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Locating an Antagonist in the 5-HT$_3$ Receptor Binding Site Using Modeling and Radioligand Binding*

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We have used a homology model of the extracellular domain of the 5-HT$_3$ receptor to dock granisetron, a 5-HT$_3$ receptor antagonist, into the binding site using AUTODOCK. This yielded 13 alternative energetically favorable models. The models fell into 3 groups. In model type A the aromatic rings of granisetron were between Trp-90 and Phe-226 and its azabicyclic ring was between Trp-183 and Tyr-234, in model type B this orientation was reversed, and in model type C the aromatic rings were between Asp-229 and Ser-200 and the azabicyclic ring was between Phe-226 and Asn-128. Residues located no more than 5 Å from the docked granisetron were identified for each model; of 26 residues identified, 8 were found to be common to all models, with 18 others being represented in only a subset of the models. To identify which of the docking models best represents the ligand-receptor complex, we substituted each of these 26 residues with alanine and a residue with similar chemical properties. The mutant receptors were expressed in human embryonic kidney (HEK)293 cells and the affinity of granisetron determined using radioligand binding. Mutation of 2 residues (Trp-183 and Glu-129) ablated binding, whereas mutation of 14 other residues caused changes in the [^{3}H]granisetron binding affinity in one or both mutant receptors. The data showed that residues both in and close to the binding pocket can affect antagonist binding and overall were found to best support model B.

The 5-HT$_3$ receptor is the only member of the 5-HT (serotonin) receptor family that is a ligand-gated ion channel. It is a member of the Cys-loop ligand-gated ion channel family, which includes nicotinic acetylcholine (nACh),[1] glycine and GABA$_A$ receptors. The receptors function as a pentameric arrangement of subunits. Each subunit has a large extracellular N-terminal region and four transmembrane domains (M1–M4). Five 5-HT$_3$ receptor subunits (A–E) have been identified although to date only homomeric (A only) or heteromeric (A and B) subunit complexes have been functionally characterized (1, 2). 5-HT$_3$ receptors may be evolutionarily the oldest members of the Cys-loop family (3), and this, combined with the ability of the A subunit to yield functional homomeric proteins, has meant that 5-HT$_3$ receptors provide a useful model system for understanding critical features of all Cys loop receptors (4). Most work on this family of proteins has been performed using nACh receptors, but despite many years of study structural details of the receptor-ligand interactions at the atomic level remain unknown. The determination of the structure of the acetylcholine-binding protein (AChBP), which is homologous to the extracellular domain of the nACh receptor, and indeed all Cys loop receptors, has significantly improved our knowledge of the ligand binding domain (5). However, some differences have emerged between the AChBP structure and cryoelectron microscopy data from the nACh receptor. For example, the extracellular domains of the nACh receptor α subunits in the closed state differ by rotations of their inner pore-facing regions when compared with the AChBP crystal structure (6). In addition, recent high resolution studies showing nACh receptor agonists bound to AChBP suggest that some details of the published homology models may need revision (7).

Nevertheless, homology models based on AChBP have proved useful for examining the binding region and predicting the orientation of ligands within the binding pockets for a range of Cys loop receptors including nACh, GABA$_A$, and 5-HT$_3$ receptors (8–12). However, it is important to combine information from such models with data from experimental studies. We have recently constructed a homology-based model of the 5-HT$_3$ receptor extracellular domain where docking 5-HT into the binding pocket yielded seven alternative possible orientations of the ligand, only two of which were supported by experimental evidence (11). In the current study we have used this model to dock the 5-HT$_3$ receptor antagonist granisetron. During the course of this work a similar study using a range of antagonists was published (10). This study supported our previous work by indicating that some of the residues that we had identified as critical for 5-HT binding and/or function in the binding pocket are also critical for antagonist binding. However, whereas these authors used previously reported experimental data to support their models, there are still many residues predicted to interact with antagonists that have not yet been examined. To provide experimental data to support our model, we have combined our modeling data with radioligand binding studies on receptors with changes in potentially critical ligand-binding amino acids to define the location of an antagonist in this site.

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[1] The abbreviations used are: nACh, nicotinic acetylcholine; AChBP, acetylcholine-binding protein; GABA$_A$, γ-aminobutyric acid, type A.

