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Pharmacology, Biochemistry and Behavior 82 (2005) 46 – 54

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Priming with BTCP, a dopamine reuptake blocker, reinstates cocaine-seeking and enhances cocaine cue-induced reinstatement

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Received 16 March 2005; received in revised form 22 June 2005; accepted 15 July 2005
Available online 11 August 2005

Abstract

N-[1-(2-benzo[*b*]thiophenyl)cyclohexyl]piperidine (BTCP), a potent dopamine reuptake inhibitor, substitutes for the reinforcing effects of cocaine and meets other criteria for possible agonist pharmacotherapeutic potential. The purpose of this study was to determine (1) whether BTCP modifies reinstatement of cocaine-seeking elicited by cocaine-related environmental stimuli and (2) whether this compound produces priming effects. Male Wistar rats were trained to associate discriminative stimuli (S^D) with cocaine availability (0.25 mg/infusion) versus non-reward and then were subjected to repeated extinction sessions during which the reinforcer and S^D were withheld. Subsequent presentation of the cocaine S^D produced recovery of cocaine-seeking. BTCP (2.5–30 mg/kg; i.p.) did not attenuate the conditioned reinstatement induced by the cocaine S^D but, rather, potentiated this effect at 10 mg/kg. To test whether BTCP, by itself, exerts priming effects, different groups of rats were trained to self-administer cocaine (0.25 mg/infusion) for 2 weeks. After a 2-week extinction period, BTCP (5, 10 and 20 mg/kg, i.p.) reinstated cocaine-seeking, showing that BTCP not only increases cocaine-seeking induced by cocaine-related stimuli but also produces priming effects following abstinence. The results suggest that, in cocaine abstinent rats, BTCP produces cocaine-like effects.

Keywords: Addiction; Conditioning; Dopamine; Drug cues; Drug-seeking behavior; Priming; Relapse

1. Introduction

Among several strategies for the treatment of drug addiction, one focus is "agonist pharmacotherapy" which refers to the use of compounds that substitute for the primary drug of abuse but have lower intrinsic abuse potential and fewer toxic side effects (e.g., Kreek, 1997b). For example, the synthetic opiate agonist methadone has proven effective for the management of opiate addiction (Kreek, 1997a). Methadone substitutes for heroin, has a slow onset of action, and has long-lasting effects when administered orally without producing a rush-like phenomenon that contributes critically to abuse liability (Kreek, 1997a). In the case of cocaine, however, no comparable agent is currently available. According to the criteria for agonist pharmacotherapeutic potential (Kreek, 1997b), treatment drugs for cocaine addiction should substitute for the subjective effects of cocaine but with a slow onset and sustained duration of action (for review, see Carroll et al., 1999; Howell and Wilcox, 2001; Platt et al., 2002).

Several compounds such as tropane analogs (Bennett et al., 1995; Lile et al., 2000; Roberts et al., 2003) and the phencyclidine derivative *N*-[1-(2-benzo[*b*]thiophenyl)cyclohexyl]piperidine (BTCP) (Martin-Fardon and Weiss, 2002, 2000) have been preclinically evaluated. Among these, BTCP may represent a promising candidate. Like cocaine, BTCP inhibits dopamine (DA) uptake (Chaudieu et al., 1989; Vignon et al., 1988), increases extracellular DA levels in the striatum and nucleus accumbens (Martin-Fardon et al., 1996; Maurice et al., 1992), stimulates locomotor activity (Koek et al., 1989; Slimani et al., 1988) and does not cross-sensitize with cocaine (Martin-Fardon et al., 2000). Of importance with respect to agonist therapeutic

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doi:10.1016/j.pbb.2005.07.010

potential, BTCP shares discriminative stimulus properties with cocaine (Koek et al., 1989) and substitutes for cocaine in self-administration tests (French et al., 1995; Martin-Fardon and Weiss, 2000). Moreover, BTCP is self-administered in drug naïve rats but, compared to cocaine, at a slower rate, indicative of a long duration of action (Martin-Fardon and Weiss, 2002). Consistent with this hypothesis, BTCP was detectable in plasma for 16 h and in brain for 4 h following intraperitoneal administration in mice (Deleuze-Masquefa et al., 2000). The similarity in the profile of behavioral effects of cocaine and BTCP, paired with the longer duration of action of BTCP, led to the hypothesis that BTCP may have potential as a pharmacotherapy for cocaine addiction.

