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# Binding of the Cocaine Analog 2β-[$^3$H] Carboxymethoxy-3β-(4-fluorophenyl)tropane to the Serotonin Transporter

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**SUMMARY**

The cocaine analog 2β-carboxymethoxy-3β-(4-fluorophenyl)-tropane (CFT) binds to platelet plasma membrane vesicles. [$^3$H] CFT binding is blocked equally well by cocaine and imipramine. Specific (cocaine-sensitive) binding requires $Na^+$ and is inhibited by $H^+$ and $Cl^-$ ions. At 150 mM $Na_2SO_4$ and pH 9.5, the $K_D$ for [$^3$H]CFT is 232 ± 71 nM. The number of specific [$^3$H]CFT binding sites on the membrane vesicles is equal to the number of serotonin transporters, as measured by [$^3$H]imipramine binding. Binding of imipramine and CFT appeared to be mutually competitive. These results suggest that [$^3$H]CFT and cocaine bind to the serotonin transporter at a site close to but distinct from the antidepressant binding site.

The $Na^+$-dependent transporters for serotonin, norepinephrine, and dopamine are responsible for inactivating synaptically released biogenic amines by transport into the nerve terminals from which these neurotransmitters are released. The serotonin transporter is believed to be the physiological target for antidepressant drugs such as imipramine (1) and fluoxetine (2). Recent evidence suggests that the dopamine transporter may be a physiological target for the reinforcing properties of cocaine (3). The usefulness of antidepressant drugs in treating cocaine addiction (4) suggests functional connections between the two transporters.

The biogenic amine transporters constitute a subset of neurotransmitter reuptake proteins that share a number of properties. They all require $Na^+$ and $Cl^-$ extracellularly and also exhibit a requirement for $K^+$, which, in the case of the serotonin transporter, is utilized intracellularly (5–7). These ions are believed to participate directly in the transport process, and accumulated evidence suggests that serotonin, for example, is co-transported with one $Na^+$ (8) and one $Cl^-$ ion (9), while one $K^+$ ion is counter-transported (10). All three $Na^+$-dependent biogenic amine transporters are inhibited by tricyclic antidepressants (11–13) and cocaine (3, 11, 12, 14, 15), although specificities and affinities vary. $Na^+$ ion is required for binding of the tricyclic antidepressant imipramine to the serotonin transporter of brain (16) and platelet plasma membrane (1) and of desipramine to the norepinephrine transporter of rat cerebral cortex membranes (17). $Cl^-$ ion, although not required, enhances imipramine binding to the serotonin transporter (1).

CFT is a derivative of cocaine in which the benzoyl ester moiety of cocaine is replaced with a fluorophenyl group attached directly to the tropane ring. CFT has been proposed as a superior radioligand for cocaine binding sites, due to its higher affinity and slower dissociation (18). Studies measuring [$^3$H] cocaine and [$^3$H]CFT binding to various brain regions have demonstrated saturable binding of both compounds, which is displaced by ligands and substrates for the $Na^+$-dependent biogenic amine transporters (18–20). In the striatum, a region rich in dopaminergic nerve endings, [$^3$H]cocaine binding was shown to be largely $Na^+$ dependent (20). A portion of this binding was independent of $Na^+$ and was attributed to serotonergic nerve endings (21). Also in platelet membranes, [$^3$H] cocaine binding was reported not to require $Na^+$ and to be noncompetitive with imipramine (22).

In previous studies, binding of serotonin, imipramine, and other antidepressant drugs to the platelet plasma membrane was uniformly $Na^+$ dependent (1, 8, 23). Moreover, each of these ligands displaced the others competitively (24). Because results reported for cocaine binding were in such contradiction to those reported for antidepressants, we undertook to study [$^3$H]cocaine binding to purified platelet plasma membrane vesicles, which are highly enriched in the serotonin transporter (5). We soon discovered that nonspecific binding of [$^3$H]cocaine to platelet membranes was much higher than that of [$^3$H]CFT, and we subsequently concentrated our attention on CFT. The results indicate that [$^3$H]CFT and [$^3$H]cocaine binding to the serotonin transporter requires $Na^+$ and is competitive with imipramine. However, [$^3$H]CFT binding differs from [$^3$H]imipramine binding in its $Cl^-$ and $H^+$ sensitivity, suggesting that different residues in the transporter binding site interact with CFT and imipramine.

