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ACCELERATED COMMUNICATION

Cocaine Receptors Labeled by [^{3}H]2β-Carbomethoxy-3β-(4-fluorophenyl)tropane

BERTHA K. MADRAS, ROGER D. SPEALMAN, MICHELE A. FAHEY, JOHN L. NEUMEYER, JAYNAKA K. SAHA, and RICHARD A. MILIUS

Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts 01772 (B.K.M., R.D.S., M.A.F.), and Research Biochemicals, Natick, Massachusetts 01760 (J.L.N., J.K.S., R.A.M.)

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SUMMARY

The potent cocaine analog 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane (CFT, also designated WIN 35,428) was tritiated and evaluated as a molecular probe for cocaine receptors in caudate putamen membranes of cynomologus monkeys. Kinetic, saturation, and competition experiments indicated that [^{3}H]CFT, like [^{3}H]cocaine, bound to at least two components. Association and dissociation of the radioligand at 0–4° occurred in two phases; the $t_{1/2}$ for dissociation of the fast and slow components was 2.5 and 23 min, respectively. Saturation analysis revealed high and low affinity binding components with affinities (K_d) of 4.7 ± 1.2 and 60 ± 12 nM (means ± SE) and densities (B_{max}) of 50 ± 18 and 290 ± 20 pmol/g of tissue, respectively. [^{3}H]CFT was displaced stereoselectively by the enantiomers of cocaine and by the diastereoisomers of the phenyltropane analog of cocaine. Most congeners displaced [^{3}H]CFT fully, with shallow competition curves (n_H, 0.69–0.81). In contrast, several monoamine uptake inhibitors structurally unrelated to cocaine (GBR 12909, Lu 19-005, and mazindol) displaced a maximum of about 90% specifically bound [^{3}H]CFT, with steeper competition curves (n_H, 0.89–1.3), suggesting that these drugs bind to a subpopulation of [^{3}H]CFT-labeled sites. The rank order of potency observed in the present study is identical to the rank order of potency at binding sites labeled by [^{3}H]cocaine: Lu 19-005 > mazindol > CFT > GBR 12909 > (−)-cocaine > bupropion > WIN 35,140 > (+)-cocaine. Moreover, there is a high positive correlation (r, 0.99, $p < 0.001$) between the affinities of drugs at sites labeled by [^{3}H]CFT and [^{3}H]cocaine. The results show that [^{3}H]CFT and [^{3}H]cocaine bind to a similar spectrum of sites in monkey caudate putamen. Because of its higher affinity and slower dissociation rate, [^{3}H]CFT appears to be a superior radioligand probe for these sites.

Despite intense study, there is still inadequate information about the molecular mechanisms mediating the behavioral effects and abuse liability of cocaine. Recent studies have identified specific binding sites for [^{3}H]cocaine in striatal tissue of rodents (1–5), humans (6), and nonhuman primates (7). These sites have properties characteristic of pharmacological receptors. First, the sites bind [^{3}H]cocaine saturably at concentrations comparable to those achieved in brain or plasma after peripheral administration of cocaine to animals or humans (8, 9). Second, they bind [^{3}H]cocaine stereoselectively, with preference for (−)-cocaine over its enantiomer (+)-cocaine or its diastereoisomer pseudococaine (7). Third, the affinities of cocaine and related drugs at [^{3}H]cocaine binding sites in striatal tissue closely parallel their potencies for inhibiting dopamine uptake in this brain region (2, 3, 6, 7), a mechanism widely implicated in the *in vivo* effects of cocaine. Lastly, and perhaps most importantly, the relative potencies of various cocaine analogs for producing cocaine-like behavioral effects, including behavioral stimulation (10), intravenous self-administration (11), and stereotypy (3) correlate highly with their relative binding affinities for [^{3}H]cocaine binding sites (3, 7, 10, 11). Together, these findings strongly suggest that specific cocaine recognition sites associated with the dopamine transport system play a fundamental role in mediating the behavioral effects and abuse of cocaine.

