Psychopharmacology (2001) 154:375–382
DOI 10.1007/s002130000616

ORIGINAL INVESTIGATION

William L. Woolverton · Gerald S. Hecht
Gregory E. Agoston · Jonathan L. Katz
Amy Hauck Newman

Further studies of the reinforcing effects of benztropine analogs in rhesus monkeys

Received: 14 July 2000 / Accepted: 26 September 2000 / Published online: 27 February 2001

Abstract *Rationale:* Several halogenated analogs of benztropine (BZT) have previously been characterized as potent DA uptake inhibitors with behavioral profiles that indicate diminished psychomotor stimulant effects relative to cocaine. In a previous study using a fixed-ratio 10 schedule, two chloro-analogs (3'-C1-BZT and 4'-Cl-BZT) maintained IV self-administration in monkeys but appeared to be weak positive reinforcers. *Objectives:* The present experiments were designed to test the hypothesis that 3'-C1-BZT and 4'-Cl-BZT are relatively weak reinforcers by evaluating reinforcing effects under increased response requirements. To examine further the effect of this halogen substitution on self-administration, 3',4''-diCl-BZT was also evaluated for reinforcing effects. *Methods:* Four rhesus monkeys self-administered cocaine (0.03 mg/kg per injection, IV) under a fixed-ratio 25 (FR25) schedule until stable responding was established. Saline, various doses of cocaine (0.003–0.2 mg/kg per injection), the BZT analogs (0.012–0.2 mg/kg per injection), GBR 12909 (0.012–0.2 mg/kg per injection), and compounds with known reinforcing effects (*d*-amphetamine, morphine, pentobarbital, ketamine) were then made available for self-administration. Various doses (0.01–0.3 mg/kg per injection) of the compounds that maintained self-administration under the FR schedule were then substituted for cocaine (0.1 mg/kg per injection) under progressive-ratio (PR) schedules. *Results:* Reinforcing effects were evident under the FR schedule for 3'-C1-BZT, 4'-Cl-BZT, GBR 12909, and the control compounds, but not by 3',4''-diCl-BZT. Results with the PR suggested that the rank order of these compounds for their effectiveness as reinforcers was cocaine>GBR 12909>3'-C1-BZT=4'-Cl-BZT>>3',4''-diCl-BZT. *Conclusions:* This study confirms and extends previous results suggesting that compounds with high DAT affinity can have strong, moderate, weak, or no effectiveness as reinforcers. The mechanisms that may underlie this variation in reinforcing effectiveness of these DAT ligands remain to be established.

Keywords Monkey · Reinforcement · Benztropine · Dopamine uptake inhibitor

W.L. Woolverton (✉) · G.S. Hecht
Department of Psychiatry and Human Behavior,
University of Mississippi Medical Center, 2500 N State Street,
Jackson, MS 39216-4505, USA
e-mail: wwoolverton@psychiatry.umsmed.edu
Tel.: +1-601-984-5898, Fax: +1-601-984-5899

G.E. Agoston · J.L. Katz · A.H. Newman
Psychobiology Section, National Institutes of Health,
National Institute on Drug Abuse, Division of Intramural Research,
P.O. Box 5180, Baltimore, MD 21224, USA

Introduction

A variety of novel, high-affinity ligands have been developed that bind selectively to the dopamine transporter (DAT; e.g., Carroll et al. 1992, 1997; Reith et al. 1997; Newman 1998; Newman and Agoston 1998). These compounds can help establish the basic neurobiology of the DAT and contribute to the development of potential pharmacotherapeutic agents for treating cocaine abuse. Benztropine [BZT, 3α-(diphenylmethoxy)-1α*H*, 5α*H*-tropane] is the parent of a series of compounds with structural similarities to both cocaine (i.e., a tropane ring) and the more selective DA uptake inhibitor GBR 12909 (i.e., a diphenyl ether system). BZT and its analogs share some pharmacological and behavioral effects with cocaine. In particular, BZT and/or many of its analogs exhibit high affinity binding to the DAT, and inhibit DA uptake in vitro. BZT has been reported to have cocaine-like effects on operant behavior in monkeys (McKearney 1982). In other respects, however, their effects appear to qualitatively differ (Newman et al. 1994, 1995; Agoston et al. 1997). For example, 4'-chloro-3α-(diphenylmethoxy)tropine (4'-chloro-benztropine, or 4'-Cl-BZT) has an affinity for the DAT that is comparable to that of cocaine, but is only marginally efficacious as a stimulant of locomotor activity in rodents, and does not have cocaine-like discriminative stimulus effects (Katz et al. 1999). In contrast to the effects of most of the BZT

analogs, 3'-chloro-3α-(diphenylmethoxy)tropane (3'-chloro-benztropine, or 3'-C1-BZT) has discriminative stimulus effects similar to those of cocaine (Kline et al. 1997).

In a previous experiment from our laboratory (Woolverton et al. 2000), BZT and its analogs 3'-C1-BZT and 4'-Cl-BZT were evaluated as reinforcers in rhesus monkeys experienced in cocaine self-administration under a fixed-ratio (FR) 10 schedule of reinforcement. A high rate of self-administration was maintained by cocaine, with lower rates maintained by both 3'-C1-BZT and 4'-Cl-BZT. BZT itself did not maintain responding above vehicle levels at any dose tested. While these results were suggestive of weak reinforcing effects of the BZT analogs, the study was not designed to assess graded reinforcing effects. The present experiment was designed to extend our evaluation of the reinforcing effects of BZT analogs with the goal of providing a more reliable estimate of the relative effectiveness of these compounds as reinforcers. To do this, the compounds in the present study were examined under conditions of increased response requirement relative to our previous study, specifically, an FR25 schedule and a progressive-ratio (PR) schedule of reinforcement (Hodos 1961). PR schedules increase the number of responses required to obtain a reinforcer until responding is no longer maintained above some minimum. This schedule has been used to rank-order drugs according to their effectiveness as reinforcers (see Richardson and Roberts 1996). In the present experiment, several PR schedules were examined to allow between-drug comparisons under multiple behavioral conditions (see Wilcox et al. 2000).

