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6 The relationship between structure and function in 5-HT_3 receptor: The transmembrane domain

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Abstract

The 5-HT_3 receptor (5-HT_3R) is a member of the Cys-loop family of neurotransmitter receptors which share similar structures and functions. The functional arrangement of the receptor components and the generation of 5-HT_3R chimaeras have helped develop the idea of these receptors as modular proteins that consist of an extracellular and a transmembrane domain. In this chapter we explore the characteristics of the transmembrane domain of the 5-HT_3R. Like other Cys-loop receptors, this domain contains four transmembrane spanning regions and a large intracellular loop. There is good evidence that the transmembrane spanning regions (M1-M4) are α-helices. M2 from each subunit lines the central ion-conducting pore of the channel and has a role in ion selectivity and the movement of ions through the membrane. It is shielded from the lipid environment of

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the membrane by the arrangement of M1, M3 and M4. The intracellular loop that lies between M3 and M4 dominates the mass of receptor protein within the cell and represents the cytoplasmic boundary for the passage of ions through the channel. This region has been implicated in channel conductance and channel modulation.

Abbreviations

LGIC; ligand-gated ion channel; 5-HT_3R; 5-HT_3 receptor; nAChR; nicotinic acetylcholine receptor; $GABA_A$R; $GABA_A$ receptor; AChBP; acetylcholine binding protein; GlyR, glycine receptor; SCAM; scanning cysteine accessibility method; SHAM; scanning histidine accessibility method.

1. Introduction

The 5-HT_3R differs from all other 5-hydroxytryptamine (5-HT; serotonin)-activated receptors as it contains an integral ion channel, and in contrast to a slow metabotropic response typical of most 5-HT receptors, the 5-HT_3R channel opens within milliseconds of agonist application (Suprenant and Crist, 1988; Yakel and Jackson, 1988; Yang et al., 1992). The cloning of the 5-HT_{3A} subunit (Maricq et al., 1991) revealed a protein of 487 amino-acids that displayed 21-30% sequence identity to homologous receptors and established this receptor as a member of the Cys-loop ligand-gated ion channel (LGIC) family. The 5-HT_{3A} subunit is unusual in that it can form a functional homo-pentameric receptor. The A-subunit can also combine with the more recently cloned 5-HT_{3B} subunit to create hetero-pentamers that display altered electrical and pharmacological properties (Davies et al., 1999; Dubin et al., 1999; Hanna et al., 2000). Genes for 5-HT_{3C}, 5-HT_{3D} and 5-HT_{3E} subunits have also been described, but to date these subunits have not been characterised (Niesler et al., 2003).

Similar to all Cys-loop LGIC members, the 5-HT_3R is a pentameric arrangement of subunits. Each subunit has both an extracellular and a transmembrane domain. The extracellular domain is highly homologous to the acetylcholine binding protein (AChBP) whose structure has been resolved to 2.1 Å (Celie et al., 2004). Structural knowledge from AChBP can be extrapolated onto other LGIC family members and has been discussed in chapter 2. The transmembrane domain consists of four membrane spanning segments (M1-M4) and a large intracellular loop between M3 and M4. It is known that the arrangement of the subunits forms a central ion-conducting pore, but the transmembrane region as a whole is considerably less understood than the extracellular domain. Studies on chimaeric receptors that combine the ligand-binding domain of either the nicotinic acetylcholine receptor (nAChR) α7 or AChBP with the transmembrane domain of the 5-HT_3R result in functional receptors, showing that these proteins can be considered as modular structures (Bouzat et al., 2004; Eiselé et al., 1993). The transmembrane domain is considered in this chapter.

