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BRIEF COMMUNICATION

Reinforcing and Discriminative Stimulus Effects of β-CIT in Rhesus Monkeys

M. R. WEED,* A. S. MACKEVICIUS,* J. KEBABIAN† AND W. L. WOOLVERTON*‡[1]

*University of Chicago, Department of Pharmacological and Physiological Sciences, 947 E. 58th Street, Chicago, IL, 60637
†Research Biochemicals International, Inc., Natick, MA 01760
‡Departments of Psychiatry and Human Behavior, Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS 39216

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WEED, M. R., A. S. MACKEVICIUS, J. KEBABIAN AND W. L. WOOLVERTON. *Reinforcing and discriminative stimulus effects of β-CIT in rhesus monkeys.* PHARMACOL BIOCHEM BEHAV 51(4) 953-956, 1995.—β-CIT (also designated RTI-55) is one of a series of 2β-carbomethoxy-3β-phenyltropane cocaine analogues that have recently been synthesized for characterizing the dopamine transporter and its function. The present study was designed to examine the behavioral effects of β-CIT in rhesus monkeys. Two monkeys were allowed to self-administer cocaine (0.01 or 0.03 mg/kg/inj, IV, fixed-ratio 10, 1 h/day) in baseline sessions. When behavior was stable, β-CIT (0.0007-0.003 mg/kg/inj, IV) was made available for self-administration for several consecutive sessions. β-CIT maintained responding above saline levels in both monkeys. Two other monkeys were trained to discriminate cocaine (0.2 or 0.4 mg/kg, IM) from saline in a two-lever, food-reinforced drug discrimination paradigm. β-CIT (0.012-0.025 mg/kg, IV) fully substituted for cocaine as a discriminative stimulus. In both preparations, β-CIT was at least eightfold more potent than cocaine and had a longer duration of action. Thus, β-CIT has cocaine-like behavioral effects indicative of a functional interaction with the dopamine transporter.

Cocaine Cocaine analogues β-CIT Dopamine transporter Self-administration Drug discrimination Rhesus monkey

β-CIT (also designated RTI-55) is one of a series of 2β-carbomethoxy-3β-phenyltropane cocaine analogues that have recently been synthesized for characterizing the dopamine (DA) and serotonin (5-HT) transporters and their functions (3,11). β-CIT has a high affinity for the DA transporter and, because it lacks the ester linkage of cocaine, β-CIT is not hydrolyzed by the serum esterases that rapidly hydrolyze cocaine. From these properties of DA and 5-HT uptake blockade and slow metabolism, one would predict a behavioral pharmacology similar to cocaine but with a longer duration of action. The behavioral studies that have been done with phenyltropanes suggest that members of this chemical class have cocaine-like effects. For instance, phenyltropanes have been reported to increase locomotor activity in mice (2,4), and several phenyltropanes, including β-CIT, have cocaine-like discriminative stimulus (DS) effects in rats (2,5). It has also been demonstrated that the behavioral potency of these phenyltropanes can be predicted by their potency as a DA uptake blocker (2,5). To characterize further the behavioral effects of β-CIT, the present study examined its reinforcing and DS effects in rhesus monkeys.

METHOD

Reinforcing Effects

The subjects were two rhesus monkeys (*Macaca mulatta*), one male (8612, 8.6 kg) and one female (9065, 4.6 kg), with a history of self-administration of various dopaminergic agents

[1] Requests for reprints should be addressed to William L. Woolverton, Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, 2500 N. State St. Jackson, MS 39216.

in the present self-administration paradigm. The method has been described in detail previously (14). Briefly, subjects were prepared with chronic indwelling IV catheters and housed continuously in self-administration chambers. Each day, injections of cocaine (0.01 mg/kg/inj for 8612 or 0.03 mg/kg/inj for 9065) were available for 1 h contingent upon lever pressing under a fixed ratio 10 (FR 10) schedule of reinforcement. When cocaine-maintained responding was stable (total injections ± 15% of the mean of three consecutive sessions, with no consistent increasing or decreasing trends), saline was substituted for cocaine until responding declined to low levels. Subsequently, responding was reestablished with cocaine and doses of β-CIT were made available for at least the same number of sessions that were required for responding to decline when saline was available and until the number of injections/session was stable. Cocaine-maintained behavior was reestablished between doses of β-CIT. A given dose of β-CIT was said to function as a positive reinforcer if the mean number of injections over the last three sessions of availability was above the corresponding mean for saline and the ranges did not overlap.