Considering the chronically relapsing nature of cocaine addiction (Leshner, 1997; O'Brien and McLellan, 1996; O'Brien et al., 1998), evaluation of the effects of BTCP on drug-seeking behavior in animal models of relapse may provide valuable additional information on the therapeutic potential of this drug. One important consideration in this regard is that agonist pharmacotherapeutic agents, while effectively substituting for the subjective effects of cocaine, should also lack priming effects that may induce craving and lead to relapse. A further consideration is whether the substitution of the reinforcing actions of cocaine by BTCP extends to the potential for BTCP to prevent craving elicited by cocaine-related stimuli, a major factor in the abuse potential of cocaine (e.g., Childress et al., 1988; O'Brien et al., 1998). Studies in animals have confirmed that environmental stimuli that have become conditioned to the reinforcing actions of drugs of abuse reliably reinstate extinguished drug-seeking behavior (For review see, See, 2002; Shaham et al., 2003; Shalev et al., 2002). Moreover, the response-reinstating effects of these stimuli show remarkable resistance to extinction with repeated exposure (Ciccocioppo et al., 2001a,b; Weiss et al., 2000, 2001) and, in the case of cocaine, can still be observed after several months of abstinence (Ciccocioppo et al., 2001b; Weiss et al., 2001). The purpose of the present experiment was to extend existing evidence on BTCP's capability to substitute for the reinforcing actions of cocaine to an understanding of the potential of this drug in the prevention of craving and relapse, with two specific goals: (1) to examine the effect of BTCP on conditioned reinstatement, an animal model of relapse, as measured by the reinstatement of cocaine-seeking produced by drug-related environmental stimuli, and (2) to examine whether BTCP is devoid of "priming" effects that may convey rather than ameliorate relapse risk.

2. Methods

2.1. Subjects

Forty male Wistar rats (Charles River, Kingston, NY) weighing 200–250 g upon arrival were used. Rats were group-housed (2–3 per cage) in a temperature and humidity controlled vivarium on a 12/12 h light/dark cycle (lights off at 6 p.m.) with ad libitum access to food and water, except during operant training for food reinforcement (see Operant Training below). All animals were handled once daily for 5 min during the first week after arrival. All procedures were conducted in strict adherence to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs

BTCP was a gift from Dr. Jean-Marc Kamenka (CNRS UPR 1086, ENSCM, Montpellier, France). BTCP was dissolved in sterile physiological saline and administered intraperitoneally (i.p.) in a volume of 1.6 ml/kg. Cocaine (National Institutes of Health) was filtered sterile and dissolved in sterile physiological saline. For intravenous (i.v.) self-administration, each infusion of cocaine was administered in 0.1 ml.

2.3. Apparatus

Animals were trained and tested in standard $29 \times 24 \times 19.5$ cm operant conditioning chambers (BRS/LVE Inc., Laurel, MD), located inside ventilated sound-attenuating cubicles (BRS/LVE Inc.). All chambers were equipped with two retractable levers, a white cue light above each lever, and a house light located at the top of the chamber's front panel. Auditory stimuli consisted of a 70 dB white noise produced by a white noise generator (The Salk Institute, San Diego, CA) presented via an 80 Ω speaker located in the center of the chamber's front panel just below the house light, and an intermittent beeping tone (7 kHz, 70 dB), generated by a tone source (Sonalert, Model SC628, 6-28 VDC, Mallory, CO) also positioned in the center of the chamber's front panel just above the speaker. Intravenous infusions were administered by a syringe pump (Razel Scientific Instruments, Stamford, CT) located outside the sound attenuating boxes. Testing equipment and data collection were controlled by an IBM-compatible microcomputer.

2.4. Operant training

Before implantation of i.v. catheters, rats were food restricted (15 g of Purina chow/day) and trained to lever-press for 45 mg food pellet reinforcers (Research Diets, New Brunswick, NJ) to facilitate subsequent acquisition of cocaine-reinforced responding. This training was conducted on a fixed ratio 1 (FR 1) time out 1 s (TO 1) schedule of reinforcement for 1 h per day. Only the right lever was available, and the TO period was signaled by illumination of the cue light above the lever. Once reliable food-reinforced operant responding was established (100 pellets/session), the TO was increased to 20 s and the 1-h

session was divided into two 30-min daily sessions. Training then continued until rats reached stable levels of responding for three consecutive sessions and earned at least 50 pellets/session. Rats designated for the priming experiment were trained to lever-press for food pellet reinforcers on a fixed ratio FR 5 TO 20 schedule. Operant training for food-maintained responding was continued until a criterion of at least 50 completed ratio requirements/30-min session over three consecutive days was reached. Food then was made available ad libitum for the duration of the experiment.

2.5. Surgery

Rats were implanted with chronic silastic catheters in the right jugular vein under isoflurane (1.0–1.5%) anesthesia as previously described (Caine et al., 1993). Catheter patency was maintained by flushing with 0.1 ml of sterile heparin/saline (33.3 USP U/ml) solution before and after each self-administration session. Rats with compromised catheters were excluded from the experiment.