2. 5-HT_3R structure

The gross structure of the 5-HT_3R remains undefined at the molecular level, but electron microscopy of 5-HT_3Rs purified from NG108-15 cells (Boess et al., 1995) and the baculovirus expression system (Green et al., 1995) have shown that these receptors share an arrangement of subunits that is similar to the nAChR (Fig. 1). Fourier transform

infrared spectroscopy and circular dichroism have also revealed that the receptor is predominantly α-helical, providing further evidence of the structural similarities between nAChRs and 5-HT_3Rs (Hovius et al., 1998; Rigler et al., 2003). Combined with evidence from molecular biological studies, these results indicate that the structure of the 5-HT_3R is well represented by the cryo-electron microscope images of the nAChR (Miyazawa et al., 2003). Consequently, it is well accepted that each membrane-spanning region of the 5-HT_3R is composed of four α-helices, a model that corresponds with predictions made using hydrophobicity analysis (Maricq et al., 1991). The helices are arranged symmetrically, forming an inner ring of M2 helices that is in direct contact with the permeating ions, and an outer ring composed of M1, M3 and M4 that may shield the inner ring from the lipid environment (De Planque et al., 2004). The structure of the large intracellular loop between M3 and M4 remains uncertain, but functionally it appears to have a role in channel conductance and phosphorylation-stimulated receptor modulation. The extracellular C-terminal region is small (~3 amino acids in length) and has not been linked to any functional properties of the receptor.

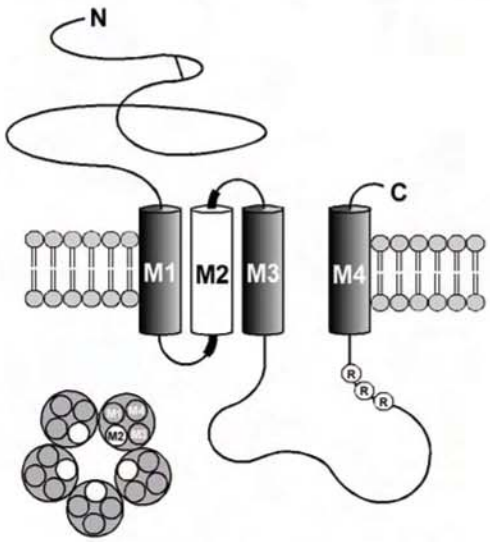

Figure 1. A schematic representation of a typical Cys-loop LGIC subunit. The diagram at the lower left is a cross section of the channel shown from above and demonstrates how five subunits associate to form the central ion-conducting pore. Attention is drawn to M2 (white circle), the regions associated with ion-selectivity (dark lines either side of M2) and the region that has been shown to influence ion conductivity (R-R-R).

Epitope tagging has provided direct experimental evidence of the transmembrane topology of the 5-HT_3R (Mukerji et al., 1996; Spier and Lummis, 2002; Spier et al., 1999) and reveals that it is comparable to other members of the LGIC family. In these studies antibodies directed against the N-terminal extracellular domain bound to intact cells whilst antibodies directed against the intracellular region only bound to permeabilised cells. The extracellular location of the C-terminal tagged domain was

consistent with the presence of a fourth transmembrane region. The observation that *Flag* and *c-myc* tags did not disrupt the agonist and antagonist properties of the channel when expressed at the C-terminus was evidence that this region was not critically involved in agonist binding or gating.

The substituted cysteine accessibility method (SCAM) has shown that the pore is lined by residues of M2 (Fig. 2). Like other LGIC receptors this region is predominantly α-helical, although the residues identified by SCAM differ between the published studies (Akabas et al., 1994; Panicker et al., 2002; Reeves, 2001; Xu and Akabas, 1996; Zhang and Karlin, 1998). A further analysis using substitution with histidines (SHAM) suggests another arrangement of M2 pore-lining residues. However, as the results all indicate an α-helical structure, these differences may be a reflection of flexibility within the protein.

