Aromatic residues play an important role in the ligand-binding domain of Cys loop receptors. Here we examine the role of the 11 tyrosines in this domain of the 5-HT$_3$ receptor in ligand binding and receptor function by substituting them for alanine, for serine, and, for some residues, also for phenylalanine. The mutant receptors were expressed in HEK293 cells and Xenopus oocytes and examined using radioligand binding, Ca$^{2+}$ imaging, electrophysiology, and immunchemistry. The data suggest that Tyr$^{90}$ and Tyr$^{91}$ are critical for receptor assembly and/or structure, Tyr$^{141}$ is important for antagonist binding and/or the structure of the binding pocket, Tyr$^{143}$ plays a critical role in receptor gating and/or agonist binding, and Tyr$^{153}$ and Tyr$^{259}$ are involved in ligand binding and/or receptor gating. Tyr$^{73}$, Tyr$^{88}$, Tyr$^{241}$, and Tyr$^{246}$ do not appear to play major roles either in the structure of the extracellular domain or in ligand binding. The data support the location of these residues on a model of 5-HT docked into the ligand-binding domain and also provide evidence for the structural similarity of the extracellular domain to AChBP and the homologous regions of other Cys loop ligand-gated ion channels.

The Cys loop family of ligand-gated ion channels plays a critical role in neuronal transmission and are also the target of many neuroactive agents; understanding their molecular details is therefore a priority in this field. Among the different family members, the 5-HT$_3$ receptor has the closest functional homology with the nicotinic acetylcholine (nACh) receptor. The nACh receptor is particularly well characterized, because although no high resolution x-ray crystal structure is known, a vast array of techniques including chemical modification, site-directed mutagenesis, electron microscopy, and molecular modeling have provided a reasonable understanding of the structure-function relationships of the receptor. Molecular details of the N-terminal extracellular domain, which harbors the ligand-binding site, have recently been further clarified following publication of the high resolution structure of the acetylcholine-binding protein (AChBP), which is homologous to this domain (1). This work has confirmed that six regions of the sequence (loops A–F), previously indicated to be involved in ligand binding, are located in the binding pocket and has provided some insight into the molecular mechanisms of ligand recognition. More recently, superimposed structures of the AChBP and the nACh receptor have also elucidated gross structural rearrangements that occur upon ligand binding (2).

AChBP also provides a good model for the extracellular domain of the 5-HT$_3$ receptor (3), although molecular details of the ACh-binding site cannot be directly extrapolated to this protein. There is, however, some overlap between pharmacophore models for the nACh and 5-HT$_3$ receptors (4), although compounds binding to both receptors can display different affinities for the two receptors or have opposing effects. For example, the 5-HT$_3$ receptor antagonist d-tubocurarine (dTC) displays differential affinities at the two nonidentical nACh receptor-binding sites, and nicotine, a nACh receptor agonist, behaves as an antagonist at the 5-HT$_3$ receptor ligand-binding site (5). Despite this, the structural variation between ligand-binding sites responsible for these differences in action may be subtle; one report suggests the replacement of just one residue in the 5-HT$_3$ receptor (F130N) is sufficient to produce a receptor responsive to ACh (6). The recently published model of the 5-HT$_3$ receptor-binding site (7), therefore, is probably broadly accurate, even though it is based on a structure than binds ACh; however, because it is purely a homology model, it requires support from experimental data.

Aromatic residues have been previously shown to be involved in ligand binding to the 5-HT$_3$ receptor (3). In particular a role for tyrosine residues has been demonstrated (8): pretreatment of 5-HT$_3$ receptors in NG108–15 cells with the tyrosine-modifying reagent tetranitromethane caused a 30% reduction in specific binding of the 5-HT$_3$ receptor antagonist [H]$^3$zacopride. In addition, mutation of Tyr$^{94}$ to alanine caused a small shift in [H]$^3$granisetron binding affinity (9), and alanine substitutions of Tyr$^{141}$, Tyr$^{143}$, and Tyr$^{153}$ have been shown to affect both agonist and antagonist affinities and 5-HT-induced currents (10). Here we extend these studies to examine the radioligand binding, electrophysiological, and cell surface expression properties of mutants of each of the 11 tyrosine residues in the mouse 5-HT$_3$ receptor extracellular domain. The data reveal that specific tyrosine residues play roles in the structure and/or function of the receptor and also support the model of the 5-HT$_3$ receptor extracellular domain (7).