Our approach was to compare self-administration of BZT analogs to cocaine and GBR 12909. Compounds were first evaluated under a FR schedule and those that functioned as reinforcers were compared under PR schedules. Cocaine is a highly effective reinforcer that reliably maintains high rates of responding under both FR and PR schedules. GBR 12909 is a 1,4-dialky(en)yl-piperazine that is more selective than cocaine for the DAT, and as noted above, shares some structural similarities with BZT. GBR 12909 has also been found to serve as a reinforcer in rhesus monkeys (Skjoldager et al. 1993; Wojnicki and Glowa 1996) and other species (Bergman et al. 1989; Roberts 1993). Although 3'- and 4'-Cl-BZT differ structurally only in the position of the chloro substituent in the meta versus the para positions on one of the phenyl rings, and have similar DAT binding affinities, there have been reported differences in their behavioral effects. Therefore, 3',4''-diCl-BZT (3',4''-dichloro-3-(diphenylmethoxy)tropane), a novel compound that incorporated the 3'-Cl substituent on one phenyl ring and a 4''-Cl substituent on the other phenyl ring, was synthesized (Katz et al. 2001). Based on structure-activity relationships derived for a large series of phenyl ring substituted BZT analogs (Newman et al. 1995), and three dimensional quantitative structure activity relations (Newman 1999), it was predicted that the 3',4''-diCl-BZT analog would have similar binding affinity at the DAT as the parent compounds. However, it was felt that this compound might shed light on the structural relationship between these compounds and their reinforcing effects, as well as other behavioral profiles (Katz et al. 2001). An assessment of the effects of this compound would provide information on whether the 3'-Cl substituent is primarily responsible for the cocaine-like effects of 3'-Cl-BZT or whether the 4'-Cl substituent interferes with cocaine-like behavioral effects.

Materials and methods

Animals and apparatus

The subjects were nine adult rhesus monkeys (*Macaca mulatta*). Four (three male and one female, 18108) responded under a fixed-ratio (FR) schedule of reinforcement. Most recently, monkeys, 18108 and AL99 had a history of self-administration of a variety of drugs under an FR10 schedule, including the same compounds studied here (Woolverton et al. 2000). Monkey Rf3 had a history drug discrimination training using DOM as the training drug and had been drug-free for 18 months (unpublished data). Three monkeys (18108; H228; AL99) also had a brief history of FR self-administration of the compounds studied in the present experiment, under slightly different conditions that those used here.

Five other monkeys (four males and one female, 11084) responded under a progressive-ratio (PR) schedule of reinforcement. Monkeys AP13, 11084 and RJu2 had a history of responding under a PR similar to the one used here. Most recently, monkeys AP13 and 11084 had a history of self-administration of cocaine/heroin combinations (Rowlett et al. 1998) while RJu2 had a history of local anesthetic self-administration (Wilcox et al. 2000). Monkeys L500 and L637 had a history of self-administration under an FR10 schedule. For monkey L500 that history included self-administration of BZT analogs under FR 10 (Woolverton et al. 2000). Monkey L637 had self-administered various monoamine uptake blockers (unpublished data). Monkey 11084 was removed from the experiment before testing all doses because of erratic responding in baseline sessions. Each was fitted with a stainless-steel restraint harness that attached to a tether. The tether was attached to the rear wall of a cubicle (1.0 m^3; Plaslabs, Lansing, Mich., USA) equipped with two response levers (BRS/LVE, PRL-001, Beltsville, Md., USA) and a peristaltic infusion pump (Cole-Parmer, Vernon Hills, Ill., USA). A force of 0.30 N upon either lever was required to close a microswitch. Above each lever were four jeweled stimulus lights, two white and two red. A Macintosh II computer with custom interface and software controlled events during the session and recorded data.

Procedure

All monkeys were fed Teklad Monkey Diet (Harlan, Indianapolis, Ind., USA) daily in an amount sufficient to maintain stable body weight. In addition, each was given a chewable multiple vitamin tablet 3 days per week and received fresh fruit 5 days per week. Water was continuously available throughout the study. Lights were on at 0600 and off at 2200 hours.

Using aseptic techniques under ketamine and isoflurane anesthesia, a silicone catheter (0.26 cm o.d.×0.076 cm i.d., Cole-Parmer Co.) was implanted into a jugular or femoral vein. Brachial veins were also implanted using a tapered micro-renethane catheter (0.08 cm o.d. ×0.04 cm i.d., Braintree Scientific, Braintree, Mass., USA). The proximal end was inserted into the vein and threaded to terminate in the vena cava near the right atrium. The distal end of the catheter was passed subcutaneously to a mid-scapular exit site. After surgery, the catheter was threaded through the tether, out the rear of the cubicle and connected to the peristaltic pump. Upon catheter failure and after confirmation of the