2.6. Cocaine-related stimuli-induced reinstatement

2.6.1. Self-administration training and conditioning

Sixteen rats were used for the conditioned reinstatement studies using a previously described procedure (Weiss et al., 2001). Briefly, rats were trained to intravenously self-administer cocaine while establishing discriminative stimuli associated with cocaine availability vs. non-availability. Ten days after recovery from surgery, cocaine self-administration began in daily 2-h sessions conducted 5 days per week for a total of 14 days. Self-administration sessions were initiated by extension of both levers and simultaneous presentation of a white noise that served as a discriminative stimulus (S^+) for cocaine availability and remained present throughout the session. Cocaine then was available for 2 h on an FR 1 schedule of reinforcement. The right lever was active, and when depressed resulted in an infusion of cocaine (0.25 mg/0.1 ml/infusion, delivered over 4 s). Completion of each ratio requirement resulted in an infusion of cocaine and concurrent illumination of the white cue light above the active right lever for a 20-s period. During this 20-s time-out period, the lever remained inactive to prevent accidental overdosing. Responses at the left lever (inactive lever) were recorded but had no scheduled consequences. After this 2-week period, daily access to cocaine was changed from a single 2 h session to two consecutive 1-h sessions separated by 20 min for 3 days. At the end of this three day period, a third daily 1-h session was added during which saline was substituted for the cocaine solution. This session was initiated by extension of both levers and illumination of the house light located at the top of the chamber's front panel, which served as a discriminative stimulus (S^-) signaling the availability of saline (i.e., non-reward) and remained present throughout the session. Each response at the active lever resulted in an infusion of 0.1 ml saline followed by presentation of a 20-s TO signal (intermittent beep; 1 s presentation at 1 s intervals) during which the lever remained inactive. Responses at the left inactive lever were recorded but had no programmed consequences. The three daily sessions (i.e., two cocaine and one saline session) were conducted in random order. Between each session, rats were removed from the self-administration chambers for 20 min. Training was continued under these conditions for a total of 30 sessions during which the rats developed stable cocaine intake (i.e., ±10% across three consecutive days) and ceased responding for saline.

2.6.2. Extinction phase

One day after the last conditioning session, the rats were placed on extinction conditions in daily 1-h sessions. Extinction sessions were initiated by the extension of both levers but without presentation of discriminative stimuli. Responses at the previously active lever had no scheduled consequences except for activation of the syringe pump motor. Extinction sessions were continued until the animals reached the criterion of 4 or fewer lever-presses/session at the previously active lever for 3 consecutive days.

2.6.3. Reinstatement phase — drug tests

One day after each animal reached the extinction criterion, reinstatement tests began. These 1 h tests were conducted once every third day for a total of 13 sessions (one S^- and 12 S^+ tests) under extinction conditions, except that the cocaine- or saline-predictive discriminative stimuli were reintroduced. Sessions began by the extension of the levers and the presentation of either the S^+ or S^-. The discriminative stimuli remained present until retraction of the levers at the end of each session. Responses at the previously active lever resulted in activation of the syringe pump and a 20-s TO (signaled by illumination of the cue light above the right lever in the S^+ condition, and the intermittent beep in the S^- condition) during which the lever remained inactive.

To verify the behavioral selectivity of the discriminative stimuli, all rats were tested in the presence of the S^- on the first day of the reinstatement phase. Two days after the S^- session (i.e., on the third day), the effect of BTCP on S^+-induced reinstatement of cocaine-seeking was tested. BTCP (2.5, 5, 10, 20, 30 mg/kg, i.p.) or its vehicle (saline, 1.6 ml/kg) were administered 10 min before the beginning of reinstatement sessions. Each animal was tested once with each dose of BTCP in random order. Rats were treated with BTCP or its vehicle before sessions 2, 4, 6, 8, 10, 12 of the reinstatement phase. During sessions 3, 5, 7, 9, 11, and 13 the rats were tested in the presence of the S^+ alone to confirm that the S^+ had retained its efficacy to elicit reliable reinstatement. Reinstatement sessions were

separated by a 2-day period during which the rats remained in their home cages.

2.7. BTCP prime-induced reinstatement

2.7.1. Self-administration training

Twenty-four rats were used at the beginning of the experiment, but one rat was lost due to health complication reducing the sample size to 23. Ten days after recovery from surgery, cocaine self-administration began in daily 2-h sessions, 5 days per week. Self-administration sessions were initiated by administration of 2 non-contingent intravenous cocaine infusions (training dose of 0.25 mg/0.1 ml/infusion, delivered over 4 s), which signaled the start of each session. Subsequently, the right lever was extended into the operant chamber at which time cocaine was made available on an FR 5 schedule of reinforcement. Completion of each ratio requirement resulted in an infusion of cocaine and concurrent illumination of the white cue light for a 20-s period. During this 20-s TO period, the lever remained inactive to prevent accidental overdosing. This training continued for a total of 14 self-administration sessions.

2.7.2. Extinction phase

The day following the last self-administration session, extinction conditions were imposed by substituting saline for cocaine. This procedure was continued for a total of 14 extinction sessions.

2.7.3. Reinstatement phase — drug tests

The day following the last extinction session, reinstatement tests began. These sessions continued to be conducted under extinction conditions (i.e., saline availability). BTCP (5, 10 and 20 mg/kg, i.p.) was administered 10 min before reinstatement sessions. The 23 rats were divided into three groups with 8 rats tested with the 5 mg/kg BTCP dose, 8 rats with the 10 mg/kg BTCP dose, and 7 rats with the 20 mg/kg BTCP dose. To check for non-specific effects of the vehicle injection on responding, all rats were treated with vehicle the day before the drug test such that each rat received a total of 2 injections (vehicle and BTCP).

