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Muscarinic preferential M_1 receptor antagonists enhance the discriminative-stimulus effects of cocaine in rats

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Abstract

Previous studies of benztropine analogues have found them to inhibit dopamine uptake like cocaine, but with less effectiveness than cocaine in producing behavioral effects related to drug abuse. Studies have assessed whether nonselective muscarinic antagonists decrease the effects of cocaine because many of the benztropine analogues are also muscarinic antagonists. As previous studies were conducted with nonselective muscarinic antagonists and the benztropine analogues show preferential affinity for the M_1 muscarinic receptor subtype, the present study examined interactions of cocaine and the preferential M_1 antagonists, telenzepine (TZP) and trihexyphenidyl (TXP) on subjective effects in rats trained to discriminate cocaine (10 mg/kg, i.p.) from saline injections. Cocaine dose-dependently increased the percentage of responses on the cocaine-appropriate lever, with full substitution at the training dose. In contrast neither TZP nor TXP produced more than 25% cocaine-appropriate responding at any dose. Both M_1 antagonists produced significant leftward shifts in the cocaine dose–effect curve, TZP at 3.0 and TXP at 0.3 and 1.0 mg/kg. The present results indicate that preferential antagonist actions at muscarinic M_1 receptors enhance rather than attenuate the discriminative-stimulus effects of cocaine, and thus those actions unlikely contribute to the reduced cocaine-like effects of BZT analogues. Published by Elsevier Inc.

Keywords: Cocaine; Drug-discrimination behavior; Muscarinic antagonist; M1, Benztropine analogues; Dopamine transporter; Rats

The behavioral effects of cocaine are believed to be primarily mediated by increased dopamine (DA) neurotransmission as a result of blockade of the DA uptake through binding to the DA transporter (DAT). It has also been hypothesized that inhibition of DA reuptake through actions at the DAT confers behavioral effects like those of cocaine (Kuhar et al., 1991). In spite of this hypothesis, several analogues of the antiparkinson drug, benztropine, that share with cocaine a similar chemical structure and a high affinity for the DAT, show reduced behavioral effects compared to cocaine (Newman et al., 1995). These behavioral effects include locomotor stimulation (Katz et al., 1999, 2004), and discriminative- (Katz et al., 1999; Tolliver et al., 1999) and reinforcing- (e.g., Woolverton et al., 2000) stimulus effects.

Several of the analogues of benztropine have high (nM) affinity for muscarinic receptors (Katz et al., 1999; Tanda et al., 2007), and it could be hypothesized that this effect contributes to the reduced cocaine-like activities of the benztropine analogues (Katz et al., 1999). Support for the hypothesis would be obtained if antimuscarinic agents decreased the effects of cocaine. Previous studies, however, have suggested otherwise. For example, Scheckel and Boff (1964) found an increase in the effects of cocaine on avoidance responding of rats after co-administration of the nonselective antagonists of muscarinic receptors, atropine, scopolamine, or the preferential antagonist of muscarinic M_1 receptors trihexyphenidyl (TXP). In addition, the discriminative-stimulus and locomotor stimulant effects of cocaine are enhanced by atropine or scopolamine (Acri et al., 1996; Katz et al., 1999).

Recent studies have suggested that the benztropine analogues have preferential activity at muscarinic M_1 receptors over the other subtypes (Katz et al., 2004; Tanda et al., 2007). Like previous results with atropine or scopolamine, it has been reported that TXP enhanced the locomotor stimulant effects of cocaine, though it antagonized place conditioning produced by methamphetamine, but curiously not that produced by cocaine (Shimosato et al., 2001). Recent studies from this laboratory

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(Tanda et al., 2007) showed an enhanced effect of cocaine on levels of dopamine in the nucleus accumbens shell, but not prefrontal cortex or nucleus accumbens core, produced by both TXP and another preferential M_1 antagonist, telenzepine (TZP). Also in that study, the locomotor stimulant effects of cocaine were enhanced by TXP, but not TZP.