**EXPERIMENTAL PROCEDURES**

**Materials—**All of the cell culture reagents were obtained from Invitrogen, except fetal calf serum which was from Labtech International (Ringmer, UK). [H]$^3$granisetron (81 Ci/mol) was from PerkinElmer Life Sciences. The 5-HT$_3$ receptor N-terminal domain antiserum, pAb120, was generated as previously described (11). 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 and 7% CO$_2$ in a humidified HEPES-buffered medium; TBS, Tris-buffered saline.
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| Mutant | Primer |
|--------|--------|
| Y50A   | TACACCCCTTCGCGGTCCGCAAGAGTGCTG |
| Y50C   | TACACCCCTTCGCGGTCCGCAAGAGTGCTG |
| Y50F   | TACACCCCTTCGCGGTCCGCAAGAGTGCTG |
| Y73A   | CTCTCACCGCTTAAATGATGGCGCAATAGATCAT |
| Y73S   | CTCTCACCGCTTAAATGATGGCGCAATAGATCAT |
| Y78A   | CCGATCTACTAAGTCGGCAGCACAGAAC |
| Y78C   | CCGATCTACTAAGTCGGCAGCACAGAAC |
| Y91A   | CAGATTCGGCCACATATTGAGT |
| Y91S   | CAGATTCGGCCACATATTGAGT |
| Y94S   | CTCATCGGCCGAGGCCCTGACCA |
| Y114A  | ATGATGACGATACATAAGGATGTCG |
| Y114S  | ATGATGACGATACATAAGGATGTCG |
| Y114F  | TTCATCGACGATACATAAGGATGTCG |
| Y114S  | TTCATCGACGATACATAAGGATGTCG |
| Y141A  | GGGCGGTCCACACATTGGCGGGCTTCCATGGCTT |
| Y141S  | GGGCGGTCCACACATTGGCGGGCTTCCATGGCTT |
| Y141F  | CAATTGCAGGTACATTCA |
| Y141S  | CAATTGCAGGTACATTCA |
| Y141F  | CAATTGCAGGTACATTCA |
| Y141S  | CAATTGCAGGTACATTCA |
| Y141F  | CAATTGCAGGTACATTCA |
| Y141S  | CAATTGCAGGTACATTCA |
| Y141F  | CAATTGCAGGTACATTCA |
| Y141S  | CAATTGCAGGTACATTCA |
| Y141F  | CAATTGCAGGTACATTCA |
| Y141S  | CAATTGCAGGTACATTCA |

Table I

Primers used to direct synthesis of mutant strands

The mutagenesis reactions were performed using the method developed by Kunkel (13) using the 5-HT\textsubscript{3} subunit DNA as previously described (14). The oligonucleotide primers were designed according to the recommendations of Sambrook et al. (15) and using some suggestions of the Primer Generator (16), mediated, and medcenter/primer/primer.cgi). A silent restriction site was incorporated in each to assist rapid identification. The primers are shown in Table I with base changes shown in bold type. The mutagenesis reactions were performed using standard electrophysiological procedures as previously described (19), with minor modifications. Briefly, a GeneClamp 500B amplifier was connected to a PC running CLAMPEx version 6.0.3 software via a DigiData1200 Series Interface (all Axon Instruments, Inc.). Glass microelectrodes were pulled from GC150TF-10 glass capillaries (Harvard Apparatus) using a P-87 micropipette puller (Sutter) to a resistance of 0.5 MΩ and back-filled with 2 M KCl. The Oocytes were maintained at a holding potential of −30 mV unless stated otherwise and perfused simultaneously with calcium-free frog ringer (115 mM NaCl, 2.5 mM KCl, 1.8 mM MgCl\textsubscript{2}, 10 mM HEPES, pH 7.5) at a rate of 3–4 µl/min. Serotonin (creatinine sulfate complex; Sigma) was diluted in calcium-free frog ringer and applied to the bath using a Valvo Bank 8 II system (Automate Scientific, Inc.). Doses of 5-HT were applied at 3-min intervals to allow for recovery from desensitization. Concentration-response curves and parameters were obtained using Prism software as described above.