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**ABBREVIATIONS:** CFT, 2β-carboxymethoxy-3β-(4-fluorophenyl)tropane; HPLC, high pressure liquid chromatography.

## Experimental Procedures

**Materials.** Platelet plasma membrane vesicles were prepared from human platelet concentrate, obtained from the American Red Cross (Farmington, CT), as described previously (25). The suspension was stored in small portions, at 8 mg/ml (by Lowry protein assay) in 0.25 M sucrose containing 10 mM Tris·HCl, pH 7.5, and 1 mM $MgSO_4$, and was thawed before each experiment. [$^3$H]CFT was obtained from New England Nuclear (Boston, MA) and [$^3$H]imipramine from Amersham (Arlington Heights, IL). Unlabeled CFT was purchased from Research Biochemicals, Inc. (Natick, MA) All other reagents were reagent grade, purchased from commercial sources.

**Binding measurements.** For [$^3$H]CFT binding, approximately 220 μg of vesicle protein, at a concentration of 8–10 mg/ml, was diluted with the indicated reaction mixture to a final volume of 300 μl, containing approximately 8 nM [$^3$H]CFT (in some cases, unlabeled CFT was added to vary the total concentration), and was incubated at 25° for 30 min. Binding measurements were typically made in triplicate. Reaction mixtures for individual experiments are given in the figure legends. Binding time course measurements indicated that binding was at equilibrium within 10 min. After this incubation, the reaction was terminated by addition of 4 ml of ice-cold 0.2 M NaCl, and the membranes in the diluted reaction mixture were collected by filtration through no. 32 glass fiber filters (Schleicher & Schuell, Keene, NH) and washing of the tube and filter with 3 × 4 ml of ice-cold 0.2 M NaCl. Dilution, filtration, and washing took between 5 and 8 sec. Separate experiments indicated that [$^3$H]CFT dissociated from membranes diluted with ice-cold 0.2 M NaCl with a $t_{1/2}$ of approximately 35 sec. Filters were placed in Optifluor (Packard, Downers Grove, IL) and counted after 5 hr.

Imipramine binding was measured at 25° using the filtration assay described previously (24). Briefly, to initiate binding, membrane vesicles were suspended at a protein concentration of 0.3 mg/ml in an assay buffer of 300 mM NaCl containing 10 mM $LiH_2PO_4$, pH 6.7, and 1 mM $MgSO_4$. The assay buffer also contained [$^3$H]imipramine (19–23 cpm/fmol). After a 15-min incubation, the reactions (300 μl/assay) were terminated by dilution with 4 ml of ice-cold isoosmotic NaCl and filtration through Schleicher & Schuell no. 32 glass fiber filters that had been pretreated with 0.3% polyethyleneimine. The tube and filter were washed three times with 4 ml of ice-cold NaCl solution, and the filter was counted as described above.

**Transport measurements.** Transport rates were measured at 25°, as described previously (10), using vesicles equilibrated with 10 mM lithium phosphate buffer, pH 6.7, containing 133 mM $K_2SO_4$ and 1 mM $MgSO_4$. Transport was initiated by diluting these vesicles 40-fold into 0.2 M NaCl containing 10 mM lithium phosphate buffer, pH 6.7, 1 mM $MgSO_4$, and 100 nM [$^3$H]serotonin (Amersham). Initial rates were measured at 20 sec after dilution. In separate experiments, we determined that transport was linear with time up to at least 20 sec.

**Specific activity determination.** Specific radioactivity of [$^3$H]imipramine was determined by HPLC. [$^3$H]Imipramine and unlabeled imipramine were applied to a 25- × 0.46-cm Ultrasphere ODS C-18 column (Beckman, San Ramon, CA) equilibrated with 3 parts acetonitrile and 1 part 100 mM ammonium formate, 0.1% triethylamine, pH 5, as the mobile phase, and were eluted with the same solvent at 1 ml/min. The percentage of $^3$H associated with imipramine and the concentration of [$^3$H]imipramine were determined by scintillation counting of the column effluent and measurement of the peak absorbance at 250 nm, respectively. The calculated specific activity was 40,000 cpm/pmol (45.5 Ci/mmol).

