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ORIGINAL INVESTIGATION

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Further evaluation of the reinforcing effects of the novel cocaine analog 2β-propanoyl-3β-(4-tolyl)-tropane (PTT) in rhesus monkeys

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Abstract 2β-Propanoyl-3β-(4-tolyl)-tropane (PTT) is a cocaine analog which has been shown in rhesus monkeys to have cocaine-like discriminative stimulus effects and a long duration of action (>8 h), yet does not function as a reinforcer when substituted for cocaine in monkeys responding under a fixed-interval 5-min schedule (Nader et al. 1997). The purpose of the present study was to evaluate the reinforcing effects of PTT under a fixed-ratio (FR) schedule and to determine if decreasing the inter-injection interval would influence the reinforcing effects of PTT. Male rhesus monkeys (n=3) were trained to respond under a multiple FR 30 food-drug-food schedule. When responding was stable, cocaine (0.003–0.3 mg/kg per injection) or PTT (0.001–0.03 mg/kg per injection) was available during the drug component for at least five consecutive sessions and until stable responding was observed. To investigate whether the inter-injection interval would influence PTT-maintained response rates, the time-out (TO) following PTT injections was reduced from 180 or 300 s to 10 s for at least five consecutive sessions. Cocaine-maintained response rates were characterized as an inverted-U shaped function of dose, with peak rates maintained by 0.03 mg/kg per injection cocaine. PTT (0.001–0.03 mg/kg per injection) maintained response rates significantly higher than rates maintained by the PTT vehicle, but significantly lower than cocaine-maintained response rates; PTT intake increased with dose. A reduction of the TO following PTT injections to 10 s did not alter PTT-maintained response rates or total session intake. Self-administered PTT was more potent than cocaine at decreasing food-maintained responding. These results suggest that for long-acting compounds like PTT, reinforcing effects are more likely to be observed when the drug is available under a ratio-based schedule, compared to an interval-based schedule.

Key words Cocaine · Tropanes · Dopamine transporter · Self-administration · Rhesus monkey

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Introduction

While cocaine is an indirect monoamine agonist, the reinforcing effects of cocaine are believed to be mediated primarily through inhibition of dopamine (DA) reuptake (Ritz et al. 1987). Understanding the behavioral and pharmacological effects of cocaine is critical to the investigation of potential pharmacotherapies to treat cocaine abuse in humans. For example, an agonist treatment would be predicted to decrease high dose self-administration, shifting the cocaine dose-response curve to the left. In addition, if the treatment agent was a DA agonist and the compound had cocaine-like reinforcing and/or discriminative stimulus effects, treatment outcome may be enhanced because compliance would be expected to be high (Mello and Negus 1996). This treatment strategy of substituting another DA agonist for cocaine is analogous to methadone maintenance treatment of heroin addiction (Dole and Nyswander 1965). For example, preclinical assessment of the indirect-acting DA agonist mazindol (Bergman et al. 1989; Kleven et al. 1990; Mansbach and Balster 1993) and the direct-acting DA D_2 agonist bromocriptine (Woolverton et al. 1984; Wise et al. 1990) indicate that these compounds can function as reinforcers and have cocaine-like discriminative stimulus effects. However, clinical studies with bromocriptine (Preston et al. 1992; Eiler et al. 1995) and mazindol (Preston et al. 1993; Stine et al. 1995) have not

proven effective in decreasing craving or reducing relapse, up to doses that induce dysphoria. Thus, continued evaluation of DA agonists, perhaps with longer durations of action that maintain lower rates of responding, is warranted.

Recently, the novel cocaine analog 2β-propanoyl-3β-(4-tolyl)-tropane (PTT) has been evaluated and compared to cocaine in several in vitro and in vivo assays. PTT has a higher affinity (8.2 versus 173 nM) for the dopamine transporter (Davies et al. 1993, 1994) and is approximately 50 times more potent at inhibiting dopamine uptake (3.2 versus 150 nM) compared to cocaine (Bennett et al. 1995). PTT contains a 2β-ethyl ketone moiety in place of the methyl ester of cocaine, and a 3β-(4 methylphenyl) group in place of the benzoate group of cocaine, thereby increasing the metabolic stability of PTT (Davies et al. 1993). In vivo microdialysis experiments in rats have found that PTT is 30 times more potent than cocaine in increasing extracellular dopamine concentrations within the nucleus accumbens (Hemby et al. 1995). In addition, in rodents, PTT elicited locomotor activity and stereotypies persisting for over 4 h compared to 30- to 60-min duration of locomotor activity seen following the peak dose of cocaine (Porrino et al. 1994, 1995). The results of these assays indicate that PTT is more potent and produces quantitatively larger and long-lasting effects upon the dopaminergic system compared to cocaine.

In monkeys self-administrating cocaine, PTT was approximately 10 times more potent at decreasing cocaine self-administration compared to pretreatments with cocaine; the effects of PTT on cocaine self-administration persisted for the entire 4-h session compared to the effects of cocaine pretreatments, which dissipated within 2 h (Nader et al. 1997). When available for self-administration, PTT did not function as a reinforcer when substituted for cocaine in monkeys responding under a fixed-interval (FI) 5-min schedule of drug presentation. However, in drug discrimination studies, PTT produced cocaine-like discriminative stimulus effects that were apparent for at least 8 h after administration (Nader et al. 1997). Thus, the behavioral profile of PTT, decreasing cocaine self-administration and producing cocaine-like discriminative stimulus effects, but not functioning as a reinforcer, is unique among indirect-acting DA agonists (e.g., Spealman et al. 1991; Glowa et al. 1995; Weed et al. 1995).

