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Binding of the Cocaine Analog 2β-Carbomethoxy-3β-(4-[^{125}I]iodophenyl)tropane to Serotonin and Dopamine Transporters: Different Ionic Requirements for Substrate and 2β-Carbomethoxy-3β-(4-[^{125}I]iodophenyl)tropane Binding

STEPHEN C. WALL, ROBERT B. INNIS, and GARY RUDNICK

Departments of Pharmacology (S.C.W., G.R.) and Psychiatry (R.B.I.), Yale University School of Medicine, New Haven, Connecticut 06510

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SUMMARY

The iodinated cocaine analog 2β-carbomethoxy-3β-(4-[^{125}I]iodophenyl)tropane (β-[^{125}I]CIT) binds with high affinity to the platelet plasma membrane serotonin transporter, as previously reported for dopamine transporters from rat brain [*Eur. J. Pharmacol.* **194:**133–134 (1991)]. Unlabeled β-CIT also inhibits serotonin transport by platelet membrane vesicles. In both rat striatal membranes and platelet plasma membranes, β-[^{125}I]CIT binding was found to be pH dependent, with a pK_a of 6.4–6.9, and did not require the presence of Cl^-. Na^+ dramatically stimulated β-[^{125}I]CIT binding to both serotonin and dopamine transporters, although a small fraction of β-[^{125}I]CIT binding to the serotonin transporter was observed in the absence of Na^+. The substrates serotonin and dopamine competed with β-[^{125}I]CIT for binding to their respective transporters. However, substrate affinity was enhanced by Cl^-, whereas β-[^{125}I]CIT binding affinity was not. [^{3}H]Imipramine binding to the platelet serotonin transporter and [^{3}H]GBR-12935 binding to the dopamine transporter were not inhibited by decreasing the pH from 8 to 6.5. Likewise, the ability of serotonin to compete with [^{3}H]imipramine binding and that of dopamine to inhibit [^{3}H]GBR-12935 binding were equal at pH 6.5 or 8. Thus, β-[^{125}I]CIT binding to biogenic amine transporters is distinct from serotonin or dopamine binding by virtue of its inhibition by H^+ and its insensitivity to Cl^-.

The plasma membrane neurotransmitter transporters are responsible for taking up released transmitter into presynaptic nerve terminals, thereby terminating the action of the transmitter in the synapse. A family of neurotransmitter transporters (1, 2) has been defined by the recent isolation of cDNAs whose introduction into mammalian cells leads to expression of high affinity transport of γ-aminobutyric acid (3), norepinephrine (4), dopamine (5–8), serotonin (9, 10), proline (11), choline (12), and glycine (13–15). Primary amino acid sequences, deduced from the sequences of these cloned cDNAs, indicate a strong homology between these transporters and suggest that they use similar mechanisms to transport their substrate amines across the presynaptic plasma membrane. The transporters for the biogenic amine neurotransmitters serotonin, norepinephrine, and dopamine represent a distinct group within this family. A common factor uniting the biogenic amine transporters is their sensitivity to inhibition by cocaine (16–19).

Of these three proteins, the serotonin transporter is the best understood in terms of its catalytic mechanism and ligand-binding properties (20). Studies with plasma membrane vesicles isolated from blood platelets demonstrated that serotonin is transported into the cell together with Na^+ (21) and Cl^- (22) and that K^+ is transported out of the cell in a separate step of the reaction cycle (23). The sequences of central and peripheral rat serotonin transporter cDNAs are almost identical (9, 10), suggesting that results obtained with the platelet serotonin transporter also apply to the brain serotonin transporter. This transporter is a target for three classes of psychoactive drugs. In addition to cocaine, the serotonin transporter is inhibited by a class of compounds, including imipramine, used in the clinical treatment of depression (24). Antidepressant binding to serotonin transporters has been a subject of intense study over the last decade (25). Amphetamine derivatives such as (3,4-methylenedioxy)methamphetamine (ecstasy) and *p*-chloroamphetamine also inhibit serotonin transport but additionally act as substrates to release serotonin by a process of transporter-mediated exchange (26, 27). The observation that a single cDNA can confer cocaine- and imipramine-sensitive serotonin transport indicates that transport and binding are

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ABBREVIATIONS: CFT, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane; CIT, 2β-carbomethoxy-3β-(4-iodophenyl)tropane; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