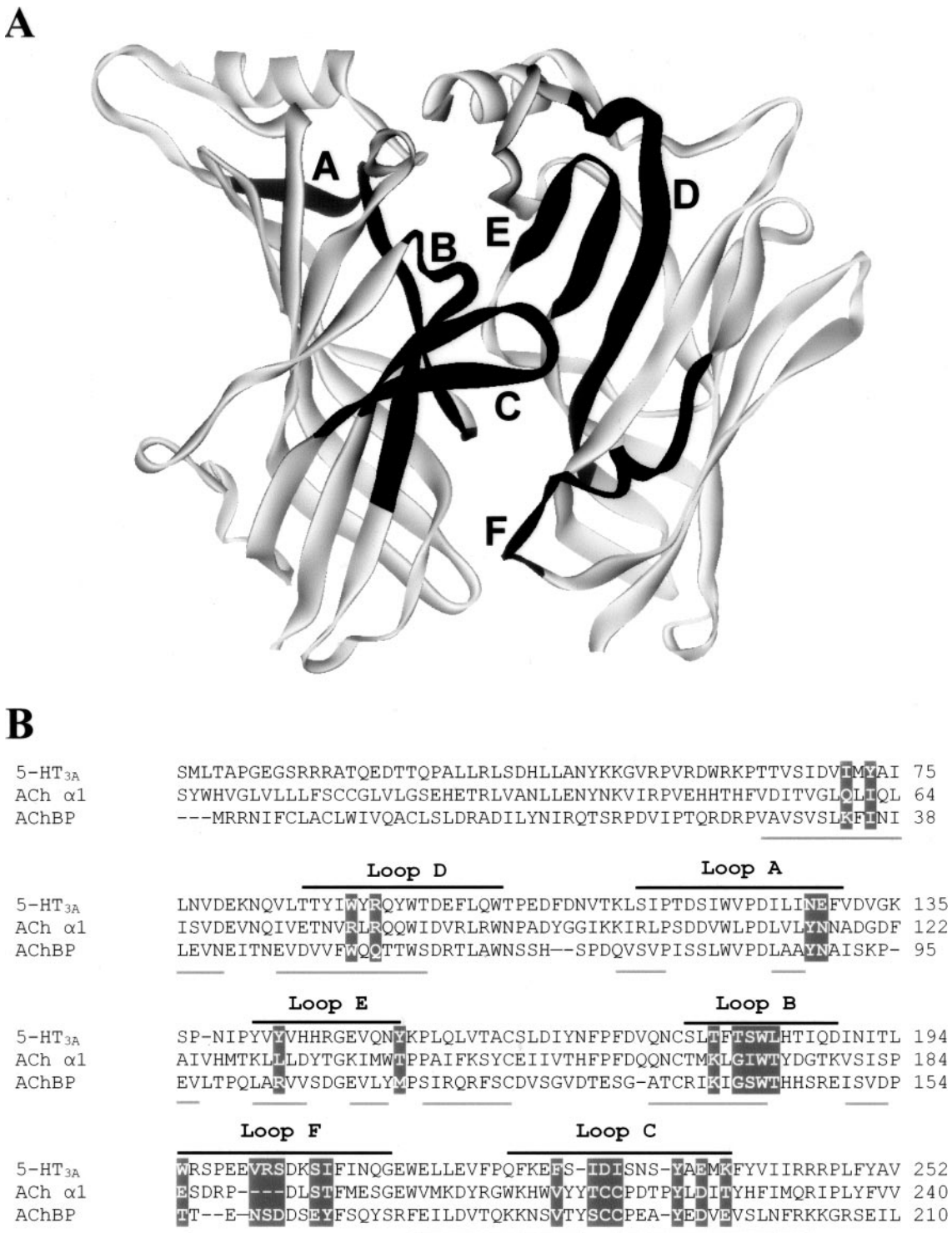


FIG. 1. **Location of the amino acid residues described in the current study.** *A*, two adjacent subunits (primary and complimentary) showing the positions of the main binding loops. *B*, alignment of the AChBP, 5-HT$_{3A}$, and nACh α1 sequences. The residues described in this study are highlighted as *white text* on a *gray* background. The loops and first two transmembrane regions of the receptors are indicated by *black lines* above the text. The positions of the β-sheets are shown by *gray lines* beneath the text and are taken from the AChBP protein crystal structure. Accession numbers for the AChBP, 5-HT$_{3A}$, and nACh α1 protein sequences are P58154, Q6J1J7, and P02710, respectively.

EXPERIMENTAL PROCEDURES

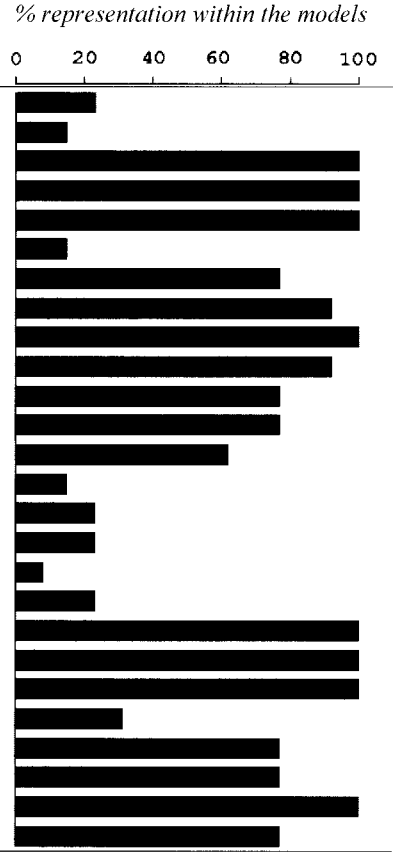
Materials—All cell culture reagents were obtained from Invitrogen (Paisley, UK), except fetal calf serum, which was from Labtech International (Ringmer, UK). [^{3}H]Granisetron (81 Ci/mmol) was from PerkinElmer Life Sciences. All other reagents were of the highest obtainable grade.