Discriminative Stimulus Effects

The subjects (8405 and 8409) were male rhesus monkeys, maintained at approximately 8.0 kg (90% of their free-feeding body weights). Both monkeys had a history of intravenous drug self-administration and cocaine discrimination in the present paradigm. The method has been described in detail previously (9). Briefly, the monkeys were seated in a primate restraint chair, injected IM with either cocaine (0.2 or 0.4 mg/kg) or 0.9% saline, and placed in a darkened experimental chamber that had two response levers. Ten minutes later, a house light and lights above both levers were illuminated and responding on the lever associated with the presession injection resulted in food delivery under a FR 30 schedule of reinforcement. Responding on the other lever did not deliver food but reset the response requirement on the correct lever. When the discrimination was stable (at least 80% injection-appropriate responses before the first reinforcer and 90% or more injection-appropriate responses over the entire session for at least seven of eight consecutive sessions), test sessions (T) were conducted according to the daily sequence (S = saline, D = drug pretreatment: STDSTDTSDT) as long as performance in the intervening training sessions remained at or above the criteria for stimulus control. If a monkey's performance fell below criteria during the intervening training sessions, tests were discontinued and training resumed until the discrimination was again at or above the criteria. Test sessions were identical to training sessions except that: a) different doses of either cocaine or β-CIT were injected IV into the sapphenous vein with pretreatment times that varied from 10 min to 8 h; b) 30 consecutive responses on either lever resulted in food delivery. When pretreatment times were longer than 1 h, monkeys were returned to their home cages after the IV injection, and were replaced in the chair 10 min prior to the start of the test session.

In both monkeys, the IV dose-response function for cocaine with a pretreatment time of 10 min was determined first. The IV dose-response function for β-CIT (0.003–0.4 mg/kg) was then determined with a 10-min pretreatment. Next, the lowest dose of β-CIT that engendered 100% cocaine-appropriate responding was administered at various pretreatment times ranging from 10 min to 8 h. Finally, the effects of cocaine were determined at 30- and 60-min pretreatment times. Generally, doses were tested once and in a nonsystematic order.

The percentage of the total responses that occurred on the drug-appropriate lever and the response rate on both levers during test sessions were calculated for each monkey. The magnitude of shifts in dose-response functions was estimated from the visibly linear portion of the dose-response functions.

Drugs

Cocaine HCl (National Institute on Drug Abuse, Rockville, MD) and β-CIT [2β-carbomethoxy-3β-(4-iodophenyl) tropane D-tartrate: Research Biochemicals Incorporated, Natick, MA] were dissolved in 0.9% saline. For self-administration, concentrations were appropriate to a 10-s injection of approximately 1.0 ml. For drug discrimination, drugs were prepared in a volume of 1.0 ml/10 kg. Drug doses refer to the salt.

RESULTS

Reinforcing Effects

The baseline doses of cocaine maintained 64.7 inj/h (range 60–71) and 36.7 (range 32–40) for monkeys 8612 and 9065, respectively (Fig. 1). Responding was evenly distributed over the session (data not shown). When saline was substituted for cocaine (Sal, Fig. 1), responding declined to low levels over six to seven sessions and was concentrated in the early segments of the session. β-CIT maintained responding above saline levels at two doses in both monkeys. Responding maintained by β-CIT tended to be concentrated in the early parts of the session. The dose-response function of β-CIT was at least one log unit to the left of cocaine's dose-response function, indicating that β-CIT was over 10-fold more potent than cocaine in this preparation.