2.7.4. Statistical analyses

Differences among responses during the Training Phase were analyzed using paired Student's *t*-test. Differences among Extinction and Reinstatement Phases, the effects of BTCP on cue-induced reinstatement, and the stability of the cocaine cue-induced reinstatement were analyzed by one-way ANOVA, followed by Newman–Keuls post hoc tests to confirm differences among individual means. To evaluate the priming effects of BTCP, the total number of lever-presses and the cumulative number of responses were analyzed by mixed-factorial ANOVA, followed by Newman–Keuls post hoc tests.

3. Results

3.1. Cocaine-related stimuli-induced reinstatement

3.1.1. Self-administration training and conditioning

All 16 rats acquired stable cocaine self-administration with minimal responding during saline availability. No differences were observed in the mean number of cocaine-reinforced responses across the last 3 days of the self-administration and conditioning phase, or between the first and second hour of cocaine availability. Therefore, the data for the 2 daily cocaine sessions of the last day of the conditioning phase were pooled for statistical analysis. In addition, the data from the 2 daily cocaine sessions were pooled across the last three days of the conditioning phase and compared to the corresponding number of saline infusions (Fig. 1A). During the last day of the conditioning phase, the number of lever-presses was significantly higher during cocaine vs. saline sessions (*t*-test, $t(15)=18.4$; $p<0.001$) (Fig. 1A). Responding at the inactive lever was negligible (Fig. 1B).

3.1.2. Reinstatement phase — drug tests

During the first extinction session, the mean (±SEM) number of responses per session was 12.7±1.2. Rats reached the extinction criterion within 13.2±3.0 days (Fig. 1C). Only minimal responding was observed at the inactive lever (Fig. 1D). For statistical analysis, data from the last day of extinction were used where the mean (±SEM) total number of lever-presses was 2.9±0.3. During reinstatement tests in the presence of the non-reward S^-, responding at the active lever remained at the extinction levels (Fig. 1C).

Two days following the S^- presentation, the effects of BTCP pretreatment on cocaine S^+-induced reinstatement were tested. Exposure to the cocaine S^+ elicited a substantial increase in responding at the previously active lever vs. extinction and S^- levels. BTCP produced changes in the effect of the S^+ compared to vehicle-treated rats and more specifically induced an enhanced S^+ effect following 10 mg/kg (Fig. 1C). Thus, a one-way ANOVA revealed a significant main effect for BTCP vs. extinction or S^- ($F(7,105)=10.26$; $p<0.001$). Newman–Keuls post hoc tests confirmed the effects of BTCP (2.5–30 mg/kg) on S^+ responses ($p<0.05$ vs. extinction and S^-) as well as the enhanced performance after the 10 mg/kg challenge ($p<0.05$ vs. vehicle; Fig. 1C). Responses at the inactive lever remained negligible during both S^- and S^+ tests (Fig. 1D). Examination of the response profiles of BTCP effects on S^+-induced responding revealed that BTCP modified the cumulative number of responses and slope of cumulative responses (Fig. 1C, Inset). A two-way ANOVA confirmed this modification and revealed a significant main effect for BTCP doses ($F(5,75)=4.5$; $p<0.01$) and a significant dose × time (10 min intervals) interaction ($F(25,375)=2.9$; $p<0.001$). Further examination of the response profile indicated that only the 10 mg/kg BTCP dose increased the