RESULTS

Radioligand Binding Properties of Wild Type and Mutant Receptors—Radioligand binding assays were initially performed with the alamone and serine mutants of the 11 tyrosine residues. Specific, saturable binding was detected for 12 of the 22 mutants but could not be detected for Tyr50A, Tyr50S, Tyr91A,
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Table II

| Receptor | $K_d$ (nM) | n | Receptor | $K_d$ (nM) | n |
|----------|------------|---|----------|------------|---|
| Wild type | 0.32 ± 0.035 | 7 | Y141S | NB | 8 |
| Y50F | NB | 3 | Y143F | 0.53 ± 0.10 | 3 |
| Y50A | NB | 10 | Y143A | 1.2 ± 0.24 | 8 |
| Y50S | NB | 13 | Y143S | 1.13 ± 0.23 | 7 |
| Y73A | 0.34 ± 0.043 | 7 | Y153F | 0.90 ± 0.20 | 5 |
| Y73S | 0.57 ± 0.086 | 5 | Y153A | 3.62 ± 1.75 | 3 |
| Y88A | 0.27 ± 0.090 | 3 | Y153S | NB | 6 |
| Y88S | 0.22 ± 0.036 | 7 | Y167A | 0.28 ± 0.053 | 3 |
| Y91F | 0.80 ± 0.036 | 3 | Y167S | 0.32 ± 0.080 | 3 |
| Y91A | NB | 15 | Y234F | 1.3 ± 0.36 | 4 |
| Y91S | NB | 19 | Y234A | NB | 5 |
| Y94A | 0.43 ± 0.058 | 8 | Y234S | NB | 5 |
| Y94S | 0.37 ± 0.070 | 4 | Y240A | 0.10 ± 0.013 | 8 |
| Y141F | 0.98 ± 0.15 | 4 | Y240S | 0.083 ± 0.016 | 4 |
| Y141A | 8.97 ± 2.44 | 4 |

*Significantly different from WT (p < 0.05).

Y91S, Y141S, Y234A, or Y234S mutants despite the use of [3H]granisetron at concentrations up to 20 nM. At least two different DNA preparations of each of these mutants were used for transfection and simultaneous transfection of WT DNA yielded cell membrane preparations that bound [3H]granisetron with high affinity. It is therefore unlikely that the failure to detect radioligand binding was a result of unsuccessful transfection. For a subset of tyrosine residues (Tyr$_{50}$, Tyr$_{91}$, Tyr$_{141}$, Tyr$_{143}$, Tyr$_{153}$, Tyr$_{234}$, and Tyr$_{240}$), radioligand binding assays using [3H]granisetron were performed on phenylalanine-substituted receptors. This more conservative mutation was able to reveal specific binding with a $K_d$ for [3H]granisetron not significantly different from WT in all mutants except one (Y50F). The dissociation constants of [3H]granisetron binding are shown in Table II.