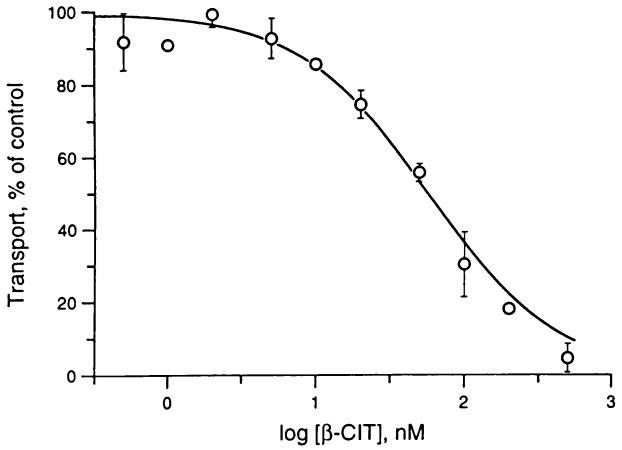

Fig. 1. Inhibition of serotonin transport by β-CIT. Serotonin transport by platelet plasma membrane vesicles was measured as described in Experimental Procedures, with the addition of the indicated concentrations of β-CIT to the assay medium. The control rate of serotonin influx in the absence of β-CIT was 52.8 pmol/min/mg. The negative control value, obtained in the presence of 1 μM imipramine, was 24.1 pmol/min/mg.

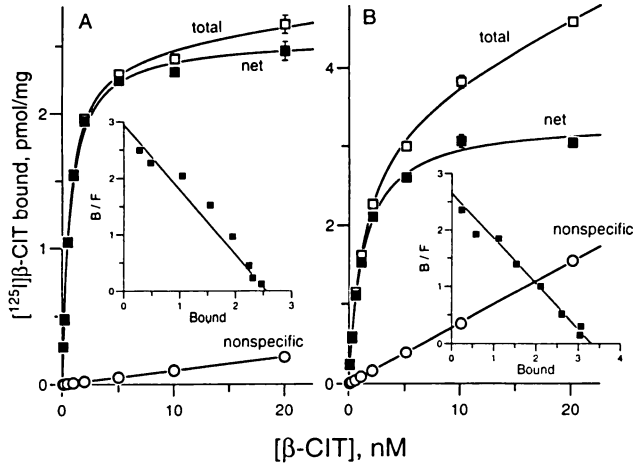

Fig. 2. β-CIT binding to serotonin and dopamine transporters. Binding to platelet (A) and striatal (B) membranes was measured as described in Experimental Procedures. The reaction mixtures contained 300 mM NaCl (A) or 150 mM Na_2SO_4 (B), 10 mM lithium borate, pH 9.5, and 1 mM $MgSO_4$, with 0.05 nM β-[^{125}I]CIT and unlabeled β-CIT added to the indicated total concentration. □, Total binding; ○, nonspecific binding measured in the presence of 100 μM cocaine; ■, net (total − nonspecific) binding. The lines are nonlinear regression fits of the data assuming a single class of binding sites.

mediated by a single protein. Thus, previous suggestions that imipramine binding was due to a modulatory receptor separate from the transporter (28, 29) are almost certainly incorrect.

Comparisons of the behavioral effects of cocaine and its congeners with their affinity for biogenic amine transporters have led to the conclusion that the dopamine transporter may be the primary target for the reinforcing properties of cocaine (30). However, inhibitors of serotonin transport influence the response to cocaine in drug discrimination trials (31), and the potential exists for norepinephrine and serotonin transporters to mediate some of the behavioral effects of cocaine.

Previous studies from this laboratory suggest that all ligands for the serotonin transporter bind to the same site (32). In particular, binding of the tricyclic antidepressant imipramine and the cocaine analog CFT were found to be mutually competitive and occupied the same number of binding sites on the platelet plasma membrane (33). Nevertheless, significant differences exist between cocaine and [^{3}H]imipramine binding to the serotonin transporter. [^{3}H]Imipramine binding is stimulated by Na^+ and Cl^- and is relatively insensitive to pH. Binding of cocaine and [^{3}H]CFT also requires Na^+, but [^{3}H]CFT binding is not stimulated by Cl^- and is strongly inhibited below pH 7 (33). These results raised the possibility that cocaine analogs and tricyclic antidepressants interact with different amino acid residues at the binding site.

β-CIT (RTI-55) (34) has recently been introduced as a high affinity ligand for dopamine transporters. To compare cocaine binding sites on serotonin and dopamine transporters and to extend our previous results with [^{3}H]CFT, we examined the binding of β-[^{125}I]CIT to serotonin and dopamine transporters. Our results suggest that the cocaine sites on these two transporters are very similar and that the ionic requirements for substrate binding are different from those for cocaine binding.