Cell Culture—Human embryonic kidney (HEK)293 cells were maintained on 90-mm tissue culture plates at 37 °C and 7% CO_2 in a humidified atmosphere. They were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mix F12 (1:1) with GlutaMAX™ I containing 10% fetal calf serum and passaged when confluent. Cells in 90-mm dishes were transfected using calcium phosphate precipitation (13, 14) at 80–90% confluency. Following transfection cells were incubated for 3–4 days before assay.

Site-directed Mutagenesis—Mutagenesis reactions were performed using the method described by Kunkel (15). The 5-HT$_{3A(b)}$ subunit DNA (accession number AY605711) has been described previously (16). Oligonucleotide primers were designed according to the recommendations

TABLE I
Residues of the 5-HT$_3$ receptor binding site within 5 Å of granisetron

Loop	5-HT$_{3A}$ Residue	nAChR α1 Residue	Models 1	2	3	4	5	6	7	8	9	10	11	12	13
	I71							+		+				+	
	Y73										+		+		
D	W90		+	+	+	+	+	+	+	+	+	+	+	+	+
D	R92		+	+	+	+	+	+	+	+	+	+	+	+	+
A	N128	Y93	+	+	+	+	+	+	+	+	+	+	+	+	+
A	E129												+		
E	Y143		+	+	+	+	+		+		+	+	+		+
E	Y153		+	+	+	+	+	+	+		+	+	+	+	+
B	T179		+	+	+	+	+	+	+	+	+	+	+	+	+
B	T181		+	+	+	+	+		+	+	+	+	+	+	+
B	S182		+	+	+	+	+		+		+	+	+		+
B	W183	W149	+	+	+	+	+		+		+	+	+		+
B	L184		+		+	+	+		+		+		+		+
F	W195							+						+	
F	V201							+		+				+	
F	R202							+		+				+	
F	S203													+	
F	S206							+		+				+	
F	I207		+	+	+	+	+	+	+	+	+	+	+	+	+
C	F226	Y190	+	+	+	+	+	+	+	+	+	+	+	+	+
C	I228	C192	+	+	+	+	+	+	+	+	+	+	+	+	+
C	D229	C193		+				+	+	+					
C	I230		+	+	+	+	+		+		+	+	+		+
C	Y234	Y198	+	+	+	+	+		+		+	+	+		+
C	E236	D200	+	+	+	+	+	+	+	+	+	+	+	+	+
C	K238		+		+	+	+	+	+	+		+		+	+
	Model Type		A	B	A	A	A	C	B	C	A	A	A	C	A



of Sambrook *et al.* (17) and some suggestions of the Primer Generator (18). A silent restriction site was incorporated into each primer to assist rapid identification.

Radioligand Binding—This was undertaken as described previously (19) with minor modifications. Briefly, HEK293 cells that had been transfected with wild type or mutant DNA were washed twice with phosphate-buffered saline at room temperature. They were then scraped into 1 ml of ice-cold HEPES buffer (10 mM, pH 7.4) containing the following proteinase inhibitors (PI): 1 mM EDTA, 50 μg/ml soybean trypsin inhibitor, 50 μg/ml bacitracin, and 0.1 mM phenylmethylsulfonyl fluoride. Harvested cells were washed in HEPES/PI and frozen at −20 °C. After thawing, they were washed twice with HEPES buffer, resuspended, and 50 μg of cell membranes were incubated in 0.5 ml of HEPES buffer containing [^{3}H]granisetron. Initially, single point radioligand binding assays were performed using 1 nM and sometimes 20 nM [^{3}H]granisetron to test for specific binding. If specific binding was present, saturation binding (8 point) assays were performed on at least three separate plates of transfected cells for each mutant. Nonspecific binding was routinely 5–10% of total binding and was determined by the addition of 1 μM D-tubocurarine or 1 μM quipazine (both potent 5-HT$_3$ receptor antagonists (19, 20)), which gave equivalent results. Reactions were incubated for 1 h at 4 °C and were terminated by rapid vacuum filtration using a Brandel cell harvester onto GF/B filters presoaked for 3 h in 0.3% polyethyleneimine (21) followed by two rapid washes with 4 ml of ice-cold HEPES buffer. Radioactivity was determined by scintillation counting (Beckman LS6000sc). Maximum specific binding was up to 70,000 dpm and routinely in the range 500–2000 fmol/mg of protein. Protein concentration was estimated using the Bio-Rad protein assay with bovine serum albumin standards. Data were analyzed by iterative curve fitting (GraphPad, PRISM, San Diego, CA) according to the equation $B = (B_{max}\cdot[L])/(K + [L])$, where B is bound radioligand, B_{max} is maximum binding at equilibrium, K is the equilibrium dissociation constant, and [L] is the free concentration of radioligand.