Immunofluorescent Localization of Wild Type and Mutant Receptors—To ascertain whether the lack of binding observed for some tyrosine mutants (Y50F, Y50A, Y50S, Y91A, Y91S, Y141S, Y153S, Y234A, and Y234S) was due to an ablation of ligand binding ability or to a lack of cell surface receptor expression, the localization of these mutant receptors was investigated using an antisera specific to the extracellular domain of the 5-HT$_3$ receptor (11). Intracellular staining was observed in all cells transfected with WT or mutant receptors (Fig. 1, left-hand panels) but not mock transfected cells. Conversely cell surface staining, visualized as a well defined ring of fluorescence and indicating that the receptors are expressed on the surface staining, visualized as a well defined ring of fluorescence and indicating that the receptors are expressed on the surface.

Functional Characterization of Wild Type and Mutant Receptors—Mutant receptors for those tyrosine residues suspected of being involved in the function of 5-HT$_3$ receptors were examined using calcium imaging of DNA expressed in HEK293 cells and, for some, also in oocytes. For WT receptors the concentration of 5-HT$_3$ required to evoke a half-maximal response (EC$_{50}$) was 1.47 ± 0.42 µM in HEK293 cells and 2.39 ± 0.23 µM (n = 5) in oocytes (Tables III and IV). The responses were blocked by the 5-HT$_3$ receptor antagonist granisetron and desensitized in the continued presence of agonist as previously described (22, 24, 25). Typical responses from WT receptors expressed in oocytes are shown in Fig. 2.

Calcium imaging studies revealed that responses to 5-HT (up to 1 mM) could not be detected in HEK293 cells transfected with the alanine and serine mutations of Tyr$_{50}$, Tyr$_{91}$, Tyr$_{141}$, or Tyr$_{234}$. In addition no responses to 5-HT were observed with cells expressing Y50F-containing receptors, but responses with EC$_{50}$ significantly greater than WT were observed for Y234F receptors. Both Y153A and Y153S receptors also had EC$_{50}$ significantly larger than WT receptors, although not as great as Y143S receptors, which had an EC$_{50}$ > 500 µM. The data are shown in Table III. The wave forms of all the functional receptors were similar to WT, suggesting no major changes in the kinetics of the response.

For mutations that we suspected may affect receptor assembly (Y50F and Tyr$_{234}$), receptors were also expressed in oocytes. Oocytes are generally more tolerant than mammalian cells to expression of ion channel proteins, which probably require longer periods to fold correctly; the difference may arise primarily from the fact oocytes are incubated at lower temperatures (26). Here responses to 5-HT could not be detected in oocytes injected with Y50A mRNA and Y50F 5-HT$_3$ receptor mRNA, but oocytes injected with Y50S mRNA responded to 5-HT with an EC$_{50}$ and Hill coefficient not significantly different from WT (Fig. 3). The responses also had similar wave forms to WT receptor, indicating no significant change in response kinetics. $I_{max}$ values for this mutant, however, were noticeably reduced, with maximal currents only 13% those of WT. A comparison of the responses obtained is shown in Table IV.

Y91A, Y91S, and Y91F mutant 5-HT$_3$ receptors expressed in Xenopus oocytes elicited inward currents upon application of 5-HT. Y91F receptors appeared similar to WT, but, as shown in Table III, the EC$_{50}$ values for the activation of Y91A and Y91S were significantly larger than WT, with a 6-fold increase in the EC$_{50}$ of Y91A and an 24-fold increase in the EC$_{50}$ of Y91S compared with WT (Fig. 3). There was no significant change in Hill coefficients or wave forms for any of these mutants.

**DISCUSSION**

To identify the roles of tyrosine residues in the extracellular domain of the 5-HT$_3$ receptor, we substituted the 11 tyrosines in this domain for alanine, for serine, and, for some residues, also for phenylalanine. The binding, function, and cell surface expression of the mutant receptors were then examined using the HEK293 and Xenopus oocyte expression systems. The data suggest that more than half of these residues play important roles in either the structure or the function of the receptor.
Thus, Tyr50 and Tyr91 are critical for correct receptor assembly and/or structure, whereas Tyr141, Tyr143, Tyr153, and Tyr234 play roles in the structure and/or function of the receptor. These residues are discussed further below.