The radioisotopic purity of [$^3$H]CFT was likewise determined by scintillation counting of the HPLC column effluent. In this case, the sample was applied to a column equilibrated with 1 part acetonitrile, 2 parts water, and 1 part 100 mM ammonium formate, 0.1% triethylamine, pH 5, and was eluted at 1 ml/min with a linear gradient of acetonitrile from 25% to 75% over 30 min. The concentration of unlabeled CFT used to dilute the [$^3$H]CFT was determined from the fluorescence of its counter-ion, naphthalene disulfonic acid (excitation, 280 nm; emission, 330 nm). The final specific activity used in binding experiments varied from 454 to 54,700 cpm/pmol (0.52 to 62.1 Ci/mmol).

**Data analysis.** Each experimental point was determined in duplicate or triplicate, and each experiment was repeated two or three times. Except where indicated otherwise, saturation and inhibition curves were analyzed by nonlinear regression using ENZFITTER (Elsevier Biosoft).

## Results

**$Na^+$ dependence and inhibitor sensitivity of imipramine and CFT binding.** Imipramine binding to the serotonin transporter and cocaine binding to other biogenic amine transporters require the presence of $Na^+$ ions (1, 20). In preliminary experiments designed to assess $Na^+$-dependent binding of cocaine and the cocaine analog CFT, we measured the amount of [$^3$H]cocaine and [$^3$H]CFT binding that was displaced by 1 mM cocaine. For both compounds, specific binding required $Na^+$ (Table 1). Nonspecific binding, however, was higher with [$^3$H]cocaine. For this reason, and because of its higher specific radioactivity, we concentrated on [$^3$H]CFT binding in the following experiments.

To evaluate more thoroughly the effect of $Na^+$ on the ability of the serotonin transporter to bind CFT, we measured the $Na^+$ dependence of [$^3$H]CFT binding and its displacement by cocaine and imipramine, using platelet plasma membrane vesicles. In parallel, we measured the $Na^+$ dependence of imipramine binding and its inhibition by cocaine and serotonin. The data in Fig. 1A show that 300 meq/liter $Na^+$ increases the amount of [$^3$H]CFT binding approximately 4-fold, under the conditions used, and that both cocaine and imipramine decrease [$^3$H]CFT binding in a concentration-dependent manner. Furthermore, the amount of cocaine- and imipramine-displaceable [$^3$H]CFT binding is identical to the $Na^+$-dependent binding, indicating that $Na^+$-dependent binding represents specific binding to the serotonin transporter. By comparison, the data in Fig. 1B demonstrate the well known $Na^+$ dependence and serotonin sensitivity of [$^3$H]imipramine binding to platelet plasma membranes (1). Fig. 1B also shows that cocaine inhibits [$^3$H]imipramine binding and that the amount of [$^3$H]imipramine displaced by cocaine is equal to the $Na^+$-dependent and serotonin-sensitive binding. These results suggest that imipramine and CFT bind to the serotonin transporter at the same, or mutually exclusive, sites. In separate experiments (not shown), cocaine and serotonin maximally inhibited [$^3$H]CFT binding to the same extent. Moreover, imipramine and cocaine inhibited [$^3$H]CFT binding to the same extent under widely

TABLE 1

**$Na^+$ dependence of cocaine and CFT binding**

[$^3$H]Cocaine and [$^3$H]CFT binding was measured as described in Experimental Procedures, using the following reaction conditions. Control incubations contained 300 mM NaCl, 1 mM $MgSO_4$, 10 mM lithium phosphate buffer, pH 6.7, and 21.6 nM [$^3$H]cocaine (28,500 cpm/pmol) or 9 nM [$^3$H]CFT (54,700 cpm/pmol). Where indicated, LiCl was substituted for NaCl, and 1 mM cocaine was added to displace specifically bound radioligand.

| | Binding | |
|---|---|---|
| | [$^3$H]Cocaine | [$^3$H]CFT |
| | *fmol/mg of protein* | |
| $Na^+$, control | 137 ± 6.2 | 87.6 ± 3.0 |
| $Na^+$ + 1 mM cocaine | 46.0 ± 7.9 | 16.5 ± 2.7 |
| $Li^+$, control | 50.7 ± 0.7 | 20.1 ± 0.5 |
| $Li^+$ + 1 mM cocaine | 45.4 ± 0.4 | 13.7 ± 0.7 |