The primary purpose of the present study was to evaluate further the behavioral effects of PTT in monkeys. It is possible that PTT did not function as a positive reinforcer when available under the FI 5-min schedule because of its long duration of action. Preliminary data in rodents indicated that when PTT was available under a fixed-ratio (FR) 10 schedule with a short time-out (TO) following each injection, rats would self-administer several injections over a short period of time followed by long pauses (Dworkin and Pitts 1994). In our earlier study, the scheduling of PTT under an FI 5-min schedule would not have allowed this high frequency of self-administration to occur. Thus, one hypothesis tested in the present study was that increasing PTT availability (by decreasing the inter-injection interval) would result in higher levels of PTT intake and rates of responding. To test this hypothesis, monkeys were initially trained to self-administer cocaine under a multiple FR 30 schedule of food and cocaine presentation. When PTT was available and the TO between injections was removed, maximal intake as well as pattern of self-administration was determined by the animal, not by the investigator. In addition, the present study also evaluated the rate-suppressing effects of self-administered PTT and cocaine on food-maintained responding.

Materials and methods

Subjects

Three adult male rhesus monkeys (*Macaca mulatta*), maintained at approximately 90% of their free-feeding weights (between 8 and 10 kg), served as subjects. All monkeys had a history of cocaine self-administration under a fixed-interval 5-min schedule (Nader and Bowen 1995; Nader et al. 1997). Monkeys were given supplemental food (Purina Monkey Chow No. 3032, Ralston-Purina Co., St Louis, Mo., USA) no sooner than 30 min post-session to maintain stable body weight. Water was continuously available and, in addition, fresh fruit and a chewable vitamin supplement were provided to each monkey at least 3 days per week. Monkeys were weighed monthly and, if necessary, their total daily food intake was adjusted to maintain stable weights. Monkeys lived in a temperature and humidity controlled colony room; lighting was maintained on an 0600:2000 on:off schedule.

Apparatus

Monkeys were individually housed in sound attenuating cubicles (91 cm wide×91 cm deep×91 cm high; Plas Labs, Lansing, Mich., USA); the front wall had a Plexiglas door that allowed visual access to the laboratory. In addition, mirrors were mounted on the wall opposite each cubicle which allowed the monkeys visual access to other monkeys in the room. During experimental sessions, the front wall was covered with a drape. Each monkey was fitted with a stainless steel restraint harness and spring arm (Restorations Unlimited, Chicago, Ill., USA) which attached to the rear wall of the cubicle. Each cubicle was equipped with two response levers (BRS/LVE, PRL-001, Beltsville, Md., USA) and a peristaltic infusion pump (7531-10; Cole-Palmer Co., Chicago, Ill., USA) for delivering injections (approximately 1.0 ml/10 s) during the drug component of the experimental session. A stainless steel food dish was mounted between the two levers. Above each lever were four stimulus lights, two covered with white lens caps and two covered with red lens caps. A food-pellet dispenser (G5210, Model A, Gerbrands Corp., Arlington, Mass., USA) was located on the front wall and delivered 1-g banana-flavored pellets (P. J. Noyes Co., Lancaster, N.H., USA) during the food components of the session. Experimental events were controlled and counted by a Macintosh II computer and associated interfaces, located in an adjacent room.

Surgery

Each monkey was anesthetized with a combination of ketamine (15 mg/kg, IM) and butorphanol (0.03 mg/kg, IM) and a chronic indwelling venous catheter was surgically implanted under sterile conditions. The proximal end of the silicone catheter (0.08 cm inside diameter; Ronsil Rubber Products, Blackstone, Va., USA)

was inserted into a major vein (internal or external jugular, femoral) or brachial vein (0.02 cm inside diameter of catheter), terminating in the vena cava. The distal end of the catheter was threaded SC and exited through a small incision (<1 cm) on the back of the animal. Monkeys were given 1–2 days to recover from surgery, prior to returning to the experiment. Antibiotics (Kefzol; cefazolin sodium, Marsam Pharmaceuticals, Inc., Cherry Hill, N.J., USA) were administered prophylactically for 5–7 days following surgery. When a catheter failed, the monkey was removed from the experiment for at least 7 days, a new catheter was implanted, and the monkey was returned to the experiment.

Procedure

During daily experimental sessions, monkeys responded under a three component multiple schedule of food and drug reinforcement. The schedule of presentation was as follows: 30-min food (FD1) component; 15-min TO; 60- (monkeys 5653 and 5664) or 100-min (monkey 5662) drug (D) component; 15-min TO; 30-min food (FD2) component. During FD1, white lights over the right response lever were illuminated to signal the availability of food pellets (1 g banana flavored pellets) under an FR 30 schedule; only responding on the right lever had scheduled consequences. The delivery of each pellet was accompanied by the termination of the white lever lights and illumination of the red lever lights for 10 s, followed by a 30-s TO, during which all lever lights were off. After completion of FD1, there was a 15-min TO period during which all stimulus lights were extinguished and responses had no programmed consequences. Following this TO period, the illumination of white lights above the left response lever signaled the availability of 0.03 mg/kg per injection cocaine under an FR 30 schedule; only responding on the left lever had scheduled consequences. During the 10-s IV injection, the white lights above the left lever were extinguished and the red lights were illuminated. Each injection was followed by a 180-s (monkeys 5653, 5664) or 300-s (monkey 5662) TO, during which all lights were extinguished and responding on either lever had no scheduled consequences. Parameters of the multiple schedule varied slightly for monkey 5662 in order to equate rates and patterns of responding across food and cocaine components. Following the D component, a 15-min TO period occurred, after which food (FD2) was again available under an FR 30 schedule, identical to the FD1 component. In all cases, the component clock did not stop during post-reinforcer time-outs.