Alanine and serine substitution of the tyrosine residues Tyr73, Tyr88, Tyr94, Tyr167, and Tyr240 resulted in mutant receptors that had [3H]granisetron binding properties not significantly different from WT receptors. This competitive antagonist binds with high affinity in the same binding pocket as 5-HT (e.g. 10) and is one of a selection of radioligands that have been used in similar conditions to probe the binding pocket (6, 9, 21); thus, we can be reasonably confident that this pocket is not significantly altered and that Tyr73, Tyr88, Tyr94, Tyr167, and Tyr240 do not play major roles in either the structure of the extracellular domain or in ligand binding.

These observations are supported by the placement of these residues on a recent model of the 5-HT3 receptor extracellular domain based on the structure of AChBP (7). Docking of 5-HT to the ligand-binding site on this model revealed seven possible agonist orientations, of which only two were supported by experimental evidence. These two only differ slightly (in the orientation of the primary amine), and because we cannot yet determine which of these is most favorable, we have chosen the orientation designated model 4 in Reeves et al. (7). This model supports our proposal that a number of tyrosine residues do not have significant roles. Thus, Tyr73 and Tyr88 are located some distance from the ligand-binding site and are not positioned in...
close proximity to any residues with which they might interact to stabilize the structure of the receptor. The same is true for Tyr94, although it is close to the subunit interface and not far from the ligand-binding site. Indeed, the WT-like $K_d$ values obtained for $[^{3}H]$granisetron binding to Y94A and Y94S mutants we obtained were unexpected in light of recent data (9), which indicated a 3-fold increase in the $K_d$ for $[^{3}H]$granisetron of the Y94A mutant (Y93A by their numbering) compared with WT, suggesting that this residue may participate in ligand binding. With this in mind, eight transfections were performed with Y94A DNA in this study, but the $K_d$ for $[^{3}H]$granisetron was consistently similar to WT. We cannot currently explain this discrepancy.

Our data suggest that Y50 mutant receptor subunits are not efficiently transported to the cell surface. No specific binding of $[^{3}H]$granisetron could be detected in HEK293 cells transfected with Y50A, Y50S, or Y50F mutant receptor DNA, and immunocytochemical experiments with pAb120, under conditions where it would only label cell surface 5-HT$_3$ receptors, revealed that few, if any, receptors were expressed at the plasma membrane.

However, expression of Y50S in oocytes revealed functional receptors, albeit at apparently low concentrations as judged by the low $I_{\text{max}}$. This supports previous studies that have shown that complex vertebrate proteins such as ion channels, particularly when modified in some way, may express more reliably in oocytes than in vertebrate expression system (28). The explanation for this is not yet clear, although it may be a result of oocytes being routinely incubated at lower temperatures than HEK293 cells, thus favoring correct folding (26). It also suggests that the assembly of such proteins is perhaps less tightly regulated in oocytes. Whatever the explanation, the data with Y50S strongly indicate that this residue plays a structural role in which the hydroxyl group is critical. Lack of a hydroxyl group, as in the Y50A and Y50F receptors, resulted in nonfunctional receptors, and we propose that removal of the hydroxyl renders them structurally inadequate to reach the plasma membrane.

This hypothesis is supported by the model. Here Tyr$^{50}$ is positioned at the end of the $\alpha$-helix at the top of the structure, where its closest neighbor (3.1 Å distant) is Asp$^{118}$. If tyrosine is replaced by serine its hydroxyl is more distant to Asp118 but is relatively close (3.2 Å distant) to S119 (Fig. 4). We postulate that a hydrogen bond interaction between Tyr$^{50}$ and Asp$^{118}$ is critical for the structural integrity of the receptor. A hydrogen bond might still be able to form when Tyr50 is substituted with serine, although it would be less favorable, because the distance between the hydroxyl and its partner residue would be less optimal. This hypothesis would explain the WT EC$_{50}$ of Y50S receptors and also the lack of expression of Y50A and
Y50F receptors; structurally inaccurate or unassembled receptors do not normally reach the cell surface (29).