Experimental Procedures

Materials

β-[^{125}I]CIT (2200 Ci/mmol) was synthesized as described previously for β-[^{123}I]CIT (35). [^{3}H]Imipramine (40.4 Ci/mmol) and [^{3}H]GBR-12935 (10–30 Ci/mmol) were purchased from New England Nuclear. Unlabeled β-CIT was purchased from Research Biochemicals Inc. (Natick, MA). All other chemicals used were reagent grade, purchased from commercial sources. Platelet plasma membrane vesicles were prepared from human platelet concentrate obtained from the American Red Cross (Farmington, CT) as described previously (36). 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. Dissected rat striata were homogenized in 20 volumes of 150 mM Li_2SO_4 buffer containing 10 mM lithium borate, pH 8.0, and 1 mM $MgSO_4$, using a Brinkmann Polytron (Westbury, NY) (setting 6 for 30 sec). The homogenate was washed twice by centrifugation for 10 min at 50,000 × *g* and resuspension in the same buffer. The final membrane suspension was distributed into microcentrifuge tubes and sedimented by centrifugation. After removal of the supernatant fluid, the pellets were frozen and stored at −80° until use.

Methods

Binding measurements. For β-[^{125}I]CIT binding, approximately 50–70 μg of platelet plasma membrane vesicle protein at a concentration of 8–10 mg/ml or approximately 15–20 μg of rat striatal membrane protein at a concentration of 1.5 mg/ml were diluted with the indicated reaction mixture to a final volume of 300 μl, containing approximately 0.05 nM β-[^{125}I]CIT (in some cases, unlabeled CIT was added to vary the total concentration), and were incubated at 25° for 30 min. In experiments with striatal membranes, 5 μM citalopram was added to prevent binding to serotonin transporters. 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 15 min. After this incubation, the reaction was terminated by addition of 4 ml of ice-cold 0.2 M NaCl. The membranes in the diluted reaction mixture were collected by filtration through no. 32 glass fiber filters that had been pretreated with 0.5% polyethyleneimine (Schleicher & Schuell, Keene, NH), and the tube and filter were washed with 2 × 4 ml of ice-cold 0.2 M NaCl. Dilution, filtration, and washing took between 5 and 8 sec. Separate experiments indicated that no β-[^{3}H]CIT dissociation occurred under these conditions within the first 60 sec after dilution. Filters were placed in Optifluor (Packard, Downers Grove, IL) and counted after 5 hr.