When rates of responding maintained by 0.03 mg/kg per injection cocaine were stable (±20% of the mean drug rate for three consecutive sessions, with no trends in responding among the three components), saline was substituted for cocaine for at least five consecutive sessions until responding in the D component declined to less than 20% of baseline. Following stable performance, the conditions were returned to baseline (i.e., 0.03 mg/kg per injection cocaine) for at least five consecutive sessions. Next, PTT dose-response functions (0.001–0.03 mg/kg per injection) were determined in each monkey, with doses tested in a random order. The minimum number of sessions that each dose was available for self-administration was individually determined and based upon the number of sessions that were required for responding to decline to less than 20% of baseline when saline was available (range of five to ten sessions). After a dose of PTT was evaluated under baseline schedule conditions (180- or 300-s TO following each injection), and responding was considered stable, the TO following each injection was reduced to 10 s and that dose of PTT was available for a minimum of five additional sessions. If, prior to changing the TO value, PTT-maintained response rates were similar to saline-maintained rates, responding was briefly (one or two sessions) re-established with cocaine (0.03 mg/kg per injection) before PTT (TO 10 s) was evaluated. After evaluating the reinforcing effects of a particular dose of PTT at the two TO parameters, there was a return to baseline conditions (0.03 mg/kg per injection cocaine) for at least five consecutive sessions prior to the evaluation of another PTT dose. Cocaine self-administration was not evaluated at the 10-s TO value.

Upon completion of the PTT dose-response-curve, various doses of cocaine (0.003–0.3 mg/kg per injection) were substituted for 0.03 mg/kg per injection cocaine in each monkey. After a dose was evaluated, there was a return to baseline conditions for at least five sessions. Sessions were conducted at the same time each day, typically 7 days/week. At the end of each session, catheters were flushed with approximately 3 ml heparinized saline (100 IU/ml), to help prevent clotting.

Data analysis

The primary dependent variables were response rate (responses per second) in each of the three components, total intake (mg/kg per session), and number of injections received in the drug component. Data are presented as the mean (±SD) of the last three sessions for a particular dose, for each monkey. For cocaine and PTT, data were analyzed separately by one-way repeated measures ANOVA, with all doses (and saline) as the main effect. To determine if there were differences between cocaine and PTT, repeated measures ANOVAs were conducted on difference scores (i.e., Coc-PTT) with drug dose as the main effect. Significant effects were followed by individual means comparisons with the Scheffé post hoc test (Scheffé 1959). For all analyses, $P<0.05$ was considered statistically significant. For the baseline dose of cocaine (0.03 mg/kg per injection), the three sessions preceding the start of the PTT substitution experiment were included in the analyses. The mean baseline rate of cocaine-maintained responding (0.03 mg/kg per injection) did not change in any systematic manner across the duration of the experiment. To evaluate the potency of drug effects on response rates in FD2, ED_{50} values for PTT and cocaine were calculated on log-transformed intake data (mg/kg per session) and were based on the linear portion of the mean FD2 dose-effect curve for each drug.

Drugs

(–)Cocaine HCl, provided by the National Institute on Drug Abuse (Rockville, Md., USA), was dissolved in sterile saline. (±)PTT was synthesized according to the procedure described by Davies et al. (1991) and dissolved in a vehicle consisting of 95% ethanol:sterile water, in a ratio of 4:1, to a concentration of 15 mg/ml. All drug concentrations available for self-administration were prepared in 250 ml sterile saline. Different doses were studied by changing the drug concentration. The PTT vehicle was 1.4% w/v ethanol. Prior to the beginning of each session, catheters were flushed for approximately 30 s with the concentration of drug available for self-administration. Because each catheter was filled with heparinized saline (approximately 2 ml), the total amount of drug solution injected into the animal prior to the start of the session was approximately 1 ml.

Results

Cocaine-maintained response rates varied as a function of dose, with mean rates of responding significantly greater than saline [$F(5, 30)=49.05$; $P<0.01$] and were characterized by an inverted-U shaped function of dose, in all monkeys (Fig. 1, open circles). Under the baseline FR 30 schedule, the highest mean rate of responding ranged from 0.46 to 0.74 responses per second across monkeys when 0.03 mg/kg per injection cocaine was available for self-administration. Cocaine intake (mg/kg per session) increased ($P<0.01$) in a dose-related manner, with the highest intakes occurring when 0.3 mg/kg per injection cocaine was available for self-administration (Table 1). The maximum number of injections received

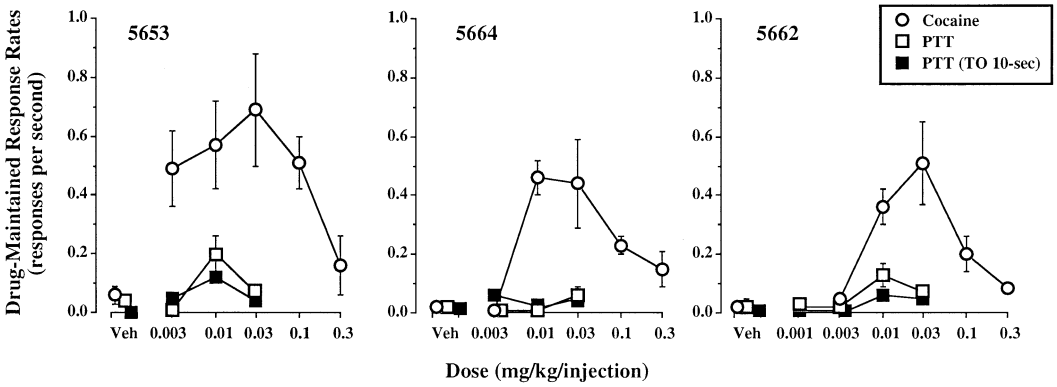

Fig. 1 Effects of cocaine (*open circles*) or PTT (*open* and *filled squares*) dose on rates of responding (responses per second) in three individual monkeys responding under a multiple FR 30 food, drug, food schedule. Data are from the drug component in which, under baseline conditions (*open symbols*), the time-out following each drug injection was 180 s (monkeys 5653 and 5664) or 300 s (monkey 5662) and when the TO following each PTT injection was 10 s (*filled squares*). Each point is the mean (±SD) of the last three sessions a particular dose was available for self-administration

