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N-Modified Fluorophenyltropane Analogs of Cocaine With High Affinity for Cocaine Receptors[1]

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MADRAS, B. K., J. B. KAMIEN, M. A. FAHEY, D. R. CANFIELD, R. A. MILIUS, J. K. SAHA, J. L. NEUMEYER AND R. D. SPEALMAN. *N-Modified fluorophenyltropane analogs of cocaine with high affinity for cocaine receptors.* PHARMACOL BIOCHEM BEHAV **35**(4) 949–953, 1990.—The binding properties of three N-modified fluorophenyltropane analogs of cocaine were compared in competition experiments with [^{3}H]cocaine. All three analogs displaced specifically bound [^{3}H]cocaine from caudate-putamen membranes of cynomolgus monkeys with affinities exceeding that of cocaine. The compound with the highest affinity, 2β-carbomethoxy-3β-(4-fluorophenyl)-N-allyl-nortropane, (N-allyl-CFNT) was about three times more potent than cocaine. N-Allyl-CFNT also had cocaine-like interoceptive effects and was about three times more potent than cocaine in squirrel monkeys trained to discriminate cocaine from vehicle in an operant drug discrimination procedure. The results suggest that N-modified fluorophenyltropane derivatives may be useful precursors for development of pharmacological probes for cocaine receptors.

Cocaine Cocaine analogs [^{3}H]Cocaine binding Drug discrimination Monkeys

SPECIFIC binding sites for cocaine have been identified in brain tissue of rodents, humans, and nonhuman primates (13, 20, 23). These binding sites have several properties characteristic of biologically relevant receptors. First, they bind cocaine saturably at concentrations similar to those found in brain after peripheral cocaine administration (13,19). Second, they are stereoselective for (−)-cocaine over its unnatural enantiomer (+)-cocaine or its diastereoisomer pseudococaine (13). Third, the affinities of cocaine and related drugs at cocaine binding sites parallel their potencies for inhibiting dopamine uptake (4, 12, 13, 19), a mechanism widely implicated in the behavioral effects of cocaine. Lastly, and perhaps most importantly, there is a high degree of correspondence between the affinities of cocaine and its congeners at cocaine binding sites (13) and their potencies for producing cocaine-like behavioral effects including intravenous self-administration, psychomotor stimulation, and drug discrimination (2, 16, 26). Together, these findings support the view that specific cocaine receptors associated with the dopamine transport system are principal neurochemical mediators of the behavioral effects and abuse of cocaine.

Further molecular characterization of cocaine receptors clearly is warranted, but has been hampered by the relative inefficiency of available pharmacological probes. In this regard, [^{3}H]cocaine binds with only modest affinity in all brain regions yet studied and dissociates rapidly from the receptor complex (4,21). Other radioligands such as [^{3}H]mazindol (11,22) or [^{3}H]GBR 12935 (1,10) are not fully satisfactory substitutes for [^{3}H]cocaine, as binding sites for these ligands are not identical to those labeled by cocaine itself (6,7).

Identification of high-affinity ligands with full cocaine-like biochemical and behavioral properties would accelerate development of efficient pharmacological probes for cocaine receptors. Previous studies have described various structural modifications of the cocaine molecule, but only a few of these analogs have potencies comparable to or exceeding that of cocaine. One such compound, 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane (CFT, also designated WIN 35,428) was originally synthesized by Clarke *et al.* (5) and more recently by Milius *et al.* (17). CFT appears to have a full spectrum of cocaine-like effects both in vitro and in vivo (8, 13, 19, 25) with the exception of weak local anesthetic activity (5). As part of our program to develop high-affinity ligands for cocaine receptors we synthesized three N-modified

[1]Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and of the "Guide for Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare, Publication No. (NIH)85-23, revised 1985. A preliminary report appeared in FASEB J. 3:A296; 1989.

derivatives of CFT (see insets in Fig. 1) and compared their binding at cocaine receptors in monkey caudate-putamen. The synthesis and chemical characterization of these fluorophenyltropane analogs as well as [^{3}H]CFT is described elsewhere (17). The most potent of these drugs 2β-carbomethoxy-3β-(4-fluorophenyl)-N-allyl-nortropane (N-allyl-CFNT) was evaluated for cocaine-like interoceptive effects in monkeys trained to discriminate cocaine from vehicle.