Because an enhanced effect of cocaine on dopamine levels was obtained with both preferential M_1 antagonists selectively in the nucleus accumbens shell, an area implicated in the abuse of drugs (Pontieri et al., 1995), and because there was some indication of antagonism of a methamphetamine conditioned place preference (Shimosato et al., 2001), we further studied the effects of combinations of the preferential M_1 antagonists, TXP and TZP, on the discriminative-stimulus effects of cocaine. The discriminative-stimulus effects of drugs of abuse are thought to be related to their subjective effects in humans, and are thus important for preclinical study of the abuse of drugs (e.g., Holtzman, 1990). Further interest in these drugs was due to their preferential activity at M_1 over other muscarinic subtypes (Bymaster et al., 1993; Doods et al., 1987; Eltze et al., 1985) and their semblance in preferential activity to profiles of several benztropine analogues (Tanda et al., 2007).

1. Materials and methods

1.1. Subjects

Experimentally naïve male Sprague Dawley rats (Taconic Farms, Germantown, NY or Charles River Laboratories, Wilmington, MA) were maintained at 325±10 g. The rats were fed 10–15 g of food (BioServ, Frenchtown, NJ) daily, 1 hr after testing to maintain their body weights and were individually housed (12-h light/dark cycle, lights on: 7am) in a temperature-and humidity-controlled room within a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

1.2. Apparatus

Experiments were conducted with subjects in a 29.2×24.2×21 cm operant-conditioning chamber (modified ENV-001, Med Associates, St. Albans, VT). The front wall of the chamber contained two response keys (levers requiring a force of 0.4 N through 1 mm to register a response), and a centrally located opening for delivery of 45 mg food pellets from a dispenser mounted behind that wall. Each press on either lever at all times produced an audible feedback click of a relay mounted behind the front wall. A pair of green and a pair of yellow light emitting diodes were arranged horizontally above each lever. The chamber was contained within a ventilated enclosure which provided light and sound attenuation. White noise to mask extraneous sounds was delivered at all times by a speaker mounted within the chamber.

1.3. Procedure

All experimental sessions were conducted between the hours of 9:00–12:00, Mondays through Fridays. Rats were initially trained with food reinforcement to press both keys, and were eventually trained to press one after cocaine (10 mg/kg, i.p.), and the other after saline (i.p.) injection. The ratio of responses to food pellets (fixed ratio or FR) was gradually increased until, under the final conditions, the completion of 20 consecutive responses on the cocaine- or saline-appropriate key produced food. Incorrect responses reset the FR response requirement. The right vs. left assignment of cocaine- and saline-appropriate keys was counterbalanced among subjects.

Subjects were placed in chambers immediately after injection. There was a 5-min timeout period during which lights were off and responses produced the feedback click but had no other scheduled consequences. Following the timeout, the house light was turned on until the completion of the FR 20 response requirement. Food presentation was followed by a 20-s timeout period. Sessions ended after 20 food presentations or 15 min, whichever occurred first. Cocaine or saline sessions were scheduled in a double-alternation sequence (cocaine, saline, saline, cocaine). Training continued until subjects met the criteria on four consecutive sessions of at least 85% cocaine- or saline-appropriate responding over the entire session, as well as the first FR of the session.

Once the criteria were met testing began, with test sessions conducted at most every third session, and followed the administration of different doses of cocaine, antimuscarinic agents, or their combination. Test sessions were conducted if the subjects met the training criteria over the two prior training sessions (one with saline and one with cocaine), and were identical to training sessions with the exception that 20 consecutive responses on either key were reinforced. All programming of behavioral contingencies and data collection was accomplished with software from Med Associates, Inc (St. Albans, VT).

1.4. Drugs

The drugs tested were telenzepine (TZP, Sigma-Aldrich), trihexyphenidyl (TXP, Sigma-Aldrich), and (−)-cocaine HCl (Sigma-Aldrich and NIDA). Drugs were dissolved in 0.9% NaCl and injected in a volume of 1.0 ml/kg. Cocaine was administered i.p., immediately before subjects were placed in the chamber. TZP and TXP were administered s.c., 15 min before the subjects were placed in the chamber. Because the session started with a 5 min timeout, the times of injection before the opportunity to respond (the session proper) were 5 and 20 min for cocaine and the M_1 antagonists, respectively. The 20-min pretreatment time for TZP and TXP was based on previous results with these drugs showing in vivo activity starting from 10 min to 30 min after injection (e.g. Bymaster et al., 1994; Ichikawa et al., 2002).