Table 1 Total drug intake (mg/kg per session) as a function of cocaine and PTT dose[a]

Monkey	Cocaine (mg/kg per injection)				
	0.003	0.01	0.03	0.1	0.3
5653	0.045(0.00)	0.16(0.01)	0.49(0.05)	1.57(0.06)	2.40(0.30)
5664	0.003(0.00)	0.15(0.01)	0.44(0.04)	1.17(0.06)	2.70(0.52)
5662	0.020(0.01)	0.16(0.01)	0.47(0.08)	1.37(0.12)	2.80(0.17)
	PTT (mg/kg per injection) baseline TO				
	0.001	0.003	0.01	0.03	
5653		0.004(0.01)	0.11(0.02)	0.16(0.02)	
5664		0.003(0.00)	0.04(0.01)	0.15(0.05)	
5662	0.002(0.00)	0.007(0.01)	0.11(0.02)	0.25(0.02)	
	PTT (mg/kg per injection) 10-s TO				
	0.001	0.003	0.01	0.03	
5653		0.02(0.00)	0.12(0.03)	0.16(0.05)	
5664		0.02(0.00)	0.02(0.01)	0.15(0.03)	
5662	0.001(0.01)	0.01(0.00)	0.12(0.02)	0.26(0.04)	

[a] Values are the mean (1 SD) of the last three sessions that dose was available

was 15–18 injections per session when 0.03 mg/kg per injection cocaine was available (maximum number of injections possible per session was 19). When saline was available, response rates ranged from 0.01 to 0.03 responses per second (Fig. 1) and the total number of vehicle injections was zero to four injections per session.

When substituted for 0.03 mg/kg per injection cocaine under baseline conditions (TO values following each injection were 180 or 300 s), PTT (0.001–0.03 mg/kg per injection) maintained response rates significantly greater [$F(3, 18)=37.15$; $P<0.01$] than drug vehicle (Fig. 1, open squares). Peak rates were observed at 0.01 mg/kg per injection PTT in two monkeys and 0.03 mg/kg per injection PTT in a third animal, resulting in response rates by each animal that ranged from 0.1 to 0.2 responses per second (Fig. 1, open squares). Post hoc analysis revealed that only 0.01 mg/kg per injection PTT resulted in significantly greater response rates than those maintained by drug vehicle ($P<0.01$). Between-drug comparisons revealed that PTT-maintained response rates were significantly lower [$F(3, 18)=47.98$; $P<0.01$] than cocaine-maintained rates (Fig. 1; open circles versus open squares). When PTT vehicle was available for self-administration, response rates were not different from rates observed when saline was available.

PTT intake increased in a dose-related manner, with the highest intakes occurring when 0.03 mg/kg per injection PTT was available for self-administration (Table 1). The maximum mean number of injections received was 11 per session when 0.01 mg/kg per injection PTT was available, which was significantly greater than when vehicle was available for self-administration ($P<0.01$).

When the TO following each PTT injection was reduced to 10 s (Fig. 1, closed squares), response rates were significantly different from vehicle-maintained re-

Table 2 FD1 rates (responses per second) as a function of drug condition[a]

[a] Rates were calculated from the three sessions that preceded each dose of cocaine and PTT. Data represent the mean (1 SD)

Monkey	Saline	Cocaine	PTT Veh (TO 180)	PTT (TO 180)	PTT Veh (TO 10)	PTT (TO 10)
5653	1.08 (0.37)	1.37 (0.38)	2.07 (0.36)	1.02 (0.22)	1.39 (0.58)	1.08 (0.24)
5664	4.53 (0.48)	2.69 (1.2)	1.44 (0.59)	2.19 (1.6)	3.55 (1.4)	2.17 (1.8)
5662	1.20 (0.42)	2.33 (0.56)	2.48 (0.43)	1.53 (1.0)	1.61 (0.79)	2.36 (0.56)

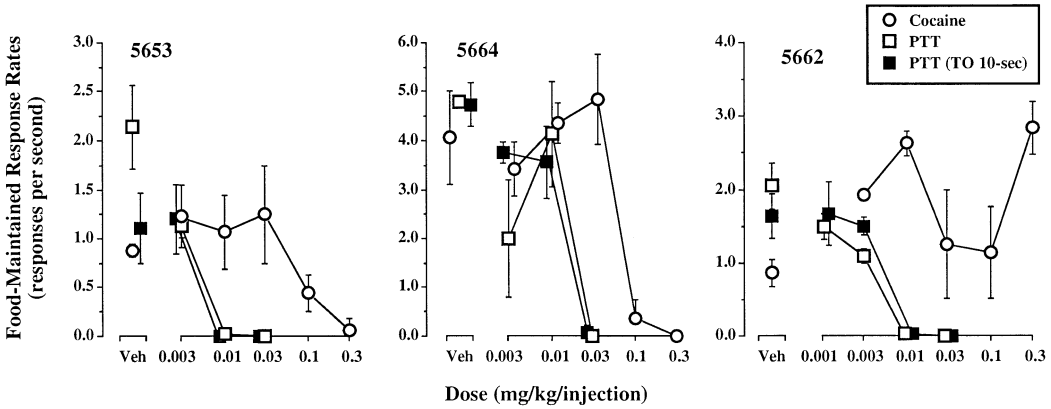

Fig. 2 Effects of cocaine (*open circles*) or PTT (*open* and *filled squares*) dose on rates of responding (responses per second) in three monkeys responding under a multiple FR 30 food, drug, food schedule. Data are from the second food component (FD2). Under baseline conditions (*open symbols*), the time-out following each drug injection was 180 s (monkeys 5653 and 5664) or 300 s (monkey 5662). *Filled squares* represent FD2 rates when the TO following each PTT injection in the drug component was 10 s. Each point is the mean (±SD) of the last three sessions a particular dose was available for self-administration