Modeling—This was performed as described previously (11). Briefly, the three-dimensional model of the extracellular region of the 5-HT$_3$ receptor was built using MODELLER 6v2 (22) based on the crystal structure of AChBP determined to a resolution of 2.7 Å. The pentamer was generated by superimposing the model on to each protomer of AChBP, with care taken not to alter the coordinate axes of reference. The generated pentameric model was energy minimized in Sybyl 6.8 using the Amber force field (23) by moving side chains alone, to relieve short contacts at the interprotomer interfaces. Electrostatic terms were included in these minimization cycles.

Ligand Docking—Granisetron was docked using AUTODOCK 3.05 (24, 25). The binding pocket is geometrically well defined. 10 genetic algorithm runs were carried out to dock each of the four stereoisomers of granisetron. For the genetic algorithm, a population size of 50 was used, and the maximum number of generations was set to 27,000. A total of 13 unique docked poses were generated. These structures were used as input for software created by one of us (see Ref. 11) that identified all amino acids that had at least one atom within 5 Å of the ligand. Potential hydrogen bonding interactions were identified using Swiss PDB Viewer (26).

RESULTS

Docking of Granisetron—AUTODOCK revealed 13 energetically favorable granisetron binding models, and 26 residues were identified as being no more than 5 Å from the docked granisetron (Fig. 1). Of these, 8 residues were found to be common to all models, with 18 others being represented in only a subset of the models (Table I). When 5-HT was docked into the binding site in our previous studies (11), it was found to be completely contained within the binding cleft, but an antagonist such as granisetron need not necessarily occupy the same location. Indeed, an antagonist might act solely by blocking the entrance to the binding pocket or by allosteric interactions at a site distant from the binding region. However, given the high affinity of granisetron binding (K_d < 1 nM), it is likely to have a well defined binding site. Combined with the competitive nature of the interactions with granisetron and 5-HT, it is

likely that granisetron will bind at least partly in the binding pocket. Thus, it was not surprising that a high proportion of the residues that we had previously identified as within 5 Å of 5-HT in the binding pocket were also within 5 Å of granisetron in most of the models that the docking procedure revealed as energetically favorable.

The models fall broadly into three groups, which we have designated A, B, and C (Table I). In group A the azabicyclic ring of granisetron is located between Trp-183 and Tyr-234, and the aromatic rings lie between Trp-90 and Phe-226. In group B the orientation of granisetron is reversed, and consequently the aromatic rings are located between Trp-183 and Tyr-234, whereas the azabicyclic ring is between Trp-90 and Phe-226. Group C is quite distinct as the azabicyclic ring lies between Phe-226 and Asn-128, and the aromatic rings between Asp-129 and Ser-206. Representatives from each of these groups are shown in Fig. 2.

Effect of Mutations—Each of the residues that were found to be within 5 Å of granisetron in the models was changed to alanine and to a residue with properties similar to those of the wild type amino acid. The experimentally derived granisetron binding affinities of these mutant receptors are shown in Table II.

Changing 10 residues resulted in no change in affinity for either mutation, suggesting these residues do not play a role in ligand binding. The results also revealed that there was no specific binding for both mutations at Trp-183, which has previously been shown to be critical for ligand binding (27, 28), and at Glu-129. In both instances mutant receptors were expressed at the cell membrane (Fig. 3). There was also no binding to receptors with mutations of Trp-90 and Tyr-234 to alanine, which again expressed at the cell membrane (Fig. 3), indicating that the characteristics of these residues are critical for binding and/or the structural integrity of the receptor. For the remaining residues there were differences in binding affinities compared with wild type for one or both of the substitutions, suggesting that these residues have some role in granisetron binding. These residues include Tyr-153 and Trp-195, which may form part of the aromatic box that appears to be critical for all ligand gated ion channels (29). The other residues are Arg-92, Thr-179, Thr-181, Ser-182, Leu-184, Ser-203, Ser-206, Ile-228, Asp-229, and Ile-230.

DISCUSSION

The structure of AChBP is a useful model for the extracellular domain of the 5-HT$_3$ receptor, which contains the binding site for agonists and competitive antagonists. Here we have used our model of the 5-HT$_3$ receptor to dock granisetron into the binding site using AUTODOCK. This yielded 13 energetically favorable models. Within these models, 26 residues were identified as being within 5 Å of granisetron, and radioligand binding data from receptors mutated at each of these 26 positions showed that as many as 16 of these residues may be involved in the binding of granisetron. These are discussed in more detail below. The data also showed that only 8 of these 26 residues were common to all 13 models (see Table I). This is in contrast to our previous studies with 5-HT docking where more than 90% of residues within 5 Å of the ligand were common to all of the binding orientations identified. Nevertheless, all 8 common residues overlap with those that were identified as being located within 5 Å of 5-HT, providing support for the hypothesis that the selective antagonist granisetron binds in the same binding pocket as 5-HT.

