Heterogeneity of Antidepressant Binding Sites on the Recombinant Rat Serotonin Transporter SERT1[†]

Patrick Schloss* and Heinrich Betz

Abteilung Neurochemie, Max-Planck-Institut für Hirnforschung, Deutschordenstrasse 46, 60528 Frankfurt, Germany

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ABSTRACT: Antidepressant drugs block the uptake of serotonin into serotonergic nerve terminals and blood platelets. Here, binding of the tricyclic antidepressant [^{3}H]imipramine to the recombinant rat serotonin transporter SERT1 expressed in human embryonic kidney cells was found to be nonhomogeneous. Scatchard analysis and competition experiments revealed the existence of two distinct antidepressant binding sites. At site 1, [^{3}H]imipramine binding was strictly sodium-dependent with an apparent K_D of ~10 nM. In contrast, [^{3}H]imipramine binding to site 2 occurred also in the absence of sodium and exhibited a lower affinity. Binding of the nontricyclic antidepressant [^{3}H]citalopram was observed only at site 2. The natural substrate of this carrier, serotonin, competitively inhibited antidepressant binding at both sites; however, its affinity to site 2 was ~5-fold lower. These data provide a molecular explanation for the distinct pharmacological actions of different antidepressants.

In the central nervous system, serotoninergic neurotransmission is terminated by high-affinity uptake of serotonin (or 5-hydroxytryptamine, 5-HT)[1] from the synaptic cleft. The 5-HT transporter is sodium-dependent and represents the target site of antidepressant drugs, which inhibit 5-HT reuptake and are widely used for the treatment of depression. In general, these drugs can be classified into two groups, the tricyclic antidepressants, such as imipramine and clomipramine, and the nontricyclic selective serotonin reuptake inhibitors, such as citalopram and fluoxetin (Brosen, 1993; Hyttel, 1994). However, the molecular basis of their pharmacological effects and the interactions between these two groups of antidepressants are only poorly understood.

So far, most investigations of antidepressant binding sites have been performed on human blood platelets, since these are a rich source of 5-HT transporter. From radioligand binding experiments, it has been proposed that the different antidepressants bind to a common recognition site on the serotonin transporter in human platelets, which then allosterically interacts with the 5-HT binding site (Segonzac et al., 1985). In contrast, chemical modification experiments suggest the existence of two distinct binding sites on the 5-HT transporter, one for tricyclic and another one for nontricyclic uptake inhibitors (Biessen et al., 1988). Furthermore, radioligand competition experiments with rat brain cortical membranes provided evidence that [^{3}H]imipramine binds to a site associated with the serotonin transporter, which differs from the site of action of nontricyclic inhibitors of neuronal uptake of 5-HT (Sette et al., 1983). Some investigators proposed the existence of two distinct [^{3}H]-imipramine binding sites on human platelets (O'Riordan et al., 1990), in mouse cerebral cortex (Reith et al., 1983), and in rat hypothalamus (Moret & Briley, 1986). All these studies, however, were unable to exclude the existence of different 5-HT transporter isoforms or of associated imipramine binding proteins in the synaptosomal or blood platelet preparations used; an unequivocal localization of the different sites on the same 5-HT transporter was therefore not possible.

Recently, cDNAs encoding the rat, human, and *Drosophila* serotonin transporters have been isolated (Hoffman et al., 1991; Blakely et al., 1991; Mayser et al., 1991; Ramamoorthy et al., 1993; Cory et al., 1994). The predicted proteins are highly homologous to each other and represent members of a larger Na^+-driven neurotransmitter transporter superfamily (Amara & Kuhar, 1993; Worrall & Williams, 1994; Schloss et al., 1994). Expression of the rat serotonin transporter (SERT1)[1] in oocytes and eukaryotic cells results in electrogenic (Mager et al., 1994), sodium-dependent high-affinity 5-HT transport, which is sensitive to several antidepressants, including citalopram and imipramine (Hoffman et al., 1991; Blakely et al., 1991). To gain further insight into the antidepressant pharmacology of the cloned 5-HT transporter, we investigated the interactions between citalopram, imipramine, and the natural substrate, serotonin, using the recombinant rat SERT1 expressed in a human kidney cell line. Our data indicate the existence of two interacting antidepressant binding sites on SERT1: an antidepressant binding site (ABS)[1] 1, which binds [^{3}H]imipramine in a strictly sodium-dependent manner, and a second site (ABS2) of lower affinity, which binds [^{3}H]imipramine also in the absence of sodium. The nontricyclic antidepressant [^{3}H]-citalopram binds exclusively to ABS2. Both binding sites also bind the natural substrate, 5-HT; however, binding to ABS2 occurs with reduced affinity. These data provide the first demonstration of two distinct antidepressant/substrate binding sites on a single mammalian 5-HT transporter.