An alternative explanation is that a hydrogen bond here forms an intersubunit contact. Tyr50 faces the subunit interface and therefore has the potential to form a head-to-tail interaction with the α-helix of the adjacent subunit. In the model adjacent subunits are not close enough to interact at this point (>5 Å distant), but because the model is based on the desensitized state of the receptor it may be inaccurate in this region, or alternatively these locations may be closer in the nascent protein, allowing this region to interact to ensure correct folding. In support of this hypothesis a potential role in receptor oligomerization of the nACh receptor 7 subunit residues Gly23–Asn46, which includes the tyrosine residue homologous to Tyr50, has recently been reported (30).

Thus, our data suggest that Tyr50 is critical for the assembly of the receptor, perhaps by allowing the formation of the correct structure at the subunit interface. In support of this important role, this residue is highly conserved among the Cys loop receptor subunits (Fig. 5; see also www.pasteur.fr/recherche/banques/LGIC/LGIC.html), suggesting a function common to all subunits in this receptor family.

Tyr91—Data obtained from Y91 mutant receptors suggest that an aromatic residue is essential here for expression in HEK293 cells; no function and poor cell surface expression were observed when it was replaced with alanine or serine, although wild type EC50 values were obtained when it was replaced by phenylalanine. All three mutant receptors, however, were functional in oocytes, perhaps indicating that this residue plays a role in receptor assembly. In the oocyte expression system there was a larger shift in EC50 for the serine mutant compared with the alanine mutant, suggesting that a hydrophobic substitution is less disruptive than a hydrophilic one, and indeed the equivalent residue is conserved as hydrophobic in all ligand-gated ion channel subunits (Fig. 5). The model of the binding site places Tyr91 at more than 5 Å from
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5-HT, and thus it is unlikely that it would participate directly in ligand binding. However, there is evidence that the adjacent residues, Trp<sup>30</sup> and Arg<sup>92</sup>, which are within 5 Å of 5-HT, are involved in such an interaction (9, 22). Previous data suggest that this region has a β-sheet composition (9); thus, if Trp<sup>30</sup> and Arg<sup>92</sup> are involved in ligand interaction, then Tyr141 would face away from the binding site. We therefore propose that Tyr141 is in a hydrophobic location and is important for the correct structure of this part of the binding pocket, possibly thereby permitting correct assembly.

**Tyr141**, **Tyr143**, and **Tyr153**—The [3H]granisetron binding data suggest that aromatic residues may be required at positions 141 and 153 for antagonist binding; large increases in K<sub>d</sub> are seen for Y141A and Y153A mutants (28-fold and 11-fold, respectively), whereas Y141S and Y153S mutants do not appear to be able to bind [3H]granisetron. Similarly Venkataraman et al. (10) observed decreases in [3H]granisetron binding affinity for Y141A (Y142A using their numbering) and Y153A (Y152A) mutant receptors. Interestingly they report a 50-fold decrease in affinity for dTC (50-fold) in Y141A receptors, the decrease in affinity for Y153A (Y152A using their numbering) and Y141S receptors did not function when expressed in HEK293 cells, as previously observed for Y141A receptors (9). Tyr141 is proposed to be more than 5 Å from 5-HT and located just outside the binding pocket; it would therefore be unlikely to participate directly in agonist binding, although it could still bind larger antagonists. It is also possible that this residue may be involved with the structure or assembly of the ligand binding microdomain. Y143A receptors were also nonfunctional, as shown previously (9), but Y143S receptors did respond to high concentrations of 5-HT. This large change in EC<sub>50</sub> (a value that incorporates both agonist binding and gating) combined with no change in antagonist binding affinity strongly suggests that this residue is involved solely in receptor gating. However, because Tyr141 is probably located within 5 Å of 5-HT and is in the binding pocket, a specific role for this residue in the binding of agonists cannot yet be excluded. Y153A and Y153S receptors were functional (as has been shown previously for Y153A receptors (9)) but showed large increases in their EC<sub>50</sub> values. Interestingly, however, the relative changes in EC<sub>50</sub> and K<sub>d</sub> compared with WT were different for the two mutants, suggesting that Tyr153 may be involved in both binding and gating.