Table 3 ED_{50} values (mg/kg) of self-administered cocaine and PTT to decrease food-maintained responding in FD2[a]

Monkey	Cocaine	PTT	PTT (10-s TO)
5653	1.31 (1.04–1.66)	0.01 (0.007–0.02)	0.05 (0.03–0.08)
5664	1.17 (0.86–1.61)	0.09 (0.057–0.15)	0.05 (0.03–0.09)
5662	1.62 (0.61–4.32)	0.01 (0.005–0.01)	0.04 (0.03–0.05)

[a] Numbers in parenthesis are 95% confidence limits

sponse rates [$F(3, 18)=49.96$; $P<0.01$]. When the TO following each PTT injection was 10 s, post hoc analysis revealed that only 0.01 mg/kg per injection PTT resulted in significantly greater response rates than those maintained by the PTT vehicle ($P<0.01$). Total PTT intake per session was not influenced by TO duration [$F(2, 12)=2.56$; $P=0.12$], with maximal intakes occurring when 0.03 mg/kg per injection PTT was available (see Table 1).

Effects of drug self-administration on food-maintained responding

FD1 response rates varied between animals, with mean rates of responding ranging from 1.02 to 4.53 responses per second (Table 2). Total food pellets delivered during the first component of the multiple schedule varied from a mean of 30 to 49. Although there were between-subject differences in FD2 response rates, there were no within-subject differences in food-maintained response rates when the baseline dose of cocaine (0.03 mg/kg per injection) was available compared to FD2 rates when saline was self-administered (Fig. 2). Increases in cocaine dose to 0.1–0.3 mg/kg per injection, resulted in dose-dependent decreases in response rates in FD2, in two of three monkeys (Fig. 2, open circles). For monkey 5662, FD2 response rates were not affected or were increased by changes in cocaine dose, perhaps due to the longer TO (300 s for monkey 5662 versus 180 s for the other two monkeys) following each cocaine injection. For all monkeys, when PTT was substituted for cocaine, responding in FD2 decreased dramatically [$F(3, 18)=43.58$; $P<0.01$] to the point of zero responding at 0.01–0.03 mg/kg per injection PTT (Fig. 2, open squares). At doses of 0.003–0.03 mg/kg per injection, there were significant differences in the rate-decreasing effects on FD2 responding between cocaine and PTT [$F(3, 18)=18.24$; $P<0.01$]. When the TO following each PTT injection was 10 s, FD2 response rates were significantly [$F(3, 18)=121.45$; $P<0.01$] decreased with increases in PTT dose (Fig. 2, closed squares).

The PTT-induced disruption in food-maintained responding during FD2 is depicted in the cumulative response records for monkey 5653 (Fig. 3). When PTT was self-administered, the majority of the injections were taken early in the 60-min drug component. Low doses of PTT only modestly decreased FD2 response rates (Fig. 3, panel b). However, when 0.03 mg/kg per injection PTT was available, responding ceased during the drug component as well as the subsequent 30-min FD2 component (approximately 60 min after the last PTT injection).

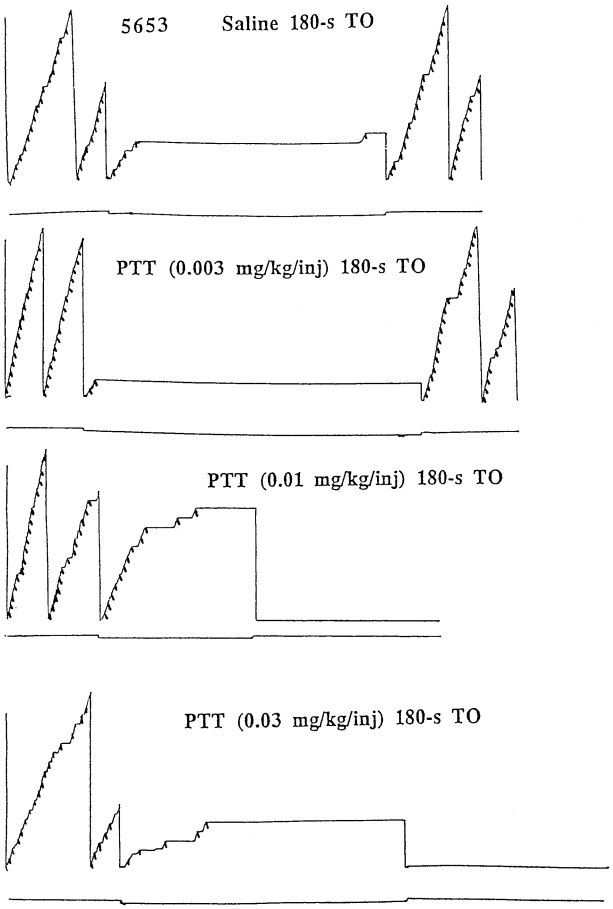

Fig. 3 Cumulative response records for monkey 5653, during sessions in which saline, and 0.003–0.03 mg/kg per injection PTT were available in the drug component of a multiple FR 30 food, drug, food schedule. The drug component, indicated by deflection of the event pen, was 60 min in duration and the TO following each injection was 180 s. The cumulative response recorder did not run during the TO, so component length along the abscissa will be longer when few injections are delivered (e.g., saline or 0.003 mg/kg per injection PTT)

The differences in potency of cocaine and PTT to decrease food-maintained responding is further depicted in Table 3. The ED_{50} for cocaine to decrease response rates in FD2 ranged from 1.17–1.62 mg/kg across animals (Table 3). PTT was 10–100 times more potent than cocaine, with ED_{50} values of approximately 0.01–0.1 mg/kg. Decreasing the TO following each PTT injection to 10 s only slightly modified the potency of PTT, with ED_{50} values of approximately 0.05 mg/kg observed in all monkeys (Table 3).