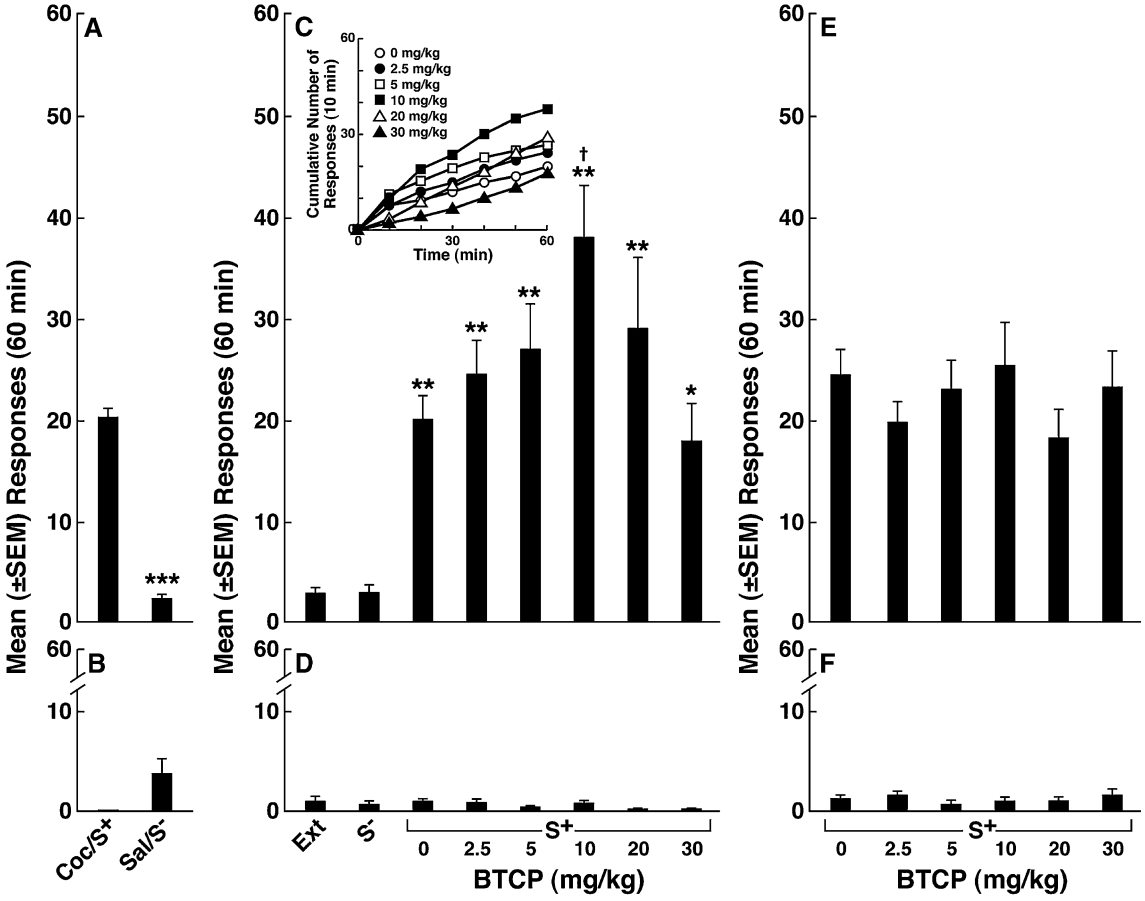

Fig. 1. Effects of BTCP on reinstatement induced by discriminative stimuli associated with cocaine. Responses during cocaine (Coc/S^+) and saline (Sal/S^-) self-administration at the active (A) and inactive (B) lever (*** $p<0.001$ vs. Coc/S^+). Responses during extinction (Ext), reinstatement tests in the presence of the saline (S^-) versus cocaine cues (S^+) and BTCP on conditioned reinstatement at the active (C) and inactive (D) lever (* $p<0.05$, ** $p<0.01$ vs. Ext and S^-; + $p<0.05$ vs. 0 mg/kg). Inset: cumulative number of responses throughout the 60 min reinstatement period (error bars were omitted for clarity). Reinstatement responses without drug or vehicle treatment 2 days after each drug test at the active (E) and inactive (F) lever.

number of responses at several time points compared to vehicle (Newman–Keuls, $p<0.05$ from $t=20$ min to $t=60$ min; Fig. 1C, Inset). At 30 mg/kg, a delayed onset of responding, lasting 10–20 min, was observed, but this effect was not statistically different from vehicle.

A one-way ANOVA revealed that the degree of responding induced by the cocaine S^+ between each drug dose test (i.e, the 6 baseline reinstatement days when the rats were tested in the presence of the S^+ alone) was unchanged ($F(5,75)=1.1$; $p>0.05$, Fig. 1E). During all of these tests, responses at the inactive lever remained negligible (Fig. 1F).

3.2. BTCP prime-induced reinstatement

3.2.1. Self-administration training

All 23 rats acquired stable cocaine self-administration. At the end of the training phase, the overall mean (±SEM) number of cocaine infusions was 39.3±1.6. No differences were found in cocaine self-administration between the groups that were to be tested with BTCP 5, 10 or 20 mg/kg ($F(2,20)=0.42$; $p>0.05$).

3.2.2. Reinstatement phase— drug tests

At the end of the 14-day extinction period, the mean (±SEM) number of responses were: 11.6±3.2 for the rats used to test BTCP at 5 mg/kg, 9.1±2.4 for the rats used to test BTCP at 10 mg/kg, and 8.0±2.0 for the rats used to test BTCP at 20 mg/kg. A saline injection before the last extinction session did not produce any effect on responding. This observation was confirmed by a two-way ANOVA showing a lack of main effect for test day ($F(1,20)=0.35$; $p>0.05$). BTCP induced reinstatement of responding but without any dose effect, reflected by a main effect of BTCP injection ($F(1,20)=21.1$; $p<0.001$) and a lack of a main dose effect ($F(2,20)=0.10$; $p>0.05$, Fig. 2). Examination of the response profiles of BTCP effects on reinstatement revealed that BTCP at each dose modified the cumulative number of responses and slope of cumulative responses compared to vehicle (Fig. 2, Inset). A three-way ANOVA revealed that while no main effect for BTCP dose was found, the time course of responding varied with time after the BTCP administration, but not following vehicle injection ($F(11,220)=8.6$; $p<0.001$). Moreover, although there were no significant differences in responding between the 3