In other Cys loop receptors, residues in this region have been suggested to play a role in gating, although their precise roles have not yet been elucidated. Data from nACh and γ-aminobutyric acid<sub>3</sub> receptors, for example, indicate that E loop residues are involved in the allosteric transitions leading to channel opening (37, 38). This region of the sequence lies adjacent to the Cys-Cys loop and could therefore provide a direct link between the receptor-ligand interactions at the binding site and the conformational rearrangement that enables channel gating. The pattern of residues identified by affinity labeling, mutagenesis, and cysteine substitution of the nACh receptor is consistent with an anti-parallel β-sheet (with the two strands linked by a turn around the central glycine residue), and such a structure is seen in the equivalent region of AChBP (1). The model and our data are consistent with a similar β-strand in the 5-HT<sub>3</sub> receptor, which would position Tyr<sup>141</sup> and Tyr<sup>153</sup> on the same face and pointing into the binding pocket. They would therefore be in a good position both to interact with agonists and also to propagate a conformational change to the nearby Cys-Cys loop. The data therefore suggest that Tyr141 plays a role in antagonist binding and/or the structure of the binding pocket, Tyr143 is critical for receptor gating and/or agonist binding, and Tyr153 is involved in both binding and gating.

**Fig. 5.** Alignment of representative subunits from the Cys loop ligand-gated ion channel superfamily. Tyr<sup>30</sup> and Tyr<sup>234</sup> are well conserved throughout the family, and Tyr<sup>41</sup> is conserved as an aromatic residue. Most other tyrosine residues are conserved only in all or some of the 5-HT<sub>3</sub> receptor family.
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native aromatic, phenylalanine, resulted in changed functional receptor characteristics (an ~10-fold increase in EC_{50}) but not in binding affinity (K_{d} not significantly different from WT), thus supporting a role for the hydroxy group in receptor gating. Extensive site-directed mutagenesis of the residue at this location has shown that it is important in the nACH receptor (39), the γ-aminobutyric acidA receptor (40), and the glycine receptor (41), although there is some debate as to whether it affects the binding affinity (42) or the gating constant (43). The binding of dTC and its analogues to the nACH receptor has been remarkably well studied, and there was evidence that these compounds might interact via cation-π interactions with Tyr^{198} (35). Subsequent incorporation of fluorinated tyrosine derivatives revealed that this does not occur for the nACH receptor (44), but interestingly the equivalent residue does appear to form such an interaction in the MOD-1 receptor (45). This points to the strong structural similarity of the extracellular domain with the 5-HT3 receptor (46), but interestingly the equivalent residue does appear to play critical for correct receptor assembly and/or structure, and Tyr^{73}, Tyr^{88}, and Tyr^{254} are not critical; nonconservative mutations do not affect [3H]granisetron binding. However, all the remaining tyrosine residues do appear important for correct receptor structure and/or function. Thus, Tyr^{73} and Tyr^{88} appear critical for correct receptor assembly and/or structure, and Tyr^{141} may also fall into this category, although it also plays a role in antagonist binding. Finally Tyr^{143}, Tyr^{153}, and Tyr^{234} are involved in binding and/or gating of the receptor. The data support the location of these residues on a model of 5-HT docked into the ligand-binding domain and also indicate the strong structural similarity of the extracellular domain with AChBP and the homologous regions of other members of the Cys loop ligand-gated ion channel family.

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