Further clarification of the mechanisms by which cocaine and related drugs alter behavior and maintain abuse will likely emerge from molecular characterization and mapping of cocaine receptor sites in brain. However, the relatively low affinity and rapid dissociation of [^{3}H]cocaine (5, 12) limit its suitability as a radioligand probe, particularly in brain regions with low receptor density or in gel filtration assays requiring long time

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ABBREVIATIONS: CFT (also designated WIN 35,428), 2β-carbomethoxy-3β-(4-fluorophenyl)tropane; WIN 35,065-2, 2β-carbomethoxy-3β-phenyltropane; WIN 35,140, 2α-carbomethoxy-3β-phenyltropane (C-2 epimer of WIN 35,065-2); GBR 12909 1-[2-[*bis*(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine; Lu 19-005, (±)-(*trans*)-3-(3′,4′-dichlorophenyl)-*N*-methyl-1-indanamine.

periods for separation of free and bound ligand (e.g., for solubilized tissue preparations). In view of these limitations, there is a need for developing improved molecular probes that label cocaine recognition sites in brain more efficiently than does [3H]cocaine. Other radioligands such as [3H]mazindol (13, 14) and [3H]GBR 12935 (15) do not appear to be fully satisfactory alternatives. Although these compounds are useful for labeling elements of the dopamine transporter, their spectrum of binding sites probably differs from the spectrum of sites labeled by [3H]cocaine. Previous studies have shown, for example, that [3H]cocaine characteristically labels multiple binding components in striatum (5, 7), whereas [3H]mazindol and [3H]GBR 12935 appear to label primarily a single site (13, 15). Along these same lines, mazindol and GBR 12909 (a close congener of GBR 12935) do not fully displace specifically bound [3H]cocaine (7). Conversely, cocaine and its congeners do not fully displace [3H]GBR 12935, and the affinities of cocaine analogs at striatal [3H]GBR 12935 binding sites are 5–10 times lower than at [3H]cocaine sites (16). Finally, the number of binding sites for [3H]cocaine in striatum has been found to approximately double following destruction of corticostriatal projections, whereas the number of sites labeled by [3H]GBR 12935 or [3H]mazindol is relatively unaffected (17).

As part of our ongoing program to develop improved radioligand probes for cocaine receptors, we have identified several fluorophenyltropane analogs with potencies exceeding that of cocaine itself.[1] The most promising of these, CFT (also designated WIN 35,428) was found to be 3–10 times more potent than (−)-cocaine as a psychomotor stimulant (18, 19) and as an inhibitor of specifically bound [3H]cocaine (3, 7). The present study describes the binding of [3H]CFT under conditions comparable to those used to characterize [3H]cocaine binding (7). The results suggest that [3H]CFT is superior to [3H]cocaine as a radioligand probe for cocaine receptors.

Materials and Methods

Tissue sources and preparation. Brain tissue was harvested within 30 min of death from adult male and female cynomolgus monkeys (*Macaca fascicularis*) that were sacrificed in the course of other studies (Charles River Breeding Laboratories, Port Washington, NY). Tissue was stored in the brain bank at the New England Regional Primate Research Center at −85° (7). At the time of regional dissection, the caudate putamen was removed from coronal slices and yielded 1–2 g of tissue.

Membranes were prepared as described previously (7). Briefly, the caudate putamen was homogenized in 10 volumes (w/v) of ice-cold Tris·HCl buffer (50 mM, pH 7.4 at 4°) and centrifuged at 38,700 × *g* for 20 min in the cold. The resulting pellet was resuspended in 40 volumes of buffer, and the entire wash procedure was repeated twice. The membrane suspension (25 mg original wet weight of tissue/ml) was diluted to 12 mg/ml in buffer just before assay and was dispersed with a Brinkmann Polytron homogenizer (setting 5) for 15 sec. All experiments were conducted in triplicate and each experiment was repeated in each of 2–6 individual brain preparations.

Kinetic studies. For association experiments, each assay tube contained 0.2 ml of buffer (Tris·HCl, 50 mM, pH 7.4 at 0–4°, containing NaCl, 100 mM), 0.2 ml of [3H]CFT (0.3 nM), and 0.2 ml of tissue, (final concentration, 4 mg original wet weight of tissue/ml). The final volume of each tube was 0.6 ml. The tubes were incubated at 0–4° for various times (1 min to 4 hr), and incubation was terminated by rapid filtration over Whatman GF/B glass fiber filters that were presoaked for at least 40 min in 0.1% bovine serum albumin (Sigma Chemical Co.). The filters were washed twice with 5 ml of Tris·HCl buffer (50 mM) and incubated overnight in scintillation fluor (Beckman Ready-Value, 5 ml); cpm were converted to dpm following determination of radioactivity by scintillation counting and of counting efficiency (49–53%) by external standardization. Total binding was defined as dpm of [3H]CFT bound at each time point. Nonspecific binding was defined as dpm of [3H]CFT bound in the presence of 30 μM unlabeled (−)-cocaine. Specific binding was the difference between the two values. Total binding at equilibrium was approximately 1800 dpm, and specific binding was 90–95% of the total. Differences in [3H]CFT bound within triplicate samples averaged about 3% of the mean at levels of radioactivity ≥400 dpm.