METHOD

[^{3}H]Cocaine Binding

Brain tissue of adult male and female cynomolgus monkeys *(Macaca fascicularis)* was obtained from the brain bank of the New England Regional Primate Research Center (14). The caudate-putamen was dissected from coronal slices and homogenized in 10 volumes (w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4 at 4°C). The homogenate was then centrifuged at 38,700 × g for 20 min at 0–4°C, and the pellet was resuspended in 40 volumes of buffer. The wash procedure was repeated twice. After washing, the membrane suspension (25 mg original wet weight of tissue/ml) was stored in small aliquots at −85°C until use, generally within two weeks. Immediately before assay, the suspension was thawed, diluted to 12 mg/ml in buffer, and dispersed with a polytron for 15 sec.

The [^{3}H]cocaine binding assay used in the present experiment is identical to the one described by Madras *et al.* (13). [^{3}H]Cocaine (28.5 Ci/mmole; DuPont-New England Nuclear Corp., Boston, MA) was stored at −20°C and diluted in buffer immediately prior to assay. Stock solutions of drugs were made in distilled water containing ethanol (≤20 ml/100 ml) and HCl (≤0.01 M), if required. The stock solutions were diluted serially in Tris-HCl buffer to yield ten or more concentrations, each of which was studied in triplicate in 3 or 4 individual brains.

Glass tubes (12 × 75 mm) received in the following order: 0.2 ml Tris-HCl buffer (50 mM) containing NaCl and test drug, 0.2 ml [^{3}H]cocaine (2.7 nM), and 0.2 ml tissue suspension (12 mg/ml original wet tissue weight). The final concentration of tissue was 4 mg/ml and the final concentration of NaCl was 100 mM in a total volume of 0.6 ml. Tubes were incubated for 60 min at 0–4°C. Incubation was terminated by rapid filtration over glass fiber filters presoaked in 0.1% ice-cold bovine serum albumin. The filters were washed twice with 5 ml of Tris-HCl buffer at 4°C under reduced pressure (Brandel cell harvester), and were stored overnight at room temperature in vials containing 4 ml of liquid scintillation fluor.

Radioactivity was monitored for 5 min by liquid scintillation spectrometry, and cpm were converted to dpm following determination of the counting efficiency (45–50%) of each vial by external standardization. Nonspecific binding was defined as dpm in the presence of an excess concentration (30 μM) of unlabeled (−)-cocaine which binds to both high- and low-affinity binding components (13); specific binding was defined as the difference between total and nonspecific binding. In the present study, total binding ranged from 500–1,000 dpm, and specific binding was approximately 85% of the total.

Data were analyzed by the EBDA (Equilibrium Binding Data Analysis; Elsevier-Biosoft, U.K.) software program, which calculated IC_{50} values and pseudo-Hill coefficients (nH).

Cocaine Discrimination

Three adult male squirrel monkeys *(Saimiri sciureus)* were maintained at approximately 85% of their free-feeding body weights. Each monkey was surgically prepared with a chronic venous catheter following the general procedure of Herd *et al.* (9). Under halothane anesthesia and aseptic conditions, one end of a polyvinyl chloride catheter was passed by way of a jugular or femoral vein to the level of the right atrium. The distal end of the catheter was passed SC and exited through the skin at the mid-scapular region. Monkeys wore nylon mesh jackets at all times to protect the catheters.

During experimental sessions, monkeys were seated in a Plexiglas chair (24), which was placed in a ventilated, sound-attenuating chamber. The chair was equipped with two response levers, a food pellet dispenser and lights to provide overall illumination. Venous catheters were connected by polyvinyl chloride tubing to syringes located outside the chamber so that drugs could be injected with minimal disturbance to the monkey.

