The transmembrane domain of the 5-HT3 receptor: its role in selectivity and gating

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Abstract

The 5-HT3 (5-hydroxytryptamine) receptor is a typical member of the Cys-loop family of ligand-gated ion channels. No atomic resolution structures of these proteins have yet been published, and thus structure-function relationships in this family of proteins have been largely determined from experimental evidence combined with the use of homologous proteins and lower resolution images. Here, recent advances in our knowledge of the structure and function of the transmembranous part of the 5-HT3 receptor are reviewed. These show that the pore region, M2, is largely \( \alpha \)-helical, and ion selectivity may be controlled by charged amino acid rings at each end of this pore region. The mechanism by which the pore opens is probably similar to that proposed for the nACh receptor, i.e. a twist of M2, caused by rotation in the extracellular domains, removes hydrophobic residues from the ion path.

Introduction

The 5-HT3 (5-hydroxytryptamine Type 3) receptor is a member of the Cys-loop family of ligand-gated ion channels. The best-studied member of this family is the nicotinic acetylcholine (nACh) receptor, which is found in tremendous abundance in the electric organs of fish such as Torpedo electricus. Thus it was the first Cys-loop receptor to be cloned, and these and other studies have revealed that nACh receptors are pentamers formed by the co-assembly of 1–4 different subunits; each subunit has a large extracellular N-terminal region, four putative transmembrane domains (M1–M4) and an intracellular loop between M3 and M4 [1]. More recently, molecular information about these proteins has been obtained not only from nACh receptor, but also from other members of the family, including 5-HT3, GABA\( _A \) (\( \gamma \)-aminobutyric acid type A) and glycine receptors. Since no X-ray crystal structures have been published so far for any of these proteins, molecular details of ligand binding and channel opening had to be inferred from lower-resolution images, homologous proteins and direct experimental evidence. For the latter, the 5-HT3 receptor, which is unusual in forming functional homopentamers, is proving to be a useful model for this family of proteins [2], and this review examines the structure and function of the transmembranous part of this protein, particularly in relation to the selectivity and gating of the protein. The region most critical for these functional attributes is the region from M1 to the start of M3; this is reflected in the conservation of this region between different Cys-loop receptors, as shown in Figure 1. Considerably more information is currently available on the extracellular domain of Cys-loop proteins. Studies on this region, which contains the ligand-binding site, have been revolutionized by the publication of the crystal structure of AChBP (acetylcholine-binding protein), which is homologous with the extracellular domain of the nACh receptor. 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 AChBP has been used to create homology-based models of the extracellular domains of 5-HT3, nACh and GABA\( _A \) receptors [3–6].

Structure of the transmembrane region of the 5-HT3 receptor

A number of 5-HT3 receptor subunits (5-HT3A–3E) [7] have been identified, of which the best characterized is the A subunit, which can form functional homo-oligomeric 5-HT3A receptors. The extracellular N-terminal domain contains the ligand-binding site and is predicted to be a twisted \( \beta \)-sandwich similar to AChBP; a homology model of this region is well supported by experimental evidence [4]. The transmembranous part of the structure is less understood, since there is no high-resolution structure available for this region in any of the Cys-loop receptors; however, biophysical, biochemical and cryo-electron microscopy structural studies show that the pore-lining region is formed from M2. The best evidence for this finding is provided by studies on nACh receptors, which have, for example, identified rings of residues that alter conductance, selectivity among univalent or divalent cations and channel gating [8–10]. All the transmembrane regions are predominantly \( \alpha \)-helical. This was initially inferred from hydrophobicity plots, although, apart from studies on M2 (see below) and a few
Figure 1 | Sequence alignment of the M1, M2 (indicated by bars) and flanking regions of representative Cys-loop receptor subunits

The white sections indicate regions of the structure that protrude above the membrane. The dashed line is the M1–M2 loop. The conserved proline residue in M1 is highlighted, as are the two residues in the 5-HT3 receptor that result in a reversal of ion selectivity when mutated. The ‘labelling system in M2 is also shown.

Figure 2 | SCAM allows the identification of residues that are accessible to water-soluble reagents; in the 5-HT3 receptor, two studies have shown that this pattern is consistent with a predominantly α-helical structure

The data are from Reeves et al. [14] and show the residues that interact with MTSET in the presence of 5-HT (A), indicating that they are on the water-accessible surface of the protein. A model based on these data is shown in (B). Anomalous data include interaction with L7′, which may indicate a kink at this location, and at L15′, which suggests a water-filled crevice behind M2 (see the text for more details).

labelling studies on M4, not much evidence was available until recently to support this hypothesis. Recent studies using cryo-electron microscopy, but have now provided this evidence [11].