The dissociation rate was determined by allowing [3H]CFT binding to achieve equilibrium (2 hr at 0–4°) under conditions identical to those described above. Subsequently, dissociation of the receptor-ligand complex was initiated by adding an excess of unlabeled CFT (10 μM) and filtering samples at various times. Specifically bound [3H]CFT at equilibrium (time 0) was used to calculate the percentage of specifically bound [3H]CFT as a function of time.

Saturation and competition experiments. For saturation experiments, membranes (0.2 ml; 4 mg/ml final tissue concentration) were incubated with increasing concentrations of [3H]CFT (0.2 ml; range, 0.3–100 nM) at 0–4° for 2 hr in a total volume of 0.6 ml of Tris·HCl buffer, as described for the kinetic studies. Nonspecific binding was measured using (−)-cocaine (30 μM) in parallel assay tubes and was subtracted from total binding to obtain specific binding.

For competition experiments, each assay tube contained buffer or buffer plus test drug (0.2 ml), [3H]CFT (0.2 ml; 0.3 nM), and tissue (0.2 ml; final assay concentration, 4 mg/ml) to a final volume of 0.6 ml. Total binding was determined in the presence of ineffective concentrations of the particular test drug (1 pM to 1 nM, depending on affinity), nonspecific binding was determined with (−)-cocaine (30 μM), and specific binding was the difference between the two values. Incubation at 0–4° was terminated at 2 hr by rapid filtration, and all subsequent steps were identical to those described above.

Data analysis. Data were analyzed by the KINETIC, EBDA, and LIGAND computer software programs (Elsevier-Biosoft, U.K.) modified for the IBM/PC/XT. Kinetic rate constants were computed with the KINETIC program from dissociation experiments, by nonlinear curve fitting. Mono- and biexponential models were tested statistically by the partial F test. Final estimates of IC_{50} values and pseudo-Hill coefficients (n_H) were computed by the EBDA program. Baseline values for the individual drugs were established from the displacement curves by this curve-fitting program. The LIGAND program provided final parameter estimates for the density of sites (B_{max}) and the dissociation constant (K_d) by iterative nonlinear curve-fitting. One- and two-component binding models were compared using the root mean square error of each fit and the F test.

Drugs. [3H]CFT [2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-[3H] methyltropane; 81.3 Ci/mmol] was synthesized by Dupont-New England Nuclear (Boston, MA). The following drugs were obtained from the sources listed: (−)-cocaine hydrochloride and (+)-cocaine base (National Institute on Drug Abuse, Bethesda, MD); WIN 35,065-2 tartrate, WIN 35,140 hydrochloride, CFT (WIN 35,428) naphthalene disulfonate, and GBR 12909 hydrochloride (Research Biochemicals, Inc., Natick, MA); mazindol base (Sandoz, Inc. East Hanover, NJ); bupropion hydrochloride (Burroughs-Wellcome, Research Triangle Park, NC); and Lu 19-005 hydrochloride (Lundbeck A/S, Copenhagen, Denmark).

Results

Kinetics of [3H]CFT binding. [3H]CFT (0.3 nM) bound rapidly and reversibly to caudate putamen membranes. Binding

[1] B. K. Madras, 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 [3H]cocaine binding sites. Submitted for publication.

achieved equilibrium within 2 hr and was stable for at least 4 hr (Fig. 1a). Analysis of the data using the KINETIC computer program revealed that association occurred in two phases, with corresponding half-times ($t_{1/2}$ = ln $2/K_{obs}$) of 0.66 ± 0.21 and 12.0 ± 2.2 min (mean ± SD; two experiments). Dissociation of [^{3}H]CFT also occurred in two phases, with corresponding dissociation rate constants (K_{-1} and K_{-2}) of 0.28 ± 0.019 and 0.032 ± 0.0076 min^{-1} (Fig. 1b). The calculated half-times for dissociation ($t_{1/2}$ = ln $2/K_{-1}$) were 2.5 ± 0.17 min for the first phase and 23 ± 5.3 min for the second phase. A biexponential model for both association and dissociation phases was statistically preferred to a monoexponential model ($p < 0.001$).

