Comparative Behavioral Pharmacology of Cocaine and the Selective Dopamine Uptake Inhibitor RTI-113 in the Squirrel Monkey

LEONARD L. HOWELL, PAUL W. CZOTY, MICHAEL J. KUHAR, and F. IVY CARROL

Yerkes Regional Primate Research Center (L.L.H., P.W.C., M.J.K.), and Departments of Psychiatry and Behavioral Sciences (L.L.H.) and of Pharmacology (L.L.H., M.J.K.), Emory University, Atlanta, Georgia; and Research Triangle Institute, Research Triangle Park, North Carolina (F.I.C.)

Accepted for publication October 13, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

The behavioral effects of 3β-(4-chlorophenyl)tropane-2β-carboxylic acid phenyl ester hydrochloride (RTI-113; 0.03–1.0 mg/kg), a selective dopamine uptake inhibitor, were compared with those of cocaine (0.03–3.0 mg/kg) and 1-[2-[(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12909; 0.03–3.0 mg/kg) in squirrel monkeys. Intermediate doses of each drug produced significant increases in response rate maintained by a fixed-interval (FI) 300-s schedule of stimulus termination, but RTI-113 was less effective than cocaine or GBR 12909. The order of potency for increasing response rate was RTI-113 ≥ cocaine > GBR 12909. In drug time course determinations, RTI-113 and GBR 12909 had longer durations of action than cocaine. RTI-113 substituted completely for cocaine in subjects trained to discriminate cocaine and saline under a two-lever drug-discrimination procedure maintained by food delivery. RTI-113 also reliably maintained self-administration behavior in subjects trained under a second-order FI 900-s schedule of i.v. cocaine delivery. Pretreatment with RTI-113 significantly decreased responding for cocaine at the highest pretreatment dose, but RTI-113 had similar effects on responding maintained by a second-order FI 900-s schedule of stimulus termination. The results indicate that the behavioral pharmacology of RTI-113 is similar to that of cocaine, further implicating a prominent role for dopamine uptake inhibition in the behavioral effects of cocaine. Its longer duration of action in conjunction with less pronounced behavioral-stimulant effects are desirable properties for a substitute pharmacotherapy for cocaine abuse. RTI-113 effectively decreased cocaine self-administration behavior, although its direct rate-altering effects may have contributed to the interactions obtained.

The dopamine transporter is a critical recognition site for cocaine and likely mediates its acute behavioral and reinforcing effects that contribute to significant abuse liability (Ritz et al., 1987; Kuhar et al., 1991). In vitro studies have demonstrated that cocaine blocks the presynaptic uptake of the monamines dopamine, serotonin, and norepinephrine (Heikkila and Manzino, 1984; Reith et al., 1986; Wilcox et al., 1999), but the behavioral effects of cocaine have been linked more closely to enhanced dopaminergic activity because of inhibition of dopamine uptake (Wise, 1984; Ritz et al., 1987; Woolverton and Kleven, 1988; Howell and Byrd, 1991, 1995). Evidence to support this conclusion is derived from various behavioral studies characterizing the acute effects of dopamine uptake inhibitors and dopamine antagonists administered alone or in combination with cocaine. Many drugs that inhibit dopamine uptake can maintain self-administration in laboratory animals (Ritz et al., 1987). Cocaine and the selective dopamine uptake inhibitor 1-[2-[(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 129090; Heikkila and Manzino, 1984; Anderson, 1989) exert similar effects on schedule-controlled behavior and are reliably self-administered in nonhuman primates (Bergman et al., 1989; Howell and Byrd, 1991; Howell et al., 1997). In drug-discrimination studies, dopamine uptake inhibitors substitute completely for cocaine (Melia et al., 1989; Melia and Spealman, 1991). Preclinical studies have demonstrated a significant correlation between dopamine transporter occupancy and the locomotor-stimulant effects of cocaine analogs (Cline et al., 1992; Kuhar, 1993), and recent neuroimaging studies in human cocaine users have demonstrated a direct relationship between dopamine transporter occupancy and drug intake.

Received for publication March 23, 1999.