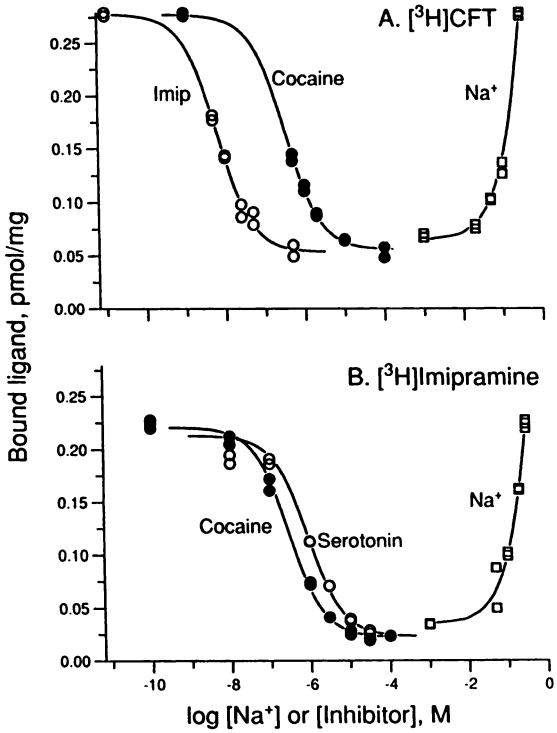


**Fig. 1.** $Na^+$ dependence and inhibitor sensitivity of radioligand binding. A, [$^3$H]CFT binding. $Na^+$ dependence (□): binding of 8 nM [$^3$H]CFT was measured in 10 mM lithium borate, pH 9.5, containing 1 mM $MgSO_4$ and 0–150 mM $Na_2SO_4$, with $Li_2SO_4$ added to maintain isotonicity. Inhibitor sensitivity: at 150 mM $Na_2SO_4$, cocaine (●) or imipramine (○) was added, at the indicated concentration, before [$^3$H]CFT. B, [$^3$H]Imipramine binding. $Na^+$ dependence (□): binding of 0.36 nM [$^3$H]imipramine was measured in 10 mM lithium borate, pH 9.5, containing 1 mM $MgSO_4$ and 0–300 mM NaCl, with LiCl added to maintain isotonicity. Inhibitor sensitivity: at 300 mM NaCl, cocaine (●) or serotonin (○) was added, at the indicated concentration, before [$^3$H]imipramine. All binding measurements were carried out as described in Experimental Procedures, with no subtraction for nonspecific binding.

varying conditions of pH and $Cl^-$ concentration.

To evaluate further the nature of this interaction, we measured the ability of imipramine to inhibit [$^3$H]CFT binding over a range of CFT concentrations. The results are shown in Fig. 2 in the form of a Dixon plot. As the [$^3$H]CFT concentration is raised, the inhibition by imipramine is less dramatic, as evidenced by a smaller slope in the Dixon plot. Furthermore, the lines intersect in the upper left quadrant, consistent with competitive inhibition (26). Likewise, CFT inhibition of [$^3$H]imipramine binding appears competitive (Fig. 3). As judged by the slopes in the Dixon plot, CFT is a more effective inhibitor at a low (0.5 nM) imipramine concentration than at a higher concentration (10 nM). Again, the lines intersect in the upper left quadrant, suggesting competitive inhibition.

In cases of competitive inhibition, the point at which lines in a Dixon plot intersect is located at $1/B_{max}$ on the ordinate and $-K_D$ for the inhibitor on the abscissa (26). The plus symbol in Fig. 2 is located at $1/B_{max}$ and $-K_D$ for [$^3$H]imipramine binding, as determined in separate experiments performed under similar conditions (see Fig. 7 below). Similarly, the plus symbol in Fig. 3 is located at $1/B_{max}$ and $-K_D$ for [$^3$H]CFT binding (see Fig. 5A below). The close agreement between the predicted and observed points of intersection strongly suggests that imipramine and CFT bind to the serotonin transporter in a mutually competitive manner. Moreover, the $K_i$ values for