Density and affinity of [^{3}H]CFT binding sites. Saturation of the binding sites was measured by incubating tissue with increasing concentrations of [^{3}H]CFT (range, 0.3–100 nM) in the presence and absence of a fixed concentration of unlabeled (−)-cocaine (30 μM), which served to measure nonspecific binding. Scatchard transformation of six independent experiments revealed a curvilinear plot suggestive of two binding components (Fig. 2a, *inset*). When data were fit to either a one- or two-component model, the two-component model was statistically preferred (F value range, 8.82–34.06; p value range, <0.02–0). The dissociation constants corresponding to the high and low affinity components averaged 4.7 and 60 nM, respectively (Table 1). The densities corresponding to the high and low affinity components averaged 50 and 290 pmol/g of tissue. About 87% of total binding was associated with the low affinity component.

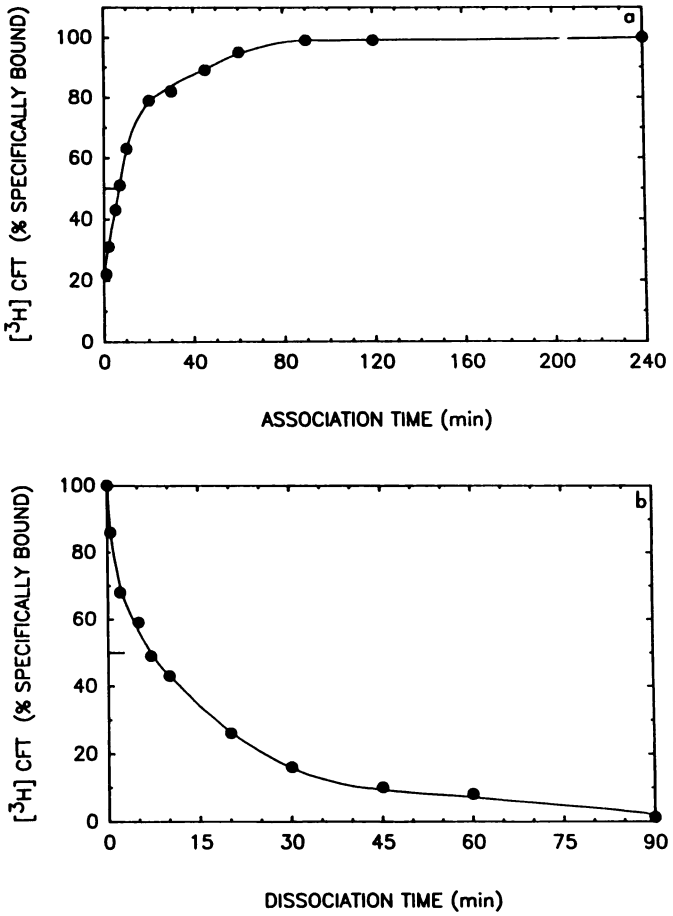

Fig. 1. Association (a) and dissociation (b) of [^{3}H]CFT to monkey caudate putamen membranes. For association experiments, [^{3}H]CFT (0.3 nM) was incubated with membranes (4 mg/ml) at 0–4° for various times. Nonspecific binding was determined with 30 μM (−)-cocaine at each time point and subtracted from total binding to determine specifically bound [^{3}H]CFT. Dissociation of [^{3}H]CFT was initiated by 10 μM CFT after equilibrium was achieved by incubating [^{3}H]CFT (0.3 nM) with membranes at 0–4° for 2 hr. The data from both association and dissociation experiments were fitted by the KINETIC program to a biexponential model. Each *point* is the mean of triplicate determinations in a representative brain.