1 This research was supported in part by U.S. Public Health Service Grants DA-01161, DA-05346, DA-10344, and RR-00165 (Division of Research Resources, National Institutes of Health) and Contract Grant OND-6069 (Office of National Drug Control Policy). The Yerkes Regional Primate Research Center is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

ABBREVIATIONS: GBR 12909, 1-[2-[(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride; FI, fixed interval; FR, fixed ratio; RTI-113, 3β-(4-chlorophenyl)tropane-2β-carboxylic acid phenyl ester hydrochloride.
and the subjective effects of cocaine (Volkow et al., 1997). Conversely, dopamine antagonists can attenuate specific behavioral effects of cocaine, including its psychomotor-stimulant effects (Scheel-Krüger et al., 1977), reinforcing effects (Woolverton, 1986), discriminative-stimulus effects (Kleven et al., 1988; Barrett and Appel, 1989; Callahan et al., 1991), and effects on schedule-controlled behavior (Bergman and Speelman, 1988; Speelman, 1990; Howell and Byrd, 1991, 1992). Collectively, the results obtained in behavioral studies provide compelling evidence that dopamine plays a major role in the neuropharmacology of cocaine.

Despite more than two decades of intensive research into the molecular, cellular, and behavioral effects of cocaine, no uniformly effective pharmacotherapy for cocaine abuse has demonstrated efficacy for long-term use. Of the various types of medications that are being pursued, substitute agonists represent a promising approach in drug development. Effective candidate medications will likely have a mechanism of action and pharmacological profile similar to cocaine but have a slower onset, longer duration of action, and lower abuse liability. In this regard, selective dopamine uptake inhibitors may be useful pharmacological adjuncts in the treatment of cocaine addiction. The phenyl-substituted piperazine derivative GBR 12909, which exhibits a high affinity and selectivity for the dopamine reuptake site (Heikkila and Manzino, 1984; Anderson, 1989), also has behavioral effects similar to those of cocaine, including psychomotor-stimulant (Baldo and Kelly, 1991; Howell and Byrd, 1991), discriminative-stimulus (Melia and Speelman, 1991; Witting et al., 1991), and reinforcing (Bergman et al., 1989; Howell and Byrd, 1991; Howell et al., 1997) effects. Note that pretreatment with GBR 12909 can selectively decrease cocaine self-administration in nonhuman primates (Glowa et al., 1995) and attenuate cocaine-induced increases in extracellular dopamine in rodents (Baumann et al., 1994).

Tropane analogs of cocaine represent another structural class of drugs that are potent and selective dopamine uptake inhibitors. Like GBR 12909, 2β-propanoyl-3β-(4-tolyl)tropane (PTT) produces cocaine-like locomotor effects in rodents (Hemby et al., 1995) and decreases cocaine self-administration in nonhuman primates (Nader et al., 1997). Similarly, the phenyltropane analog 3β[4-chlorophenyl]tropane-2β-carboxylic acid phenyl ester hydrochloride (RTI-113) (Carroll et al., 1995) decreases cocaine self-administration in rodents at doses that exhibit high occupancy of dopamine transporters (Dwarkin et al., 1998). Collectively, these preclinical studies provide evidence that selective inhibitors of dopamine uptake may be useful substitute agonist medications in the treatment of cocaine abuse.

Our study extended previous research with the phenyltropane analog RTI-113 by characterizing its behavioral pharmacology in nonhuman primates. The effects of RTI-113 were compared directly with those of cocaine on several measures of operant behavior including schedule-controlled behavior, drug discrimination, and drug self-administration in squirrel monkeys. Both RTI-113 and cocaine had a similar profile of behavioral effects. However, RTI-113 had a longer duration of action and less pronounced behavioral-stimulant effects, thereby exhibiting desirable properties for a substitute pharmacotherapy for cocaine abuse. RTI-113 significantly decreased cocaine self-administration behavior, but the same dose disrupted operant behavior maintained by a nondrug reinforcer. Hence, behaviorally active doses of RTI-113 were required to decrease cocaine-maintained behavior.

Materials and Methods

Subjects. Sixteen adult male squirrel monkeys (Saimiri sciureus) weighing 820 to 1350 g served as subjects. Between daily sessions, subjects lived in individual cages and had access to food (Harlan Teklad Diet, fresh fruit and vegetables) and water. Nine subjects were surgically prepared with a chronically indwelling venous catheter for i.v. administration of drugs. While anesthetized with ketamine hydrochloride (10.0 mg/kg, supplement to effect) and diazepam (0.1 mg/kg), polyvinyl chloride tubing (0.38 mm i.d., 0.75 mm o.d.) was inserted via the left or right femoral or external jugular vein with sterile surgical technique. The catheter was filled with heparinized saline (20 U/0.2 ml saline) and sealed with a stainless steel obturator when not in use. A nylon-mesh jacket protected the externalized end of the catheter. All animal-use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Emory University.