EXPERIMENTAL PROCEDURES

Chemicals. [^{3}H]Imipramine (21 Ci/mmol) was obtained from Amersham; and [^{3}H]citalopram (86.5 Ci/mmol) from

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[1] Abbreviations: ABS, antidepressant binding site; 5-HT, 5-hydroxytryptamine; SERT1, serotonin transporter; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid; PBS, phosphate-buffered saline.

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New England Nuclear. Unlabeled imipramine was purchased from ICN Biochemicals; unlabeled citalopram was a gift from J. Hyttel, H. Lundbeck A/S, Copenhagen-Valby, Denmark.

Expression of SERT1 cDNA in HEK-293 Cells. HEK-293 cells were grown and transfected as described elsewhere (Sontheimer et al., 1989; Chen & Okayama, 1987). For heterologous expression, the coding sequence of SERT1 including 163 bp of 5′ untranslated sequence and 653 bp of 3′ untranslated sequence (Mayser et al., 1991) was subcloned into the *XhoI*/*Hpa*I sites of the eukaryotic expression vector pCIS (Gormann et al., 1989). The resulting clone, termed pSERT, was used throughout this study.

Preparation of Membranes. For binding experiments, cells were plated into 10 cm tissue culture dishes and transfected with pSERT1 or insertless pCIS (control) DNA (10 μg/dish) as described (Chen & Okayama, 1987); 28–36 h after transfection, the cells from each dish were harvested into 2 mL of phosphate-buffered saline (PBS)[1] and homogenized on ice in a Potter homogenizer. Nuclei and debris were removed by centrifugation at 1000*g* for 5 min at 4 °C, and membranes were collected by centrifugation at 20000*g* for 20 min at 4 °C. After resuspension in PBS containing 5% (w/v) glycerol at a protein concentration of about 1 mg/mL, the membranes were frozen on liquid nitrogen and stored in aliquots at −70 °C. Protein concentrations were determined according to Lowry et al. (1951), using bovine serum albumin as a standard.

[^{3}H]Imipramine and [^{3}H]Citalopram Binding. For the determination of antidepressant binding, 10–20 μg of membrane protein was incubated in a final volume of 200 μL of buffer TB1 (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.5) with the indicated concentrations of radioactive ligands. After 30 min at room temperature, i.e., a time sufficient to reach binding equilibrium, the samples were diluted with 4 mL of ice-cold TB1 and rapidly filtered under vacuum through Whatman GF/C glass fiber filters presoaked in 0.3% (w/v) polyethylenimine. The filters were washed with another 4 mL of TB1, and the radioactivity retained on the filters was determined by scintillation counting using a Beckman LS60001IC scintillation counter. Specific binding was defined as the difference between the binding to membranes from pSERT transfected cells and the binding to membranes from control cells, which were transfected with the pCIS vector alone. Competition experiments with a >100-fold excess of unlabeled ligand showed that the unspecific binding defined by control membranes was identical to that seen by ligand competition (not shown, but see Figure 2). Each binding experiment was repeated at least twice; all data represent the means of triplicate determinations, and standard deviations were routinely <7%. Fits for Scatchard plots were derived from linear regression analysis of the data; a nonlinear regression analysis program (XACT, Atari) was used to analyze competition experiments.

RESULTS

[^{3}H]Citalopram and [^{3}H]Imipramine Binding to the Recombinant SERT1 Protein. Functional expression of the SERT1 cDNA was achieved by transfecting HEK-293 cells with the pSERT plasmid, using pCIS as a negative control. Membrane preparations of the transfected cells then were incubated with radioactively labeled antidepressant in the absence or presence of a competing drug. To evaluate the affinities of [^{3}H]citalopram and [^{3}H]imipramine binding to recombinant SERT1, we analyzed the concentration dependence of specific ligand binding (Figure 1). The K_D values calculated from Scatchard plots (insets) were 2.0 ± 1.9 nM (n = 6) for [^{3}H]citalopram and 9.6 ± 2.7 nM for [^{3}H]-imipramine (n = 5). The corresponding numbers of binding sites (B_{max}) varied from 4 to 40 pmol/mg of protein, depending on transfection efficiency (~10–80% in different experiments as determined by parallel transfections with a LacZ carrying expression vector). However, the ratio of the B_{max} values for [^{3}H]citalopram and [^{3}H]imipramine in single membrane preparations consistently was close to 1 (B_{max}[imipramine]/B_{max}[citalopram] = 0.92 ± 0.03, n = 4).