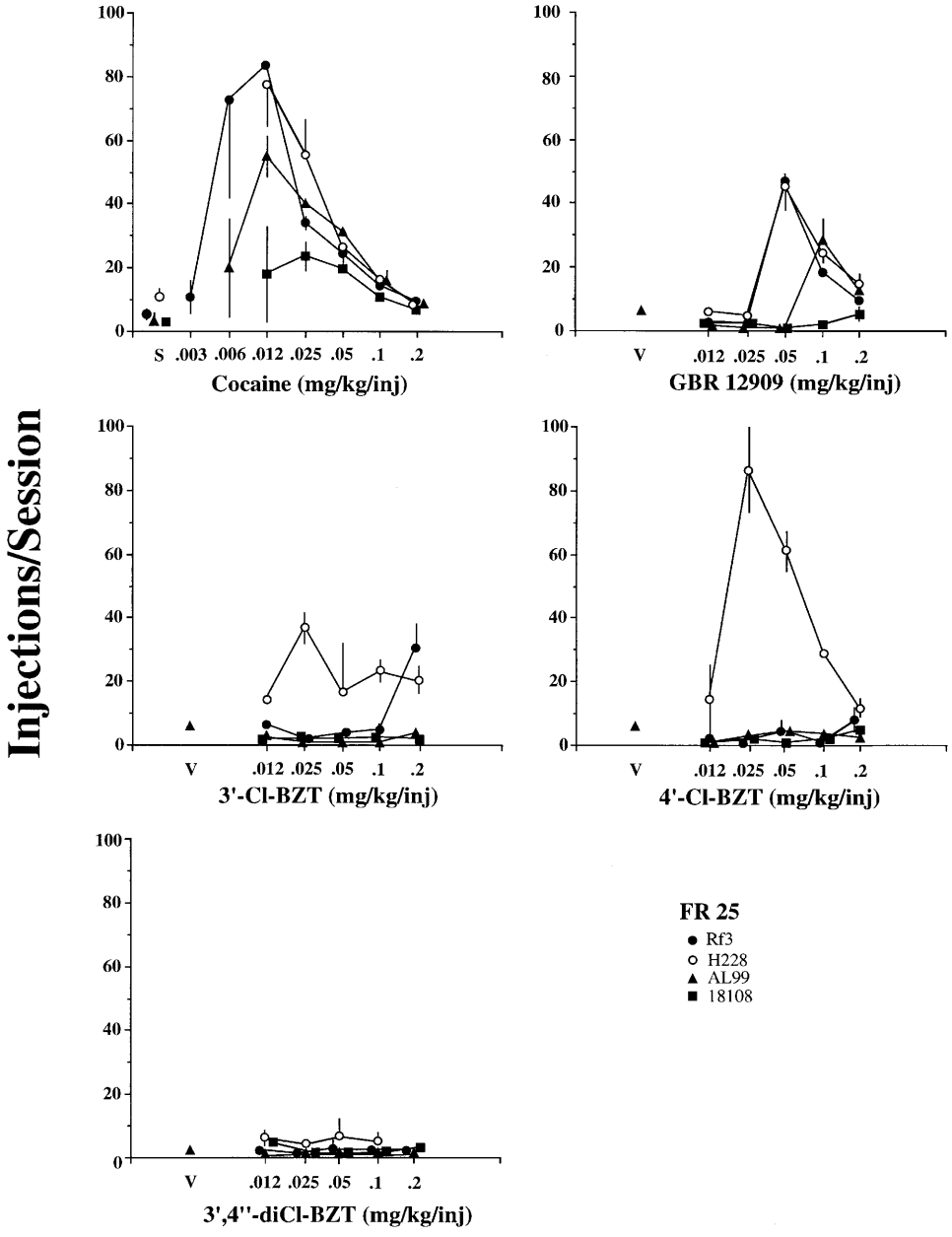

Fig. 1 Self-administration of cocaine, GBR 12909 and benztropine analogs by rhesus monkeys responding under a fixed-ratio 25 schedule of reinforcement. *Symbols* refer to different monkeys and represent the mean of two or three test sessions. *Vertical lines* represent the range of values over two test sessions. Where vertical lines do not appear, the range is contained within the symbol. *x-Axis* is drug dose and *y-axis* is injections/2-h session. *S* and *V* refer to self-administration of 0.9% saline and vehicle, respectively

monkey's health by a veterinarian, surgery was repeated using another vein.

Experimental sessions were conducted seven days a week beginning at noon with the illumination of the white lights above both levers. Responses on the left lever had no consequences. Completion of the response requirement on the right lever extinguished the white lights, illuminated the red lights and began a 10-s injection of drug or vehicle. During an injection, responding on either lever had no programmed consequences. The catheters were flushed after the session with a solution of 30 IU/ml heparin (mixed in saline) to prevent clotting at the catheter tip. For the FR schedule, a baseline dose of cocaine (0.03 mg/kg per injection) was available under conditions requiring 25 lever presses/injection (fixed-ratio 25; FR25 schedule) in daily 2-h sessions (Fig. 1). Once responding was stable (three consecutive sessions with the number of injections within ±15% of the three-session mean and no upward or downward trends), saline was substituted until responding declined to low levels (fewer than ten injections/session). Next, a double alternation schedule was begun in which two consecutive sessions of cocaine availability alternated with two consecutive sessions of saline availability. This schedule was continued until stable responding was re-established.

The PR schedule was essentially identical to one we have used previously (e.g., Rowlett et al. 1998; Wilcox et al. 2000). In baseline sessions, cocaine (0.1 mg/kg per injection) or saline was available for injection. The schedule consisted of five components, each made up of four trials, for a total of 20 trials/day. The response requirement for each of the four trials of the first component was 100 (monkeys AP13, L637, 11084) or 200 (monkeys L500, RJu2), and doubled for each successive component. The same response requirement was in effect for each trial in a component, and a trial ended with a 10-s drug injection or the expiration of a 30-min limited hold (LH). There was a 30-min time-out after each drug injection or the expiration of a LH. If the response requirement was not completed (i.e., the LH expired) for two consecutive trials, or the animal took all 20 injections, the session ended. The sequence of sessions was comparable to that used with the FR schedule and continued until responding was stable (mean ±2 injections).