Examination of the average number of residues that altered binding affinity when mutated was 12 for model A and 12 for model B but only 10 for model C. This suggests that model C is less likely to be correct. Many of the other data would fit both

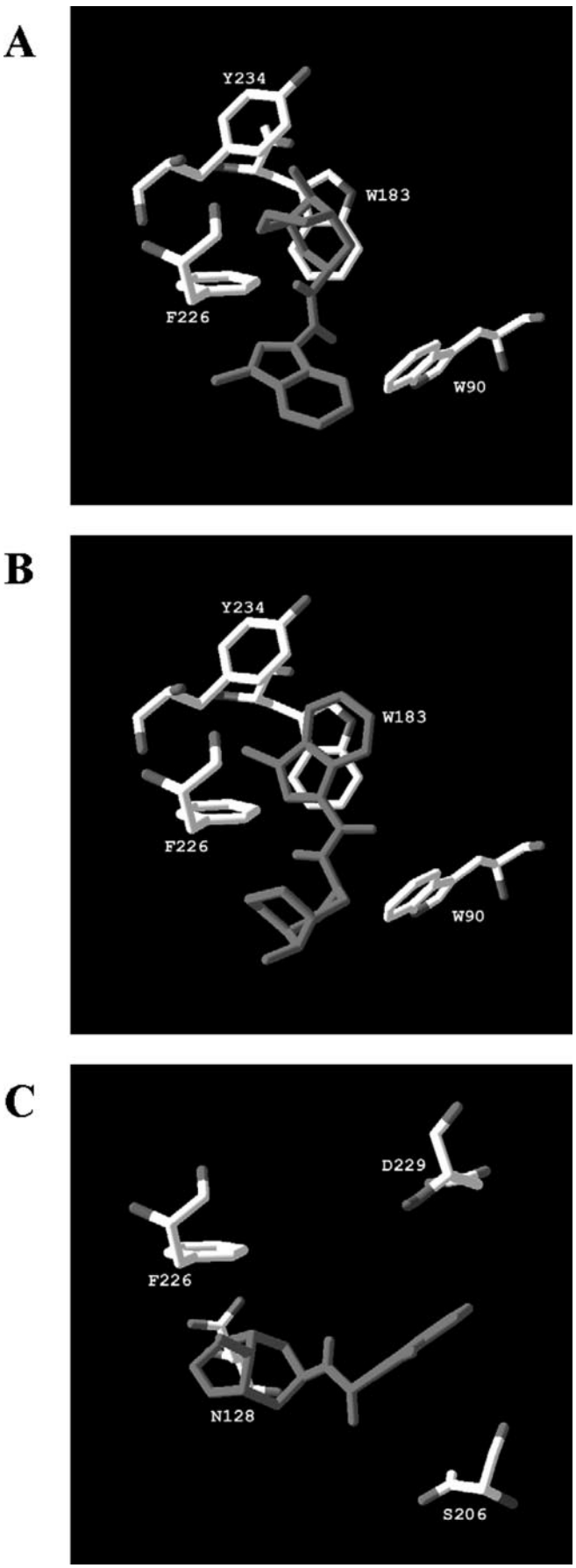


FIG. 2. **Representative examples of the three model types showing the orientation of the four main residues that define these models.** Granisetron is shown in the center of each image.

TABLE II
Effects of alanine and conserved amino acid changes on [^{3}H]granisetron binding to the 5-HT$_3$ receptor

Alanine mutant	K_d (mean ± S.E.)	n	Conserved mutant	K_d (mean ± S.E.)	n
	nM			nM	
Wild type	0.31 ± 0.04	3	Wild type	0.31 ± 0.03	3
I71A	0.37 ± 0.10	3	I71L	0.31 ± 0.08	3
Y73A	0.34 ± 0.04	7	Y73S	0.57 ± 0.09	5
W90A[a]	No binding	5	W90Y[a]	0.90 ± 0.06	3
R92A[a]	1.80 ± 0.40	3	R92K	1.00 ± 0.30	3
N128A	0.40 ± 0.10	3	N128D	0.50 ± 0.10	3
E129A[a]	No binding	3	E129D[a]	No binding	3
Y143A	1.20 ± 0.24	8	Y143F	0.53 ± 0.10	3
Y153A[a]	2.36 ± 0.53	7	Y153F	0.90 ± 0.20	5
T179A[a]	3.20 ± 0.10	3	T179S	0.38 ± 0.20	6
T181A[a]	0.12 ± 0.04	3	T181S	0.58 ± 0.10	3
S182A[a]	1.00 ± 0.20	3	S182T[a]	1.80 ± 0.09	7
W183A[a]	No binding	5	W183Y[a]	No binding	3
L184A[a]	4.11 ± 0.94	4	L184I[a]	0.71 ± 0.05	6
W195A[a]	5.08 ± 0.88	4	W195Y[a]	8.70 ± 2.40	3
V201A	0.65 ± 0.12	3	V201R	0.69 ± 0.21	3
R202A	0.32 ± 0.09	3	R202K	0.14 ± 0.10	3
S203A[a]	0.08 ± 0.02	3	S203T	0.26 ± 0.11	3
S206A[a]	1.67 ± 0.27	4	S206T[a]	4.40 ± 0.49	4
I207A	0.39 ± 0.07	3	I207L	0.62 ± 0.20	3
F226A	0.18 ± 0.06	3	F226Y	0.57 ± 0.21	5
I228A[a]	1.40 ± 0.30	3	I228N	0.30 ± 0.05	3
D229A[a]	3.80 ± 0.26	4	D229E[a]	0.11 ± 0.03	3
I230A	0.30 ± 0.10	3	I230N[a]	1.70 ± 0.40	3
Y234A[a]	No binding	5	Y234F	1.30 ± 0.36	4
E236A	0.80 ± 0.20	3	E236D	0.70 ± 0.30	3
K238A	0.30 ± 0.10	6	K238R	0.81 ± 0.19	11