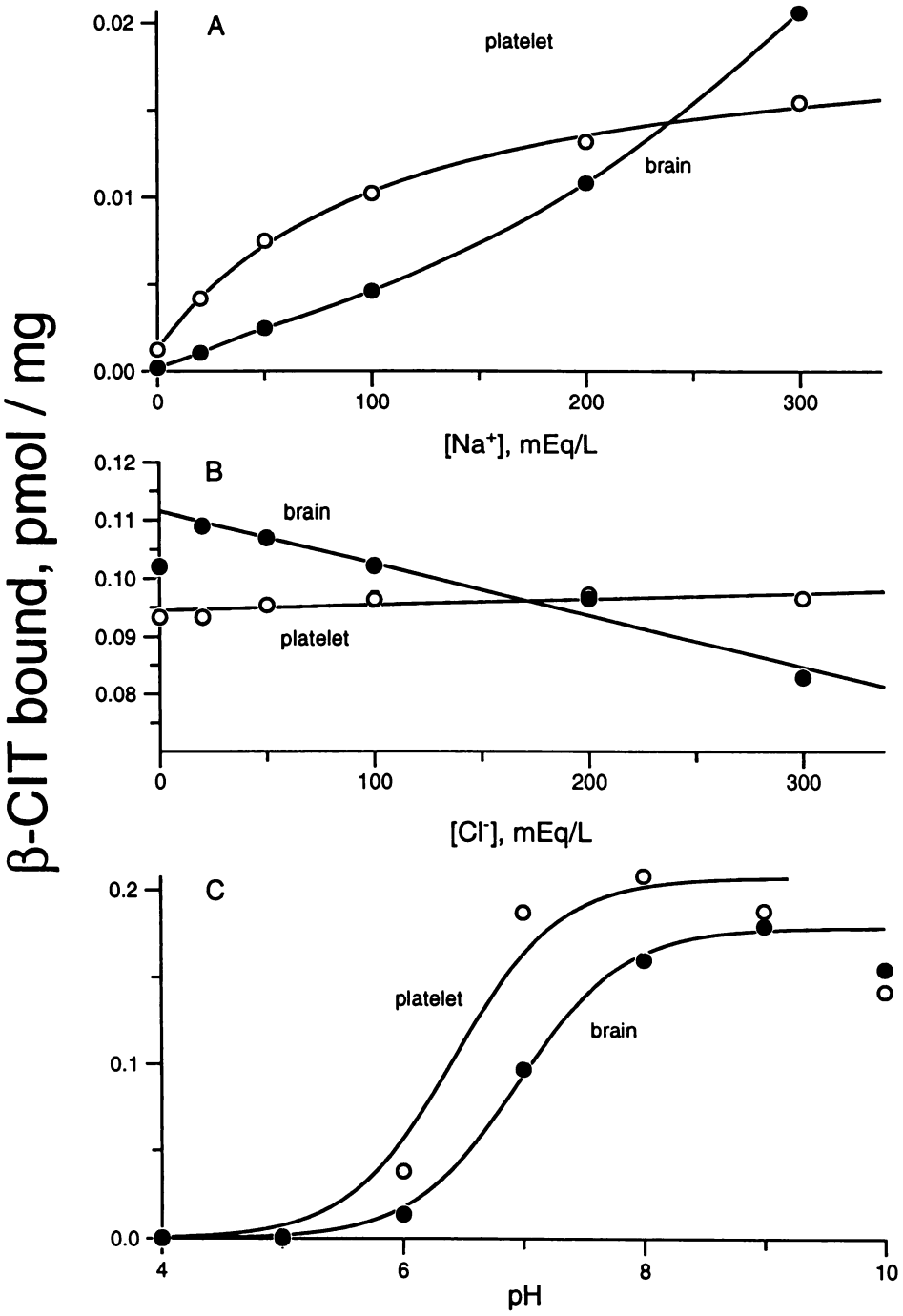

Fig. 3. Ion dependence of β-CIT binding. A, Na^+ dependence. β-[^{125}I]CIT binding was measured as described in Experimental Procedures, using a reaction mixture consisting of 150 mM Na_2SO_4, 10 mM lithium borate, pH 9.5, and 1 mM $MgSO_4$, with 0.05 nM β-[^{125}I] CIT. The indicated concentrations of Na^+ were obtained by isoosmotic replacement of Na_2SO_4 with Li_2SO_4. B, Cl^- dependence. Binding was measured as in the experiment described in A, except that the Cl^- concentration was adjusted by replacing Na_2SO_4 with isotonic NaCl. C, pH dependence. Binding was measured as described in A with reaction mixtures adjusted to the indicated pH with 10 mM sodium citrate (pH 4 and 5), sodium *N*-(2-acetamido)-2-iminodiacetate (pH 6 and 7), sodium *N*-(2-hydroxyethyl)piperazine-*N'*-(3-propanesulfonic acid (pH 8), or sodium borate (pH 9 and 10).

Imipramine binding was measured at 25° using the filtration assay described previously (36). Briefly, to initiate binding, membrane vesicles were suspended at a protein concentration of 0.3 mg/ml in an assay buffer of 200 mM NaCl containing 10 mM lithium borate, pH 8.0, or lithium HEPES, pH 6.5, and 1 mM $MgSO_4$. The assay buffer also contained [^{3}H]imipramine (19–23 cpm/fmol) at the indicated concentration. After a 30-min incubation, the reactions (300 μl/assay) were terminated by dilution with 4 ml of ice-cold iso-osmotic NaCl and were filtered through Schleicher & Schuell no. 32 glass fiber filters that had been pretreated with 0.5% polyethyleneimine. The tube and filter were washed twice with 4 ml of ice-cold NaCl solution and the filter was counted as described above.

GBR-12935 binding was determined using the same procedure described for imipramine binding measurements, except that the reaction mixtures contained 300 mM NaCl.

Transport measurements. Transport rates were measured at 25° as described previously (36), 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, Arlington Heights, IL). Initial rates were measured 20 sec after dilution. In separate experiments we determined that transport was linear with time for at least 20 sec.

Data analysis. Saturation data for transport, binding, and inhibition were analyzed by nonlinear regression using the Simplex algorithm and are presented with calculated standard errors.