In the nAChR, a kink at the centre of the M2 helices lies within the most constricted region of the channel pore and coincides with a collar of hydrophobic side groups centred around Leu 9'. Interestingly this leucine is conserved between most subunit types, with the notable exception of the 5-HT_{3B} subunit (Fig. 3). The incorporation of five

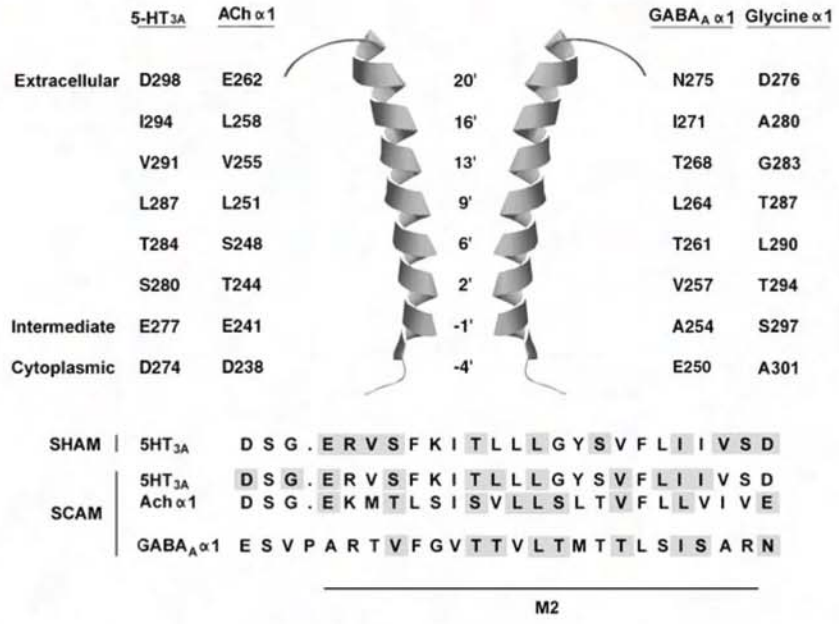

Figure 2. M2 channel lining residues in four representatives of the ligand-gated ion-channel family. Proposed pore lining residues are shown next to the M2 α-helix (PDB ID; 1OED). Members of the family that have been studied using SCAM and SHAM analysis are shown in the alignment below. Amino acids that have been identified as accessible by modifying sulfhydryl reagents or transition metal cations are highlighted by boxes in the 5-HT_3R (Kaneez and White, 2004; Reeves et al., 2001), nAChR (Akabas et al., 1994) and $GABA_A$R (Xu and Akabas, 1993). Charged rings of amino acids are located at positions -4', -1' and 20'. Accession numbers for the alignment are as follows: Mouse 5-HT_{3A} Q6J1J7, Electric ray Ach α1 P02710, Human $GABA_A$ α1 P14867, Human Glycine α1 P23415.