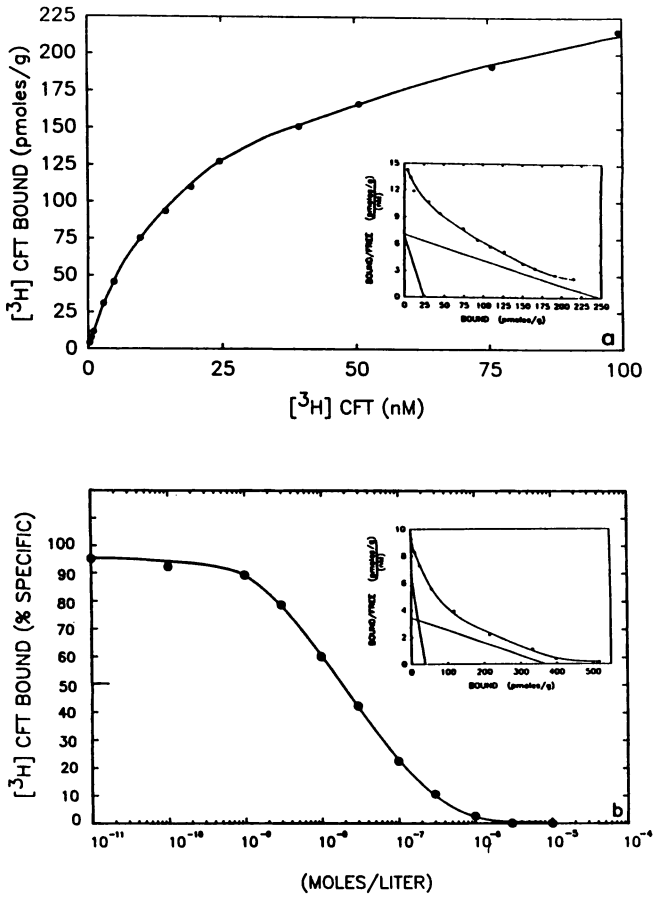

Fig. 2. Saturation of [^{3}H]CFT binding sites measured by increasing the concentration of [^{3}H]CFT (0.3–100 nM) (a) or by fixing the concentration of [^{3}H]CFT (0.3 nM) and increasing the concentration of unlabeled CFT (b). Data are means of triplicate determinations of representative experiments. In a, specific binding is plotted as a function of [^{3}H]CFT concentration. In b, the percentage of specifically bound [^{3}H]CFT (0.3 nM) is plotted as a function of increasing concentrations of unlabeled CFT. *Insets*, Scatchard transformations.

Two binding components were also observed in saturation experiments using a fixed concentration of [^{3}H]CFT (0.3 nM) and increasing concentrations of unlabeled CFT (1 pM to 100 μM). Scatchard transformation of the data revealed a curvilinear plot (Fig. 2b, *inset*), similar to the one obtained using increasing concentrations of radioligand (compare with Fig. 2a). Analysis of the data using the LIGAND program confirmed that a two-component binding model was statistically preferred over a one-component model (F values, 40.95 and 11.73; p values, 0 and <0.002). The dissociation constants for the high and low affinity binding components averaged 4.70 and 66 nM, respectively (Table 1). The corresponding densities were 28 and 360 pmol/g of tissue. Low affinity binding constituted approximately 93% of the specific binding sites.

Pharmacological specificity of [^{3}H]CFT binding sites. In order to evaluate the pharmacological specificity of binding

TABLE 1

Affinity (K_d) and density (B_{max}) of [^{3}H]CFT binding components in monkey caudate putamen

Values are estimated by LIGAND from saturation experiments. Membranes (4 mg/ml) were incubated with 8–13 concentrations of [^{3}H]CFT (0.3–75 or 100 nM) in the presence and absence of (−)-cocaine (30 μm) to measure nonspecific binding (means ± standard errors; six experiments) (Expt. A) or with a fixed concentration of [^{3}H]CFT (0.3 nM) in the presence of increasing concentrations of unlabeled CFT (1 pM to 100 μM) (means ± standard deviations; two experiments) (Expt. B). Each independent experiment was performed in triplicate as described in Materials and Methods.

$K_{0.50}$	High affinity			Low affinity	
	K_d	B_{max}	Proportion of high affinity sites	K_d	B_{max}
nM	nM	pmol/g	%	nM	pmol/g
Expt. A					
19 ± 1.4	4.7 ± 1.2	50 ± 18	13	60 ± 12	290 ± 20
Expt. B					
16 ± 5.2	4.7 ± 2.3	28 ± 10	7	66 ± 33	360 ± 8

sites labeled by [^{3}H]CFT, competition experiments were conducted with several compounds previously studied in comparable experiments involving [^{3}H]cocaine (7). Cocaine and its congeners displaced specifically bound [^{3}H]CFT in a concentration-dependent and parallel manner (Fig. 3a). [^{3}H]CFT binding was displaced stereoselectively by (−)-cocaine, which was 200 times more potent than its enantiomer (+)-cocaine, and by WIN 35,065-2, which was 45 times more potent than its diastereoisomer WIN 35,140. The rank order of potency of various cocaine congeners was CFT > WIN 35,065-2 > (−)-cocaine > WIN 35,140 > (+)-cocaine (Table 2). The competition curves for each of these drugs had relatively shallow slopes (n_H, 0.69–0.81), and computer resolution of the binding isotherms confirmed that the data best fit a two-component binding model (p value range, <0.01 to 0). All drugs that recognized two components bound approximately 66% of the high and 34% of the low affinity component labeled with 0.3 nM [^{3}H]CFT. The high and low affinities (K_d) of the drugs for each component are given in Table 2.