Apparatus. During experimental sessions, subjects were seated comfortably in a Plexiglas chair within a sound-attenuating enclosure (Byrd, 1979). Illumination was provided by either of two pairs of 7-W a.c. colored lights (red and white) mounted on the front wall of the chair just above eye level. A response lever (E21-03; Coulbourn Instruments, Allentown, PA) mounted on the wall facing the monkey registered a response and operated a feedback relay when depressed with a downward force of >0.2 N. In experiments with a stimulus-termination schedule, the subject’s tail was held motionless in a small stock, and two brass plates rested on a shaved portion near the end. Electrode cream (The Lumiscopic Co., Inc., Edison, NJ) applied to the tail minimized changes in impedance between the tail and the brass plates when a 4-mA electric stimulus of 200-ms duration was delivered. In drug-discrimination experiments, the subjects faced two retractable response levers (E-23-07; Coulbourn Instruments) placed 10 cm apart horizontally. Food pellets (190-mg sucrose pellets with fruit punch flavor; P.J. Noyes Co., Lancaster, NH) were delivered individually into a tray positioned between the levers. In drug self-administration experiments, the distal end of the venous catheter was connected via polyvinyl chloride tubing to a motor-driven syringe located outside the test chamber. The syringe was driven by a 100-V a.c. motor that was controlled by electronic circuitry to yield a precise injection volume of 0.2 ml. Microcomputers controlled experimental events and recorded and stored data. Continuous white noise and an exhaust fan masked extraneous sounds during all sessions, and subjects were tested 5 days per week.

Stimulus-Termination Schedule. Monkeys S-87, S-91, S-93, S-98, S-111, S-115, and S-122 were trained under a fixed-interval (FI) 300-s schedule of stimulus termination (Morse and Kelleher, 1966) with a 3-s limited hold. A red light illuminated the experimental chamber during the FI and, after 300 s elapsed, the animal had 3 s to press the lever and terminate the light that was associated with an impending electric stimulus. When the animal pressed the lever during the limited hold, a white light was illuminated for 2 s, followed by a 60-s timeout during which the chamber was darkened and responses had no scheduled consequences. In the absence of a response during the 3-s limited hold, a 4-mA stimulus was delivered once for 200 ms followed by a 60-s timeout. A daily session comprised 13 consecutive FI 300-s components, each followed by a 60-s timeout. Complete dose-effect curves were established for RTI-113 (0.03–1.0 mg/kg), cocaine (0.03–3.0 mg/kg), and GBR 12909 (0.03–3.0 mg/kg) by injecting graded doses i.v. during the 60-s timeout that preceded FI components 2, 5, 8, and 11. The time course of effects of RTI-113 (0.1 mg/kg), cocaine (0.3 mg/kg), and GBR 12909 (1.0 mg/kg) was determined by injecting a single dose i.m. 5 s before a session comprising 20 consecutive FI 300-s components. Typically, drug experi-
ments were conducted on Tuesday and Friday, and saline (control) was administered on Thursday. Each drug dose was studied at least twice in each monkey.

After drug effects were characterized on performance maintained by the FI 300-s schedule of stimulus termination, monkeys S-87, S-91, S-93, and S-98 were trained under a second-order FI 900-s schedule of stimulus termination with fixed-ratio (FR) 20 components. A red light illuminated the chamber during the FI, and every 20th response (FR 20) during the FI changed the red light to white for 2 s. During the brief presentation of white light, responding had no programmed consequences. After the 900-s FI elapsed, the animal had 10 s to complete an FR 20 and terminate the light that was associated with an impending electric stimulus. When the animal completed an FR 20 during the limited hold, a white light was illuminated for 15 s, followed by a 60-s timeout. If an FR 20 was not completed during the limited hold, a 4-mA stimuli was delivered once for 200 ms, followed by a 60-s timeout. A daily session comprised four consecutive FI 900-s intervals. To parallel the drug self-administration experiments described below, a single dose of RTI-113 (0.03 or 0.3 mg/kg) was administered i.m. 30 min before the session for 3 consecutive days. Each subject received all doses of RTI-113 on two separate occasions.