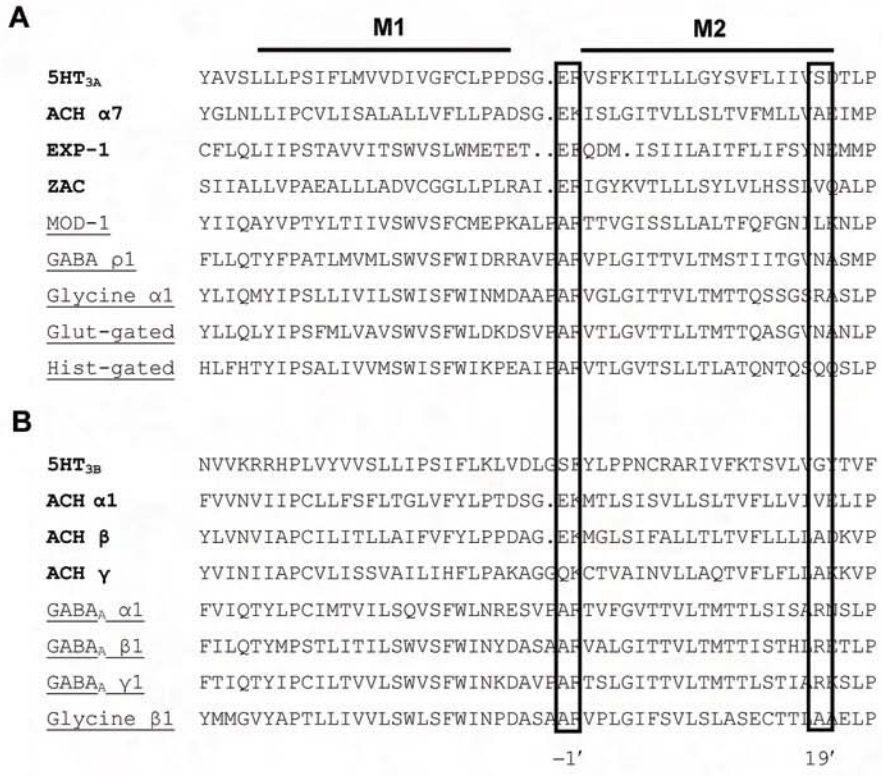

Figure 3. A. Alignment of M2 residues in representative examples of subunits that can form functional homomeric receptors. **B.** Subunits that must combine with other subunits to form functional heteromeric receptors. Attention is drawn to the -1' and 19' residues that have been identified as important features of charge selectivity in the LGIC family. Subunit names that are highlighted in bold are associated with receptors that display cationic selectivity. Subunits that are associated with anionic receptors are underlined. Accession numbers for the alignment are as follows: Mouse 5-HT_{3A} Q6J1J7, Human ACh α7 P36544, Nematode EXP-1 Q6TXQ9, Human ZAC Q86YW4, Nematode MOD-1 Q8MPU3, Pufferfish GABA ρ1 Q8UW04, Human Glycine α1 P23415, Nematode GluCl α1 Q9TZR3, Drosophila HisCl1 Q9VGI0, Human 5-HT_{3B} O95264, Electric ray Ach α1 P02710, Human Ach β P11230, Human ACh γ P07510, Human $GABA_A$ α1 P14867, Human $GABA_A$ β1 P18505, Human $GABA_A$ γ1 Q8N1C3, Human Glycine β1 P48167.

symmetrically placed M2 helices from each of the five subunits (Hucho et al, 1986) creates a region that is 3 Å at its narrowest and less than 3.5 Å over a distance of approximately 8 Å (Miyazawa et al., 2003). This constricted hydrophobic region is proposed to be the gate, as it would provide an energetic barrier to ion permeation. This region also shares many structural similarities to the gate of other membrane permeable channels, although some evidence has placed the gate closer to the intracellular side of the membrane (Chang et al., 1998; Doyle et al., 1998; Murata et al., 2000; Ren et al., 2001; Wilson and Karlin, 1998). However, the location of the gate at or close to Leu 9' is supported by photo-labelling

(Blanton et al., 1998; White and Cohen, 1992; White et al., 1991) and mutagenesis in both nAChR and 5-HT_3Rs (Buckingham et al., 1998; Chang and Weiss, 1998; 1999; Filatov and White, 1995; Labarca et al., 1995; Panicker et al., 2002).

3. 5-HT_3R function

The integral ion channel of the 5-HT_3R is formed from five M2 domains. Similar to the nAChR, binding of agonists in the extracellular domain is believed to cause movements of the extracellular domain that are translated to the M2 helices, destabilizing their hydrophobic centre and leading to opening of the pore (Grosman et al., 2000; Unwin et al., 2002; Miyazawa et al., 2003).Evidence to support this comes from a variety of studies, such as SCAM analysis (see *section 2*) and mutagenesis. For example, mutation of a conserved lysine at position 4' (Lys 4') (Figs. 2 and 3) in homomeric 5-HT_{3A}Rs has revealed slowed desensitisation and increased receptor sensitivity to the agonist 5-HT (Gunthorpe et al., 2000), whilst there are complex changes in desensitisation kinetics when the highly conserved Leu 9' is mutated (Yakel, 1996; Yakel et al., 1993). The ligand binding and transmembrane domains may interact at various locations, but the transduction of binding into channel opening appears to primarily involve the interaction of the β1-β2 and M2-M3 loops (Bouzat et al., 2004). In particular, there is evidence that proline in the M2-M3 loop may play a critical role here (Lummis et al., 2005). However, many of the molecular details of the mechanism of channel opening have yet to be determined.