The binding sites were characterized further in competition studies with monoamine uptake inhibitors that are structurally unrelated to cocaine. Lu 19-005, mazindol, GBR 12909, and bupropion all displaced [^{3}H]CFT in a concentration-dependent manner, with Lu 19-005 being the most potent compound (Fig. 3b; Table 2). Unlike the cocaine congeners, however, Lu 19-005, mazindol, and GBR 12909 did not fully displace specifically bound [^{3}H]CFT. The competition curves for these drugs consistently plateaued at approximately 90% displacement of specifically bound [^{3}H]CFT, and the residual sites were not displaced even at 100-fold higher drug concentrations. The competition curves also had steeper slopes than those for the cocaine congeners, with pseudo-Hill coefficients of 0.89–1.30. Computer analysis showed that data for Lu 19-005 and GBR 12909 consistently fit a single-component binding model but

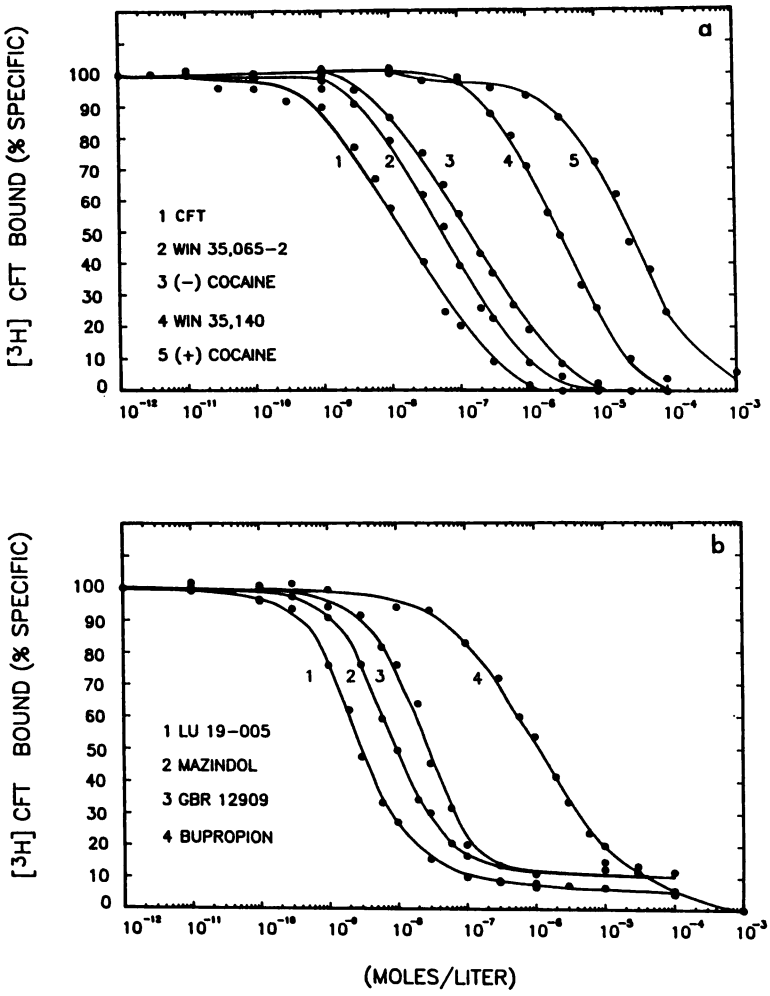

Fig. 3. Displacement of [^{3}H]CFT binding (0.3 nM) by cocaine congeners (a) and by structurally unrelated monoamine uptake inhibitors (b). Each *curve* is the mean of two to four independent experiments, each performed in triplicate.

TABLE 2

Inhibition of specifically bound [^{3}H]CFT by cocaine congeners and structurally unrelated monoamine uptake inhibitors

[^{3}H]CFT (0.3 nM) and membranes (4 mg of tissue/ml) were incubated with various concentrations of competing drugs as described in Materials and Methods. Nonspecific binding was determined with (−)-cocaine (30 μM). IC_{50} and n_H values were computed by EBDA and data were analyzed by LIGAND for a one- or two-component fit. IC_{50} and K_d values represent the means (± standard errors) of three or four independent experiments, each performed in triplicate. Approximately 66% of the binding of cocaine congeners and bupropion was associated with the high affinity component, as determined by the LIGAND program.