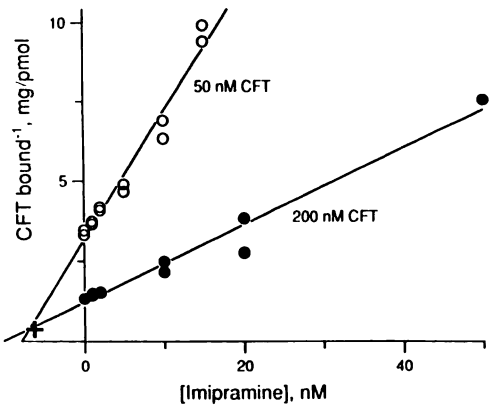


**Fig. 2.** Dixon plot of imipramine inhibition of [$^3$H]CFT binding. Equilibrium [$^3$H]CFT binding was measured as described in Experimental Procedures, at 50 nM (○) or 200 nM (●) CFT, over the indicated range of imipramine concentrations, in 300 mM NaCl containing 1 mM $MgSO_4$ and 10 mM lithium borate, pH 9.5. Nonspecific binding in the presence of 100 μM cocaine was subtracted from all measurements. +, Expected intersection position, assuming 2.67 pmol of CFT binding sites/mg of membrane protein and also assuming a $K_D$ of 6.3 nM for imipramine. The *lines* are drawn from linear least squares fits of the points.

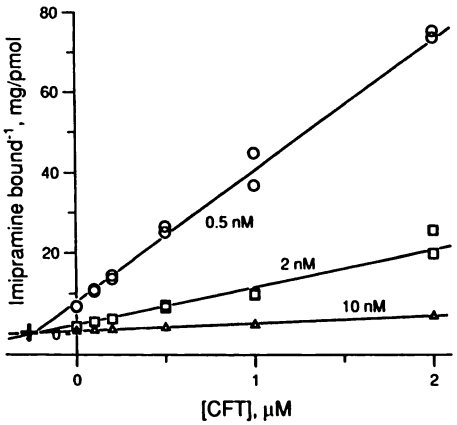


**Fig. 3.** Dixon plot of CFT inhibition of [$^3$H]imipramine binding. Equilibrium [$^3$H]imipramine binding was measured as described in Experimental Procedures, at 0.5 nM (○), 2 nM (□), or 10 nM (△) imipramine, over the indicated range of CFT concentrations, in 300 mM NaCl containing 1 mM $MgSO_4$ and 10 mM lithium borate, pH 9.5. Nonspecific binding in the presence of 100 μM serotonin was subtracted from all measurements. +, Expected intersection position, assuming 2.85 pmol of imipramine binding sites/mg of membrane protein and also assuming a $K_D$ of 290 nM for CFT in 300 mM NaCl.

imipramine and CFT defined by the points at which the lines intersect in Figs. 2 and 3 (259 and 6.94 nM, respectively) agree well with $K_D$ values for radioligand binding determined in the experiments shown below in Figs. 5 and 7.

**Inhibition of serotonin transport.** To confirm that competition between CFT and imipramine was occuring at the serotonin transporter, we measured the ability of CFT to inhibit serotonin transport. The initial rate of serotonin accumulation by platelet plasma membrane vesicles is inhibited in a concentration-dependent manner by CFT, as shown in Fig. 4. The concentration of CFT required for half-maximal inhibition was approximately 270 nM, similar to the $K_i$ for inhibition of imipramine binding. This is likely to be close to the true $K_i$ for transport inhibition, because the serotonin concentration (100 nM) was below the $K_m$ for transport (0.3–0.5 μM) (10). Moreover, transport was completely blocked at CFT concentrations

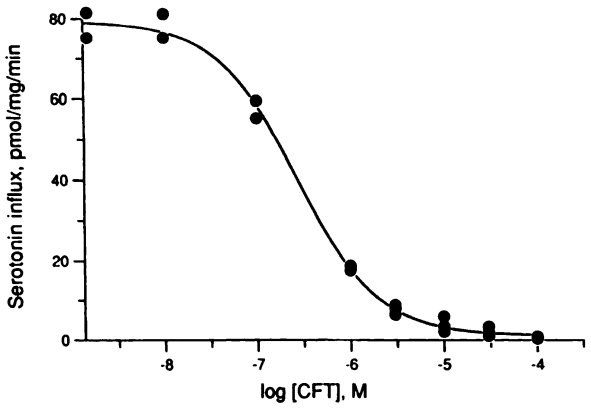


**Fig. 4.** CFT inhibition of serotonin transport. Initial rates of serotonin accumulation were measured as described in Experimental Procedures in the presence of the indicated concentrations of unlabeled CFT. The transport rate in the absence of $Na^+$ (7.38 pmol/mg/min) was subtracted from each point.

higher than 30 $\mu$M, indicating that all functional serotonin transporters were sensitive to CFT.