[a] Significantly different from wild type (Student's t test, $p < 0.05$).

models A and B, but the potential for weak interactions between granisetron and the protein is greatest in model B. In particular our data support a hydrogen bond between granisetron and Tyr-153 (shown in Fig. 4) and also aromatic interactions with Tyr-234 and Trp-183. Additionally, in this model granisetron is in the same orientation as predicted by Maksay *et al.* (10), who show that it best fits the 5-HT$_3$ receptor antagonist pharmacophore model in this orientation. Consequently, we propose that granisetron is located in the binding pocket with its aromatic rings positioned between Trp-183 and Tyr-234, and its azabicyclic ring is close to Trp-90 and Phe-226. However, it should be remembered that the model is based on the structure of AChBP, which is probably more similar to the desensitized and/or open states of the nACh receptor than its closed state (6). Granisetron, as a competitive antagonist, would be expected to bind preferentially to the closed state, and consequently a number of the residues examined in this study may be in a different location, albeit probably only a few angstroms displaced from the AChBP-like model. Therefore, we must be cautious in using distances between atoms obtained from this model to confirm the presence of atomic interactions such as hydrogen bonds, which depend critically on the distance between atoms.

The Role of Residues That Ablate Binding (Glu-129 and Trp-183)—The mutation of Glu-129 and Trp-183 to either alanines or chemically similar residues were found to eliminate [^{3}H]granisetron binding. This was to be expected for Trp-183, which has been previously identified as critical for ligand binding (27, 28, 30). Beene *et al.* (28) have shown that Trp-183 forms a cation-π interaction with the 1° amine of 5-HT, as has previously been demonstrated in the nACh receptor between acetylcholine and the homologous residue, Trp-149 (31). Aromatic residues are found in homologous positions in GABA$_A$ and glycine receptors where they have also been shown to play a role in ligand binding (32–34). Consequently, it is not unexpected that Trp-183 forms an essential part of the antagonist binding site and we suggest that it forms a π-π interaction with granisetron.

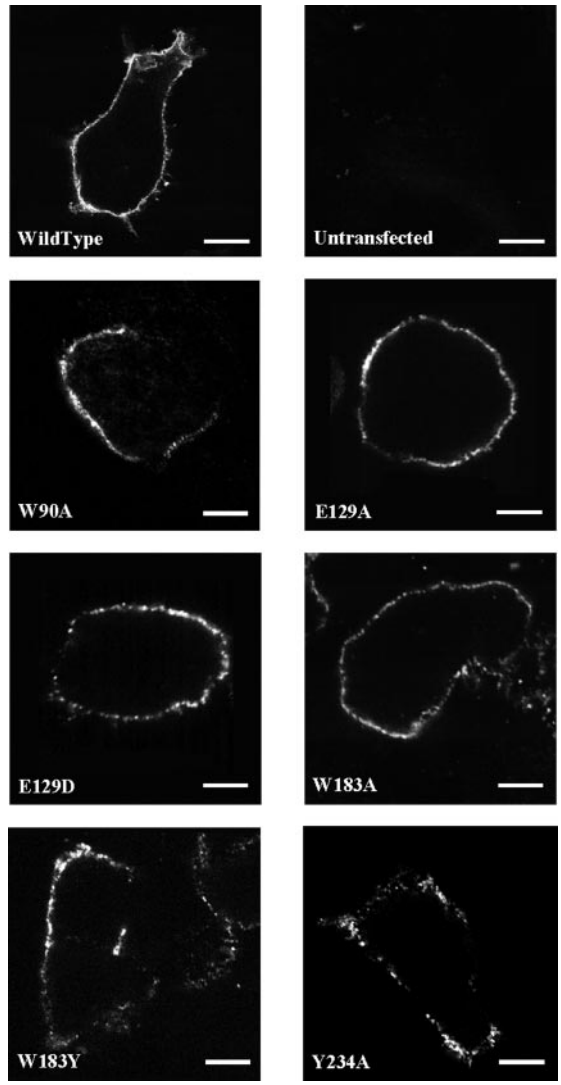


FIG. 3. **Images of immunofluorescent labeled, non-permeabilized HEK293 cells revealing cell surface expression of receptors that displayed no specific radioligand binding.** *Scale bar* = 10 μm.