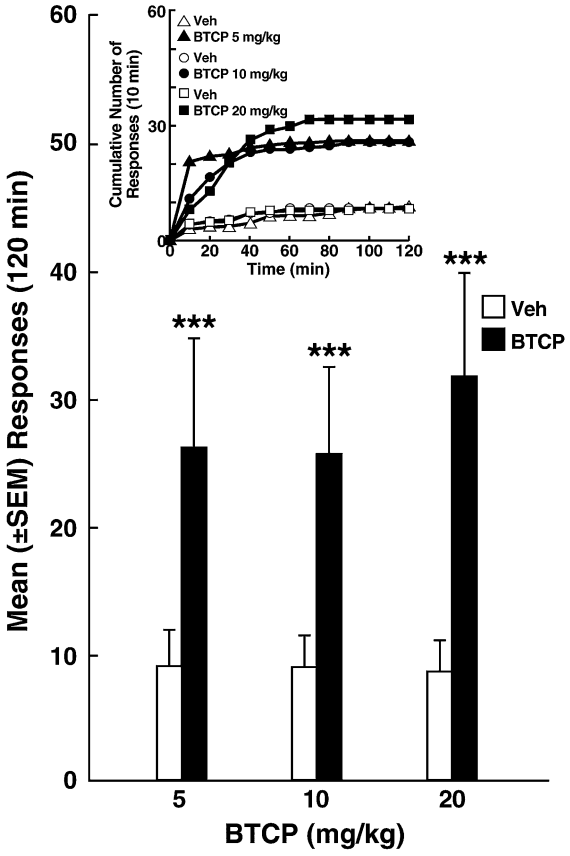

Fig. 2. Effects of BTCP-induced reinstatement of extinguished cocaine-seeking behavior. (*** $p<0.001$ vs. vehicle). Inset: cumulative number of responses throughout the 120 min reinstatement period (error bars were omitted for clarity).

BTCP doses, a significant three-way interaction was found, indicative of significant differences in the time course of responding among the BTCP doses ($F(22,220)=1.8$; $p<0.05$). To explore the three-way interaction, separate two-way ANOVAs were conducted comparing each dose of BTCP to its respective vehicle. At each dose tested, the two-way ANOVAs revealed a main effect for BTCP doses. BTCP produced an increase in responding compared to saline (5 mg/kg, $F(1,7)=7.2$; $p<0.05$; 10 mg/kg, $F(1,7)=6.4$; $p<0.05$; 20 mg/kg, $F(1,7)=5.5$; $p<0.05$). Further examination of the response profile indicated that the onset of increased responding was delayed with increased doses of BTCP (Newman–Keuls, compared to vehicle: 5 mg/kg, $p<0.05$ from $t=20$ to 120 min; 10 mg/kg, $p<0.05$ from $t=50$ to 120 min; 20 mg/kg, $p<0.05$ from $t=70$ to 120 min; Fig. 2, Inset).

4. Discussion

The results show that BTCP produced an inverted U-shaped dose–response effect on reinstatement induced by cocaine-related stimuli with significant increases in conditioned reinstatement at the 10-mg/kg dose. Moreover, BTCP produced priming effects at the three doses tested, but the effects were not dose-dependent. The results suggest that BTCP does not attenuate reinstatement induced by cocaine cues but can increase this behavior, and that BTCP can produce priming effects.

Consistent with previous observations (Weiss et al., 2000, 2001), the cocaine S^+ remained efficacious in producing high levels of conditioned reinstatement with repeated presentation under extinction conditions. The "baseline" magnitude of reinstatement under "no treatment" conditions and after vehicle injections remained highly stable throughout the 13 reinstatement sessions (the last conducted 37 days after the last extinction session or 50 days after the last cocaine self-administration session). These data confirm previous evidence that the motivating effects of stimuli previously predictive of cocaine availability are resistant to extinction and also strengthen previous findings showing that, regardless of the nature of the stimulus, drug predictive stimuli (S^+) consistently induce reinstatement with similar robustness, whereas stimuli predictive of non-reward (S^-) consistently fail to increase responding over extinction levels (Ciccocioppo et al., 2001b, 2002, 2003, 2004; Katner and Weiss, 1999; Weiss et al., 2000, 2001). It is important to note, however, that during the reinstatement phase, like in the training phase, the animals were presented with the S^+ and with the TO period (i.e., the lever light that was present for 20 s after each response at the active lever). This signaled TO period during the training phase was designed to protect the animals against any accidental overdosing, and eventually may function as a conditioned stimulus (CS^+). In a study from this laboratory where the importance of the S^+ vs. CS^+ was evaluated (Martin-Fardon, Stuempfig and Weiss, in preparation), it was shown that presentation of the S^+ (white noise) after the extinction phase drives responding. In other words, the CS^+ did not play any role in the reinstatement level of cocaine-seeking. Therefore, while the CS^+ was present during the reinstatement phase, only the S^+ is reported because it is the most salient stimulus that induces reinstatement.