**Drug-Discrimination Schedule.** Monkeys S-86, S-89, S-118, and S-134 were trained to discriminate 0.3 mg/kg of cocaine and saline under a two-lever drug-discrimination procedure maintained by food delivery. Once lever-pressing was established, the response requirement was increased until 30 responses (FR 30) were required for each food-pellet delivery. During this initial training period, the levers retracted in alternation so that only one lever was available for each FR 30, thus reducing the opportunity to develop a lever bias. Cocaine discrimination training began when response rate and pattern were comparable and stable on both levers. Subjects were administered either cocaine or saline i.m. 10 min before each session began, with both levers extended and red and blue lights illuminated. Responses on the lever associated with the presession injection were reinforced with a food pellet on the FR 30 schedule, whereas responses on the other lever did not produce a food pellet (extinction). Incorrect responses did not reset the FR value on the correct lever. The drug- and saline-paired levers were counterbalanced across subjects to control for any systematic lever bias unrelated to the injections. Cocaine (C) and saline (S) were administered in double alternation across sessions (CCSS). Each pellet delivery was accompanied by illumination of the white lights for 15 s and the retraction of the levers and was followed by a 5-s timeout during which the levers remained retracted and the lights extinguished. Sessions were conducted Monday through Friday and ended after 30 pellet deliveries (approximately 30 min).

Cocaine generalization and RTI-113 substitution experiments began for each subject once responding on the injection-associated lever was equal to or >90% of all responses during the first FR 30 for 10 consecutive training sessions. Test sessions for cocaine generalization or RTI-113 substitution were conducted on Tuesday and Friday if the previous two training sessions produced >90% correct responding. If this criterion was not met and a training session was conducted instead, the test session was postponed until a following Tuesday or Friday when the criterion was again attained. During test sessions, a food pellet was delivered after the first FR 30 was completed on either lever, and the session ended after the first reinforcer or when a 30-min limited hold expired. Only one drug dose was studied during each test session, and each dose was tested at least twice in each subject. Cocaine doses were administered i.m. 10 min before the session, and RTI-113 doses were administered i.m. 30 min before the session to allow adequate time for absorption and distribution.

**Drug Self-Administration Schedule.** Monkeys S-124, S-126, S-129, S-130, S-135, and S-140 were trained initially under a second-order FI 900-s schedule of stimulus termination as described above. When rates and patterns of responding were stable for each animal, subjects were prepared with chronically indwelling venous catheters. Subsequently, the stimulus-termination schedule was replaced with the drug self-administration procedure, and the first FR completed after the 900-s FI elapsed produced a single i.v. injection of drug. Otherwise, all schedule parameters remained the same.

Responding was maintained by a 0.1-mg/injection training dose of cocaine for several months (>50 sessions) before substitution with saline or different doses of cocaine (0.03–1.0 mg/injection) and RTI-113 (0.1 and 0.3 mg/injection). Saline and each drug dose were substituted for the 0.1-mg/injection maintenance dose of cocaine for at least five consecutive sessions on two separate occasions. In drug pretreatment experiments, a single dose of RTI-113 (0.03, 0.1, or 0.3 mg/kg) was administered i.m. 30 min before the session for 3 consecutive days. Each subject received all drug combinations.  

**Data Analysis.** Response rate maintained by the FI 300-s schedule of stimulus termination was computed separately for each FI component by dividing the total number of responses in a component by the total time the red light was present. Mean control rate was determined for each monkey by averaging response rate for all saline (control) sessions. Mean rate of responding during cumulative-dosing studies was determined for each monkey by averaging data from selected individual FI components of cumulative dose-response curves. Although three FI components followed each dose, data were derived for computation from the last two components to compensate for the time required for absorption and distribution of the drug. A repeated-measures t test was used to determine the statistical significance of differences in maximal rate-increasing effects produced by the drugs studied. Mean rate of responding maintained by the second-order FI 900-s schedule of stimulus termination was determined for individual monkeys by averaging together all FI components during a session. In drug-discrimination experiments, percent responding on the cocaine lever was computed by dividing the number of responses on the cocaine-associated lever by the total responses during the test session and multiplying by 100. Data are presented for individual subjects, with partial substitution defined as 20 to 80% cocaine-lever responding and full substitution defined as >80% cocaine-lever responding. Mean rate of responding during drug self-administration studies was determined for individual monkeys by averaging rate in the presence of the red light during all components of a session. Data are presented for the group of monkeys as mean response rate ± S.E. during all sessions when a particular drug dose was self-administered. A repeated-measures ANOVA with Tukey’s post hoc multiple comparisons was used to determine the statistical significance of drug treatment conditions. Statistical significance was accepted at the 95% level of confidence (P < .05).

**Drugs.** The drugs studied were RTI-113 (Carroll et al., 1995) and cocaine HCl (National Institute on Drug Abuse, Rockville, MD) and GBR 12909 (Research Biochemicals, Inc., Natick, MA). RTI-113 and cocaine were dissolved in 0.9% saline, and doses were determined in terms of the salt. GBR 12909 was dissolved in a small volume of 95% alcohol and emulphor and diluted with 0.9% saline.