Drug	IC_{50}	K_d		n_H
		High	Low	
	nM	nM		
Cocaine congeners				
CFT (WIN 35,428)	18 ± 1.9	6.5	100	0.73
WIN 35,065-2	65 ± 12	29	390	0.76
(−)-Cocaine	150 ± 18	52	890	0.70
WIN 35,140	2,900 ± 310	1,500	45,000	0.81
(+)-Cocaine	30,000 ± 2,100	16,000	420,000	0.69
Other monoamine uptake inhibitors				
Lu 19-005[a]	2.5 ± 0.04	2.1		1.05
Mazindol[b]	7.7 ± 0.75	8.1		0.89
GBR 12909[a]	22 ± 2.2	17		1.34
Bupropion	1,300 ± 310	560	40,000	0.64

[a] A single-component binding model provided a statistically preferred fit in all cases.
[b] A single-component binding model provided a statistically preferred fit in two of four cases.

the results with mazindol fit a single-component model in two of four experiments. In contrast, bupropion displaced [^{3}H]CFT fully, with a shallow competition curve similar to those of the cocaine congeners. Computer resolution of the binding isotherm confirmed that bupropion consistently recognized two binding components labeled by [^{3}H]CFT ($p < 0.001$).

Discussion

Previous studies have shown that [^{3}H]cocaine binds to specific recognition sites in mammalian brain (1–7), and accumulating evidence indicates that these receptors can be important mediators of the behavioral effects and self-administration of cocaine and its congeners in monkeys (7, 10, 11). Although [^{3}H]cocaine had been indispensable for characterizing binding sites in brain regions with high receptor density (3, 5–7), its relatively low affinity and rapid dissociation (5, 12) are limiting factors for mapping the regional distribution of receptors or for molecular characterization in solubilized tissue preparations. It was of interest, therefore, to develop novel radioligands that selectively bind cocaine receptors but with increased affinity and improved kinetic properties. The present results suggest that [^{3}H]CFT meets these requirements.

Under experimental conditions similar to those in the present study, [^{3}H]cocaine has been found to label multiple binding components in caudate putamen membranes of monkeys (7). Computer resolution of the binding isotherms in the earlier study revealed distinct high and low affinity binding components, with the latter comprising about 90% of specifically bound [^{3}H]cocaine. In the present study, analysis of kinetic, saturation, and competition experiments indicates that [^{3}H]CFT also labels at least two components in caudate putamen, with the low affinity component comprising about 87–93% of the total number of binding sites.

In addition to labeling a similar proportion of high and low affinity binding sites, both [^{3}H]CFT and [^{3}H]cocaine bind to sites with similar pharmacological specificity. In this regard, the rank order of potency of cocaine congeners and other monoamine uptake inhibitors at [^{3}H]CFT binding sites is identical to their rank order of potency at [^{3}H]cocaine binding sites (7). Moreover, as shown in Fig. 4, there is a high positive correlation (r, 0.991; $p < 0.001$) between IC_{50} values of drugs at [^{3}H]cocaine and [^{3}H]CFT binding sites.

Although the present results clearly demonstrate a similar binding profile for [^{3}H]CFT and [^{3}H]cocaine, some differences are noteworthy. First, the total number of specific binding sites labeled by [^{3}H]CFT in the present study is about 16% less than the number of sites labeled by [^{3}H]cocaine determined under comparable conditions (7). In previous studies, we found that CFT and other phenyltropane analogs of cocaine, which lack an ester link between the phenyl and the tropane moieties, often do not fully inhibit binding of [^{3}H]cocaine (7).[1] As a result, competition curves for the phenyltropane analogs characteristically plateau at levels corresponding to 80–90% of complete inhibition. The residual 10–20% of [^{3}H]cocaine binding sites appear to be relatively inaccessible to the phenyltropane analogs and, in the present study, apparently were not labeled by [^{3}H]CFT. Although speculative, these residual sites may be associated with the local anesthetic effects of cocaine. In this regard, phenyltropane analogs are weak local anesthetics (20) and bind poorly to sites on sodium channels that are considered to be important for local anesthetic activity (21). It is also possible that the residual sites are associated with enzymes involved in the deesterification of cocaine and its ester-linked congeners.