BTCP substantially increased conditioned reinstatement compared to the effects of the S^+ alone. Examination of the time course of responding shows that higher doses of BTCP (and particularly 30 mg/kg) induced a delay in the onset of responding but did not alter overall responding for the session (see Fig. 1C). One possible interpretation of this finding is that the motivating impact of the cocaine cue was initially reduced because of the subjective hedonic effect produced by BTCP pretreatment rather than being the result of non-specific behavioral inhibition. This hypothesis is consistent with an earlier study showing that BTCP pretreatments (16 and 32 mg/kg) induce a compensatory delay in the onset of cocaine self-administration without affecting responding for water in water-restricted animals, leading to the conclusion that BTCP substitutes for cocaine (Martin-Fardon and Weiss, 2000). Interestingly, at the

highest dose, BTCP seemed to delay responding, without producing a secondary increase in drug-seeking (see Fig. 1C, Inset). While BTCP under the present conditions failed to significantly attenuate conditioned reinstatement, the latter observation could perhaps be exploited by examining whether manipulations that, for example, decrease the rate of absorption of BTCP provide longer-lasting inhibitory effects on drug-seeking associated with cocaine cue exposure.

It is well established that "priming" doses of drugs of abuse elicit craving in drug addicts (Jaffe et al., 1989) and reinstate drug-seeking after extinction in animals (e.g., de Wit and Stewart, 1981; Shaham et al., 2003). Although the two procedures used to measure the reinstatement effects of BTCP after extinction were significantly different (i.e., different length of training, different FR, different exposure to cocaine), these two procedures are complementary. The conditioned reinstatement procedure and the prime-induced reinstatement procedure are designed to answer two different questions. In conditioned reinstatement, the ability of BTCP to modify conditioned reinstatement induced by cocaine-related stimuli is assessed. In prime-induced reinstatement, the ability of BTCP to trigger cocaine-seeking behavior in abstinent subjects is assessed. Thus, levels of responding in these procedures are not intended to be directly compared. Priming with BTCP reinstated cocaine-seeking at all three doses tested. This raises the possibility that reinstatement is produced by BTCP because this compound shares discriminative stimulus properties with the self-administered drug (see for example, Schenk and Partridge, 1999). This hypothesis is consistent with earlier studies showing that BTCP shares discriminative stimulus properties with cocaine (Koek et al., 1989) and also substitutes for cocaine in self-administration tests (French et al., 1995; Martin-Fardon and Weiss, 2000). However, the behavioral effects of BTCP on reinstatement (cues and prime) were unexpectedly low considering the high affinity of this drug for the DA transporter (DAT) compared to that of cocaine (Deleuze-Masquefa et al., 2000; Lebel et al., 1994). In vitro, BTCP is approximately 20 times more potent than cocaine in binding to the DAT (Deleuze-Masquefa et al., 2000; Lebel et al., 1994) leading to the prediction that BTCP would exert strong priming and additive effects with the cocaine-related stimuli. This discrepancy might be the result of a higher lipophilicity of BTCP compared to cocaine, a characteristic that would reduce the overall bioavailability of BTCP. For example, it is known that BTCP's parent molecule phencyclidine and its metabolites are stored in fat, lungs and liver as long as 2 weeks after an acute injection (Martin, 1982). Consistent with this hypothesis, after an acute injection in mice, only 0.7% of the dose was recovered in plasma, urine and brain (Deleuze-Masquefa et al., 2000). Therefore, the accumulation of BTCP in different organs may account for a reduction of its bioavailability. Moreover, while BTCP had priming actions at all doses, this effect was additive with the conditioned effects of the cocaine cues only at the 10 mg/kg but not the 5 and 20 mg/kg doses. Furthermore, at 5 mg/kg, BTCP had no effects on conditioned reinstatement, but at 20 mg/kg, a trend toward a delay of responding was observed.

One possible explanation for the limited actions of BTCP on conditioned reinstatement and prime-induced reinstatement is that BTCP has different discriminative stimulus characteristics than cocaine. Although the discriminative stimulus effects of cocaine and BTCP are similar (Kleven et al., 1999; Koek et al., 1989), the subjective effects of BTCP may be produced by norepinephrine (NE) uptake inhibition, whereas the subjective effects of cocaine depend primarily on DAT inhibition (Katz et al., 2000; Kleven et al., 1999). It is known for example that cocaine and BTCP cross-substitute in drug self-administering rats, and that DA reuptake blockers induce a dose-related drug lever selection in both cocaine and BTCP trained rats. However, NE reuptake inhibitors produce greater correct lever selection in BTCP-trained rats than cocaine-trained rats (Kleven et al., 1999). Therefore, the noradrenergic component of the discriminative stimulus effects of BTCP may make this stimulus sufficiently different from that produced by cocaine, reducing its efficacy to elicit reinstatement.

