conditioned activating effects of environments previously associated with amphetamine can be seen as an example of drug-related stimuli controlling behaviour that is relevant to the study of drug abuse. These considerations led us to test the hypothesis that BP 897 would attenuate conditioned activity produced by environmental stimuli associated previously with amphetamine.

MATERIALS AND METHODS

Subjects: Treatment of rats was in accordance with guidelines of the Animals for Research Act, the Canadian Council on Animal Care, and was approved by the Queen's University Animal Care Committee. Experimentally naive male albino Wistar rats ($n = 60$) weighing 225–275 g upon arrival from Charles River Canada were housed in pairs in clear Plexiglas shoebox-style cages. The colony room was temperature controlled ($21 \pm 1^{\circ}C$) with lights on from 07.00 to 19.00 h. Rats were handled for 1 week prior to starting the experiment and were maintained on a free feeding and drinking schedule (Purina rodent laboratory chow 5001).

Drugs: BP 897 (Bioprojet, Paris, France) in a dose of 1.0 mg/kg and amphetamine (USP, Rockville, MD) in a dose of 2.0 mg/kg were prepared fresh each day in saline (1.0 mg/ml). The dose of BP 897 was selected as the most effective dose for lessening cocaine seeking in the study of Pilla *et al*. [3] and the dose of amphetamine was selected as one that has been found to reliably produce conditioned activity in our hands [5].

Apparatus: Activity was measured as the number of breaks across 14 pairs of photocells positioned at a height of 5.0 and 15.0 cm above the metal rod floor in each of six experimental chambers ($50 \times 40 \times 40$ cm) constructed from Plexiglas and housed in wooden, Styrofoam-insulated outer boxes. Each chamber was illuminated with a 2.5 W bulb and ventilated by a small fan that also provided background noise. Beam breaks were recorded on an experimenter-controlled circuit board connected to a Macintosh computer. For further details of the apparatus see Beninger *et al*. [9].

Procedure: Rats received five 1 h habituation trials, one each day, over 5 days during which no drug was administered. The conditioning phase began on the next day and consisted of three 1 h sessions, one each day. Injections were given 30 min prior to each conditioning session. The single 1 h test session followed the last conditioning session and also was preceded by injections 30 min earlier. Sessions were conducted at about the same time each day.

There were five groups ($n = 12$) that received the following treatments during conditioning and testing: saline/saline; amphetamine (2.0 mg/kg)/saline; amphetamine (2.0 mg/kg) plus BP 897 (1.0 mg/kg)/saline; saline/BP 897 (1.0 mg/kg); and amphetamine (2.0 mg/kg)/BP 897 (1.0 mg/kg). All injections were given i.p.

RESULTS

The dependent measure was the number of beam breaks made during a 60 min session in the activity monitor. Only the last day of habituation was used for the purposes of statistical analyses. The mean ($\pm$ s.e.m.) habituation scores for the groups were quite consistent (Fig. 1). As expected, the test day score for the group conditioned with amphetamine and tested following saline was higher than that of the saline/saline control group. When BP 897 was administered with amphetamine during conditioning, it had no apparent effect on establishment of the conditioned activity effect. Animals receiving BP 897 during the test following conditioning with saline showed little change in activity. Conditioned activity was not seen in the group conditioned with amphetamine and given BP 897 in the test (Fig. 1).

Habituation and test results were subjected to a two-variable mixed design ANOVA with repeated measures on phase and independent groups. Results yielded a significant group by phase interaction ($F(4,55) = 42.98$, $p < 0.001$), showing that the phase effect differed among the groups. Subsequent tests of simple effects of phase for each group confirmed that a significant conditioned activity effect was seen in the amphetamine/saline ($F(1,55) = 97.76$, $p < 0.001$), and amphetamine plus BP 897/saline groups ($F(1,55) = 160.37$, $p < 0.001$). The phase effect was not significant for the remaining groups.