1.5. Data analysis

Overall response rates and percentages of responses occurring on the cocaine-appropriate lever for the entire session were calculated. The mean values for groups of subjects were calculated at each drug dose. Dose-effect curves for response rates and per cent drug response were analyzed using standard

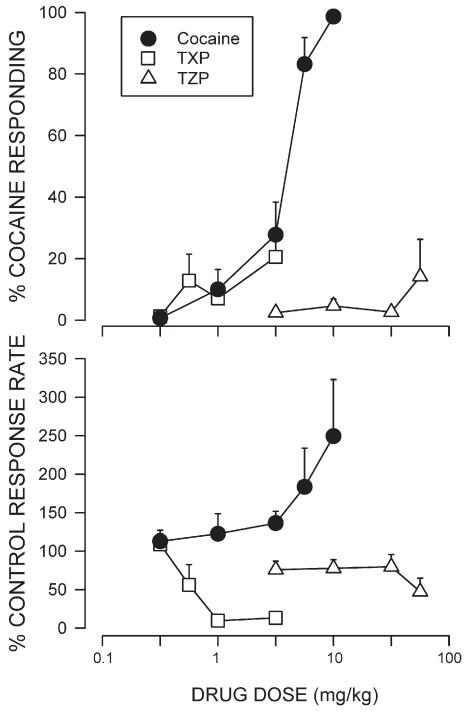

Fig. 1. Effects of cocaine, TZP, and TXP in rats trained to discriminate injections of cocaine (10 mg/kg) from saline. Ordinates for top panel: percentage of responses on the cocaine-appropriate key. Ordinates for the bottom panel: rates at which responses were emitted (as a percentage of response rate after saline administration). Abscissae: drug dose in mg/kg (log scale). TZP and TXP were each studied in 6 rats and cocaine was studied in 12 rats. The percentage of responses emitted on the cocaine-appropriate key was considered unreliable, and not plotted, if fewer than half of the subjects responded at that dose. Note that only cocaine produced substitution above 25%.

analysis of variance (ANOVA) and nonlinear regression techniques, with post-hoc Dunnett multiple comparisons tests. The data for cocaine effects from the two groups of subjects were combined for analyses and graphical representation because a two-way ANOVA on the percentage of responses on the drug lever after cocaine injection indicated a significant effect of dose, but neither a significant effect of group ($F_{1,30}=0.214$; $p=0.654$) nor a group-by-dose interaction ($F_{3,30}=1.856$; $p=0.158$). ED_{50} values were calculated as the doses producing 50% cocaine-appropriate responding from using nonlinear regression and a sigmoidal model (four parameter logistic equation with bottom and top set to 0 and 100, respectively) using GraphPad Prism software. The data were further analyzed to determine the dose ratios and their 95% confidence limits. The dose ratio is the dose of cocaine alone producing an effect equivalent to that produced by 1.0 mg/kg of cocaine in combination with the M_1 antagonist. Response-rate data were analyzed by a two-way repeated measures ANOVA with treatment and cocaine dose as factors for all cocaine doses and all of the doses of an antagonist. Separate analyses were conducted for each antagonist.

2. Results

When administered alone, cocaine produced a dose-dependent increase in the percentage of responses on the cocaine-appropriate key, as has been shown in several previous studies (Fig. 1, top panels, filled symbols). The ED_{50} value for cocaine (Table 1) was 3.78 (95% CL: 3.28–4.36). Neither 0.1 to 56.0 mg/kg of TZP nor 0.3 to 3.0 mg/kg of TXP produced substitution for cocaine that was significantly different from vehicle (Dunnett's test) and none of the doses produced an effect that averaged greater than 25% (Fig. 1, top panels, triangles for TZP and squares for TXP). The doses of the M_1 antagonists studied ranged from those having no effect to those producing a substantial decrease in response rates (Fig. 1, bottom panels). In contrast, cocaine produced a dose-related increase in response rates (One-way ANOVA, $F_{5,58}=4.025$, $p=0.0033$). A post-hoc Dunnett test indicated that doses of 3.0 to 10 mg/kg ($t>2.63$; $p<0.05$) significantly increased rates of responding greater than those obtained with vehicle.