For both schedules, after responding was stable test sessions were conducted every third session, as long as baseline responding

Table 1 Self-administration of control compounds under an FR25 schedule of reinforcement with a double alternation schedule of daily sessions. Saline levels are mean (range) of values from saline test sessions. Numbers after drug names are the dose range tested over all monkeys. Numbers under monkey numbers are the maximum number of injections/session over the dose-response function and, in parentheses, the dose (mg/kg per injection) that maintained maximum responding. (–) Drug was not self-administered above saline levels at any dose

Drug (mg/kg per injection)	Monkey				
	AL99	Rf3	H228	18108	Total
Saline	2.5 (2–3)	6 (5–7)	10.5 (9–12)	3 (2–4)	
d-Amphetamine (0.003–0.1)	43 (0.006)	52 (0.012)	43.5 (0.012)	10.5 (0.05)	4/4
Morphine (0.012–0.2)	12 (0.05)	35 (0.012)	23.5 (0.2)	9.5 (0.05)	4/4
Pentobarbital (0.03–2.0)	24 (0.5)	49.5 (0.25)	16 (0.062)	(–)	3/4
Ketamine (0.03–0.25)	27 (0.12)	23 (0.25)	72 (0.03)	(–)	3/4

was stable. For the FR, stability in cocaine sessions continued to be ±15% of the running mean number of injections while stability in saline sessions was defined as within ±2 injections/session with a maximum of ten injections/session. Stability criteria were comparable for the PR schedule with fewer than four saline injections in baseline sessions. A test session was identical to a training session, except that vehicle or a different dose of cocaine or of a novel drug was made available. Various doses of cocaine, GBR 12909, 3'-Cl-BZT and 4'-Cl-BZT were tested in all animals. In addition, 3',4''-diCl-BZT was tested under the FR schedule only. For the FR, doses were tested over a range that included a dose low enough not to maintain responding and a dose high enough to demonstrate the biphasic nature of the dose-response function. For 3'-Cl-BZT, 4'-Cl-BZT and GBR 12909 doses were between 0.012 and 0.2 mg/kg per injection, a range that included doses found to be self-administered in the Woolverton et al. (2000) study. Doses of 3',4''-diCl-BZT were the same as those of 3'-Cl-BZT and 4'-Cl-BZT. For the PR, 3'-Cl-BZT and 4'-Cl-BZT were tested up to doses 3- to 10-fold higher than those found to maintain responding in the Woolverton et al. (2000) study, i.e., up to 0.3 mg/kg per injection. Higher doses were not tested because of solubility limitations. Each dose could be evaluated under five PR sequences: (1) 50, 100, 200, 400, 800; (2) 100, 200, 400, 800, 1600; (3) 200, 400, 800, 1600, 3200; (4) 400, 800, 1600, 3200, 6400; (5) 800, 1600, 3200, 6400, 12,800. The PR sequences were tested in ascending order beginning with 50 (PR50) and increased until responding was not maintained at any dose. Each dose of a test drug was made available twice, once with cocaine available in the previous session and once with saline available in the previous session. Occasionally, when the two tests were inconsistent, a dose was tested a third time. Drug and dose orders were irregular. Since the alternation sequence and single-session testing regimen used here has not been studied previously for the FR schedule, standard compounds [*d*-amphetamine (0.003–0.1 mg/kg per injection), pentobarbital (0.03–2.0 mg/kg per injection), ketamine (0.03–0.25 mg/kg per injection) and morphine (0.012–0.2 mg/kg per injection)] were tested to examine the reliability of the procedure for detecting positive reinforcers.

Drugs

3'-C1-BZT was synthesized as described by Kline et al. (1997). The synthesis of 3',4''-diCl-BZT is described by Katz et al. (2001). 4'-C1-BZT was purchased from Research Biochemicals, Inc. (Natick, Mass., USA). GBR 12909 was supplied by Novo Industri (Denmark). All were prepared in a vehicle of 2% ethanol and 98% saline. Cocaine HC1, morphine sulfate and *d*-amphetamine sulfate were provided by the National Institute on Drug Abuse (Rockville, Md., USA) and were dissolved 0.9% saline. Pentobarbital solutions were prepared by dilution of Nembutal (Abbott Laboratories, Chicago, Ill., USA) with 0.9% saline. Ketamine solutions were prepared by dilution of ketamine hydrochloride (Abbott Laboratories) with 0.9% saline. All solutions were filtered with 0.22 micron filter unit (Millipore, Bedford, Mass., USA). Doses are expressed as the salt forms of the drugs. Injections were delivered over 10 s and volumes approximating 1.0 ml/injection. Concentrations of drugs were adjusted to give the reported mg/kg per injection dose.

Data analysis

For both schedules, the mean number of injections for the two test sessions of each dose of each drug was compared to the comparable mean of two sessions of availability of saline and/or the appropriate vehicle. A dose of a drug was considered to be a positive reinforcer for an individual monkey if the mean number of injections it maintained was greater than the mean maintained by saline and/or the drug vehicle, and the ranges did not overlap. Additionally for the PR, the maximum number of injections of each drug, regardless of dose, was determined for each animal (see Rowlett et al. 1996). Maximums for all drugs were compared using a repeated measures ANOVA. Maximums for individual drugs were compared using a paired *t*-test. Statistical significance was set at $P<0.05$. Since monkey 11084 did not finish the experiment, her data were excluded from this analysis.