As mentioned above, there has been good evidence for many years that M2 is predominantly α-helical. Early labelling studies with the nACh receptor channel blocker chlorpromazine indicated an α-helical conformation (see e.g. [12]), and these results were later supported by further labelling and mutagenesis experiments in a variety of Cys-loop receptors. However, it was not until the publication of the 9 Å-resolution structure (1 Å = 0.1 nm) of the nACh receptor, which revealed an α-helical structure lining the pore region, that there was direct evidence that this region is α-helical in nature [13]. To date, there have been no similar direct structural studies on the 5-HT3 receptor, but studies using SCAM (substituted cysteine accessibility method) show convincingly that the 5-HT3 receptor M2 region is also predominantly α-helical [14,15]. Results of one of these studies are shown in Figure 2.

There are, however, some anomalous SCAM data that reveal interesting features of the 5-HT3 receptor pore. In
The thiol reagent MTSET ([2-(trimethylammonium)ethyl] methanethiosulphonate bromide), which suggests that the centre of M2 is not α-helical. Similarly, SCAM studies on GABA_A and nACh receptors, and the 9 Å-resolution cryo-electron microscopy structure of the 
*Toad* nACh receptor, suggested that there may be a kink in the middle of M2 [13,16,17]. It was hypothesized that the 9′ leucine residue, which is conserved in almost all ligand-gated ion channel subunits, is located at this bend and forms the channel gate. More recent cryo-electron microscopy studies suggest that the 9′ leucine residue does not form a physical block in this region, but in conjunction with the structurally adjacent 13′ valine residue, it forms an ion-impermeant hydrophobic barrier [11].

Another section of M2 that does not appear to be α-helical is in the region surrounding L293 (15′), which is towards the extracellular end. L15′C mutant receptors reacted with MTSET, indicating that this residue is located in a water-accessible position. This supports previous studies on the GABA_A receptor M3 segment, which suggest that the rear of the M2 segment faces a water-filled crevice that extends into the interior of the membrane-spanning domain [18]; the recent nACh cryo-electron microscopy studies also indicate such a crevice [11].

### Ion selectivity of 5-HT_3 receptors

The ion channel coupled with the 5-HT_3 receptor pore is cation-selective, as is the nACh receptor [19]. The determinants for this in 5-HT_3 receptors must lie predominantly towards the middle or intracellular end of the pore, since SCAM studies have shown that both anions and cations can enter the extracellular end of the 5-HT_3 channel [14]. This is interesting in view of the three rings of negatively charged residues proposed by Imoto et al. [8] to play a major role in ion selectivity; indeed, the fact that rings of negatively charged residues play a role in determining conductance and cation selectivity in the nACh receptor is now well established (see e.g. [8,20–22]). One of Imoto’s rings, however, is on the extracellular side of the membrane, and the SCAM data described above and other studies (see below) suggest that this ring does not play a major role in ion selectivity. One of these was the elegant study by Galzi et al. [20], which showed that changing only three amino acids could convert the channel to anionic; two of these were in the M1–M2 loop and one was towards the middle of M2 at position 13′ (see Figure 1). Subsequently, similar studies were performed in glycine [23], 5-HT_3 [24] and GABA_A [25] receptors, and they confirmed that changing the equivalent amino acids in these receptors also resulted in a change in ion selectivity.

There were, however, some unusual features in these ‘selectivity transition mutants’, which exhibited additional substantial differences in properties compared with the corresponding wild-type receptors; these included changes in desensitization and activation rates, and increased agonist affinity. Since all the mutants had an additional or deleted proline residue, these changes in function may have resulted from substantial structural alterations within the channel. Further studies on the nACh receptor [26] showed that the introduction of a proline residue in a variety of positions in the M1–M2 loop could produce functional anionic α7 nACh receptors; thus its insertion may have caused a structural change in the protein that resulted in a change in selectivity. More recent studies on the 5-HT_1 receptor have shown that neutralization of the ring of negative charge at the −1′ position and adding a ring of positive charge at position 19′ (Figure 1) creates an anionic receptor that has similar response kinetics and agonist affinity as wild-type receptors [27], thus supporting the above hypothesis. Interestingly, replacement of the glutamate residue at position −1′ (E-YA) alone results in a receptor that is non-selective, indicating the importance of this residue, and also demonstrating that electrostatic factors are of primary importance in controlling ion selectivity. This has been further highlighted by studies on the anion-selective receptors: results from the GABA_A receptor are very similar to those from the 5-HT_1 receptor, since changing the −1′ residue from alanine to glutamate resulted in a non-selective channel [25]. However, changing the −1′ residue in the glycine receptor reversed its ion selectivity, suggesting that there may be subtle differences in the mechanism of selectivity between different ligand-gated ion channels [28]. In addition, such a mechanism may be subunit-specific; a recent study in the GABA_A receptor indicates that changing the M1–M2 loop residues in the β3 subunit alone can reverse ion selectivity [29]. Thus all these studies show that the M1–M2 loop contains the major determinant of ion selectivity, and the data are currently not inconsistent with a simple electrostatic mechanism although some details of its molecular control remain to be determined.