**Effect of $K^+$, $Cl^-$, and $H^+$ on [$^3$H]CFT binding.** In addition to external $Na^+$, serotonin transport into platelets also requires $Cl^-$ externally (9) and either $H^+$ or $K^+$ internally (27). Imipramine binding is enhanced by $Cl^-$ (1) but is insensitive to $K^+$ (28). To test the effect of $Cl^-$ and $H^+$ on [$^3$H]CFT binding, we determined the [$^3$H]CFT binding isotherm at high (9.5) and low (6.6) pH, in the presence and absence of $Cl^-$. The results shown in Fig. 5, A and B, indicate that $Cl^-$ inhibits [$^3$H]CFT binding at both pH values. In at least three other experiments, the inhibition by $Cl^-$ was always observed, although the extent of inhibition varied from 30 to 50%. In contrast, Fig. 5C, which shows imipramine binding isotherms at low and high $Cl^-$, demonstrates the dramatic stimulation of imipramine binding by $Cl^-$. As will be described elsewhere,[1] $Cl^-$ increases imipramine affinity, with no effect on the maximal number of sites. From the results shown in Fig. 5, A and B, it is clear that the affinity of [$^3$H]CFT binding is increased at high pH, both in the presence and in the absence of $Cl^-$. The maximal number of [$^3$H]CFT binding sites under optimal conditions (2.67 ± 0.83 pmol/mg, in replicate experiments) is equal to the number of imipramine binding sites determined using the same membranes (2.85 ± 0.10 pmol/mg) (see Fig. 7 below).

[1]C. J. Humphreys, J. M. Kootsey, and G. Rudnick. Manuscript in preparation.

The effect of pH on [$^3$H]CFT binding is shown more clearly in Fig. 6. Binding is barely detectable at pH 4 but rises, with a p$K$ of 6.79 ± 0.41, to a maximum at pH 9–10. The tertiary amino group of cocaine has a p$K_a$ of 5.59, and CFT is expected to be identical in this respect. It is possible that the p$K_a$ observed for binding represents dissociation of an $H^+$ ion from CFT, which is enhanced by its binding to the transporter. Alternatively, this titration curve is also consistent with there being a weakly basic residue on the transporter whose protonation causes a decrease in CFT affinity. In either case, the neutral form of CFT is likely to be the species that is bound. This pH dependence represents another difference between imipramine and CFT binding. The data in Fig. 7 demonstrates that imipramine binding is unaffected by raising of the pH from 6.6 to 9.5. The results of saturation isotherms at these two pH values are essentially superimposable.

We tested the effect of $K^+$ on [$^3$H]CFT binding by replacing some of the $Na^+$ with either $Li^+$ (which is known to be inert in the serotonin transport reaction) or $K^+$ (Table 2). Maximal effects of external $K^+$ on transport are observed at concentrations as low as 30 mM (10). There was no effect of 35 or 50 meq/liter $K^+$ (in $Cl^-$ or $SO_4^{2-}$ medium, respectively) on CFT binding that could not be accounted for by a decrease in the $Na^+$ concentration.

## Discussion

The results presented here indicate that the cocaine analog CFT binds stoichiometrically to the serotonin transporter, that CFT and imipramine bind competitively to the same site or to mutually exclusive sites, and that $Na^+$ is required for binding of both ligands. Despite these similarities between imipramine and CFT binding, the two processes are distinguished by their response to $Cl^-$, which enhances imipramine binding and inhibits CFT binding. Furthermore, CFT binding is sensitive to pH over a range that has no effect on imipramine binding.

The differences between CFT and imipramine binding suggest that CFT and cocaine interact with amino acid residues on the serotonin transporter that are not part of the imipramine binding site. Previous studies from this laboratory suggested that $Na^+$, $Cl^-$, and serotonin are bound together to the transporter before translocation (8), and binding studies indicate that $Na^+$ and $Cl^-$ are also bound together with imipramine (1, 8). From these data, we infer that a large binding pocket exists on the transporter, with subsites for $Na^+$, substrate, and $Cl^-$. The inability of $Cl^-$ to stimulate CFT binding ($Cl^-$ actually