Administration of TZP (1.0 mg/kg) did not significantly alter the discriminative-stimulus effects of cocaine (Fig. 2; top left panel, compare filled circles to open upward triangles), and the ED_{50} value was not significantly different from that of cocaine alone (Table 1). At a higher dose (3.0 mg/kg), there was a significant 1.6-fold leftward shift (Fig. 2; top left panel, compare filled circles to open downward triangles), with an ED_{50} value of 2.32 (95% CL: 1.71–3.15) mg/kg. The highest dose (10 mg/kg) was not effective in changing the discriminative-stimulus effects of cocaine (Fig. 2; top left panel, compare filled circles to open diamonds).

When TZP was administered in combination with cocaine, the increases in rates of responding were diminished (Fig. 2, bottom left panel; compare open to filled symbols). The decreases in this effect of cocaine occurred with all doses of TZP.

Administration of TXP (0.3 and 1.0 mg/kg) before cocaine also produced a leftward shift in the discriminative-stimulus effects of cocaine (Fig. 2, top right panel; compare open to filled symbols). At 0.3 mg/kg TXP there was a 2.67-fold change in the potency of cocaine reflected in an ED_{50} value of 1.42 mg/kg of cocaine. At 1.0 mg/kg of TXP the cocaine ED_{50} value was significantly decreased to 1.70 (Table 1).

Table 1
ED_{50} values and changes in cocaine dose effects produced by the muscarinic antagonists, telenzepine and trihexyphenidyl

Treatment	ED_{50} value (mg/kg)	Dose ratio	Significance
Cocaine	3.78 (3.28–4.36)	–	
Cocaine and 1.0 telenzepine	3.30 (1.59–6.86)	1.15 (0.781–2.15)	N.S.
Cocaine and 3.0 telenzepine	2.32 (1.71–3.15)	1.63 (1.39–1.96)	p=0.0015
Cocaine and 10.0 telenzepine	4.30 (1.53–12.1)	0.877 (0.581–1.83)	N.S.
Cocaine and 0.3 trihexyphenidyl	1.42 (0.594–3.38)	2.67 (1.97–4.13)	p=0.0030
Cocaine and 1.0 trihexyphenidyl	1.70 (1.16–2.49)	2.23 (1.92–2.65)	p<0.0001

ED_{50} values are in mg/kg of cocaine, and dose ratio is the dose of cocaine alone producing an effect equivalent to that produced by 1.0 mg/kg of cocaine in combination with the M_1 antagonist.

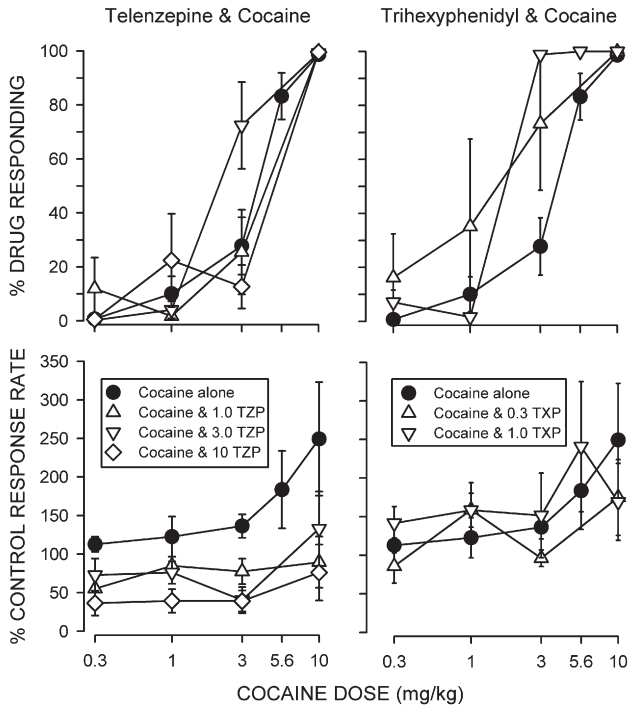

Fig. 2. Changes in the cocaine dose–effect curve produced by pretreatments with TZP or TXP administered as mg/kg before cocaine. Ordinates: percentage of responses on the cocaine-appropriate key. Abscissae: cocaine dose in mg/kg (log scale). Interactions of either TZP or TXP were each studied in different groups of 6 rats. The percentage of responses emitted on the cocaine-appropriate key was considered unreliable, and not plotted, if fewer than half of the subjects responded at that dose. Note that there was a leftward shift in the cocaine dose–effect curve produced by at least one dose of either muscarinic antagonist.