Results

Fixed-ratio schedule

There was substantial individual variability in the time required for responding to stabilize under the double alternation. Mean time to begin testing was 136 (±46.5 SEM) sessions. Test sessions occurred at rates between one for every 3.9 sessions (AL99 and Rf3) and one for every 4.75 sessions (18108). Cocaine (0.03 mg/kg per injection) maintained an average of between 28 (18108) and 48.2 (H228) injections/session in baseline sessions while saline maintained an average of between three (AL99) and eight (H228) injections/session. Over the course of the experiment, responding in baseline cocaine sessions increased slightly in H228, AL99 and 18108 did not change in Rf3. Cocaine dose-response functions were biphasic or injections/session decreased with dose (H228) with maximum responding at 0.03 mg/kg per injection in all monkeys. All monkeys self-administered *d*-amphetamine, and morphine (Table 1). Three of the four monkeys self-administered PB and ketamine. Interestingly, the single exception for these

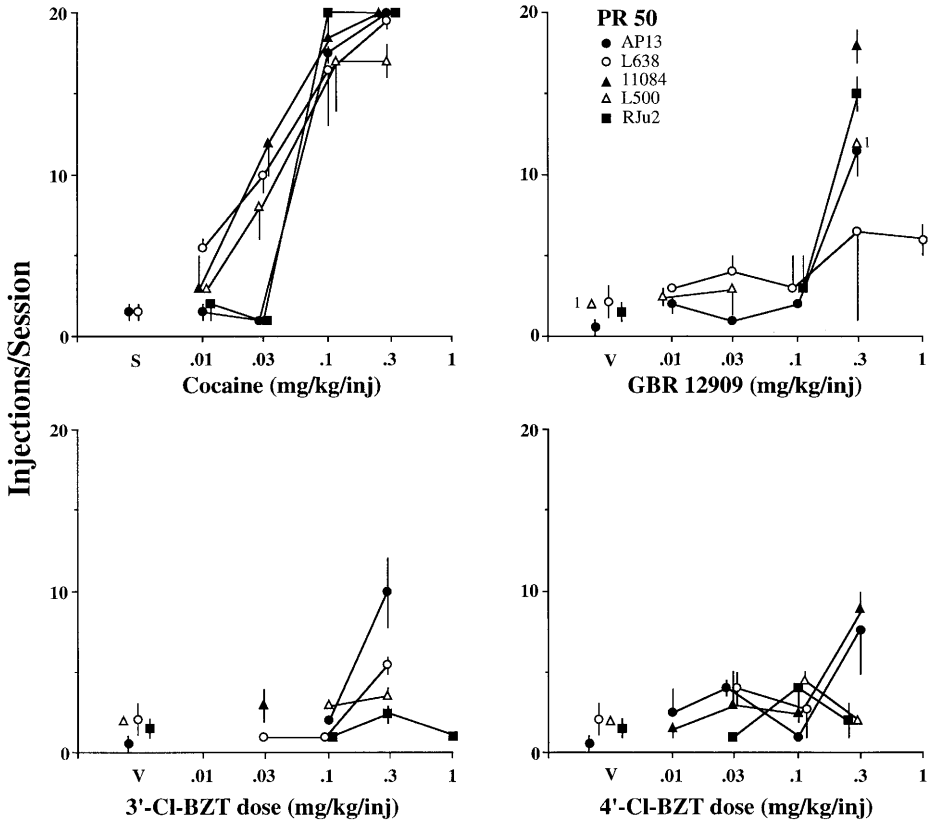

Fig. 2 Self-administration of cocaine, GBR 12909 and benztropine analogs by rhesus monkeys responding under a progressive-ratio schedule of reinforcement. The response requirement began at 50 and doubled after every four injections (see Materials and methods). *Symbols* refer to different monkeys and represent the mean of two or three test sessions. *Vertical lines* represent the range of values over two test sessions. Where vertical lines do not appear, the range is contained within the symbol. Points labeled *1* were determined one time. *x-Axis* is drug dose and *y-axis* is injections/2-h session. *S* and *V* refer to self-administration of 0.9% saline and vehicle, respectively

latter two drugs was the female monkey in the group, 18108. When drugs were self-administered, the dose-response functions were biphasic. Doses that maintained maximum responding varied across monkeys suggesting individual differences in sensitivity to the reinforcing effects of these drugs.

Cocaine and GBR 12909 functioned as reinforcers, at least at one dose and generally at several doses, in all monkeys. Dose-response functions were, in general, biphasic and shifted downward for GBR 12909 relative to cocaine. The lowest rates of self-administration of both drugs were seen in the female, 18108, and GBR 12909 exceeded our definition of positive reinforcer by only one injection at one dose (0.2 mg/kg per injection). Responding was maintained by 3'-Cl-BZT in two of four monkeys, also at relatively low rates. Responding was maintained by 4'-Cl-BZT only in monkey H228 and maximum rate was comparable to that seen with cocaine. No monkey self-administered 3',4''-diCl-BZT above vehicle levels over a dose range of 0.012–0.2 mg/kg per injection. Generally, responding was stable in baseline sessions during testing of all drugs. There was no effect of any compound on cocaine self-administration the day after a test session. The effects of a dose did not vary systematically with the baseline condition that was in effect the previous session.

Progressive-ratio schedule

For monkeys naive to the PR, the number of baseline sessions before testing began was 50 for L637 and 74 for L500. Experienced monkeys began testing immediately upon beginning the experiment. Once testing began, test sessions were conducted at rates between one for every 3.2 (RJu2) and one for every 4.4 (L500) sessions. In baseline sessions, cocaine (0.1 mg/kg per injection) maintained an average of between 11.7 (11084) and 14.7 (AP13) injections/session, while saline maintained between 1.6 (RJu2) and 2.9 (11084) injections/session. Over the course of the experiment, responding in baseline sessions increased or decreased slightly in individual monkeys but did not change systematically across monkeys (data not shown).