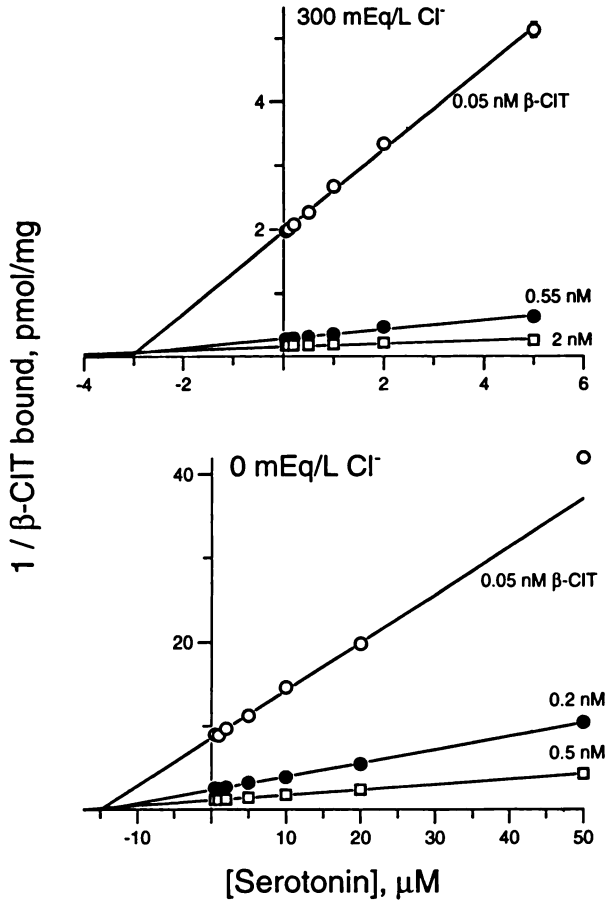

Fig. 4. Competitive inhibition of β-CIT binding to platelet membranes by serotonin at 0 and 300 meq/liter Cl^-. β-[^{3}H]CIT binding was measured as described in the legend to Fig. 3B, using added unlabeled β-CIT to adjust the concentration. At each β-CIT concentration, a range of serotonin concentrations was used to inhibit binding. The *x*-axis value at which all three lines intersect represents the negative of the K_i for serotonin. This K_i was 2.93 ± 0.05 μM under control conditions (*upper*) and 14.95 ± 0.24 μM under conditions where Cl^- was replaced by sulfate (*lower*).

Results

As a high affinity ligand for the dopamine transporter, the cocaine analog β-CIT was expected to inhibit dopamine transport. The similarity between serotonin and dopamine transporters suggested that serotonin transport may also be a target for this compound. We measured the ability of β-CIT to inhibit serotonin transport into plasma membrane vesicles isolated from human platelets. As shown in Fig. 1, β-CIT was a potent inhibitor of serotonin transport, with half-maximal inhibition occurring at 75 ± 13 nM.

Using platelet plasma membrane vesicles and rat striatal membranes, we measured binding of β-[^{125}I]CIT to serotonin and dopamine transporters, respectively. Fig. 2A shows an equilibrium binding isotherm for β-[^{125}I]CIT binding to platelet plasma membranes in the presence and absence of 100 μM cocaine. The cocaine-sensitive binding saturated with a K_d of 0.73 ± 0.10 nM and a B_{max} of 2.55 ± 0.09 pmol of CIT bound/mg of membrane protein. Although the number of binding sites was similar to that measured in these membranes using [^{3}H]imipramine or [^{3}H]CFT (33), the affinity was significantly higher. In rat striatal membranes (Fig. 2B), we obtained similar results, with the exception that nonspecific binding represented a larger fraction of the total. Citalopram was added at 5 μM to inhibit binding to striatal serotonin transporters. The cocaine-sensitive binding saturated with a K_d of 1.25 ± 0.08 nM and a B_{max} of 3.31 ± 0.06 pmol of CIT bound/mg of membrane protein. In both membrane preparations, cocaine-sensitive β-[^{125}I]CIT binding in the 0.1–20 nM concentration range followed a simple saturation profile consistent with a single class of binding sites.

Because of the difference in apparent affinity calculated from direct binding (0.73 nM) (Fig. 2) and transport (75 nM) (Fig. 1) measurements, we investigated the possibility that β-[^{125}I]CIT binding was too slow to come to equilibrium within the 20-sec transport assay. The time course of β-[^{125}I]CIT binding indicated that, under the conditions used, the binding $t_{1/2}$ was 2.99 min (data not shown). Accordingly, we inferred that during the time of the transport measurement much higher concentrations of β-CIT, relative to its K_d, would be required to inhibit transport.

To examine the ionic dependence of β-[^{125}I]CIT binding, we measured binding to platelet plasma membranes and striatal membranes in media of different Na^+ and Cl^- concentrations and at different pH values. Na^+ and Cl^- were replaced isotonically with Li^+ and sulfate, respectively. With both the serotonin and dopamine transporters, binding was dramatically stimulated in the presence of Na^+ (Fig. 3A). The Na^+ dependence of β-CIT binding to the dopamine transporter was sigmoidal, suggesting the possibility that more than one Na^+ ion is involved in binding. In contrast to the effect of Na^+, Cl^- did not stimulate binding and actually inhibited binding slightly in the case of the striatal dopamine transporter (Fig. 3B). We previously reported that [^{3}H]CFT binding is maximal at high pH and decreases dramatically below pH 7 (33). The data in Fig. 3C demonstrate a similar pH dependence for β-[^{125}I]CIT binding to both serotonin and dopamine transporters. The pH dependence for binding to the serotonin transporter in platelet plasma membrane showed a pK_a of approximately 6.42, whereas in rat brain the pK_a was approximately 6.95.