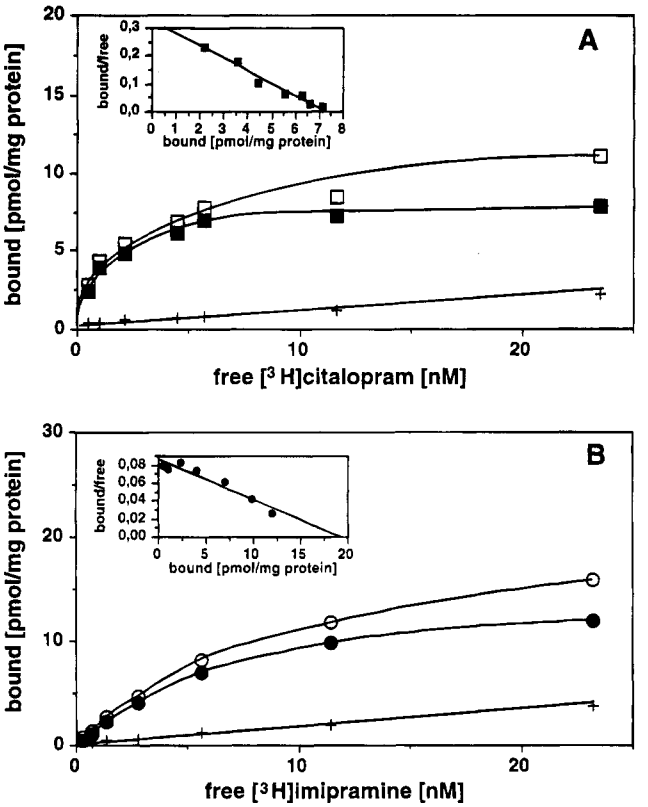

FIGURE 1: Saturation analysis of [^{3}H]citalopram (A) and [^{3}H]-imipramine (B) binding to HEK-293 cell membranes. Ligand binding to SERT containing membranes (□/○) and to control membranes (+) was determined as described in Experimental Procedures. The difference between those was defined as specific binding (■/●). K_D and B_{max} values obtained in these particular experiments were 1.2 nM and 7.2 pmol/mg of protein for [^{3}H]-citalopram and 11.0 nM and 19.0 pmol/mg of protein for [^{3}H]-imipramine binding, respectively. The insets show the corresponding Scatchard plots; the difference in the B_{max} values reflects the use of different batches of transfected cell membrane preparations.

To determine the inhibitory potencies of citalopram, imipramine, and 5-HT on [^{3}H]citalopram and [^{3}H]imipramine binding, we performed competition experiments at varying inhibitor concentrations. As shown in Figure 2, the binding of both [^{3}H]citalopram and [^{3}H]imipramine was completely inhibited by all substances tested. The calculated IC_{50} values are summarized in Table 1. Interestingly, the inhibitory potencies of all ligands were higher in [^{3}H]imipramine binding than in [^{3}H]citalopram binding assays. This suggested that the two antidepressants may bind to distinct sites on the SERT1 protein.