The 5-HT_3R is able to form functional channels as a homomeric receptor when introduced into expression systems, but some of its functional properties such as channel rectification and single channel conductance do not resemble those observed for some native 5-HT_3Rs. The presence or absence of the 5-HT_{3B} subunit may explain why the current-voltage relationship has been described as both linear (Higashi and Nishi, 1982; Neijt et al., 1989) and inwardly rectifying (Lovinger and White, 1991; Peters et al., 1993; Robertson and Bevan, 1991; Yang et al., 1992) in native tissues, although this may also be due to variation between species (Davies et al., 1999; Dubin et al., 1999; Hanna et al., 2000). Certainly, studies of heteromeric 5-HT_{3A} and 5-HT_{3B} receptors reveal a single channel conductance that more closely resembles the conductance displayed by some native receptors (Davies et al., 1999; Kelley et al., 2003). In the absence of the 5-HT_{3B} subunit, homomeric 5-HT_{3A}R responses have a conductance that is typically less than 1 pS and can only be measured indirectly using fluctuation analysis. When the B-subunit is introduced the conductance rises to around 20 pS and can be discerned using standard patch clamp techniques. Specific amino acids that are responsible for this change in conductance have been identified (Kelley et al., 2003) and are discussed in more detail below. As the sequence similarity of the 5-HT_{3A} and 5-HT_{3B} subunits (41%) is comparable to the sequence similarity observed between the subunits of other receptor classes within the LGIC family, it is likely that these two subunits at least partially account for the functional variability observed in native tissue.

Structural heterogeneity resulting from alternative splicing has also been identified. Receptors that are missing a six amino acid stretch in the M3-M4 loop (short splice form) have been isolated in human (Belelli et al., 1995; Miyake et al., 1995), rat (Miquel et al., 1995) and guinea pig (Lankiewicz et al., 1998) tissue as well as some mouse neuronal cell lines (Fig. 4) (Hope et al., 1993; Werner et al., 1994). In rat, the distribution of this short

Figure 4. Conservation of residues in 5-HT_3Rs from five different species. Residues that are completely conserved are highlighted as white text in red boxes. The remaining residues are highlighted as follows: 4/5 = red text, 3/5 = light blue text, 2/5 = dark blue text and 1/5 = black text. High levels of conservation are seen within the 5-HT_3Rs of the species shown. The major differences are seen in the ligand binding loops C and F and in the intracellular M3-M4 region. The text box shows the additional six amino acids that are found in the guinea pig sequence. Accession numbers for the alignment are as follows: Guinea Pig O70212, Rat P35563, Ferret Q9N0F4, Human P46098, Mouse Q6J1J7.

splice variant varies depending upon its location in the animal (Miquel et al., 2002) and the stages of development (Miquel et al., 1995). Splice variants studied in HEK293 cells and oocyte expression systems have also revealed some small functional differences (Downie et al., 1994; Hubbard et al., 2000; Niemeyer and Lummis, 1998) that are comparable to native systems (Lankiewicz et al., 1998; Van Hooft and Vijverberg, 1995; Yakel and Jackson, 1988).

In humans, the splice acceptor site that is responsible for the long form of the receptor is missing (Bruss et al., 2000b; Werner et al., 1994) and consequently the long variant that is found in rodents is not expressed. However, in humans a truncated (h5-HT_{3AT}) and an alternative long (h5-HT_{3AL}) form have been identified (Bruss et al., 2000a). The truncated version consists of 238 amino acids and contains only a single transmembrane (M1) region whilst the long form contains an additional 32 amino acids in the M2-M3 loop (Fig. 5). Whilst these two subunit variants cannot form functional homomeric receptors when expressed alone, they are able to co-assemble with 5-HT_{3A} subunits and modulate the 5-HT response. The presence of splice variants may in part explain the functional variation seen in nature, although they are unlikely to account for all the functional diversity of native 5-HT_3Rs.