Discriminative Stimulus Effects

In training sessions, cocaine engendered 99–100% cocaine-appropriate responding whereas saline engendered less than 10% cocaine-appropriate responding. When cocaine was administered IV 10 min before test sessions, there was a dose-

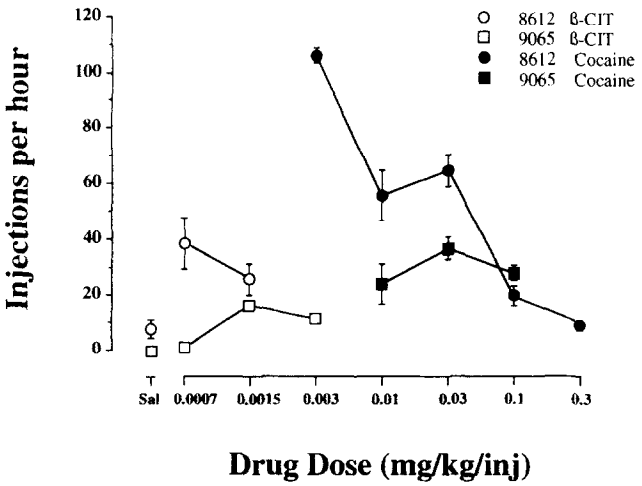

FIG. 1. Reinforcing effects of β-CIT in rhesus monkeys (8612 and 9065). Each point is the mean of the last three sessions of availability of β-CIT and vertical bars are the range. Points above Sal represent self-administration of 0.9% saline.

related increase in cocaine-appropriate responding that reached 100% at the training dose in both monkeys (Fig. 2, open circles). When the pretreatment time for cocaine was increased, cocaine-appropriate responding decreased to less than 20% at 1 h (8405) or 30 min (8409). Full drug lever responding was restored at these time points by increasing the cocaine dose to 0.2 mg/kg in 8405 and 0.4 mg/kg in 8409. β-CIT administered 10 min presession also engendered a dose-related increase in cocaine-appropriate responding in both monkeys, reaching 99–100% cocaine-appropriate responding at 0.012 mg/kg (8405) and 0.025 mg/kg (8409; Fig. 2, open squares). When pretreatment times for these doses of β-CIT were increased, cocaine-appropriate responding remained at 100% at 4 h and decreased to less than 20% at 8 h for both monkeys. Full drug lever responding was restored at 8 h by increasing β-CIT dose to 0.05 mg/kg (Fig. 2, closed squares). β-CIT appeared to be approximately eightfold more potent than cocaine in producing cocaine-like DS effects. Neither cocaine nor β-CIT affected rate of responding at any pretreatment time at the doses tested (data not shown).

DISCUSSION

The results of the present experiment confirm and extend previous findings of the behavioral effects of β-CIT. As has been found in rats (2,5), β-CIT had cocaine-like DS effects in rhesus monkeys. Moreover, β-CIT functioned as a positive reinforcer in rhesus monkeys experienced in the self-administration of cocaine. These results are consistent with previous reports that the fluorinated analogue CFT had cocaine-like DS effects and could function as a positive reinforcer in squirrel monkeys (12,13). Thus, these phenyltropanes have cocaine-like behavioral effects that are consistent with abuse liability. β-CIT was at least eightfold more potent than cocaine as both a DS and a positive reinforcer, a result that is consistent with its increased potency at the DA uptake carrier relative to cocaine (2,3). It seems likely, therefore, that binding to the DA transporter and blockade of DA uptake is the mechanism that underlies these behavioral effects of β-CIT. Additionally, β-CIT was longer acting than cocaine. The pattern of self-administration, with rate of injection above the rate maintained by saline and with responding concentrated early in the session, is typically seen with a reinforcing drug that has a long duration of action. The rapid accumulation of long-acting drugs is consistent with the comparitively low rates of responding seen with β-CIT. In the drug discrimination paradigm, increasing the pretreatment time to 30 or 60 min shifted the dose–response function for the DS effect of cocaine approximately twofold to the left whereas a comparable shift was not seen with β-CIT until the pretreatment time was increased to 8 h. Furthermore, the pattern of responding maintained by β-CIT in the self-administration paradigm (i.e., injections concentrated early in the session) is consistent with a long duration of action and a suppression of responding later in the session. Both of these results are consistent with an extended half-life of β-CIT resulting from the absence of the ester linkage found in cocaine.