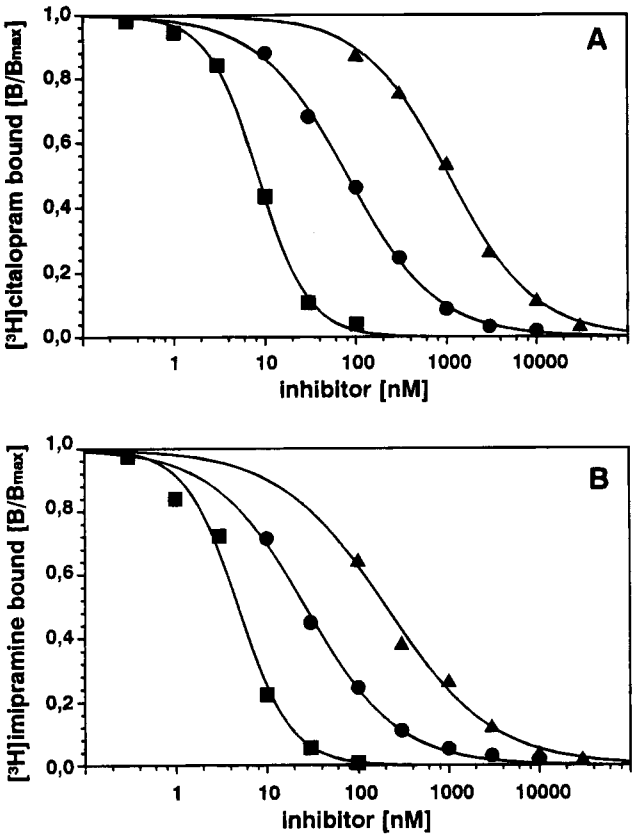

FIGURE 2: Inhibition of [^{3}H]citalopram (A) and [^{3}H]imipramine (B) binding by unlabeled SERT1 ligands. Binding was performed in the presence of the indicated amounts of citalopram (■), imipramine (●), and 5-HT (▲). The sigmoidal curves shown represent fits to the equation $B/B_{max} = IC_{50}^n/(I^n + IC_{50}^n)$, where B/B_{max} represents normalized binding, I the inhibitor concentration, and n the Hill coefficient. The radioligand concentrations were 3 nM for [^{3}H]citalopram and 12 nM for [^{3}H]imipramine, respectively. The IC_{50} values obtained in these experiments were as follows: (A) citalopram, 8.4 nM; imipramine, 79 nM; 5-HT, 1058 nM. (B) citalopram, 4.8 nM; imipramine, 26.3; 5-HT, 201 nM.

Table 1: Inhibition of Antidepressant Binding by Unlabeled SERT1 Ligands[a]

	IC_{50} (nM)	
competitor	[^{3}H]citalopram	[^{3}H]imipramine
citalopram	7.6 ± 0.8	3.7 ± 1.2
imipramine	83.2 ± 11	27.2 ± 4.8
5-HT	1183 ± 154	252 ± 44

[a] Drug concentrations producing half-maximal inhibition (IC_{50}) of [^{3}H]citalopram and [^{3}H]imipramine binding to the recombinant SERT1 were determined as described in Experimental Procedures using 3 nM [^{3}H]citalopram or 12 nM [^{3}H]imipramine, respectively, in the binding assays. Data represent the means ± SD of three independent experiments.

Heterogeneity of Antidepressant Binding Sites. Scatchard analysis of [^{3}H]citalopram binding in the presence of different concentrations of imipramine (Figure 3A) revealed two inhibitory mechanisms. Competition by 10 nM imipramine, i.e., a concentration corresponding to the K_D value found in our binding experiments with [^{3}H]imipramine, consistantly ($n = 4$) reduced the B_{max} of [^{3}H]citalopram binding by 12.2% (±4.2% $p < 0.05$) without affecting the apparent radioligand affinity, indicating a noncompetitive inhibition mechanism. At 100 nM imipramine, however, no further reduction in the B_{max} of [^{3}H]citalopram (85.8 ± 6.9% of control, $p < 0.05$), but a decrease in radioligand affinity was observed

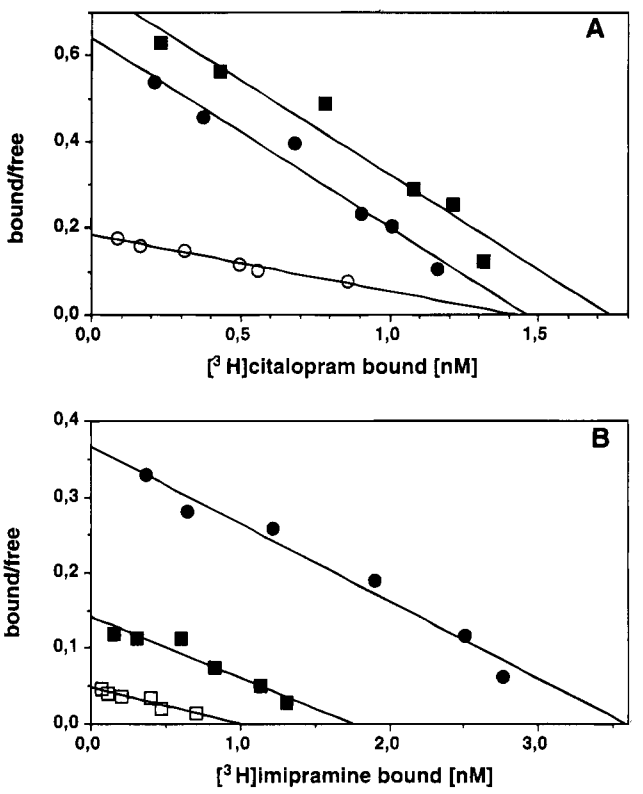

FIGURE 3: Effect of imipramine on [^{3}H]citalopram binding (A) and of citalopram on [^{3}H]imipramine binding (B). (A) [^{3}H]Citalopram binding was performed in the absence (■) and presence of 10 (●) or 100 nM (○) imipramine. The apparent binding parameters obtained in this experiment were as follows: (■), $K_D = 2.2$ nM and $B_{max} = 31.1$ pmol/mg of protein; (●) $K_D = 2.2$ nM and $B_{max} = 26.6$ pmol/mg of protein; (○), $K_D = 7.7$ nM and $B_{max} = 25.4$ pmol/mg of protein. (B) [^{3}H]Imipramine binding was performed in the absence (●) and presence of 1 (■) or 2 nM (□) citalopram. Corresponding binding parameters were as follows: (●) $K_D = 10$ nM and $B_{max} = 36$ pmol/mg of protein; (■), $K_D = 12.5$ nM and $B_{max} = 17.5$ pmol/mg of protein; (□) $K_D = 21$ nM and $B_{max} = 10$ pmol/mg of protein. These experiments were repeated four times with similar results (see text).