Each monkey was trained to respond differentially on the left and right levers depending on whether cocaine or saline was injected. After injection of cocaine, 10 consecutive responses (FR 10) on one lever (left for monkeys S-94 and S-496; right for monkey S-329) produced food, whereas after an injection of saline, 10 consecutive responses on the other lever produced food. Responses on the inappropriate lever (e.g., the saline-associated lever when cocaine was injected) reset the FR requirement. Training sessions consisted of a variable number (n = 1–4) of components of the FR schedule. Each component ended after the completion of 10 FRs or after 10 min, whichever occurred first, and each was preceded by a 10-min timeout period, during which the lights were off and responses had no programmed consequences. During most training sessions, saline was injected during timeout periods preceding the first n-1 components and cocaine was injected before the nth component of the session. During some sessions, however, saline was injected before all four components to prevent an invariant association between the last component and cocaine. Training continued until a criterion of at least 80% of responses were made on the injection-appropriate lever for 4 out of 5 consecutive sessions (total training: 64–117 sessions for the different monkeys). The training dose of cocaine initially was 0.3 μmol/kg for each monkey, but subsequently was increased to 0.9 μmol/kg for monkey S-329 to achieve criterion-level performance. Due to a dislodged catheter, the route of administration was changed from IV to IM for monkey S-496; all of the data reported in the present study is from the latter route.

Drug test sessions were conducted once or twice per week, providing that performance during the five preceding training sessions met criterion. Test sessions consisted of four components, during which 10 consecutive responses on either lever produced food. Drugs were studied using the cumulative dosing procedure described previously (24). Briefly, incremental doses of test drugs were injected during the timeout periods preceding sequential components, permitting a complete cumulative dose-response curve to be determined in a single session. Saline served as control injections.

RESULTS

[^{3}H]Cocaine Binding

All three N-modified fluorophenyltropane analogs displaced [^{3}H]cocaine in a concentration-dependent manner (Fig. 1). The competition curves for these drugs as well as cocaine were characterized by relatively shallow slopes, with pseudo-Hill coefficients ranging from 0.64–0.70. Although cocaine itself displaced [^{3}H]cocaine fully, plateaus corresponding to 85 to 90% displacement were apparent in the competition curves for the three fluorophenyltropane derivatives.

Comparison of the IC_{50} values and the competition curves (Fig. 1) shows that the three fluorophenyltropane derivatives had greater