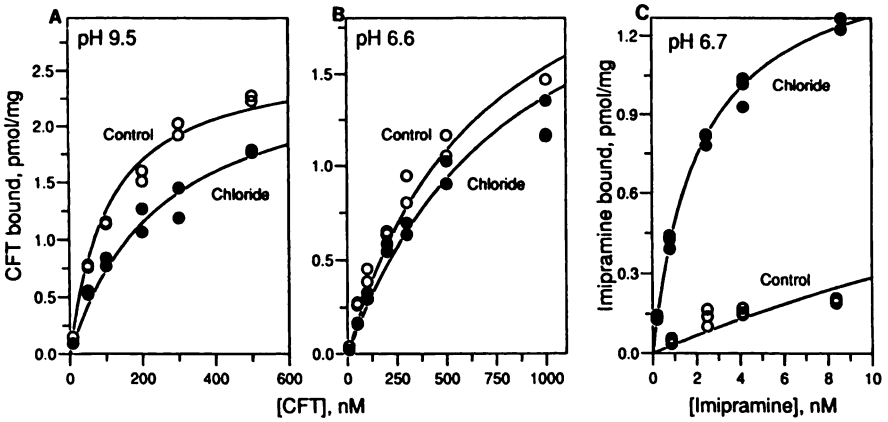


**Fig. 5.** Effect of $Cl^-$ and pH on [$^3$H]CFT (A, B) and [$^3$H]imipramine (C) binding. Binding isotherms were measured at the pH indicated, in the presence or absence of $Cl^-$. The reaction mixture consisted of 150 mM $Na_2SO_4$ (○) or 300 mM NaCl (●) containing 1 mM $MgSO_4$ and either 10 mM lithium borate, pH 9.5, or 10 mM lithium phosphate, pH 6.6. Binding was measured as described in Experimental Procedures, with controls for nonspecific binding (100 $\mu$M cocaine) subtracted from each measurement.

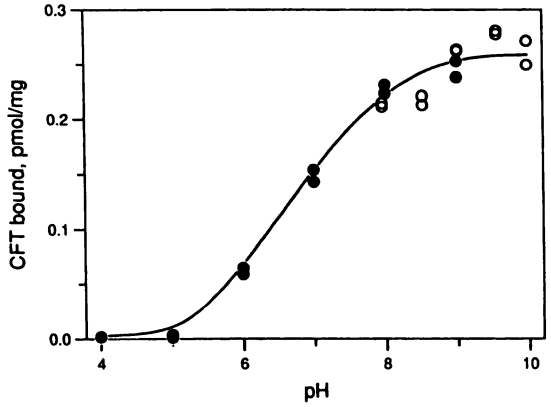


**Fig. 6.** pH dependence of [$^3$H]CFT binding. The reaction mixture consisted of 150 mM $Na_2SO_4$ containing 1 mM $MgSO_4$ and either 10 mM sodium citrate (pH 4–5), 10 mM lithium *N*-2-acetamidoiminodiacetate (pH 6–7), 10 mM lithium *N*-2-hydroxyethylpiperizine-*N'*-3-propanesulfonate (pH 8), or 10 mM lithium borate (pH 9–10). Binding was measured as described in Experimental Procedures, using 8 nM [$^3$H]CFT, with controls for nonspecific binding (100 μM cocaine) subtracted from each measurement. Data are from two experiments (○, ●).

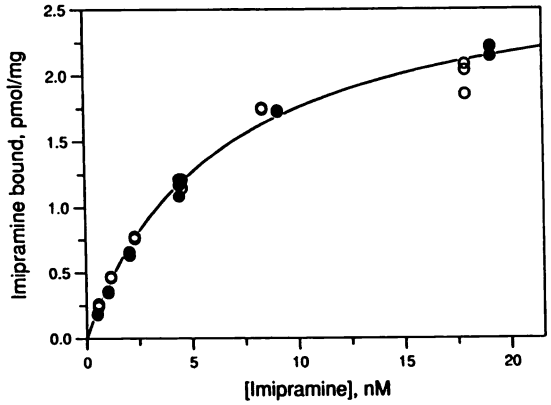


**Fig. 7.** [$^3$H]Imipramine binding isotherm at pH 6.6 and 9.5. Imipramine binding was measured as described in Experimental Procedures, in medium consisting of 300 mM NaCl containing 1 mM $MgSO_4$ and either 10 mM lithium borate, pH 9.5 (●), or 10 mM lithium phosphate, pH 6.6 (○). Controls for nonspecific binding (100 μM serotonin) were subtracted from each measurement.