(apparent $K_D = 8.9 \pm 1.6$ nM). This points to an additional competitive effect of imipramine on [^{3}H]citalopram binding.

Different results were obtained when Scatchard analysis of [^{3}H]imipramine binding in the presence of citalopram (Figure 3B) was performed. Here, increasing amounts of citalopram caused only a decrease in apparent [^{3}H]imipramine B_{max} values without affecting the affinity of [^{3}H]-imipramine. Thus, citalopram inhibits [^{3}H]imipramine binding purely noncompetitively, whereas imipramine blocks [^{3}H]citalopram binding in a mixed fashion. These complex interactions are most easily explained by the existence of two interacting antidepressant binding sites on the recombinant SERT1: ABS1, which binds imipramine with high affinity ($K_D \approx 10$ nM), and ABS2, which binds imipramine with a lower affinity. [^{3}H]Citalopram recognizes only ABS2, and thus its binding is allosterically inhibited by 10 nM imipramine (this corresponds to a half-maximal saturation of ABS1 but <10% saturation of ABS2), whereas at 100 nM imipramine competitive inhibition is observed in addition. The inhibition by citalopram of [^{3}H]imipramine binding to ABS1 in contrast is entirely noncompetitive.

To further understand the nature of these binding sites, we also analyzed the interactions between 5-HT and the radiolabeled antidepressants (Figure 4). Addition of different

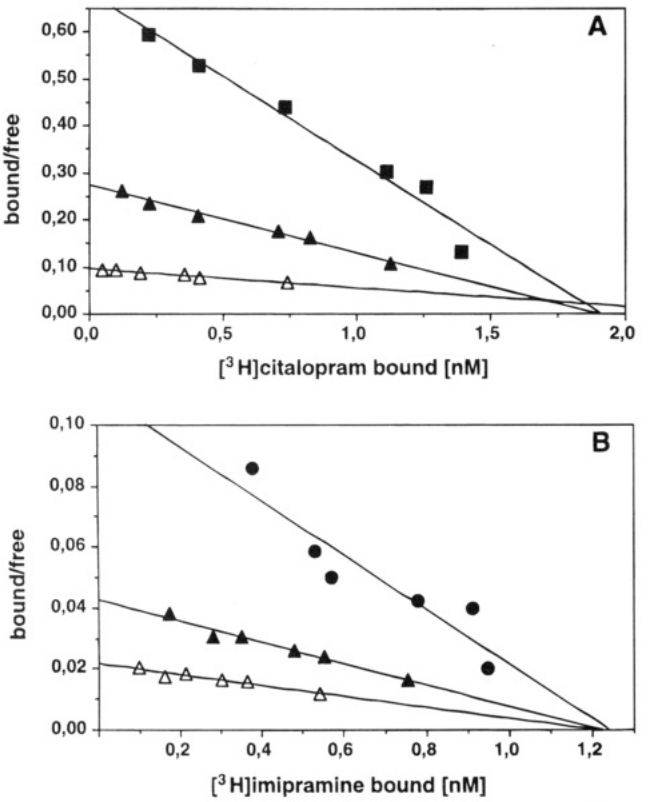

FIGURE 4: Inhibition by 5-HT of [^{3}H]citalopram (A) and [^{3}H]-imipramine (B) binding. (A) [^{3}H]Citalopram binding was performed in the absence (■) and presence of 1 (▲) or 5 μM (△) 5-HT. The corresponding binding parameters were as follows: (■) K_D = 2.8 nM and B_{max} = 26.6 pmol/mg of protein; (▲) K_D = 6.9 nM and B_{max} = 26.8 pmol/mg of protein; (△) K_D = 25 nM and B_{max} = 32.9 pmol/mg of protein. (B) [^{3}H]Imipramine binding was performed in the absence (●) and presence of 300 nM (▲) or 1 μM (△) 5-HT. The parameters obtained in this experiment were as follows: (●) K_D = 11.3 nM and B_{max} = 17.5 pmol/mg of protein; (▲) K_D = 28 nM and B_{max} = 16.8 pmol/mg of protein; (△), K_D = 58 nM and B_{max} = 17.1 pmol/mg of protein.

concentrations of 5-HT reduced the apparent affinities of both [^{3}H]citalopram and [^{3}H]imipramine without affecting the corresponding B_{max} values. Thus, 5-HT inhibits the binding of antidepressants in a purely competitive manner, which indicates the existence of heterogeneous substrate binding sites on the mammalian SERT1 protein.