Co-assembly of the 5-HT_{3A} subunit with the nAChR α4 subunit in HEK293 and oocyte expression systems has also been observed (Kriegler et al., 1999; van Hooft et al., 1998), but in nerve terminals these two subunits do not appear to interact despite their co-localisation (Nayak et al., 2000). Purification of 5-HT_{3A} subunits from pig brain has identified four proteins that co-purify (unidentified 52, 57, 63 and 71 kD), but these may correspond to other Cys-loop receptor subunits or may be proteins from other receptor families (Fletcher and Barnes, 1997). Studies have also shown that 5-HT_3Rs are influenced by interactions with other ligand-gated receptors (Boue-Grabot et al., 2003; Boue-Grabot, 2000; Morales and Backman, 2002), an observation that has been seen in other members of the family (Boue-Grabot et al., 2004; Khakh et al., 2000; Li and Yang, 1998; Li and Xu, 2002; Searl et al., 1998; Sokolova et al., 2001).

4. The first (M1) and second (M2) transmembrane domains of the 5-HT_3R

Studies of M1 have shown that this region is important for correct channel function. Of particular interest is the highly conserved Pro in M1, which has been shown to be essential. Changing this to any other amino acid results in loss of function, although radioligand binding characteristics are preserved (Deane and Lummis, 2001). Pro has a number of unusual properties, including a lack of ability to act as a hydrogen bond donor due to its ring structure and a study using unnatural amino acid mutagenesis has shown that it is this ability that is critical for 5-HT_3R function (Dang et al., 2000).

Mutations in other regions can also affect function. For example, the subtle change of Ser^{248} to Thr modifies desensitisation kinetics in the human 5-HT_3R so that it more closely resembles that found in the guinea pig 5-HT_3R (Lobitz et al., 2001). The reverse mutation (Thr^{254} to Ser) makes the guinea pig receptor more closely resemble the human receptor. The extracellular part of the M1 transmembrane helix has also been implicated as an important functional element of the gating process. Mutation of Arg^{222} caused changes in the apparent affinity of both full and partial agonists and as mutagenesis of