Cocaine functioned as a reinforcer in all monkeys at all PR values. Responding increased with dose in all monkeys at PR50, 100 (Figs 2 and 3) and at PR200, 400 and 800 (data not shown). Injection maximums decreased as beginning response requirement increased (Table 2). Similarly, GBR 12909 functioned as a reinforcer in all monkeys at PR50 and 100 (Figs 2 and 3), in four of five monkeys at PR200 and 400 (data not shown), and in two of five monkeys at PR800 (data not shown). As with cocaine, injection maximums decreased with response requirement (Table 2). Maximum responding maintained by GBR 12909 was consistently lower than that maintained by cocaine. Responding was not as strongly maintained by 3'- and 4'-Cl-BZT under PR schedules. At PR50, 3'-Cl-BZT functioned as a reinforcer clearly in two monkeys, marginally in a third (L500), and failed to maintain responding in the fourth (2). At PR100, results were similar but injection rates were lower. Results with

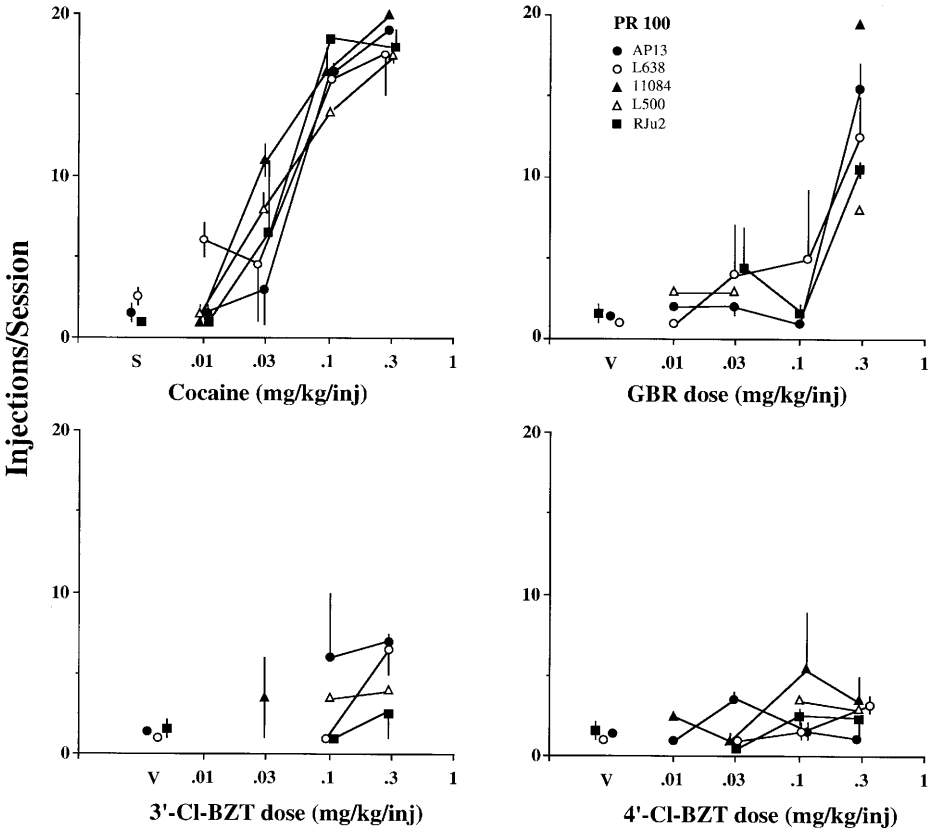

Fig. 3 Self-administration of cocaine, GBR 12909 and benztropine analogs by rhesus monkeys responding under a progressive-ratio schedule of reinforcement. The response requirement began at 100 and doubled after every four injections (see Materials and methods). Other details are as in Fig. 2

Table 2 Maximum number of injections maintained by each drug under each PR sequence. Numbers are mean value (SD) from of between three and five monkeys. Where fewer than three monkeys were tested, mean were not calculated and number of subjects tested is presented. *n.t.* not tested

	PR50	PR100	PR200	PR400	PR800
Cocaine	19.3 (1.3)	18.5 (1.06)	15.5 (2.2)	11.9 (1.9)	8.3 (1.5)
GBR 12909	12.5 (4.5)	13.2 (4.5)	8.7 (4.6)	7 (2.55)	4.1 (2.5)
3'-Cl-BZT	5.4 (3.3)	5 (2.1)	*n*=2	n.t.	n.t.
4'-Cl-BZT	5.2 (3)	3.5 (2.2)	3.3 (0.76)	*n*=1	*n*=1
Saline	1.5 (0)	1.3 (0.25)	2.1 (0.95)	2.2 (1.26)	*n*=2

4'-Cl-BZT were similar to those with 3'-Cl-BZT at PR50 (Fig. 2) with responding maintained in two monkeys. At PR100, injections rates were lower than at PR50, and comparable to or lower than those seen with 3'-Cl-BZT at PR100. Neither 3'- nor 4'-Cl-BZT functioned as a reinforcer up to 0.3 mg/kg per injection in those monkeys tested at PR200 or above (data not shown). As with the FR, the effects of a dose did not vary systematically with the baseline condition that was in effect the previous session.

Differences between drugs in injection maximums were statistically significant at both the PR50 [$F(3,9)=24.8$; $P<0.05$] and the PR100 sequence [$F(3,9)=30$; $P<0.05$] (Table 2).. Maximums for cocaine were higher than those for GBR 12909 at all sequences up to and including 800 ($P<0.05$ in all cases). Maximum maintained by GBR was higher than that maintained by 3'-Cl-BZT at the PR100 but not at the PR50. Maximums maintained by 3'-Cl-BZT and 4'-Cl-BZT were not significantly different in any case.

Discussion

In a previous study (Woolverton et al. 2000) using an FR10 schedule both 3'-Cl- and 4'-Cl-BZT functioned as reinforcers. However, the results suggested that they were relatively weaker reinforcers than cocaine. The results of the present experiment provide further support for that conclusion. Whereas all monkeys tested ($n=4$) self-administered cocaine and GBR 12909 under the FR25 schedule, only two of four self-administered 3'-Cl-BZT and only one self-administered 4'-Cl-BZT. In comparison, four of four monkeys self-administered 3'-Cl-BZT under an FR10 schedule in the Woolverton et al. (2000) experiment, while three of four monkeys self-administered 4'-Cl-BZT in that experiment. It seems likely that the increase in response requirement from 10 to 25 in the present experiment substantially weakened the reinforcing effect of the BZT analogs while having no apparent effect on the reinforcing effect of cocaine (see, e.g., Goldberg et al. 1970).