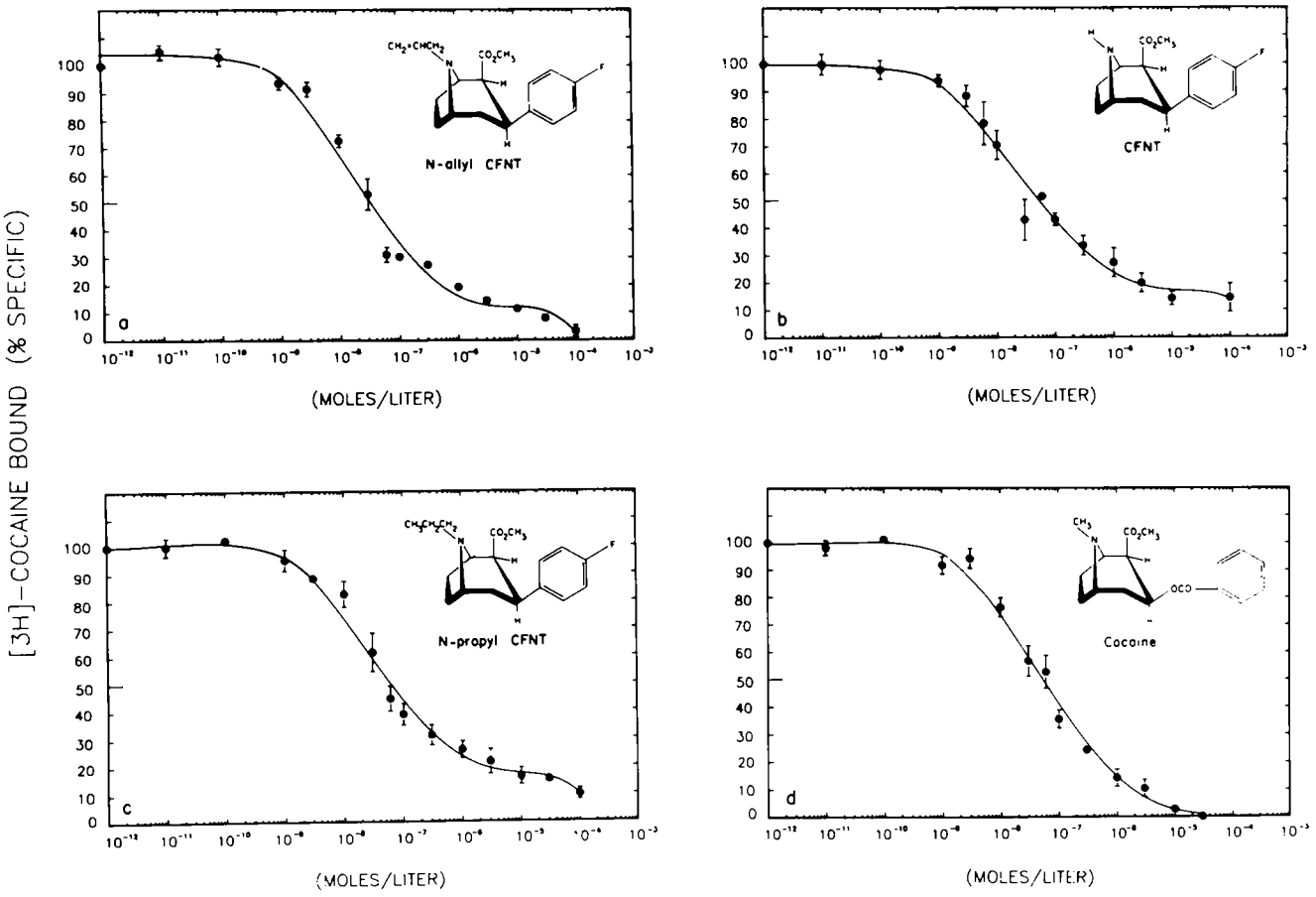

FIG. 1. Displacement of [^{3}H]cocaine by N-modified fluorophenyltropane analogs of cocaine (a, b, c) and by cocaine (d). Abscissae: drug concentration, log scale; ordinates: percentage of specifically bound [^{3}H]cocaine. Nonspecific binding was measured with 30 μM (−)cocaine. Data are means ± S.E.M. from 3 or 4 independent experiments performed in triplicate.

affinity for [^{3}H]cocaine binding sites than did cocaine itself. The compound with the highest affinity, N-allyl-CFNT, (IC_{50}: 22.6 ± 2.9 nM, S.E.M.) was three times more potent than cocaine (IC_{50}: 67.8 ± 8.7 nM) in displacing specifically bound [^{3}H]cocaine. The affinities of CFNT (IC_{50}: 36.4 ± 1.5 mM) and N-propyl CFNT (IC_{50}: 43.0 ± 17.7 nM) were higher than that of cocaine but lower than that of N-allyl CFNT.

Cocaine Discrimination

During training sessions that preceded drug test sessions, individual monkeys made an average of 95–100% of responses on the injection-appropriate lever. Overall response rates averaged 1.2–2.8 responses per sec for individual monkeys after injection of saline and 0.6–3.1 responses per sec after injection of the training dose of cocaine.

During test sessions, cocaine produced dose-related increases in percentage of responses on the cocaine-associated lever, with 98–100% cocaine-appropriate responding observed after 0.9 μmol/kg in all three monkeys (Fig. 2, open circles). N-Allyl-CFNT also produced dose-related increases in the percentage of responses on the cocaine-associated lever (Fig. 2, filled circles). For all monkeys, 99–100% cocaine-appropriate responding was observed after 0.3 μmol/kg N-allyl-CFNT.