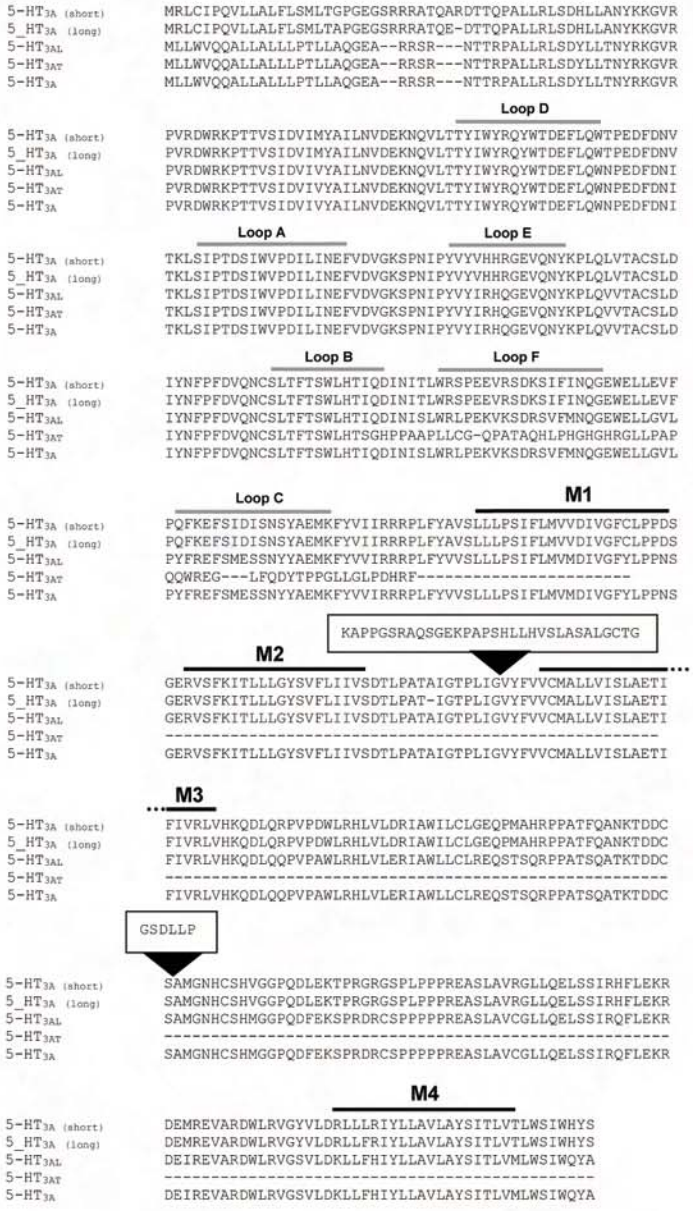

Figure 5. Alignment of 5-HT$_3$R splice variants. The first two sequences represent mouse short (5-HT$_{3A\ (short)}$) and mouse long (5-HT$_{3A\ (long)}$) isoforms. For comparison, the human alternative long (5-HT$_{3AL}$) and human truncated (5-HT$_{3AT}$) forms are shown next to a typical human sequence (5-HT$_{3A}$). The boxes show the additional residues found in 5-HT$_{3AL}$ (32 amino acids) and 5-HT$_{3\ (long)}$ (6 amino acids) variants. Accession numbers for the alignment are as follows: Mouse 5-HT$_{3A\ (short)}$ Q6J1J7, Mouse 5-HT$_{3A\ (long)}$ P23979, Human 5-HT$_{3A}$ P46098. Sequences for the human 5-HT$_{3AL}$ and 5-HT$_{3AT}$ were taken from Bruss et al., 2000a.

closely located residues in glycine, $GABA_A$ and nACh receptors has also revealed changes in function, the authors suggest this region is involved in transmitting information from the ligand binding domain to the M2 region (Hu et al., 2003).

The M2 α-helices line the channel pore and influence ion selectivity. The ion-selectivity displayed by the 5-HT_3R is cation specific and is predominantly composed of a mixed sodium and potassium conductance (Peters et al., 1992; 1993; Yang, 1990). Ion substitution experiments have also established that the channel is permeable to calcium, magnesium, and small organic cations (Yakel et al., 1990; Yang, 1990; Yang et al., 1992).

Like other Cys-loop LGIC channels, the residues that run along the ion-accessible inner face of the 5-HT_3R channel are predominantly non-polar except for rings of charged amino-acids (Fig. 2) (Akabas et al., 1992; Akabas et al., 1994; Reeves et al., 2001; Zhang and Karlin, 1998; Panicker et al., 2002; Xu and Akabas, 1993; Xu et al., 1995). An evaluation of the differences between the α7 nAChR and the α1 glycine receptor (GlyR) M2 regions has revealed contenders for the amino acids responsible for ion selectivity and initial experiments showed that, replacement of all the pore facing α7 AChR residues with those from the α1 GlyR resulted in an anionic channel (Galzi et al., 1992). As the number of mutations was reduced, the smallest number of residues required to reverse ion selectivity was identified, namely a Val to Thr (Val^{251}Thr or $Val^{9'}$Thr), neutralisation of a glutamate (Glu^{237}Ala or $Glu^{-1'}$Ala) and the insertion of a Pro (Pro^{236} or $Pro^{-2'}$) in the M1-M2 loop. Homologous changes have been made to amino acids in 5-HT_{3A} (Gunthorpe and Lummis, 2001) and α1 GlyRs (Keramidas et al., 2000) and have similarly reversed charge selectivity. However, these studies also generated significant changes in the functional properties of all the receptor, including differences in activation, desensitisation, agonist EC_{50}, Hill coefficient and spontaneous channel openings (Corringer et al., 1999; Wotring et al., 2003) that suggest gross structural changes to the channel. This study also showed that the altered selectivity was dependent upon the coordinated presence of all three mutations and was not due to an incremental influence of each mutation.