**Results.**

**Stimulus Termination.** In experiments with an FI 300-s stimulus-termination schedule, responding during nondrug sessions was characteristic of typical performance maintained under FI schedules of reinforcement in squirrel monkeys. Response rate was low early in the interval and increased as the interval elapsed. All subjects terminated most stimuli, and electric stimulus presentation was infrequent. Dose-effect curves for RTI-113 (0.03–1.0 mg/kg), cocaine (0.03–3.0 mg/kg), and GBR 12909 (0.03–3.0 mg/kg) were compared in a group of three subjects (Fig. 1). There was no systematic change in response rate over the course of control sessions. Therefore, mean session rate was used to determine
drug effects on response rate. Mean ± S.E. response rate during saline control sessions (*n* > 10) was 0.51 ± 0.11 responses/s for the group. Intermediate doses of each drug produced significant increases in response rate, whereas the highest doses decreased response rate well below control values. RTI-113 was the least effective in increasing FI response rate, whereas cocaine and GBR 12909 were equally effective as behavioral stimulants. The order of potency for the three dopamine uptake inhibitors was RTI-113 ≥ cocaine > GBR 12909.

Drug-time course effects for doses of RTI-113 (0.1 mg/kg), cocaine (0.3 mg/kg), and GBR 12909 (1.0 mg/kg) that produced peak behavioral-stimulant effects were compared in a group of four subjects (Fig. 2). Mean ± S.E. response rate during saline control sessions (*n* > 15) was 0.44 ± 0.09 responses/s for the group. Each of the dopamine uptake inhibitors markedly increased response rate in all subjects during sessions comprising 20 consecutive FI 300-s components. However, cocaine had a shorter duration of action than RTI-113 and GBR 12909. Peak effects for all drugs occurred within 10 to 20 min after administration. Response rate returned to control levels within 40 min after cocaine administration, whereas response rate remained above control values for the duration of the session after RTI-113 or GBR 12909 administration. Behavior was typical of control performance when subjects were tested the day after drug administration.

**Drug Discrimination.** Subjects met the criterion to begin drug testing after 36 to 62 sessions of discrimination training. Once the training criterion was reached, performance during maintenance sessions was consistently maintained at >90% responding on the injection-associated lever in all subjects.

During test sessions, substitution with different doses of cocaine (0.03–0.3 mg/kg) in four subjects occasioned dose-dependent increases in cocaine-lever responding (Fig. 3). The training dose (0.3 mg/kg) occasioned >95% responding on the cocaine-associated lever, whereas saline administration occasioned responding almost exclusively on the saline-associated lever. Similarly, substitution within different doses of RTI-113 (0.03–0.3 mg/kg) also occasioned dose-dependent increases in cocaine-lever responding. The lowest dose of RTI-113 (0.03 mg/kg) failed to substitute for cocaine in any subject, whereas the two higher doses (0.1–0.3 mg/kg) substituted completely (>90% cocaine-lever responding) in all subjects. Although the highest dose of RTI-113 (0.3 mg/kg) had pronounced rate-decreasing effects in two of four subjects (Table 1), there was no significant main effect of dose on mean response rate in the group of four subjects.

**Drug Self-Administration.** When responding was maintained by i.v. self-administration of cocaine (0.1 mg/injection), rates and patterns of responding were characteristic of performance under second-order FI schedules with FR components as reported previously (Goldberg, 1973; Katz, 1980; Howell and Byrd, 1991, 1995). Characteristic FR rates and patterns were maintained during each FR component by the 2.0-s presentation of white light on the completion of each FR 20. A brief pause in responding after each presentation of white light was followed by an abrupt change to a steady, high rate of responding. Mean response rates during individual FR components typically were lower during the start of each 900-s interval and increased during subsequent FR components as the interval elapsed. The increase in mean response rates during successive FR components was due primarily to a decrease in the pause duration at the beginning of each FR component. Mean ± S.E. response rate during maintenance sessions (*n* > 50) was 0.46 ± 0.11 responses/s for the group of three monkeys.
When other doses of cocaine (0.03–1.0 mg/injection) were substituted for the 0.1-mg/injection maintenance dose for at least five consecutive sessions on two separate occasions, response rate was a direct function of cocaine dose (Fig. 4). Responding was not well maintained by the lowest dose (0.03 mg/injection), and performance was typically erratic throughout the session. The 0.1-mg/injection maintenance dose reliably maintained responding across sessions, and the 0.3-mg/injection dose maintained a higher rate compared with the maintenance dose. The highest dose (1.0 mg/injection) consistently disrupted performance during the latter components of a session, ostensibly resulting from an accumulation of drug. Hence, response rate increased as the unit dose of cocaine increased over a range of cocaine doses (0.03–0.3 mg/injection), whereas the highest dose (1.0 mg/injection) disrupted performance.