Sodium Dependency of Ligand Binding. Since 5-HT uptake by serotonin transporters is driven by extracellular sodium ions, we investigated the influence of Na^+ on antidepressant binding. Exchanging Na^+ by Li^+ only reduced the apparent affinity of [^{3}H]citalopram binding ($K_D[Li^+]$ = 6.4 ± 3.1 nM) without affecting its B_{max} value (data not shown). In contrast, in the absence of Na^+, binding of 10 nM [^{3}H]imipramine was nearly abolished (Figure 5A) and the binding of 48 nM [^{3}H]imipramine was reduced by 90% in Li^+ as compared to Na^+-containing buffer. This suggests that Na^+-free conditions abolish [^{3}H]imipramine binding to ABS1 and also reduce the apparent affinity of [^{3}H]imipramine binding to ABS2. Consistant with this interpretation, saturation of [^{3}H]imipramine binding to SERT1 transfected HEK-293 cell membranes could not be achieved in Li^+-containing buffer even when [^{3}H]imipramine concentrations up to 200 nM were used (data not shown). Moreover, in the absence of Na^+, 10 nM imipramine did not inhibit [^{3}H]citalopram binding and 100 nM imipramine produced only a 30% reduction (Figure 5B). Finally, [^{3}H]-

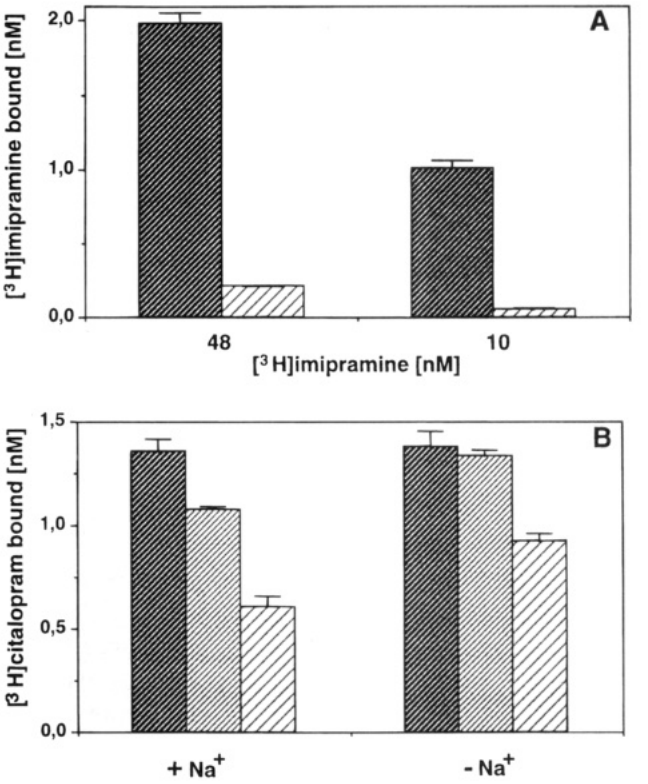

FIGURE 5: Sodium-dependent [^{3}H]imipramine binding (A) and imipramine inhibition of [^{3}H]citalopram binding (B). (A) Binding of [^{3}H]imipramine was measured at 10 and 48 nM in the presence (dark hatched bars) or absence (slightly hatched bars) of sodium. Binding in the absence of sodium was 5.3% at 10 nM and 11.0% at 48 nM, as compared to binding in the presence of sodium ions. (B) Binding of 6 nM [^{3}H]citalopram was measured in the absence (dark hatched bars) and presence of 10 (medium hatched bars) or 100 nM (slightly hatched bars) imipramine in Na^+-containing and Na^+-free buffer, as indicated. Here, 10 nM imipramine reduced [^{3}H]citalopram binding to 79% in the presence of sodium, but only to 98% when sodium was omitted. Imipramine, at a concentration of 100 nM, reduced [^{3}H]citalopram binding to 45% and 68% of the control values in the presence and absence of sodium, respectively.

citalopram binding in Na^+-free buffer was inhibited by imipramine (100 and 200 nM) in a purely competitive fashion (Figure 6). This indicates that the noncompetitive inhibition of citalopram binding mediated by ABS1 (compare Figure 3A) also requires Na^+ ions (compare Figure 5B), which again underlines the sodium dependence of this site.