TABLE 2

**Replacement of $Na^+$ by $Li^+$ and $K^+$**

[$^3$H]CFT binding was measured as described in Experimental Procedures, using the following reaction conditions. Control incubations contained 300 meq/liter $Na^+$ as either NaCl or $Na_2SO_4$, 1 mM $MgSO_4$, 10 mM sodium *N*-2-acetamidoiminodiacetate, pH 6.6, and 9 nM [$^3$H]CFT. Where indicated, 35 mM LiCl or KCl was substituted for NaCl, and 25 mM $Li_2SO_4$ or $K_2SO_4$ was substituted for $Na_2SO_4$. Control values for nonspecific binding determined in the presence of 100 μM cocaine were subtracted from each measurement.

| | [$^3$H]CFT binding | |
|---|---|---|
| | Chloride medium | Sulfate medium |
| | *fmol/mg of protein* | |
| Control | 26.5 ± 1.3 | 53.2 ± 3.5 |
| $Li^+$ | 21.0 ± 2.8 | 42.8 ± 1.8 |
| $K^+$ | 18.7 ± 4.0 | 43.5 ± 1.8 |

inhibits binding) suggests that CFT binds to a subsite on the transporter that is accessible in the absence of $Cl^-$. It would not be surprising if the major determinants of cocaine and CFT affinity were outside the substrate binding site, in a region common to other biogenic amine transporters. Cocaine and CFT inhibit serotonin, norepinephrine, and dopamine transporters (11, 12, 15). Moreover, all three transporters require $Cl^-$ to function (6, 29). The cocaine site of the dopamine and norepinephrine transporters may also demonstrate the same lack of $Cl^-$ stimulation.

The $K_D$ for CFT binding measured in this work, 232 ± 71 nM, is significantly higher than 4.7 ± 1.2 nM measured in brain (18). The most likely reason for this difference is that the brain measurements, made in monkey caudate putamen, reflected binding to the dopamine transporter. In that system, approximately 90% of the binding was displaced by specific inhibitors of dopamine transport, and the contribution of serotonin transporters was obviously minor. Thus, it is likely that the difference in affinity reflects the difference between serotonin and dopamine transporters, rather than a difference between serotonin transporters in platelet and brain.

Previous studies have suggested that [$^3$H]cocaine binding to brain and platelet serotonin transporters was not competitive with imipramine and was independent of $Na^+$ (21, 30). Those reports, however, used concentrations of $Na^+$ up to only 50 mM. Our results (Fig. 1) clearly indicate that $Na^+$ is required for cocaine- and imipramine-sensitive [$^3$H]CFT binding. The effect of $Na^+$ is weak below 100 mM and increases as $Na^+$ is raised up to 300 mM, the highest concentration tested. Although it is likely that insufficient $Na^+$ was responsible for the failure of previous workers to detect $Na^+$-dependent binding, alternative explanations are also possible. These include the possibility that [$^3$H]cocaine binds to additional sites unavailable to [$^3$H]CFT, although the close structural similarity between cocaine and CFT renders such a possibility unlikely. It is also possible that a $Na^+$-independent [$^3$H]cocaine-binding component is present in brain and platelets but not in purified platelet plasma membrane vesicles. The advantage of using platelet vesicles for binding studies is that essentially all biogenic amine transport in these vesicles occurs through the serotonin system (31). Consequently, all of the specific ($Na^+$-dependent and cocaine-sensitive) [$^3$H]CFT binding represents the serotonin transporter. This is evidenced by the close agreement between the number of [$^3$H]CFT and [$^3$H]imipramine binding sites and the complete inhibition of serotonin transport by CFT measured in this work. Because the comparison of [$^3$H]CFT and [$^3$H]imipramine site density requires accurate determination of radioligand specific activity, we determined specific radioactivity, using HPLC, for both compounds (see Experimental Procedures).

The $Na^+$ dependence of [$^3$H]CFT binding represents an additional similarity among the $Na^+$-dependent transporters for serotonin, norepinephrine, and dopamine. All three require $Na^+$ and $Cl^-$ for transport (6, 29), and all are inhibited by cocaine (11, 12, 15) and tricyclic antidepressants (11, 12, 13). The serotonin transporter, whose mechanism is well understood (5), can now provide a useful model system for insight into the details of CFT and cocaine binding to this family of biogenic amine transport proteins.

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