Results with the PR schedule provide further support for the conclusion that these BZT analogs are relatively weak reinforcers. Cocaine maintained self-administration in all monkeys up to a PR schedule that began at 800 while GBR 12909 maintained self-administration in all

monkeys up to a PR that began at 400 and in a portion of the monkeys at PR800. For both 3'-Cl-BZT and 4'-Cl-BZT, responding was only weakly maintained under a PR that began with a response requirement of 50, and more weakly maintained under a PR schedule that began at 100. Responding was not maintained at all under schedules with higher response requirements. That is, cocaine and GBR 12909 were clearly more effective reinforcers than the BZT analogs. It is interesting to note that 3'-Cl-BZT has been found to engender full cocaine-like activity as a discriminative stimulus in rats while 4'-Cl-BZT has not (Newman et al. 1994; Kline et al. 1997; Katz et al., submitted). Based on that finding, one might propose that 3'-Cl-BZT would be a stronger reinforcer than 4'-Cl-BZT. Although there was the suggestion of a difference between these compounds, variability in the present results does not allow such a conclusion to be firmly made.

The 3',4''-diCl analog of BZT did not maintain responding despite DAT affinity comparable to those of 3'-Cl-BZT and 4'-Cl-BZT, and of high affinity cocaine binding (Katz et al., submitted). Thus, dichloro substitution preserved the DAT affinity of the mono-substituted compounds but appeared to eliminate reinforcing effects. Considered together with our previous experiment (Woolverton et al. 2000), the present experiment supports the conclusion that BZT and analogs can be rank ordered according to their effectiveness as reinforcers as follows: cocaine>GBR 12909>3'-Cl-BZT=4'-Cl-BZT>>3',4''-diCl-BZT=BZT. That is, chloro substitution at either the 3'- or 4'-position of the BZT molecule was associated with weak reinforcing effects while both the unsubstituted parent compound BZT, and the 3',4''-dichloro substituted compound failed to maintain self-administration. Moreover, despite relatively high affinity for the DAT a compound may have strong (cocaine), moderate (GBR 12909), weak (3'-Cl-BZT and 4'-Cl-BZT) or no (3',4''-diCl-BZT, BZT) effectiveness as a reinforcer. Clearly, some mechanism(s) other than, or in addition to, simple affinity for the DAT contributes to reinforcing effects of these compounds. A number pharmacodynamic (e.g., different DAT binding domain, anticholinergic actions) and pharmacokinetic (slow onset, long duration) possibilities have been suggested that could contribute to this apparent disconnect between DAT actions and reinforcing effects (Katz et al. 1997, 1999; Woolverton et al. 2000). Further research with these compounds may provide insights into the relationship between the DAT and reinforcing effects.

In evaluating these conclusions, it is important to comment on the validity of the testing conditions. Although similar rapid paradigms have been published (see Aigner and Balster 1978; Woods 1978), self-administration has not been studied under the present FR schedule and with this sequence of sessions. Therefore, we studied compounds known to be self-administered under FR schedules (see Johanson and Balster 1978) as positive controls. Since these compounds were reinforcers, it seems likely, that the testing conditions used here provide reliable measures of reinforcing effects. The PR schedule varies somewhat from typical PR schedules in being a trials procedure with a TO after each injection. Cocaine, heroin, procaine and other local anesthetics have all been found to maintain self-administration under conditions similar to those of the present experiment (Rowlett et al. 1998; Wilcox et al. 2000). Advantages of the present approach include its rapidity and the consumption of relatively small amounts of drug. In terms of rapidity, stability took longer to establish than the 3–4 weeks reported by Woods (1978), perhaps because two sessions were conducted each day in that study compared to a single daily session in the present study. Once responding was established, rate of testing was comparable across the different methods. In the present study and the study of Wilcox et al. (2000), drugs were available under multiple PR sequences. The advantage of this approach is that it allows comparisons of drugs under multiple behavioral conditions, strengthening conclusions about relative effectiveness as reinforcers. The obvious disadvantage to be considered is that comparison under multiple conditions takes more time than a single set of conditions.

In considering the behavioral effects of these BZT analogs, the compounds with 3-Cl-substituents are less effective than cocaine but more effective than those with 4-Cl-substituents in stimulating locomotor activity and substituting for cocaine as a discriminative stimulus (Newman et al. 1994, 1995; Acri et al. 1996; Kline et al. 1997; Katz et al. 2001). Interestingly, 3',4''-diCl-BZT has been found to have at least partial cocaine-like discriminative stimulus effects in rats (Katz et al. 2001). The results in these other behavioral procedures contrast to the lack of reinforcing effectiveness of 3',4''-diCl-BZT relative to 3'- and 4'-Cl-BZT. When considered together, then, these results suggest mechanistic distinctions between the reinforcing effects and these other behavioral effects of BZT analogs.

Acknowledgements We gratefully acknowledge the excellent technical assistance of Kenneth Pace. We also gratefully acknowledge J. Cao for synthesizing multigram quantities of both 3'-Cl BZT and 3',4''-diCl BZT. This study was supported by NIDA Grants DA-10352. W.L.W. is the recipient of NIDA grant K02-DA-00161. The experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals from the National Institutes of Health. Experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Mississippi Medical Center.