Scatchard analysis of [^{3}H]citalopram binding in the presence of 5 μM 5-HT failed to reveal any significant inhibition of radioligand binding in Na^+-free buffer (data not shown). Thus, the interaction of 5-HT with ABS2 appears also to be sodium-dependent. This is consistent with an absolute requirement for Na^+ of 5-HT transport.

DISCUSSION

To elucidate the nature of the interactions between the binding sites of tricyclic and nontricyclic antidepressants on mammalian serotonin transporters, we performed a detailed radioligand binding analysis of recombinant SERT1 expressed in a human fibroblast-like cell line, HEK-293. Since HEK-293 cells do not display endogeneous binding sites for antidepressants, all drug interactions in the transfected cells result from expression of the SERT1 protein. The SERT1 cDNA has been previously shown to induce Na^+-dependent

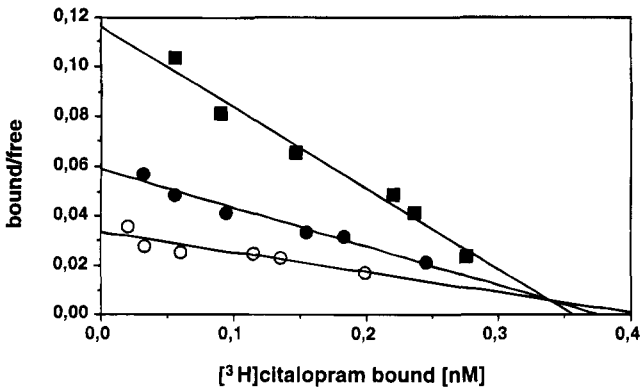

FIGURE 6: Effect of imipramine on Scatchard plots of [^{3}H]-citalopram binding in Na^+-free buffer. [^{3}H]Citalopram binding was performed in the absence (■) and presence of 100 (●) or 200 nM (○) imipramine in Na^+-free buffer. The parameters obtained in this experiment were as follows: (■) K_D = 3.0 nM and B_{max} = 7.2 pmol/mg of protein; (●) K_D = 6.3 nM and B_{max} = 7.6 pmol/mg of protein; (○) K_D = 12.1 nM and B_{max} = 8.0 pmol/mg of protein, respectively.

serotonin transport in nonneuronal mammalian cells (Blakely et al., 1991) and *Xenopus* oocytes (Mager et al., 1994). The data presented here demonstrate the presence of two distinct antidepressant binding sites on the recombinant SERT1. Both sites bind 5-HT and the tricyclic antidepressant imipramine; however, the nontricyclic antidepressant citalopram binds only to ABS2. In the presence of sodium, high-affinity binding of imipramine to ABS1 inhibits the binding of [^{3}H]-citalopram to ABS2 by a noncompetitive mechanism. Elevated concentrations of imipramine in addition directly block ABS2, as revealed by its competitive inhibition of citalopram binding. The interactions between ABS1 and ABS2 demonstrated here must be complex, since the inhibition of [^{3}H]imipramine binding to ABS1 by citalopram is very potent and leads to a complete displacement of [^{3}H]-imipramine from this site. In contrast, imipramine binding to ABS1 affects citalopram binding at ABS2 much less efficiently. In the absence of sodium, imipramine does not bind to ABS1 and thus only competitive binding of the antidepressants to ABS2 is observed. These data are consistent with a model of two distinct, but interacting or partially overlapping antidepressant binding sites, but may also be explained by SERT1 existing in two different conformational states. Accordingly, one conformation allows drug binding exclusively to ABS2, whereas a second conformational state, which is favored in the presence of Na^+ ions, allows imipramine to also bind to ABS1. At present, we have no means to neatly distinguish between these alternatives; however, protein modification studies [Biessen et al. (1988) and P. Schloss, unpublished data] support the existence of distinct tricyclic and nontricyclic binding sites.

This interpretation is in good agreement with the proposed binding of [^{3}H]imipramine to two classes of binding sites on the 5-HT carrier of human platelets as revealed by kinetic studies (O'Riordan et al., 1990). In the latter study, the different affinities were shown to result from 3−5-fold differences in dissociation rate constants, although equilibrium binding of [^{3}H]imipramine exhibited monophasic association kinetics. Different binding affinities of the deduced [^{3}H]imipramine sites are not inconsistent with the apparently linear Scatchard plot reported by others (Tarrant & Williams, 1995), as nonlinearity is difficult to detect with equilibrium dissociation constants differing by a factor of only 3−5. A low-affinity binding site for [^{3}H]imipramine (K_D ~700 μM) has been found in mouse cerebral cortical membrane preparations which, however, is thought to be unrelated to the neuronal serotonin uptake system (Reith et al., 1983). Moreover, additional [^{3}H]imipramine binding proteins may exist in nervous tissues. D'Amato et al. (1987) have shown that the selective labeling of 5-HT uptake sites in rat brain by [^{3}H]citalopram does not completely coincide with the distribution of [^{3}H]imipramine binding sites. Also, in rat cortex and hippocampus, the pattern of [^{3}H]imipramine binding differs from that of [^{3}H]paroxetine, another selective serotonin uptake inhibitor (Hrdina et al., 1990).