Because the Cl^- dependence of [^{3}H]CFT binding to platelet serotonin transporters and β-[^{125}I]CIT binding to both serotonin and dopamine transporters was so different from that of [^{3}H]imipramine binding to platelet membranes (33, 37), we measured the Cl^- dependence of serotonin and dopamine binding to their respective transporters. The relative Cl^- insensitivity of β-[^{125}I]CIT binding allowed us to measure the substrate affinity of the transporters at both high and low Cl^- concentrations. Fig. 4 shows the competitive inhibition of β-[^{125}I]CIT binding by serotonin at 0 and 300 meq/liter Cl^-. In these Dixon plots, increasing concentrations of serotonin progressively inhibited β-[^{125}I]CIT binding, as evidenced by the increase in 1/CIT bound. For competitive inhibition, the slope of the line should decrease as the CIT concentration increases, and lines representing different β-CIT concentrations should intersect at a point to the left of the *y*-axis. The K_i for serotonin, given by the negative of the *x*-value at the point where the lines intersect, was 14.9 ± 0.2 μM in the absence of Cl^- and 2.93 ± 0.05 μM at 300 meq/liter Cl^-. Similarly, in the case of rat striatal membranes dopamine competitively displaced β-[^{125}I]CIT from the dopamine transporter (Fig. 5). The K_i determined for dopamine was 517 ± 80 μM in the absence of Cl^- and 80.5 ± 0.6 μM at 300 meq/liter Cl^-. In both cases, Cl^- increased the substrate affinity >5-fold.

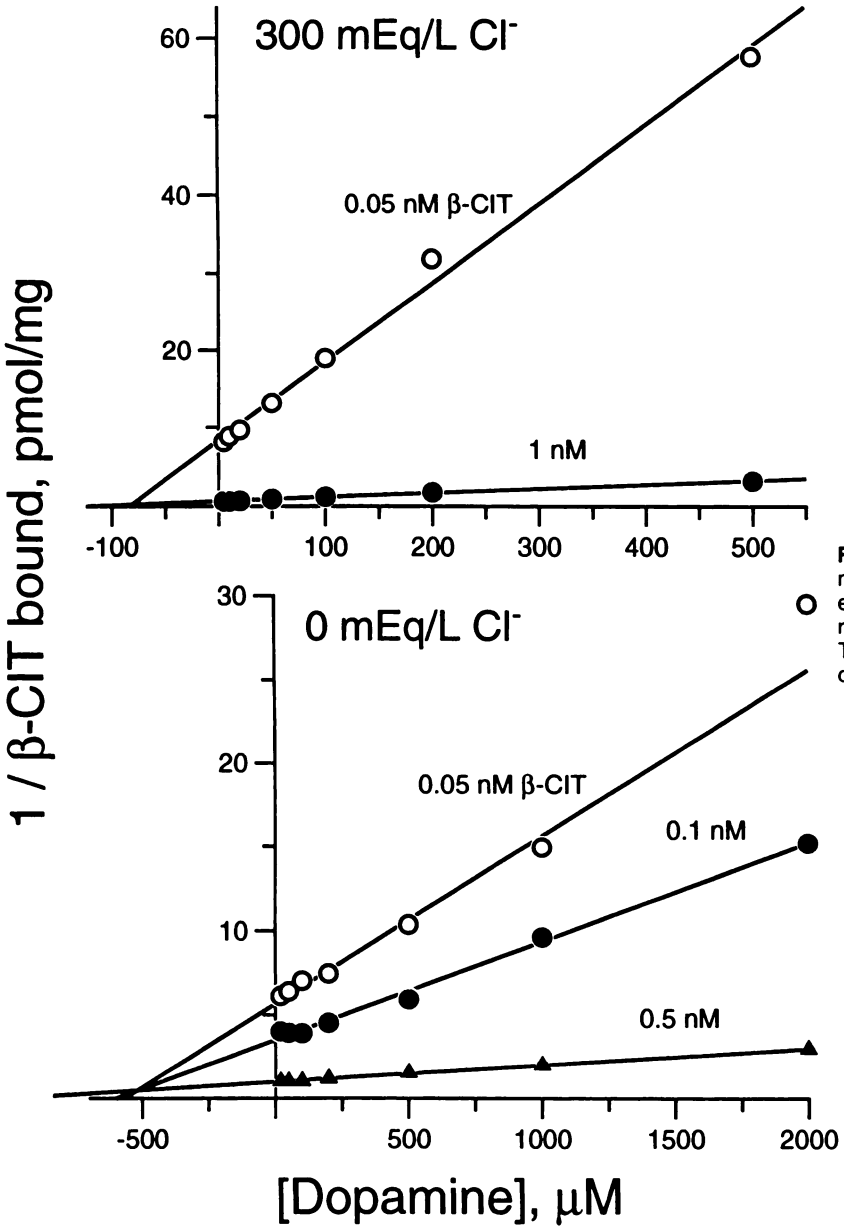

Fig. 5. Competitive inhibition of β-CIT binding to striatal membranes by dopamine at 0 and 300 meq/liter Cl^-. This experiment was performed as described for Fig. 4, using rat striatal membranes and displacing CIT with dopamine. The K_i calculated for dopamine was 80 ± 0.6 μM in the control and 517 ± 80 μM in the absence of Cl^-.