References

Acri JB, Sedleck BK, Witkin JM (1996) 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)-piperizine. J Pharmacol Exp Pharm 277:198–206

Agoston GE, Wu JH, Izenwasser S, George C, Katz J, Kline RH, Newman AH (1997) Novel N-substituted 4', 4''-difluoro-3α-(diphenylmethoxy)tropane analogs: selective ligands for the dopamine transporter. J Med Chem 40:4329–4339

Aigner TG, Balster RL (1979) Rapid substitution procedure for intravenous drug self-administration studies in rhesus monkeys. Pharmacol Biochem Behav 10:105–112

Bergman J, Madras BK, Johnson SE, Spealman RD (1989) Effects of cocaine and related drugs in nonhuman primates. III. Selfadministration by squirrel monkeys. J Pharmacol Exp Ther 251:150–155

Carroll FI, Lewin AH, Boja JW, Kuhar MJ (1992) Cocaine receptor: biochemical characterization and structure-activity relationships of cocaine analogues at the dopamine transporter. J Med Chem 35:969–981

Carroll FI, Lewin AH, Kuhar MJ (1997) Dopamine transporter uptake blockers: structure-activity relationships. In: Reith MEA (ed) Neurotransmitter transporters: structure, functions and regulation. Humana Press, Totowa, N.J., pp 263–295

Goldberg SR, Hoffmeister F, Schlichting UU, Wuttke W (1970) A comparison of pentobarbital and cocaine self-administration in rhesus monkeys: effects of dose and fixed-ratio parameter. J Pharmacol Exp Ther 179:163–169

Hodos W (1961) Progressive-ratio as a measure of reward strength. Science 134:943–944

Johanson CE, Balster RL (1978) A summary of the results of a drug self-administration study using substitution procedures in rhesus monkeys. Bull Narc 30:43–55

Katz JL, Newman AH, Izenwasser S (1997) Relations between heterogeneity of dopamine transporter binding and function and the behavioral pharmacology of cocaine. Pharmacol Biochem Behav 57:505–512

Katz JL, Izenwasser S, Kline RH, Allen AC, Newman AH (1999) Novel 3-diphenylmethoxytropane analogs: selective dopamine uptake inhibitors with behavioral effects different from those of cocaine. J Pharmacol Exp Ther 288:302–315

Katz JL, Agoston GE, Alling KL, Kline RH, Forster MJ, Woolverton WL, Kopajtic TA, Newman AH (2001) Dopamine transporter binding without cocaine-like behavioral effects: Synthesis and evaluation of benzotropine analogs alone and in combination with cocaine in rodents. Psychopharmacology DOI 10.1007/s002130000667

Kline RH, Izenwasser S, Katz JL, Joseph DB, Bowen WD, Newman AH (1997) 3'-Chloro-3-(diphenylmethoxy)tropane but not 4'-chloro-3-(diphenylmethoxy)tropane produces a cocaine-like behavioral profile. J Med Chem 40:851–857

McKearney JW (1982) Effects of dopamine uptake inhibitors on schedule-controlled responding in the squirrel monkey. Psychopharmacology 78:377–379

Newman AH (1998) Novel dopamine transporter ligands: the state of the art. Med Chem Res 8:1–11

Newman AH, Agoston GE (1998) Novel benztropine [3-(diphenylmethoxytropane)] analogs as probes for the dopamine transporter. Curr Med Chem 5:301–315

Newman AH, Allen AC, Izenwasser S, Katz JL (1994) Novel 3-(diphenylmethoxy) tropane analogs: potent dopamine uptake inhibitors without cocaine-like behavioral profiles. J Med Chem 37:2258–2261

Newman AH, Kline RH, Allen AC, Izenwasser S, George C, Katz JL (1995) Novel 4'- and 4', 4''-substituted-3-(diphenylmethoxy) tropane analogs are potent and selective dopamine uptake inhibitors. J Med Chem 38:3933–3940

Newman AH, Robarge M, Izenwasser S, Kline RH (1999) A comparative molecular field analysis (CoMFA) study of novel ring-substituted 3a-(diphenylmethoxy)tropane analogs at the dopamine transporter. J Med Chem 42:3502–3509

Reith MEA, Xu C, Chen N-H (1997) Pharmacology and regulation of the neuronal dopamine transporter. Eur J Pharmacol 324:1–10

Richardson NR, Roberts DCS (1996) Progressive-ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Meth 66:1–11

Roberts DC (1993) Self-administration of GBR 12909 on a fixed ratio and progressive ratio schedule in rats. Psychopharmacology 111:202–206

Rowlett JK, Massey BW, Kleven MS, Woolverton WL (1996) Parametric analysis of cocaine self-administration under a progressive-ratio schedule in rhesus monkeys. Psychopharmacology 125:361–370

Rowlett JK, Wilcox KM, Woolverton WL (1998) Self-administration of cocaine-heroin combinations by rhesus monkeys: antagonism by naltrexone. J Pharmacol Exp Ther 286:61–69

Skjoldager P, Winger G, Woods JH (1993) Effects of GBR 12909 and cocaine on cocaine-maintained behavior in rhesus monkeys. Drug Alcohol Depend 33:31–39

Wilcox KM, Rowlett JK, Paul IA, Ordway GA, Woolverton WL (2000) On the relationship between the dopamine transporter and the reinforcing effects of local anesthetics: practical and theoretical concerns. Psychopharmacology 153:139–147

Wojnicki FH, Glowa JR (1996) Effects of drug history on the acquisition of responding maintained by GBR 12909 in rhesus monkeys. Psychopharmacology 123:34–41

Woods JH (1978) Narcotic-reinforced responding: a rapid evaluation procedure. Drug Alcohol Depend 1980 5:223–30

Woolverton WL, Rowlett JK, Wilcox KM, Paul IA, Kline RH, Newman AH, Katz JL (2000) 3'- and 4'-chloro-substituted analogs of benztropine: intravenous self-administration and in vitro radioligand binding studies in rhesus monkeys. Psychopharmacology 147:426–435