Discussion

The primary purpose of the present study was to evaluate further the reinforcing effects of the novel cocaine analog PTT in rhesus monkeys. When substituted for cocaine in monkeys responding under an FR schedule, PTT (0.01 mg/kg per injection) maintained response rates significantly higher than rates maintained by the drug vehicle and significantly lower than cocaine-maintained response rates. In addition, decreasing the TO following each PTT injection (and increasing PTT availability) did not alter total session intake of PTT. Behaviorally active doses of PTT were studied, since response rates in the food component that followed PTT self-administration were significantly decreased relative to baseline rates. These results extend earlier findings and provide additional evidence that under several reinforcement schedules, PTT maintains lower rates of responding compared to cocaine.

In an earlier study (Nader et al. 1997), PTT was directly compared to cocaine in drug self-administration and drug discrimination paradigms. When substituted for cocaine in monkeys responding under an FI 5-min schedule, PTT did not function as a reinforcer. However, in another group of animals, PTT produced cocaine-like discriminative stimulus effects that persisted for 8 h in two monkeys and 24 h in one subject. This unique behavioral profile can perhaps be understood by the neuropharmacological characteristics of PTT. In vitro assays have shown that PTT binds with higher affinity than cocaine to the DA transporter (Davies et al. 1993, 1994) and, unlike cocaine, PTT is selective for the DA transporter relative to the serotonin and norepinephrine transporters (Bennett et al. 1995). PTT also has been shown to bind to the DA transporter for a longer duration than cocaine (Bennett et al. 1997). In vivo microdialysis studies have demonstrated that PTT-induced increases in extracellular DA in the nucleus accumbens are quantitatively larger than cocaine-induced effects and the duration of action is 2–3 h longer for PTT compared to cocaine (Hemby et al. 1995). Thus, one possible explanation for the low rates of PTT self-administration is the drug's long duration of action compared to cocaine.

While PTT's long duration of action has been well characterized, the data demonstrating the onset of PTT-induced DA elevation have not been clearly elucidated. Drug discrimination data indicate that PTT has cocaine-like effects within 10 min of administration, although earlier time points were not examined (Nader et al. 1997). With this in mind, and because of preliminary data in rodents demonstrating high rates of PTT self-administration under an FR schedule (Dworkin and Pitts 1994), one hypothesis tested in the present study was whether animals would self-administer PTT in rapid succession, given the opportunity. In other words, it is possible that the lack of robust PTT self-administration under the FI 5-min schedule reported earlier (Nader et al. 1997) was due to the fairly long inter-injection interval. In the present study, increasing PTT availability, by decreasing the TO following injections, did not increase PTT-maintained response rates or PTT intake during the 60-min drug component. This lack of effect of a schedule manipulation that would allow rapid drug intake suggests that monkeys were either self-administering PTT up to a specific dose or that PTT's rate-decreasing effects interfered with responding. Intake values shown in Table 1 suggest

that monkeys were not responding to a specific intake. Rather, data from the second food component support the hypothesis that the direct rate-decreasing effects of PTT limited intake, since intakes of PTT greater than approximately 0.15 mg/kg completely eliminated food-maintained responding.

Earlier work by Winger (1993) has shown that TO duration following IV injections can substantially modify rates of drug self-administration and shift the dose-response curve in an orderly fashion. For short acting drugs like cocaine and methohexital, Winger (1993) found that increasing the TO increased response rates and shifted the self-administration dose-response curve to the right. Similarly, decreasing the TO following each cocaine or methohexital injection decreased response rates. The generality of these findings for drugs with longer durations of action has not been systematically evaluated. In the present experiment, PTT was available for self-administration under an FR 30 schedule with TO values varying from 180 s (or 300 s) to 10 s. These results can be compared to earlier findings in which responding was maintained under an FI 5-min schedule of PTT presentation (Nader et al. 1997). Taken together, under both interval and ratio schedules, PTT availability resulted in low rates of responding which did not appear to be modified by altering the inter-injection interval from 300 s to 10 s. However, it is possible, as suggested by Winger (1993) for other drug reinforcers, that longer TO values following each injection would enhance the reinforcing effects of PTT. In fact, when PTT was studied under an FI 5-min schedule (Nader et al. 1997), the mean inter-injection interval across the entire 4-h session was approximately 25 min. This pattern was not similar to saline, in that the mean inter-injection interval for saline was approximately 17 min.