It is also probable that regions outside the pore-lining domains play a role in ion selectivity. Recently, these regions have been shown to play a major role in the conductance of 5-HT_3 receptors [30] and are probably also important in concentrating the appropriately charged species as suggested by Unwin [31] for the nACh receptor.

### Gating in 5-HT_3 receptors

5-HT_3 receptor gating [an agonist-induced transition between a resting (closed) state and a conducting (open) state of the pore] occurs within 10–40 ms of agonist application, and the response generated rapidly desensitizes in the continued presence of agonist (see [2] for a review). Functional studies with 5-HT_1 receptors reveal Hill coefficient values >1, and many are >2, indicating that two or possibly three molecules of agonist are required for channel opening. Thus, even though there are five potential binding sites in the 5-HT_3 receptor, it is probable that all of them need not be occupied for channel gating to occur. The α subunits in the nACh receptor are not adjacent, and the binding of an agonist molecule to each appears to result in a twist of the structure, which is then transduced to the pore
Figure 3 | A model of receptor gating based on that proposed by Miyazawa et al. [11]
5-HT binding to the extracellular domain (ECD) causes a rotation which is then propagated via the β1–β2 and M2–M3 loops to M2 in the transmembrane domain (TMD). This would result in breaking of the hydrophobic interactions at the centre of M2, allowing ions to flow.

region [11]. These non-adjacent rotations may provide the greatest torsional force to open the channel; thus, probably at least two non-adjacent subunits in 5-HT3 receptor need to bind agonist before this channel can open. Indeed, a recent study suggests that homomeric 5-HT3 receptors require the binding of at least three molecules of agonist to open [32].

After binding, the conformational change is postulated to be transduced to the pore through the interaction of the extracellular-domain β1–β2 loops and transmembrane-domain M2–M3 loops [11]. Results from mutations in the M2–M3 loop in many Cys-loop receptors have indicated that this region forms a critical link between binding and function, as does the recent model from the cryo-electron microscopy studies [11,33–37]. Some studies suggest that it is a change in the interactions of the residues that form the Cys loop that propagates channel opening, whilst others suggest that its role is primarily structural. Further experiments suggest that the pre-M1 region and yet another loop (loop 9 or the β9 loop) may be involved [38–40]. More data are clearly required to determine the precise role and/or interactions of all of these segments.

These structural changes are then proposed to open the gate. As described above, results of cryo-electron microscopy studies [11] suggest that the 9' leucine residue and structurally adjacent 13' valine residue form a hydrophobic girdle in the channel, which acts as an energetic barrier to ions. A twist of M2 would allow these residues to be replaced by the adjacent hydrophilic residues, and a model of this is shown in Figure 3. It is possible that M1 too plays a role. This transmembrane region, which also forms a part of the channel lining (see e.g. [41]), contains a highly conserved proline residue. Experiments using unnatural amino acid substitutions have shown that the lack of ability of this proline residue to act as a hydrogen bond donor is necessary for normal gating transitions in both 5-HT3 and nACh receptors [37,42,43]. A speculative interpretation of these results is that the twist of M2 not only provides the energy required to break the hydrophobic interactions, but also causes one of the inter- or intra-chain hydrogen bonds in M2 to break. This might then reform with the uncompensated hydrogen bond acceptor provided by the essential proline residue in M1 and therefore help to destabilize the helical nature around the conserved 9' leucine residue, allowing it to swing away from the channel lumen and form the open state.

To summarize, in the 5-HT3 receptor, as in other Cys-loop ligand-gated ion channels, the transmembrane region from M1 to M3 provides the residues critical for ion selectivity and gating of the receptor. In this brief review, some details and potential molecular mechanisms controlling these features in this family were reviewed, and, although some good hypotheses have emerged, it is clear that more experimental evidence is required. The 5-HT3 receptor may prove to be a useful tool in providing this evidence.

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