During saline substitution (extinction) sessions, responding was not well maintained, and performance was erratic throughout the session (Fig. 5). However, substitution with RTI-113 (0.1 mg/injection) reliably maintained self-administration behavior in all subjects. Although there was variability in response rate for individual subjects, mean rate of responding for the group was similar to that obtained with the maintenance dose of cocaine (0.1 mg/injection). A higher dose of RTI-113 (0.3 mg/injection) consistently disrupted performance during the latter components of a session, similar to the results obtained with the highest dose of cocaine (1.0 mg/injection).

When the maintenance dose of cocaine (0.1 mg/injection) was self-administered after pretreatment with several doses of RTI-113 (0.03–0.3 mg/kg), response rate increased at the two lower doses (Fig. 6, left). However, the latter effect was not statistically significant because of marked variability among individual subjects during drug-interaction experiments. In contrast, there was a significant decrease in responding for cocaine at the highest dose of RTI-113 (0.3 mg/kg). When the unit dose of cocaine was increased from 0.1 to 0.3 mg/injection, no dose of RTI-113 enhanced responding, and the highest dose of RTI-113 (0.3 mg/kg) significantly decreased responding for cocaine (Fig. 6, left).

To determine the specificity of effects of RTI-113 pretreatment on cocaine-maintained responding, a separate group of

![Drug Discrimination](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cocaine</th>
<th>RTI-113</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>0.03</td>
</tr>
<tr>
<td>86</td>
<td>2.15</td>
<td>1.51</td>
</tr>
<tr>
<td>89</td>
<td>3.76</td>
<td>3.03</td>
</tr>
<tr>
<td>118</td>
<td>3.73</td>
<td>2.96</td>
</tr>
<tr>
<td>134</td>
<td>2.34</td>
<td>2.88</td>
</tr>
<tr>
<td>Group, mean ± S.E.</td>
<td>3.00 ± 0.50</td>
<td>2.60 ± 0.42</td>
</tr>
</tbody>
</table>
three subjects was maintained under a second-order FI 900-s schedule of stimulus termination. Mean ± S.E. response rate during control sessions (n = 15) was 0.77 ± 0.06 responses/s for the group of three monkeys. RTI-113 had effects on responding maintained by the schedule of stimulus termination that were similar to those obtained during cocaine self-administration (Fig. 6, right). Response rate increased at the intermediate doses of RTI-113 (0.03 and 0.1 mg/kg), whereas there was a significant decrease in response rate at the highest dose (0.3 mg/kg).

Discussion

Direct comparisons between the effects of cocaine and RTI-113 on schedule-controlled operant behavior suggest that their behavioral effects were mediated through a common mechanism. Both drugs increased FI response rates at intermediate doses and decreased response rates at the highest doses studied. In monkeys trained to discriminate cocaine from saline, RTI-113 produced dose-related increases in cocaine-appropriate responding, with complete substitution for cocaine achieved at the highest doses of RTI-113. An extensive literature on drug discrimination indicates that drugs from the same pharmacological class typically cross-generalize to one another. Hence, the finding that RTI-113 has prominent cocaine-like discriminative-stimulus effects provides strong evidence that RTI-113 and cocaine have similar neurochemical effects in vivo. Finally, in monkeys trained to self-administer cocaine i.v., substitution with RTI-113 reliably maintained self-administration behavior in all subjects. The latter results provide clear evidence that RTI-113 has prominent reinforcing effects comparable with those of cocaine. Collectively, these findings demonstrate a similar pharmacological profile for cocaine and RTI-113 in nonhuman primates and are consistent with a common mechanism of action involving inhibition of dopamine uptake.