While it is possible that the low rates of PTT self-administration compared to cocaine-maintained responding are due to differences in onset of action, recent findings from Volkow and colleagues studying cocaine and methylphenidate in human subjects suggest that duration of action is an important determinant of abuse liability (Fowler et al. 1989; Volkow et al. 1995, 1997). In these studies, positron emission tomography was utilized to determine the pharmacokinetics of cocaine and methylphenidate and to correlate the uptake and washout of the tracers to subjective drug effects. These investigators found that uptake of [^{11}C]cocaine and [^{11}C]methylphenidate were nearly identical, but the rate of washout of methylphenidate was much slower than the rate of washout of cocaine. The ratings of “high” paralleled the uptake of the tracer to the DA transporter, not the clearance of the drug from brain. These investigators concluded that methylphenidate had lower abuse liability than cocaine because of its slow rate of washout from the brain (Volkow et al. 1995). Thus, compounds that have a slower dissociation from the DA transporter compared to cocaine, as is true for PTT (Bennett et al. 1997), would be predicted to have lower reinforcing effects and maintain lower rates of self-administration.

The fact that monkeys did not self-administer PTT in rapid succession when the TO was 10 s is not consistent with the earlier findings in rodents (Dworkin and Pitts 1994). There are several possibilities to account for these discrepant findings. One possibility is species differences. However, this seems unlikely since other in vivo assays in rodents (Porrino et al. 1994, 1995; Hemby et al. 1995) demonstrate similar potency differences between PTT and cocaine as what is seen with primates (Nader et al. 1997; present study). A more likely explanation involves the use of single session substitution procedures in the rodent experiment (Dworkin and Pitts 1994). However, no data from saline substitution sessions were presented, which would have provided an indication of the sensitivity of the behavior to changes in drug dose (Dworkin and Pitts 1994). In the present study, we allowed self-administration to stabilize over at least five consecutive sessions. If we had only examined data from the first session of substitution, no differences from saline would have been observed. Thus, the discrepancy in results between the rodents and the primates may involve procedural details, rather than species differences or schedules of drug availability.

In addition to assessing the reinforcing effects of PTT under an FR schedule, a second purpose of this study was to evaluate the effects of PTT on a non-drug reinforcer. In the present study, self-administered PTT was 10–100 times more potent at decreasing food-maintained responding than was cocaine. These potency comparisons support previous in vivo data showing that PTT was more potent than cocaine in several behavioral paradigms (Hemby et al. 1995; Porrino et al. 1995; Nader et al. 1997). Further comparisons with our previous study, in which PTT or cocaine was administered as a pretreatment to cocaine self-administration sessions (Nader et al. 1997), suggests that PTT is equipotent at decreasing food-maintained responding and cocaine-maintained performance, although this comparison is indirect. For example, when peak effects were observed, the ED_{50} for *non-contingent* PTT to decrease cocaine self-administration (0.1 mg/kg per injection cocaine) was 0.06 mg/kg (Nader et al. 1997). In the present study, the ED_{50} values for *self-administered* PTT to decrease food-maintained responding ranged from 0.01 to 0.09 mg/kg. For cocaine, these values were 1.14 mg/kg (non-contingent; Nader et al. 1997) and 1.17 to 1.62 mg/kg (self-administered; present study) for decreasing cocaine- and food-maintained responding, respectively.

The apparent lack of selectivity of PTT in decreasing cocaine- versus food-maintained responding is in contrast to recent findings with the DA transport inhibitor GBR 12909 (Glowa et al. 1995). However, GBR 12909 functions as a reinforcer in monkeys under several conditions (Bergman et al. 1989; Skjoldager et al. 1993; Wojnicki and Glowa 1996) and substitutes for cocaine in drug discrimination paradigms (Kleven et al. 1990; Melia and Spealman 1991; Spealman 1993). It should be pointed out that PTT substitutes for cocaine’s discriminative stimulus effects at doses that do not decrease re-

sponse rates (Nader et al. 1997), indicating that cocaine-like effects can be obtained before other behaviorally disruptive effects are observed.

While rates of PTT-maintained responding are significantly lower than cocaine-maintained responding, it is nevertheless possible that PTT has greater reinforcing efficacy compared to cocaine, because of the quantitatively larger increases in synaptic DA following PTT compared to cocaine, as determined from microdialysis experiments. In preliminary studies using progressive-ratio schedules in rats, the break point for PTT is greater than that for cocaine (D. C. S. Roberts, personal communication). Consistent with our results, rats self-administered PTT at low rates, averaging one injection per hour and taking 16–18 h to reach their break point, compared to 2 h to reach the cocaine break point. Taken together, these results suggest that PTT may be preferred over cocaine (i.e., have greater reinforcing efficacy), yet maintain substantially lower rates of self-administration. How these measures of reinforcing efficacy and the earlier findings comparing subjective effects and dissociation from the receptor can be integrated, as well as whether such a behavioral profile predicts greater clinical efficacy, remains to be determined.

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References

Bennett BA, Wichems CH, Hollingsworth CK, Davies HML, Thornley C, Sexton T, Childers SR (1995) Novel 2-substituted cocaine analogs: uptake and ligand binding studies at dopamine, serotonin and norepinephrine transport sites in the rat brain. J Pharmacol Exp Ther 272: 1176–1186

Bennett BA, Hollingsworth CK, Martin RS, Childers SR, Ehrenkaufer RE, Porrino LJ, Davies HML (1997) Prolonged dopamine and serotonin transporter inhibition after exposure to tropanes. Neuropharmacology (in press)

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

Davies HML, Saikali E, Young WB (1991) Synthesis of (±)-ferrugine and (±)-anhydroecognine methyl ester by a tandem cyclopropananation/cope rearrangement. J Org Chem 56: 5696–5700

Davies HML, Suikali E, Sexton T, Childers SR (1993) Novel 2-substituted cocaine analogs: binding properties at dopamine transport sites in rat striatum. Eur J Pharmacol 244: 93–97

Davies HML, Suikali E, Huby NJ, Gilliatt VJ, Matasi JJ, Sexton T, Childers SR (1994) Synthesis of 2β-acyl-2β-aryl-8-azabicyclo[3.2.1] octanes and their binding affinities at dopamine and serotonin transport sites in rat striatum and frontal cortex. J Med Chem 37: 1262–1286