Similarly, we took advantage of the fact that imipramine binding is similar at pH 6.5 and 9.5 (33) to measure the pH dependence of serotonin binding to the platelet plasma membrane serotonin transporter. Fig. 6 shows the competitive displacement of equilibrium [^{3}H]imipramine binding by serotonin at pH 6.5 (Fig. 6A) and 8.0 (Fig. 6B). At both pH values, the apparent K_d (negative reciprocal slope in the Scatchard plot) was increased by the presence of 2 μM serotonin, and the B_{max} (x-axis intercept) was essentially unchanged. From these data, the K_i for serotonin was 0.75 ± 0.06 μM at pH 6.5 and 0.92 ± 0.12 μM at pH 8. We also tested [^{3}H]GBR-12935 binding to rat striatal membranes to determine its pH dependence. Essentially the same amount of binding was observed at the two pH values (Fig. 6C). We therefore measured the ability of dopamine to displace equilibrium [^{3}H]GBR-12935 binding at pH 6.5 and 8.0, to determine the influence of pH on dopamine binding to the dopamine transporter. The results in Fig. 6C suggest that dopamine binds more avidly at low pH, where β-[^{125}I]CIT binding is markedly inhibited. Half-maximal inhibition of [^{3}H]GBR-12935 binding occurred at 12.2 ± 2.7 μM dopamine at pH 6.5 and at 27.9 ± 5.3 μM at pH 8.0.

Discussion

The results presented here strongly suggest that the serotonin and dopamine transporters bind cocaine analogs at essentially similar sites and that cocaine analogs bind in a manner clearly distinguishable from that of the natural substrates serotonin and dopamine. These findings establish the functional similarity between the serotonin and dopamine transporters, a similarity expected in light of their homologous primary sequences (5–7, 9, 10). Members of the plasma membrane biogenic amine transporter family, including the dopamine, norepinephrine, and serotonin transporters, are expected to have much in common, including similar mechanisms of transport and inhibitor binding. Although much is known about the mechanism of serotonin transport (20) and somewhat less

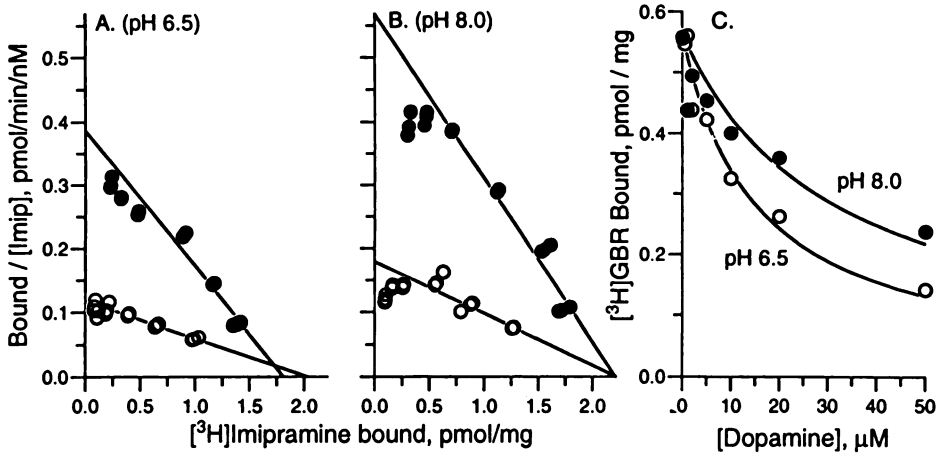

Fig. 6. Effect of pH on displacement of ligand binding by serotonin and dopamine. A and B, Scatchard plots showing competitive inhibition by serotonin of [^{3}H]imipramine binding at pH 6.5 (A) and 8.0 (B). The K_i values calculated for serotonin were 0.75 ± 0.06 μM at pH 6.5 and 0.92 ± 0.12 μM at pH 8. ●, Absence of serotonin; ○, presence of 2 μM serotonin. C, Inhibition of [^{3}H]GBR-12935 binding to rat striatal membranes at pH 6.5 (○) and 8.0 (●). Equilibrium binding was performed as described in Experimental Procedures.

is known about norepinephrine transport (38, 39), the mechanism of dopamine transport, aside from its external ion requirements, is almost completely unknown.