Knowledge of the molecular pharmacology of tricyclic and nontricyclic antidepressants on the 5-HT transporter is of great clinical importance. Cross-over studies have revealed that 60−65% of tricyclic antidepressant nonresponders respond to the selective nontricyclic uptake inhibitors, and conversely, a similar percentage is only sensitive to tricyclic antidepressant (Cole, 1992). Unlike the latter compounds, the selective uptake inhibitors do not display anticholinergic and antihistaminic effects or cardiotoxicity. Also, these drugs are well tolerated and have a low lethality in overdose (Westwick, 1990). Further elucidation of the complex interactions of imipramine and citalopram at the recombinant SERT1 should provide the basis for a better understanding of the mechanisms by which different antidepressants act on the binding and transport of the physiological substrate of the 5-HT transporter, serotonin, and thus exert their therapeutic effects in depression.

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REFERENCES

Amara, S. G., & Kuhar, M. J. (1993) *Annu. Rev. Neurosci. 16*, 73−93.

Biessen, E. A. L., Norder, J. A., Horn, A. S., & Robillard, G. T. (1988) *Biochem. Pharmacol. 37*, 3959−3966.

Blakely, R. D., Berson, H. E., Fremeau, R. T., Jr., Caron, M. G., Peek, M. M., Prince, H. K., & Bradley, C. C. (1991) *Nature 354*, 66−70.

Brosen, K. (1993) *Clin. Invest. 71*, 1002−1009.

Chen, C., & Okayama, H. (1987) *Mol. Cell. Biol. 7*, 2745−2751.

Cole, J. O. (1992) *J. Clin. Psychiatry 53*, 333−340.

Corey, J. L., Quick, M. W., Davidson, N., Lester, H. A., & Guastella, J. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 1188−1192.

D'Amato, R. J., Largent, B. L., Snowman, A. M., & Snyder, S. (1987) *J. Pharmacol. Exp. Ther. 242*, 364−371.

Gormann, C. M., Gies, D., McCray, G., & Huang, M. (1989) *Virology 171*, 377−385.

Hoffman, B. J., Mezey, E., & Brownstein, M. J. (1991) *Science 254*, 579−580.

Hrdina, P. D., Foy, B., Hepner, A., & Summers, R. J. (1990) *J. Pharmacol. Exp. Ther. 252*, 410−418.

Hyttel, J. (1994) *Int. Clin. Psychopharmacol. 9*, 19−26.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265−275.

Mager, S., Min, C., Henry, D. J., Chavkin, C., Hoffman, B. J., Davidson, N., & Lester, H. A. (1994) *Neuron 12*, 845−859.
Mayser, W., Betz, H., & Schloss, P. (1991) *FEBS Lett. 295*, 203−206.
Moret, C., & Briley, M. (1986) *J. Neurochem. 47*, 1609−1613.
O'Riordan, C., Phillips, O. M., & Williams, D. C. (1990) *J. Neurochem. 54*, 1275−1280.
Ramamoorthy, S., Bauman, A. L., Moore, K. R., Han, H., Yang-Feang, T., Chang, A. S., Ganapathy, V., & Blakely, R. D. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 2542−2546.
Reith, M. E. A., Sershen, H., Allen, D., & Lajtha, A. (1983) *J. Neurochem. 40*, 389−395.
Schloss, P., Püschel, A. W., & Betz, H. (1994) *Curr. Opin. Cell Biol. 6*, 595−599.
Segonzac, A., Raisman, R., Tateishi, T., Schoemaker, H., Hicks, P. E., & Langer, S. Z. (1985) *J. Neurochem. 44*, 349−356.
Sette, M., Briley, M. S., & Langer, S. Z. (1983) *J. Neurochem. 40*, 622−628.
Sontheimer, H., Becker, C.-M., Prichett, D. B., Schofield, P. R., Grenningloh, G., Kettenmann, H., Betz, H., & Seeburg, P. (1989) *Neuron 2*, 1491−1497.
Tarrant, H. M., & Williams, D. C. (1995) *Biochem. Soc. Trans. 23*, 41S.
Westwick, F. (1990) *Br. J. Hosp. Med. 44*, 367.
Worrall, D. M., & Williams, D. C. (1994) *Biochem. J. 297*, 425−436.

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