[^{3}H]CFT and [^{3}H]cocaine also differ with respect to affinity and dissociation rates, properties critical for reproducible radioligand binding assays. The integrated K_d ($K_{0.50}$) for [^{3}H]CFT in the present study, 16 nM (Table 1), was 1 order of magnitude lower than the $K_{0.50}$ for [^{3}H]cocaine, 283 nM, determined under comparable conditions (7). Similarly, the K_d values for the high and low affinity binding components of CFT were about 9 times lower than the corresponding values for cocaine (Table 2). Equally significant, the $t_{1/2}$ values for dissociation of [^{3}H]CFT were up to 100 times longer than the $t_{1/2}$ values reported for [^{3}H]cocaine (5, 12), although differences in assay conditions may constrain direct comparisons. The higher affinity and slower dissociation of [^{3}H]CFT compared with [^{3}H]cocaine are important factors determining the potential broader utility of the novel radioligand. Finally, although not anticipated, non-

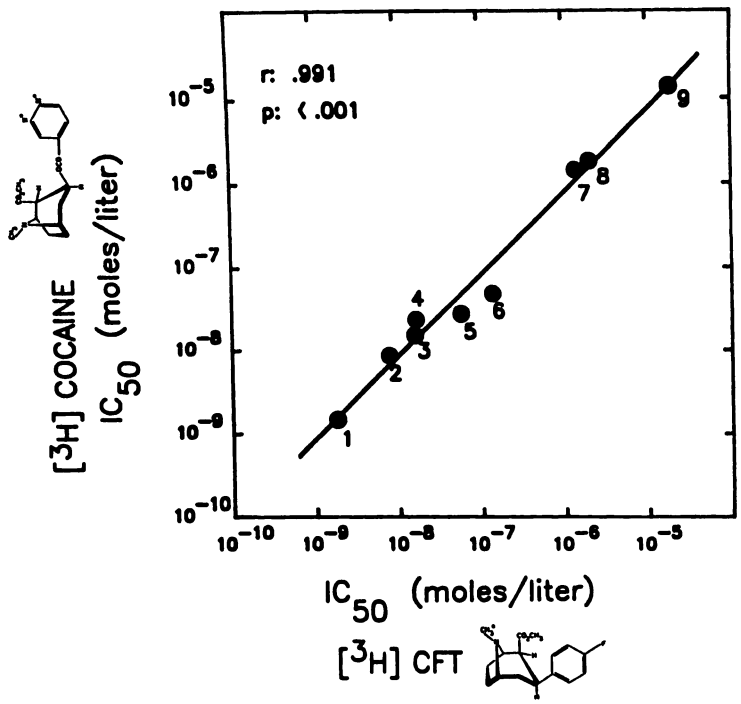

Fig. 4. Relationship between the potencies of drugs for displacing specifically bound [³H]CFT and for displacing specifically bound [³H] cocaine in monkey caudate putamen. The IC_{50} values for [³H]CFT binding sites are from Table 1 of the present study; the IC_{50} values for [³H]cocaine binding sites are from Table 1 of Ref. 7. The individual drugs are coded as follows: **1**, Lu 19-005; **2**, mazindol; **3**, CFT; **4**, GBR 12909; **5**, WIN 35,065-2; **6**, (−)-cocaine; **7**, bupropion; **8**, WIN 35,140; **9**, (+)-cocaine.

specific binding of [³H]CFT (5–10% of the total) was less than nonspecific binding of [³H]cocaine (about 15%) (7).

Because of the superior binding properties of [³H]CFT, data from competition experiments in the present study were more reproducible than in our earlier studies using [³H]cocaine, permitting better resolution of differences in the binding of different drugs. Specifically, the competition curves for the cocaine congeners as well as bupropion were shallow, reached full displacement of [³H]CFT, and were characterized by at least two binding components. In contrast, competition curves for Lu 19-005, mazindol, and GBR 12909 were steeper, plateaued at levels corresponding to about 90–93% displacement of [³H]CFT, and, with the possible exception of mazindol, were characterized by a single binding component. These findings show that the binding of Lu 19-005, mazindol, and GBR 12909 is not identical to that of the cocaine congeners and suggest that the former drugs recognize a subset of [³H]CFT binding sites.

To summarize our principal findings, [³H]CFT labels high and low affinity binding components similar to those previously reported for [³H]cocaine in monkey caudate putamen and the potency relationships observed in the two assays are virtually identical. Based on its high affinity and slow dissociation, [³H] CFT appears to be superior to [³H]cocaine as a radioligand probe for cocaine receptors.

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Send reprint requests to: B. K. Madras, Ph.D., Harvard Medical School, New England Regional Primate Research Center, One Pine Hill Drive, Southborough, MA 01772.