Dole VP, Nyswaynder ME (1965) A medical treatment for diacetylmorphine (heroin) addiction. JAMA 193: 646–650

Dworkin SI, Pitts RC (1994) Use of rodent self-administration models to develop pharmacotherapies for cocaine abuse. In: Erinoff L, Brown R (eds) Neurobiological models for evaluating mechanisms underlying cocaine addiction and potential pharmacotherapies for treating cocaine abuse. NIDA Research Monograph No. 145. US Government Printing Office, Washington, pp 88–112

Eiler K, Schaefer MR, Salstrom D, Lowery R (1995) Double-blind comparison of bromocriptine and placebo in cocaine withdrawal. Am J Drug Alcohol Abuse 21: 65–79

Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ, MacGregor RR, Hitzemann R, Logan J, Bendriem B, Gatley SJ, Christman D (1989) Mapping cocaine binding sites in human and baboon brain in vivo. Synapse 4: 371–377

Glowa JR, Wojnicki FHE, Matecka D, Bacher JD, Mansbach RS, Balster RL, Rice K (1995) Effects of dopamine reuptake inhibitors on food- and cocaine-maintained responding: I. Dependence on unit dose of cocaine. Exp Clin Psychopharmacol 3: 219–231

Hemby SE, Co C, Reboussin D, Davies HML, Dworkin SI, Smith JE (1995) Comparison of a novel tropane analog of cocaine, with cocaine HCl in rats: nucleus accumbens extracellular dopamine concentration and motor activity. J Pharmacol Exp Ther 273: 656–666

Kleven MS, Anthony EW, Woolverton WL (1990) Pharmacological characterization of the discriminative stimulus effects of cocaine in rhesus monkeys. J Pharmacol Exp Ther 254: 312–317

Mansbach RS, Balster RL (1993) Effects of mazindol on behavior maintained or occasioned by cocaine. Drug Dev Res 31: 183–191

Melia KF, Spealman RD (1991) Pharmacological characterization of the discriminative stimulus effects of GBR 12909. J Pharmacol Exp Ther 258: 626–632

Mello NK, Negus SS (1996) Preclinical evaluation of pharmacotherapies for treatment of cocaine and opioid abuse using drug self-administration procedures. Neuropsychopharmacology 14:375–424

Nader MA, Bowen CA (1995) The effects of different food-reinforcement histories on cocaine self-administration by rhesus monkeys. Psychopharmacology 118: 287–294

Nader MA, Grant KA, Davies HML, Mach RH, Childers SR (1997) The reinforcing and discriminative stimulus effects of the novel cocaine analog 2β-propanoyl-3β-(4-tolyl)-tropane (PTT) in rhesus monkeys. J Pharmacol Exp Ther 280: 541–550

Porrino LJ, Migliarese K, Davies HML, Saikali E, Childers SR (1994) Behavioral effects of the novel tropane analog, 2β-propanoyl-3β-(4-tolyl)-tropane (PTT). Life Sci 54: PL511–PL517

Porrino LJ, Davies HML, Childers SR (1995) Behavior and local cerebral metabolic effects of the novel tropane analog, 2β-propanoyl-3β-(4-tolyl)-tropane. J Pharmacol Exp Ther 272: 901–910

Preston K, Sullivan JT, Strain EC, Bigelow GE (1992) Effects of cocaine alone and in combination with bromocriptine in human cocaine abusers. J Pharmacol Exp Ther 262: 279–291

Preston K, Sullivan JT, Berger P, Bigelow GE (1993) Effects of cocaine alone and in combination with mazindol in human cocaine abusers. J Pharmacol Exp Ther 267: 296–307

Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science (Washington DC) 237: 1219–1223

Scheffé H (1959) The analysis of variance. Wiley, New York

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

Spealman RD (1993) Modification of behavioral effects of cocaine by selective serotonin and dopamine uptake inhibitors in squirrel monkeys. Psychopharmacology 112: 93–99

Spealman RD, Bergman J, Madras BK (1991) Self-administration of the high-affinity cocaine analog 2β-carbomethoxy-3β-(4-fluorophenyl)tropane. Pharmacol Biochem Behav 39: 1011–1013

Stine SM, Krystal JH, Kosten TR, Charney DS (1995) Mazindol treatment for cocaine dependence. Drug Alcohol Depend 39: 245–252

Volkow ND, Ding Y-S, Fowler JS, Wang G-J, Logan J, Gatley JS, Dewey S, Ashby C, Liebermann J, Hitzemann R, Wolf AP (1995) Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. Arch Gen Psychiatry 52: 456–463

Volkow ND, Wang G-J, Fischman MW, Foltin RW, Fowler JS, Abumrad NN, Vitkun S, Logan J, Gatley SJ, Pappas N, Hitzemann R, Shea CE (1997) Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 386: 827–830

Weed MR, Mackevicius AS, Kebabian J, Woolverton WL (1995) Reinforcing and discriminative stimulus effects of β-CIT in rhesus monkeys. Pharmacol Biochem Behav 51: 953–956

Winger G (1993) Fixed-ratio and time-out changes on behavior maintained by cocaine or methohexital in rhesus monkeys: 1. Comparison of reinforcing strength. Exp Clin Psychopharmacol 1: 142–153

Wise RA, Murray A, Bozarth MA (1990) Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing in rats. Psychopharmacology 100: 355–360

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

Woolverton WL, Goldberg LI, Ginos JZ (1984) Intravenous self-administration of dopamine receptor agonists by rhesus monkeys. J Pharmacol Exp Ther 230: 678–683