The findings of this are consistent with a growing literature implicating the dopamine transporter as a primary mechanism involved in the behavioral pharmacology and addictive properties of cocaine (Ritz et al., 1987; Kuhar et al., 1991). Various tropane derivatives with high affinity and selectivity for the dopamine transporter have reported cocaine-like behavioral effects in animal models. Several substituted analogs of cocaine have prominent psychomotor-stimulant effects on spontaneous activity in rodents (Cline et al., 1992; Hemby et al., 1995) and on schedule-controlled performance in nonhuman primates (Spealman et al., 1977, 1989). Moreover, these compounds demonstrate similar potency in behavioral tests and in transporter-binding assays (Spealman et al., 1989; Cline et al., 1992; Kuhar, 1993). Similarly, tropane derivatives selective for the dopamine transporter have been shown to have cocaine-like discriminative-stimulus effects (Spealman et al., 1991b; Weed et al., 1995; Nader et al., 1997) and to maintain drug self-administration (Bergman et al., 1989; Spealman et al., 1991a; Weed et al., 1995) in nonhuman primates. Cocaine-like behavioral effects also have been reported for selective dopamine uptake inhibitors with chemical structures distinct from the tropanes. As shown in this study and reported previously, the phenyl-substituted piperazine derivative GBR 12909 has

![Fig. 4. Mean rate of responding maintained by i.v. injection of cocaine (0.03–1.0 mg/injection) under a second-order FI 900-s (FR 20: S) schedule in a group of three monkeys (S-129, S-136, and S-140). Data for each dose of cocaine were derived from at least five consecutive sessions on two separate occasions. Error bars represent ±S.E. for the group and ±S.D. for individual subjects. Abscissae, dose, log scale. Ordinates, mean response rate expressed as responses per second.](image1)

![Fig. 5. Mean rate of responding maintained by i.v. injection of saline (extinction), cocaine (0.1 mg/injection), or RTI-113 (0.1 and 0.3 mg/injection) under a second-order FI 900-s (FR 20: S) schedule in a group of three monkeys (S-124, S-126, and S-138). Data for saline (SAL) and each dose of cocaine or RTI-113 were derived from at least five consecutive sessions on two separate occasions. *P < .05 versus cocaine. **P < .05 versus saline. Otherwise, as in Fig. 4.](image2)
psychomotor-stimulant effects (Spealman et al., 1989; Howell and Byrd, 1991; Howell et al., 1997), discriminative-stimulus effects (Kleven et al., 1990; Melia and Spealman, 1991), and reinforcing effects (Bergman et al., 1989; Howell and Byrd, 1991; Howell et al., 1997) similar to those of cocaine in nonhuman primates. Hence, there is a close relationship between inhibition of dopamine reuptake and cocaine-like behavioral effects induced by various drugs from distinct chemical classes. The relevance of the dopamine transporter in the behavioral pharmacology of cocaine is supported further by recent clinical studies. Neuroimaging studies in human cocaine users have found a significant correlation between dopamine transporter occupancy and the subjective high reported after administration of cocaine (Volkow et al., 1997) or methylphenidate (Volkow et al., 1999).

Given the apparent importance of the dopamine transporter in the addictive properties of cocaine, the development and use of compounds that target the dopamine transporter would seem to be a reasonable approach for the pharmacological treatment of cocaine abuse. The therapeutic approach of replacement or substitute agonist medication has been successful, as shown with methadone maintenance for heroin dependence and nicotine replacement for tobacco use. Preclinical studies with dopamine transporter inhibitors provide evidence that substitute agonists may also be used effectively to reduce cocaine use. Note that pretreatment with GBR 12909 decreased cocaine self-administration in rhesus monkeys (Skjoldager et al., 1993; Glowa et al., 1995) and attenuated cocaine-induced increases in extracellular dopamine in rats (Rothman et al., 1991; Baumann et al., 1994). Similarly, the tropane analog PTT decreased cocaine self-administration in rhesus monkeys (Nader et al., 1997), and RTI-113 decreased cocaine self-administration in rats (Dworkin et al., 1998). In this study, RTI-113 exhibited desirable properties for a substitute agonist medication. It had a profile of behavioral effects that was similar to cocaine, but it had a longer duration of action and less pronounced psychomotor-stimulant effects. Although the relative positioning of the dose-effect curves indicated that RTI-113 was approximately 3-fold more potent than cocaine and 10 times more potent than GBR 12909 as a behavioral stimulant, cocaine’s shorter duration of action in conjunction with the cumulative dosing procedure may have underestimated the potency of cocaine relative to RTI-113 and GBR 12909. At the highest dose tested, RTI-113 also effectively decreased cocaine self-administration, and it maintained its effectiveness when the unit dose of cocaine was increased from 0.1 to 0.3 mg/injection. However, the same dose of RTI-113 caused a general disruption of operant behavior maintained by a comparable schedule of stimulus termination, thereby demonstrating that behaviorally active doses of RTI-113 were required to decrease cocaine-maintained behavior. It is unclear why RTI-113 had selective effects on cocaine-maintained behavior in the rat (Dworkin et al., 1998) but not in the squirrel monkey. A possible explanation is the use of a complex, second-order schedule of limited drug access in our study, which may have engendered behavioral performances that are more resistant to drug pretreatment effects. Hence, behavior maintained by the second-order FI schedule of drug self-administration may provide a more conservative assessment of medication effectiveness than simple FR schedules of unlimited drug delivery.