β-[^{125}I]CIT binds tightly to both serotonin transporters in platelet plasma membrane and dopamine transporters in rat striatum. We previously determined (33) that [^{3}H]CFT bound less well to the serotonin transporter than had been reported for the dopamine transporter (40). The substitution of iodine for fluorine apparently changes the relative affinity for the two transporters so that β-CIT binds more tightly to the serotonin transporter than to the dopamine transporter (Fig. 2), although the opposite order of affinity was found with CFT (33, 40).

As reported previously for CFT binding to the platelet serotonin transporter (33), Cl^- does not stimulate β-[^{125}I]CIT binding to either serotonin or dopamine transporters (Fig. 3B). This is in contrast to the absolute requirement for Cl^- in transport (22, 41–43) and the dramatic stimulation of imipramine binding by Cl^- (33, 37). The relative insensitivity of β-[^{125}I]CIT binding to Cl^- allowed us to determine the Cl^- dependence of substrate binding to the two transporters by measuring the ability of serotonin or dopamine to displace β-[^{125}I]CIT at high and low Cl^- concentrations. The results in Figs. 4 and 5 represent the first demonstration of Cl^--dependent substrate binding to the serotonin and dopamine transporters. Thus, Cl^- ion participates in transport both by increasing substrate affinity and by serving as a cotransported substrate in the subsequent translocation step (22).

β-[^{125}I]CIT binding to both serotonin and dopamine transporters is highest at pH 8–9 and decreases dramatically below pH 7 (Fig. 3C). The apparent pK_a for CIT binding is approximately 6.7, a value similar to the pK_a for [^{3}H]CFT binding to the serotonin transporter (33). We previously suggested that this represented the pK_a of the cocaine analog, but the pK_a of cocaine is approximately 8.6 (44–46) and not 5.9 as stated in the Merck Index and reported in our earlier study (33). CFT and β-CIT are expected to have the same pK_a as cocaine. The titratable group responsible for inhibition of β-[^{125}I]CIT and [^{3}H]CFT binding is, therefore, likely to be an amino acid residue of the transporter and not the amino group of the ligand. Protonation of this residue, although it prevents [^{3}H]CFT binding to the serotonin transporter and β-[^{125}I]CIT binding to both the serotonin and the dopamine transporters, does not inhibit [^{3}H]imipramine binding to the serotonin transporter (Fig. 6A and Ref. 33) or [^{3}H]GBR-12935 binding to the dopamine transporter (Fig. 6B). Furthermore, serotonin and dopamine binding to their respective transporters is similar at pH 6.5 and 8.0, as judged by their ability to compete with imipramine or GBR-12935 binding (Fig. 6).

In the absence of added Na^+, we detected a small but significant amount of β-[^{125}I]CIT binding to the serotonin transporter. Results to be reported elsewhere indicate that this binding is displaced by serotonin, imipramine, and citalopram. β-CIT is the first high affinity ligand for the serotonin transporter shown to have significant affinity in the absence of Na^+, although Reith *et al.* (47, 48) reported that lower affinity [^{3}H]cocaine binding to brain and platelet membranes was Na^+ independent.

The binding site for cocaine and its analogs, as defined by the work presented here, demonstrates properties distinctly different from those of the site where substrates and antidepressant drugs bind to the serotonin and dopamine transporters. The sensitivity to pH and the lack of stimulation by Cl^- stand out in contrast to the relative pH insensitivity and striking stimulation by Cl^- that are characteristic of dopamine, serotonin, and imipramine binding. As model compounds for studying the substrate binding site, antidepressants like imipramine would seem to mimic substrate more accurately than do cocaine analogs, although differences between serotonin and imipramine binding have been noted (21). The ability of serotonin and dopamine to competitively displace [^{3}H]CFT and β-[^{125}I]CIT argues that the substrate and cocaine sites are closely linked, despite their differing ion requirements. It is likely that the binding sites for substrate and cocaine are formed, in part, by the same amino acid residues but that each ligand additionally interacts with unique residues. Substrates may interact with one or more residues whose accessibility is dependent on Cl^-, whereas cocaine and its derivatives may interact with a protonatable residue. From the amino acid sequences predicted from the cDNAs (4, 7, 10), 35-amino acid residues are identical in the cocaine-sensitive serotonin, dopamine, and norepinephrine transporters and different from corresponding residues in other members of the gene family that are insensitive to co-

caine. Of these 35 residues, eight (including Asp-98, Glu-322, and Asp-524) are protonatable. We previously proposed that antidepressants bind to the serotonin transporter by interacting with a collection of contiguous subsites within the binding pocket of the transporter (32). The identities of these substrate- and cocaine-binding residues will now be the subject of intense investigation.

Acknowledgments

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Send reprint requests to: Gary Rudnick, Department of Pharmacology, Yale University School of Medicine, Sterling Hall of Medicine, P.O. Box 3333, New Haven, CT 06510-8066.