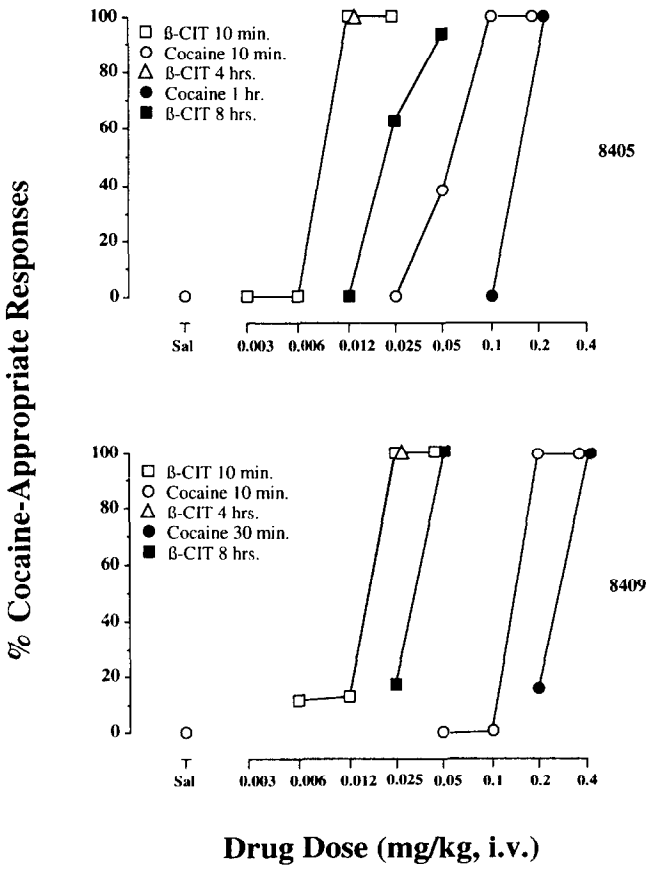

FIG. 2. Discriminative stimulus effects of cocaine and β-CIT in rhesus monkeys (8405 and 8409). Open symbols: 10-min pretreatment time; closed symbols: pretreatment times noted on graph. Each point represents a single determination of the effect of that dose. Points above Sal represent test sessions with saline injections. The training dose of cocaine was 0.2 mg/kg in 8405 and 0.4 mg/kg in 8409.

β-CIT can be prepared with ^{123}I for SPECT imaging (1,6, 7,11) and its *N*-methyl group can be prepared for ^{11}C labeling in PET studies (10). As such, labeled β-CIT has proven useful for the noninvasive study of DA neurons in humans (7) and baboons (6). As in the present study, SPECT β-CIT has a relatively long duration of action (t_12 = 27 h) (6). Such an imaging agent might be applied to the diagnosis of DA neuron dysfunction [e.g., Parkinson's disease (8)], as well as to the study of the neurobiology of drug abuse.

It has been suggested that a long-acting cocaine substitute might aid in the treatment of cocaine abuse in the same way that methadone is used in the treatment of opioid abuse. β-CIT or a similar compound might prove useful in this regard. Despite their increase in potency at the dopamine uptake site, phenyltropane analogues had a fourfold lower LD_{50} than cocaine in rodents (4). Obviously, low toxicity is important in any human usage, whether as an imaging ligand or as a therapeutic agent. As a treatment for cocaine abuse, the positive reinforcing effects and long duration of action of β-CIT may encourage patient compliance. On the other hand, cocaine-like reinforcing and DS effects might also reinstate cocaine use in abstinent individuals. Therefore, additional research may be necessary to further characterize the abuse liability of β-CIT or similar compounds.

ACKNOWLEDGEMENTS

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