The role of Glu-129 is not yet clear, although our data show it has an essential role in either ligand binding or in the structure of the receptor and/or binding pocket. Given that radioligand binding was completely ablated when Glu-129 was replaced with the chemically similar aspartate, yet the receptors reached the membrane, we propose that this residue is critical for the local structure of the binding pocket. Our data are somewhat surprising given previous experiments that reported both radioligand binding (of a different antagonist GR65630) and function of an E129D mutant 5-HT$_3$ receptor (Glu-106 by their numbering) (35). These authors reported poor expression of Glu-129 mutants (E129A and E129Q receptors expressed too poorly to characterize them) and used a different splice variant of the receptor, which might explain these differences. Although intuitively we would not anticipate that this splice variant (which contains an extra six amino acids in the M3-M4 intracellular loop) would affect receptor function, this change is sufficient to convert *m*-chlorophenylbiguanide from a partial agonist to a full agonist (36) and may have as yet unexplained long distance effects on the binding pocket.

The Roles of Aromatic Residues That May Interact with Granisetron (Trp-90, Tyr-153, Trp-195 and Tyr-234)—The results from these mutations suggest that these residues have

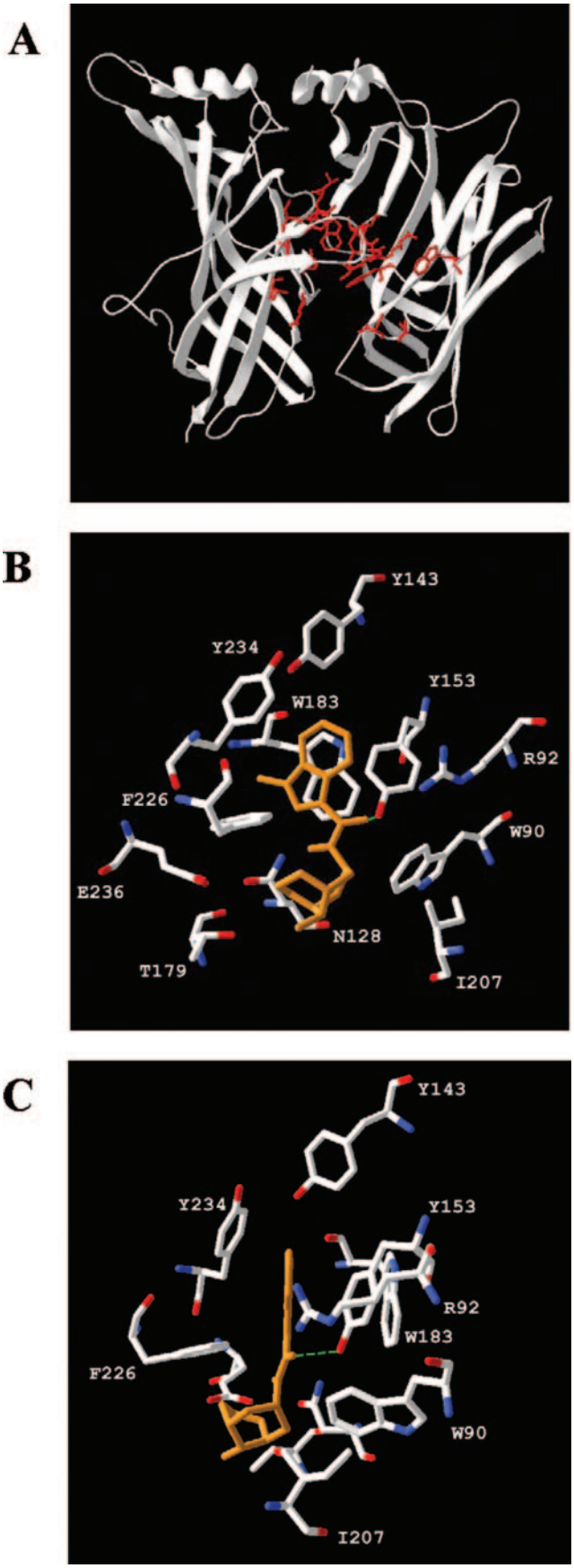


FIG. 4. *A*, location of all amino acids that caused changes in the affinity of [^{3}H]granisetron (in *red*), showing that most are within the binding pocket. *B* and *C*, two views of granisetron (*orange*) docked in the binding pocket in our preferred orientation (Model B) and indicating the hydrogen bond formed with Tyr-153 (in *green*).

specific roles in the binding of granisetron and/or the structure of the binding pocket. There is already evidence from previous studies that the aromatic residues Trp-90, Tyr-153, Trp-195, and Tyr-234 are important in antagonist binding to the 5-HT$_3$ receptor (27, 30, 37). The importance of Trp-90 appears to be primarily because of its aromatic nature as removal of the aromatic ring, as in W90A (present study) and W90S (27), eliminates granisetron binding. The role of this residue is probably similar to the equivalent residue in AChBP (W53), which provides aromatic character in the binding pocket but only makes limited or no contact with agonists.

Both an aromatic and a hydroxyl group are important at position 153, and we propose that the hydroxyl group of Tyr-153 forms a hydrogen bond with granisetron, as previously discussed (30). Conversely, it is difficult to assign a specific role to Trp-195, as changing it to another large aromatic residue (Tyr) or to a small uncharged residue (Ala) results in a similar large effects on granisetron binding. We suggest that Trp-195 plays an indirect role in antagonist binding and may, for example, assist the formation of the correct structure of the binding pocket.