The lack of a dose-dependent effect of BTCP prime-induced reinstatement was also surprising, and the results suggest that, at a dose as low as 5 mg/kg, BTCP produced a plateau effect. One may hypothesize that this effect was the result of BTCP action on the NE and serotonin (5-HT) transporters. While the potency of BTCP for inhibition of 5-HT and NE transporters is about the same as that for DA reuptake (Lebel et al., 1994), there is much evidence that inhibition of DA reuptake specifically accounts for the behavioral cocaine-like effects of BTCP (Katz et al., 2000; Roberts et al., 1999). Moreover, while using different procedural approaches, earlier studies have shown that cocaine, also a DA, 5-HT and NE uptake inhibitor, produces dose-dependent priming effects (e.g., de Wit and Stewart, 1981; Schenk and Partridge, 1999). Nonetheless, future studies using specific NE and 5-HT reuptake inhibitors will be needed to confirm this hypothesis as well as test DA involvement in this phenomenon using compounds with higher affinity for the DAT than BTCP itself, such as BTCP's active metabolites (Deleuze-Masquefa et al., 1997; Martin-Fardon et al., 2001, 2003).

An important goal of the development of medications to treat cocaine addiction is the identification of pharmacotherapies that prevent withdrawal symptoms, normalize physiological functions disrupted by chronic cocaine use, and prevent craving that contributes critically to relapse vulnerability. One therapeutic approach that has been proven effective in opiate addiction is maintenance pharmacotherapy with the long- lasting opiate agonist methadone (Kreek, 1997a). Effective maintenance medications for cocaine addiction are likely to have a pharmacological profile that overlaps significantly with the pharmacological profile of cocaine. A cocaine maintenance medication

should substitute to some extent for the subjective effects of cocaine but have limited abuse liability and toxic side effects. By partially reproducing cocaine-like subjective effects, maintenance medications would be expected to alleviate cocaine craving (Carroll et al., 1999; Howell and Wilcox, 2001; Platt et al., 2002).

BTCP's profile of behavioral effects, as characterized in previous work, identifies this compound as a potential maintenance medication. BTCP reduces cocaine self-administration in a dose-dependent manner by substituting for the reinforcing actions of cocaine without affecting behavior maintained by a non-drug reinforcer (Martin-Fardon and Weiss, 2000), has a substantially longer duration of action than cocaine (Martin-Fardon and Weiss, 2000, 2002), and does not elicit sensitized locomotor responses in cocaine-sensitized rats (Martin-Fardon et al., 2000). However, BTCP is readily self-administered in drug-naïve rats and may have greater reinforcing actions than cocaine (Martin-Fardon and Weiss, 2002). Moreover, the present results show that BTCP increases conditioned reinstatement and has a priming effect in abstinent rats. Under the present conditions, these findings certainly disqualify BTCP as being a candidate for maintenance therapy because this compound, when administered to an abstinent individual, may induce intense craving and leads to relapse. However, the observation that increased doses of BTCP produced both a delay in the onset of responding (Fig. 1C Inset and Fig. 2 Inset) and a trend towards a decrease in conditioned reinstatement over time may indicate that in certain conditions this drug can block reinstatement. Nonetheless, several important issues remain for future research. One question to be addressed is whether manipulations that modify BTCP's rate of absorption and bioavailability would modify the pharmacological profile of this agent (i.e., a decreased priming effect and a decreased effect on conditioned reinstatement). One way to change the bioavailability of BTCP would be to use a different route of administration, such as orally, and a different formulation for BTCP, such as a sustained release. This method has been proven effective for the nicotine abuse treatment bupropion (e.g., Hays and Ebbert, 2003; Tonstad and Johnston, 2004). This stimulant shows behavioral effects indicative of abuse liability similar to cocaine when administered i.v. or i.p. (Nomikos et al., 1992; Tella et al., 1997), but its apparent abuse liability is mitigated when given under a sustained release formulation orally (Hays and Ebbert, 2003; Tonstad and Johnston, 2004). Another question to be addressed is whether BTCP will remain effective with chronic administration. Repeated daily exposure will be needed to characterize peak effectiveness and also to document continued effectiveness over multiple sessions. Finally, understanding of the toxic side effects produced by BTCP relative to cocaine will require systematic exploration.

In conclusion, considering the importance of the DAT in the addictive properties of cocaine, the development of compounds that target the DAT is a logical approach for the pharmacological treatment of cocaine abuse. However, while the agonist therapy approach has been successful in the case of heroin abuse using the opiate agonist methadone, together with previous studies, the present findings suggest that using a potent DAT blocker such as BTCP might have some limitations. BTCP may have abuse potential and a "cocaine-like" profile in animal models of relapse, two undesirable properties for a medication.

Acknowledgements

This is publication number 15931-NP from The Scripps Research Institute. The authors thank M. Michaud for the synthesis of BTCP, Dr. M.A.S. Baptista, Dr. H. Aujla, Dr. C.V. Dayas and M. Arends for assistance with preparation of the manuscript. This research was supported by NIH/NIDA grant DA 07348 and DA 08467 (F.W.).

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