Activity during each day of conditioning and for the 3 days averaged together for each group is shown in Table 1. As expected, groups treated with amphetamine showed increased motor activity. A two-variable mixed design ANOVA with repeated measures on days and independent groups revealed only a main effect of group ($F(4,55) = 69.42$, $p < 0.001$). *Post hoc* (Tukey) comparisons of the 3-day averages revealed that the amphetamine/saline, amphetamine plus BP 897/saline and amphetamine/BP 897 groups differed from the saline/saline and saline/BP 897 groups and not from each other. It is notable that although the increase in activity during conditioning in the amphetamine/BP 897 group was similar to that seen in the other groups conditioned with amphetamine, conditioned activity was not seen in that group.

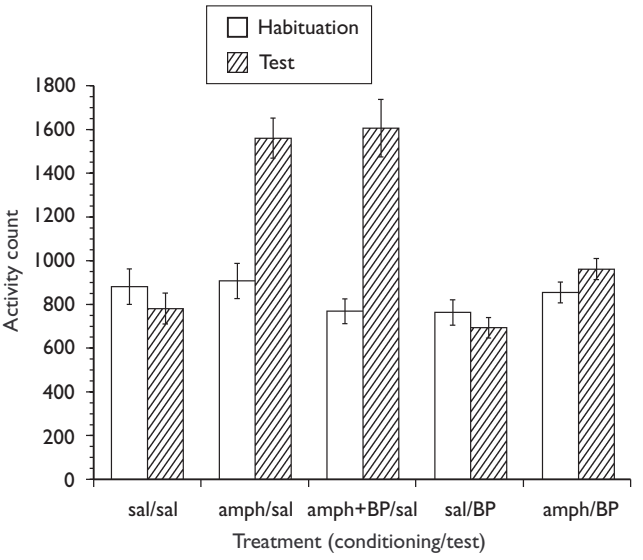

Fig. 1. Mean ($\pm$ s.e.m.) activity counts (per session) for each group on the last day of habituation and on the test phase. Test phase activity was significantly ($p < 0.001$) higher for the amph/sal and amph+BP/sal groups by simple effects tests following a significant interaction in ANOVA. Amph, amphetamine; BP, BP 897; sal, saline.

Table 1. Activity ($\pm$ s.e.m.) during conditioning days 1–3.

Treatment	Day 1	Day 2	Day 3	Average
Sal/sal	874.4 $\pm$ 83.2	888.3 $\pm$ 95.5	810.5 $\pm$ 45.8	857.8 $\pm$ 59.6
Amph/sal	2098.7 $\pm$ 157.7	2154.3 $\pm$ 149.0	2368.5 $\pm$ 130.4	2207.2 $\pm$ 109.3*
Sal/BP	732.9 $\pm$ 54.0	780.0 $\pm$ 64.6	689.8 $\pm$ 51.2	734.3 $\pm$ 41.6
Amph + BP/sal	2346.7 $\pm$ 175.4	2454.3 $\pm$ 182.6	2505.3 $\pm$ 182.2	2435.4 $\pm$ 128.7*
Amph/BP	2065.4 $\pm$ 172.4	2204.2 $\pm$ 147.5	2325.7 $\pm$ 199.9	2198.4 $\pm$ 120.9*

Amph, amphetamine; BP, BP 897; sal, saline.
*Significantly ($p < 0.001$) different for sal/sal and sal/BP by Tukey HSD *post hoc* tests following ANOVA.

DISCUSSION

The results can be summarized as follows. Animals receiving amphetamine during conditioning and saline in the test were more active in the test than control animals that had never received amphetamine in the test environment. Co-administration of amphetamine plus BP 897 during conditioning had no significant effect on the increase in activity produced by amphetamine nor did it affect the establishment of conditioned activity. Treatment with BP 897 during the test session following conditioning with saline had no effect on activity but treatment with BP 897 during the test session following conditioning with amphetamine blocked the conditioned activity effect.