Some reductions in response rate were evident after high doses of either drug (data not shown). Except after the highest

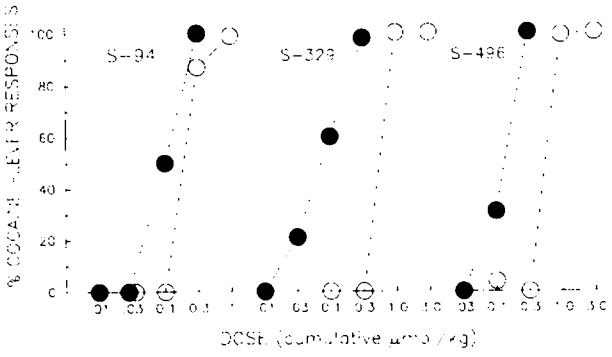

FIG. 2. Effects of cocaine (open circles) and N-allyl-CFNT (filled circles) in individual monkeys trained to discriminate cocaine from saline. Abscissa: cumulative dose, log scale; ordinate: percentage of responses on the cocaine-associated lever. Saline injections under test conditions occasioned an average of 8% cocaine-appropriate responding in monkey S-94 and 0% in monkeys S-327 and S-496. The training dose was 0.3 μmol/kg IV for monkey S-94, 0.9 μmol/kg IV for monkey S-329, and 0.3 μmol/kg IM for monkey S-496. ●: N-allyl-CFNT; ○: cocaine.

doses of cocaine in monkey S-496, however, each monkey completed all 10 possible FRs in each component of the test session.

DISCUSSION

Previous studies have shown that the cocaine analog CFT (WIN 35,428) has pronounced psychomotor-stimulant effects in laboratory animals (25), inhibits uptake of monoamine neurotransmitters (8), and displaces [^{3}H]cocaine from binding sites in brain tissue (13,19). These findings prompted us to synthesize several N-modified derivatives of CFT as potential high-affinity ligands for cocaine receptors. All three N-modified derivatives displaced specifically bound [^{3}H]cocaine with potencies exceeding that of cocaine itself. The shallow competition curves and low pseudo-Hill coefficients for the drugs are similar to those seen with cocaine. Such findings may be indicative of multiple binding components, as has been suggested previously for cocaine (3,13). It should be noted, however, that multiple binding components for cocaine have not always been reported (19,20), possibly reflecting differences in assay conditions.

In our previous study involving [^{3}H]cocaine binding, distinctive plateaus were observed in the competition curves for several phenyltropane analogs of cocaine, including CFT (13). Likewise, in the present study, plateaus corresponding to 85–90% displacement of [^{3}H]cocaine were observed for the three N-modified derivatives of CFT. Based on the apparent structural requirements for full displacement of [^{3}H]cocaine, the phenyltropane-inaccessible sites may represent a recognition site for the ester group and possibly be associated with local anesthetic activity [cf. (13)]. In this regard, CFT and other phenyltropane analogs are weak local anesthetics (5) and, unlike cocaine, have low affinity for sodium channel binding sites labeled with [^{3}H]batrachotoxinin (18). It is unlikely, however, that these sites are crucial for expression of cocaine-like behavioral activity as the discriminative-stimulus effects of cocaine were fully mimicked by N-allyl-CFNT.

The potency of N-allyl-CFNT was greater than that of cocaine in both the [^{3}H]cocaine binding assay and the cocaine-discrimination study and compares favorably with that of CFT, the most potent cocaine analog yet identified. Recently, we have shown that a tritiated form of CFT can be used to label cocaine receptors in vitro and, by several criteria, is superior to [^{3}H]cocaine as a pharmacological probe (15). Based on its high in vitro and in vivo potency N-allyl-CFNT also may be a suitable candidate for development as a radioligand for cocaine receptors. Moreover, because of its hydrogen-rich substituent, N-allyl-CFNT (as well as N-propyl-CFNT) may be particularly amenable to multiple tritiation as a means of increasing specific activity.

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