As with TZP co-administration, the 1.0 mg/kg dose of TXP produced a trend towards a decrease in the response-rate stimulating effects of cocaine (Fig. 2, bottom right panel; compare filled symbols to open upward triangles). The decreases in this effect of cocaine however, were not significant.

3. Discussion

In the present study, as previously reported, rats trained to discriminate injections of cocaine from saline showed a dose-dependent increase in responding on the cocaine-appropriate key, as dose of cocaine was increased from an inactive dose to the dose at which the subjects were trained. In contrast, neither of the preferential M_1 antagonists, TZP and TXP, produced responding on the cocaine-appropriate key that was substantially different from that produced by vehicle, across the entire range of behaviorally active doses. When administered in combination with cocaine both M_1 antagonists produced significant leftward shifts in the cocaine dose–effect curve; the greatest magnitude of shift however never exceeded three fold.

The results of this study are similar to several previously reported findings of an enhancement of the behavioral effects of stimulant drugs by anticholinergic agents. As mentioned above, Scheckel and Boff (1964) found an enhanced cocaine-induced increase in avoidance responding of rats after co-administration of atropine, scopolamine, or TXP. With regard to the discriminative-stimulus effects, we previously reported a leftward shift in the cocaine dose–effect curve with atropine or scopolamine (Katz et al., 1999). Much of the literature on interactions of stimulant drugs and antimuscarinic agents has focused on the nonselective antagonists, atropine and scopolamine. Because many of the analogues of benztropine have preferential affinity for M_1 over the other subtypes of muscarinic receptors, studies of the nonselective antagonists in combination with cocaine are not optimal for addressing whether antimuscarinic actions of benztropine analogues interfere with what would otherwise be a cocaine-like behavioral effect. Because the preferential affinity of TXP and TZP for M_1 receptors over the other subtypes approaches that for several benztropine analogues, the present study examined these drugs in combination with cocaine.

We recently reported that both TZP and TXP selectively increased the effects of cocaine on concentrations of dopamine in the nucleus accumbens shell (Tanda et al., 2007). However, despite that enhancement, only TXP enhanced the effects of cocaine on locomotor activity (Tanda et al., 2007). The different effects of combinations of cocaine and TXP or TZP on locomotor activity might be reconciled if TZP with cocaine produced a greater induction of stereotyped behavior than did TXP with cocaine, however, there was no evidence to support that interpretation of the differences between the drugs (Tanda et al., 2007). In the present study, cocaine with both TZP and TXP produced effects that were generally similar: a significant but small enhancement of cocaine's effects. That modest enhancement is consistent with the reported effects of combinations of TXP with cocaine on locomotor activity (Shimosato et al., 2001; Tanda et al., 2007), avoidance responding (Scheckel and Boff, 1964), though a lack of effect of TXP on cocaine place conditioning was also reported (Shimosato et al., 2001). In addition, genetic deletion of muscarinic M_1 receptors produces increases in dopaminergic tone and locomotor activity, and an increased responsiveness to the stimulant effects of amphetamine and cocaine (Gerber et al., 2001). Thus, the past results taken together suggest that preferentially interfering with M_1 muscarinic receptor action increases sensitivity to psychomotor stimulant drugs with many of their prototype effects.

As mentioned in the introduction, benztropine analogues bind to the dopamine transporter and block the uptake of dopamine, but most do not have maximal effects that are similar to those of cocaine or other standard dopamine uptake inhibitors (e.g. Katz et al., 1999). Because many of the benztropine analogues have affinity for muscarinic receptors, and that affinity is preferential for the M_1 subtype (Tanda et al., 2007), the present study relates to the potential of M_1 antagonist actions of BZT analogues to interfere with what would otherwise be cocaine-like effects. In the present study there was clearly no evidence for any antagonism of the discriminative-stimulus effects of cocaine by TZP and TXP. Thus the present results are consistent with previous ones with nonselective muscarinic antagonists (Katz et al., 1999). Because TZP and TXP have preferential affinities for M_1 over other muscarinic receptors approximating those of many of the BZT

analogues, the present study further suggests little if any support for the hypothesis that M_1 muscarinic receptor antagonism could be a factor contributing to a reduction in the cocaine-like behavioral effects of BZT analogues.