The observation of conditioned activity following a saline injection in a test environment previously paired with amphetamine is consistent with previous reports of conditioned activity [5]. Previous studies have shown that additional control groups that received environment–saline pairings and also received injections of amphetamine on conditioning days but in a different environment, did not show increased activity in the test session [5]. Thus, the observation of increased activity in rats having received environment–amphetamine pairings can be attributed to the association of environmental stimuli with amphetamine rather than to a non-associative effect of a previous history with amphetamine.

The finding that BP 897, when injected prior to the test session into rats that had received environment–amphetamine pairings, blocked the expression of conditioned activity is in good agreement with the findings of Pilla *et al*. [3]. These researchers found that drug seeking during the initial interval of a second order schedule was selectively attenuated by BP 897. Like Pilla *et al*. [3], who reported that BP 897 failed to affect responding for cocaine once the drug was given, we found that BP 897 given during conditioning had no significant effect on the motor activating effect of amphetamine. BP 897 also had no effect on motor activity in the test session when given to animals that had never experienced environment–amphetamine pairings. The present results extend the finding that BP 897 attenuates drug seeking to its ability to block conditioned activity effects and they extend the potential therapeutic use of BP 897 for reducing the strength of cocaine-related stimuli to reduction of the strength of amphetamine-related stimuli.

The mechanism underlying the effects of BP 897 remains unknown. BP 897 is a selective and partial dopamine D3 receptor agonist both *in vitro* and *in vivo* [3], but has also been reported to be a D3 receptor antagonist *in vitro* [10,11] and therefore may produce its effects by acting on D3 receptors. The present observation of decreased conditioned activity with BP 897, but no effect on amphetamine-stimulated activity or normal levels of activity in undrugged animals makes it unlikely that BP 897 simply reduces locomotor activity. Furthermore, previous studies have shown that conditioned activity based on amphetamine or cocaine is resistant to the effects of dopamine receptor antagonists [7,12,13], suggesting that during conditioning dopamine produces learning by changing the strength of non-dopaminergic synapses [14–17]. It is unclear how an action of BP 897 at D3 receptors can be reconciled with these findings. One possibility is that BP 897 acts on D3 receptors located on non-dopaminergic cells in the nucleus accumbens to modulate their activity either there or at terminals in the ventral tegmental area [18]; these may be the cells that undergo modified inputs through the action of dopamine [19]. Another possibility is that BP 897, acting as a receptor agonist, reduces the activity of dopaminergic cells in the ventral tegmental area [18,20], that project to forebrain targets. As a mixed agonist and antagonist, BP 897 may exert its action at multiple sites.

In recent studies, Grimm and See [21] have shown that tetrodotoxin (TTX)-induced inactivation of the basolateral amygdala blocked responding for cocaine-related stimuli but not the cocaine-produced reinstatement of previously extinguished responding for cocaine self-administration. This can be seen as analogous to the ability of BP 897 to block responding for cocaine cues, but not for cocaine itself [3]. TTX in the nucleus accumbens, on the other hand, failed to affect responding for cocaine-related stimuli but blocked the reinstatement of responding produced by cocaine. See *et al*. [22] showed that it was the blockade of dopamine D1 or D1 plus D2 receptors in the amygdala, not the blockade of glutamate receptors, that produced the same effect as TTX. In studies using systemic drug administration, Khroyan *et al*. [23] similarly showed that the ability of cocaine and cocaine-related cues to reinstate responding was blocked by dopamine D1- or D2-like receptor antagonists. How these findings and those with BP 897 will eventually be integrated is not clear.

In conclusion, the present findings show that conditioned activity based on environment–amphetamine pairings is blocked selectively by BP 897. This supports and extends previous findings that this compound attenuates the behavioural effects of cocaine-paired stimuli and continues to support its potential as a therapeutic agent for the prevention of relapse to abuse of various drugs.

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