A possible limitation to the use of selective dopamine transporter inhibitors as medications for cocaine addiction is their potential for abuse liability given their demonstrated reinforcing effects in animal models. In this study, RTI-113 was reliably self-administered by nonhuman primates, and it maintained rates of responding comparable with those maintained by cocaine. Similarly, previous reports have demonstrated that GBR 12909 can maintain rates of i.v. self-administration comparable with those of cocaine in nonhuman primates (Dworkin et al., 1998; Howell et al., 1997). However, both RTI-113 and GBR 12909 have a fairly rapid onset of action that cannot be distinguished from cocaine. Structural modifications that limit absorption and entry into the brain, resulting in slower onset and longer duration of action, could effectively reduce the abuse potential of candidate compounds. For example, the selective dopamine uptake inhibitor PTT has a slower onset and much longer duration of action than cocaine (Hemby et al., 1995; Nader et al., 1997), and it reliably decreases cocaine self-administration without maintaining self-administration behavior under FI schedules in nonhuman primates (Nader et al., 1997). Although PTT maintains response rates significantly higher than those maintained by vehicle under FR schedules, response rates are significantly lower than those maintained by cocaine (Birmingham et al., 1998). In addition to pharmacokinetic considerations, an alternative approach may be the development of mixed-action medications that target both dopamine and serotonin transporters. In vitro studies have demonstrated that cocaine blocks the reuptake of dopamine and serotonin (Heikkila and Manzino, 1984; Reith et al., 1986), and there is a negative relationship between the potencies of several cocaine analogs in self-administration studies and their binding potencies to serotonin.

Fig. 6. Left, mean ± S.E. rate of responding maintained by i.v. injection of cocaine (0.1 and 0.3 mg/injection) after pretreatment with RTI-113 (0.03–0.3 mg/kg) in a group of three monkeys (S-129, S-136, and S-140). Right, mean ± S.E. rate of responding maintained by a second-order FI 900-s (FR 20: S) schedule of stimulus termination after pretreatment with RTI-113 (0.03–0.3 mg/kg) in a group of three monkeys (S-87, S-93, and S-98). Subjects were pretreated with each dose of RTI-113 for three consecutive sessions, and each subject received all drug combinations on two separate occasions. Abscissae, dose, log scale. Ordinates, mean response rate expressed as responses per second. *P < .05, significant effect of RTI-113 pretreatment.
uptake sites (Ritz et al., 1987). Moreover, studies in nonhuman primates demonstrate that selective serotonin uptake inhibitors can attenuate the behavioral-stimulant and reinforcing effects of cocaine and related psychomotor stimulants (Kleven and Woolverton, 1993; Howell and Byrd, 1995; Czoty et al., 1997) with no evidence of abuse liability (Howell and Byrd, 1995).

In summary, RTI-113 and cocaine had a similar profile of behavioral effects on several measures of operant behavior including schedule-controlled performance, drug discrimination, and i.v. drug self-administration. The results support a growing literature implicating the dopamine transporter as a primary mechanism involved in the addictive properties of cocaine. Moreover, RTI-113 had a longer duration of action and less pronounced behavioral-stimulant effects than cocaine, and pretreatment with the highest dose of RTI-113 decreased cocaine self-administration. The latter pharmacological effects are desirable properties for a substitute pharmacotherapy for cocaine abuse. However, RTI-113 had similar effects on cocaine self-administration behavior and on behavioral performances maintained by a second-order schedule of stimulus termination. Hence, behaviorally active doses of RTI-113 were required to decrease cocaine-maintained behavior. RTI-113 also reliably maintained self-administration behavior, demonstrating the potential for abuse liability.

Acknowledgments

We gratefully acknowledge the technical assistance of J.E. Majors, A.M. McDonough, C.M. Webb, and P.M. Plant.

References


Send reprint requests to: Dr. Leonard L. Howell, Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322. E-mail: leonard@rmy.emory.edu