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References

Acri JB, Siedleck BK, Witkin JM. Effects of benztropine on behavioral and toxic effects of cocaine: comparison with atropine and the selective dopamine uptake inhibitor 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenyl-propyl)-piperazine. J Pharmacol Exp Ther 1996;277(1):198–206.

Bymaster FP, Heath I, Hendrix JC, Shannon HE. Comparative behavioral and neurochemical activities of cholinergic antagonists in rats. J Pharmacol Exp Ther 1993;267(1):16–24.

Doods HN, Mathy MJ, Davidesko D, van Charldorp KJ, de Jonge A, van Zwieten PA. Selectivity of muscarinic antagonists in radioligand and in vivo experiments for the putative M1, M2 and M3 receptors. J Pharmacol Exp Ther 1987;242(1):257–2562.

Eltze M, Gonne S, Riedel R, Schlotke B, Schudt C, Simon WA. Pharmacological evidence for selective inhibition of gastric acid secretion by telenzepine, a new antimuscarinic drug. Eur J Pharmacol 1985;112(2):211–24.

Gerber DJ, Sotnikova TD, Gainetdinov RR, Huang SY, Caron MG, Tonegawa S. Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor-deficient mice. Proc Natl Acad Sci USA 2001;98(26):15312–7.

Holtzman SG. Discriminative stimulus effects of drugs: relationship to potential for abuse. In: Adler MW , Cowan A, editors. Modern methods in pharmacology. Testing and evaluation of drugs of abuse. New York: Wiley-Liss Inc.; 1990. p. 193–210.

Ichikawa J, Chung YC, Li Z, Dai J, Meltzer HY. Cholinergic modulation of basal and amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. Brain Res 2002;958(1):176–84.

Katz JL, Izenwasser S, Kline RH, Allen AC, Newman AH. Novel 3alpha-diphenylmethoxytropane analogues: selective dopamine uptake inhibitors with behavioral effects distinct from those of cocaine. J Pharmacol Exp Ther 1999;288(1):302–15.

Katz JL, Kopajtic TA, Agoston GE, Newman AH. Effects of N-substituted analogs of benztropine: diminished cocaine-like effects in dopamine transporter ligands. J Pharmacol Exp Ther 2004;309(2):650–60.

Kuhar MJ, Ritz MC, Boja JW. The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci 1991;14(7):299–302.

Newman AH, Kline RH, Allen AC, Izenwasser S, George C, Katz JL. Novel 4'- and 4',4-substituted-3α-(diphenylmethoxy)tropane analogues are potent and selective dopamine uptake inhibitors. J Med Chem 1995;38:3933–40.

Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc Natl Acad Sci USA 1995;92(26):12304–8.

Scheckel CL, Boff E. Behavioral effects of interacting imipramine and other drugs with D-amphetamine, cocaine, and tetrabenazine. Psychopharmacologia 1964;13:198–208.

Shimosato K, Watanabe S, Kitayama S. Differential effects of trihexyphenidyl on place preference conditioning and locomotor stimulant activity of cocaine and methamphetamine. Naunyn Schmiedebergs Arch Pharmacol 2001;364(1):74–80.

Tanda G, Ebbs AL, Kopajtic TA, Elias L, Campbell B, Newman A, et al. Effects of muscarinic M1 receptor blockade on cocaine-induced elevations of brain dopamine levels and locomotor behavior in rats. J Pharmacol Exp Ther 2007. doi:10.1124/jpet.106.118067 Jan 25; [Epub ahead of print].

Tolliver BK, Newman AH, Katz JL, Ho LB, Fox LM, Hsu Jr K, et al. Behavioral and neurochemical effects of the dopamine transporter ligand 4-chlorobenztropine alone and in combination with cocaine in vivo. J Pharmacol Exp Ther 1999;289(1):110–22.

Woolverton WL, Rowlett JK, Wilcox KM, Paul IA, Kline RH, Newman AH, et al. 3'-and 4'-chloro-substituted analogues of benztropine: intravenous self-administration and in vitro radioligand binding studies in rhesus monkeys. Psychopharmacology 2000;147(4):426–35.