Mutation of Tyr-234, which is located on the opposite side of the binding pocket to Trp-183, has previously revealed that this residue has an important role in ligand binding and/or receptor structure. In particular the aromatic group is essential, as replacement with either serine or alanine resulted in non-binding receptors (30). This residue does not appear to form a cation-π bond with 5-HT, although the equivalent tryptophan residue in MOD-1 receptors (Trp-226) does form this type of bond with 5-HT (29). We propose that in the 5-HT$_3$ receptor Tyr-234 forms a π-π interaction with the aromatic rings of granisetron.

The data from Phe-226 show that an aromatic residue is not essential here, as alanine could substitute reasonably satisfactorily for phenylalanine. This is surprising as the aligning position in the nACh receptor α1 subunit (Tyr-190) and in AChBP (Tyr-185) have important roles in ligand binding (7). Nevertheless our data indicate that Phe-226 does not play a role in granisetron binding.

The Roles of Non-aromatic Residues That May Interact with Granisetron (Arg-92, Thr-179, Ser-182, Leu-184, Ser-203, Ser-206, Ile-228, Asp-229, and Ile-230)—Arg-92 has the potential to hydrogen bond with granisetron in our model and previous data support its role in antagonist binding (38). It has also been suggested that Arg-92 forms a salt bridge with Asp-229 and/or Glu-200 (10), which would give it a role in the local structure of the binding pocket. Our data show that D229A receptors have a lower affinity than wild type, which provides some support for a hydrogen bond. However, the higher affinity of D229E receptors compared with wild type may indicate a different interaction here, although it is also possible that the atomic distances are more favorable for such a bond in this mutant.

The data from Thr-179, Ser-182, and Leu-184 are surprising given that these residues have not been previously reported as being important in either the structure or the function of 5-HT$_3$ receptors. Radioligand binding of the Thr-179 mutants indicate that the hydroxyl group is important as replacement by serine has no effect, but removal of the hydroxyl in T179A mutant receptors results in a greatly decreased binding affinity. In support of a role of the Thr-179 hydroxyl, the model indicates it has the potential to hydrogen bond with Asn-128 and/or Glu-236. We propose that Thr-179 does not directly bind granisetron but is important in the local structure of the binding site.

The model suggests that Ser-182 also has the potential to hydrogen bond with granisetron, via its backbone carbonyl, and the data support this, as the decreased binding affinity we

observed occurs for both mutations. However, the aligning residue in AChBP (S142) has been proposed to have a structural role (discussed in more detail below), and we cannot yet eliminate this possibility.

Leu-184 is equivalent to AChBP residue Thr-144, whose backbone carbonyl has recently been shown to hydrogen bond with Asp-85 (a highly conserved aspartate residue), which lies just outside the ligand binding pocket (7). This aspartate forms hydrogen bonds with Ser-142 and is important for the structure of the binding pocket, although its major role is in polarizing Trp-143 (the residue equivalent to Trp-183), which allows a favorable interaction with the positively charged ligand. The present data do not show whether or not a hydrogen bond similar to the Thr-144–Asp-85 bond occurs between Leu-184 and the equivalent aspartate residue in the 5-HT$_3$ receptor, but these data do suggest that a large hydrophobic residue is strongly preferred at this location. We propose that such a residue is important for the structure of the binding pocket and may specifically be involved in correctly locating the adjacent residue, Trp-183.

The side chains of Ile-228 and Ile-230, which the model places less than 4 Å from granisetron, are probably important for shaping the binding pocket and/or for creating hydrophobicity. The radioligand binding data suggest that Ile-228 is important for creating bulk, as replacing this with the similarly bulky asparagine yields receptors with a similar K_d for [^{3}H]granisetron binding, whereas the smaller alanine decreases affinity. Hydrophobicity is the more critical feature at position 230. At this position, replacement with alanine renders the K_d unchanged but replacement with asparagine decreases binding affinity. These observations support the model, as Ile-228 is close to the center of granisetron and may play a role in its correct location, whereas Ile-230 is close to granisetrons hydrophobic aromatic rings.

The locations of Ser-203 and Ser-206 in our model are currently only tentative as they are part of loop F, which has not yet been clearly resolved in the structure of AChBP. Indeed our model suggests that these residues are located some distance from granisetron, whereas the binding data indicate they play a role. As loop F residues have been shown to play an important function in both GABA$_A$ and nACh receptor binding pockets (39, 40) we believe this region of sequence is also likely to be involved in 5-HT$_3$ receptor function. We await further high resolution structural data to determine the exact role of these residues.

Conclusion—We have identified a possible location of the specific 5-HT$_3$ receptor antagonist granisetron in the 5-HT$_3$ receptor binding pocket using homology modeling, ligand docking, and radioligand binding. Our results suggest that the orientation of granisetron in the 5-HT$_3$ receptor binding pocket is with its aromatic rings between Trp-183 and Tyr-234 and its azabicyclic ring between Trp-90 and Phe-226. Further high resolution structural data, particularly of the closed state of the receptor, will allow us to test our hypothesis.

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