In contrast to the experiments described above, charge selectivity has been reversed in the 5-HT_3R (Thompson and Lummis, 2001; Thompson and Lummis, 2003) by mutating just two amino acids ($Glu^{-1'}$Ala and $Ser^{19'}$Arg). This was achieved without the other associated changes in receptor properties and in an additive manner. The -1' position forms a ring of charged residues in M2 originally identified as the intermediate or intracellular ring (Imoto et al., 1988) (Fig. 2). This ring has been shown to have a far greater influence on ion selectivity than the extracellular or cytoplasmic rings (Imoto et al., 1988) and its proposed location at the narrow region of the pore supports this function. In the 5-HT_3R and nAChR this ring is largely formed by negatively charged Glu residues (Fig. 3) (Corringer et al., 1999; Galzi et al., 1992; Imoto et al., 1988; Konno et al., 1991). In contrast, the anionic $GABA_A$R and GlyR have a ring that is composed of uncharged amino acids, usually Ala (Keramidas et al., 2002; Wotring et al., 2003). In the 5-HT_3R, replacement of the native Glu at this position with Ala results in a largely non-selective channel (Thompson and Lummis, 2001; Thompson and Lummis, 2003). When this mutation is combined with changing a neutral Ser at the extracellular 19' position to a positively charged Arg, the receptor becomes predominantly anion selective. These results show that in the 5-HT_3R, and possibly in all Cys-loop receptors, the control of ion selectivity is dominated by charged rings of residues at one or both ends of M2.

It is also possible that charged residues in the channel vestibules and at the channel mouth concentrate the relevant ions and play a significant role in ion selectivity in the LGIC family (Unwin, 2000). Similar mechanisms have been identified in other receptor families (Doyle et al., 1998; Dutzler et al., 2002; Yue et al., 2002). In particular, there are many charged residues in the amphipathic helix that forms part of the M3-M4 loop and which may influence ion selectivity. This region is discussed in more detail below.

5. The third (M3) and fourth (M4) transmembrane domains of the 5-HT_3R

The M3-M4 linker is responsible for most of the mass that lies at the cytoplasmic side of the receptor and presents a potential barrier to the passage of ions through the channel (Miyazawa et al., 1999). Electron microscopy of the nAChR has revealed an area of electron density that forms a closed cage with openings that are presumed to allow the movement of ions between the cytoplasm and the intracellular mouth of the pore (Miyazawa et al., 1999). A detailed analysis of this region in the 5-HT_3R has shown it to be a major determinant of channel conductance (Kelley et al., 2003). In the 5-HT_3R, A-subunits can form functional homomeric channels with a conductance that is so small (sub-pS) that it cannot be resolved directly. Whilst the 5-HT_{3B} subunit cannot form homomeric channels, it can be combined with A-subunits to generate functional heteromeric receptors that display a much larger conductance (9-17 pS) (Brown et al., 1998; Davies et al., 1999; Derkach et al., 1989; Hussy et al., 1994). By exchanging parts of the A-subunit sequence with homologous regions from the B-subunit, three Arg residues were identified as the source of the difference (Kelley et al., 2003; Peters et al., 2004). These three positively charged residues lie within a predicted amphipathic α-helix (Finer-Moore and Stroud, 1984; Miyazawa et al., 1999) within the M3-M4 loop. This region may be responsible for the binding of accessory proteins to the base of the channel (Miyazawa et al., 1999) which would hinder the flow of ions in the homomeric receptor (Fig. 6). It is also possible that the amino acids line the inner surface of the cytoplasmic region of the pore, enabling the charged groups to influence the ions passing through the channel (Unwin, 2000). A more likely proposition places these residues on the outer rim of apertures that exist at the meeting of cytoplasmic regions from adjacent subunits and which allow ions to pass from the cytoplasm and into the inner vestibule that exists at the base of the pore (Miyazawa et al., 1999). The widest region of these apertures is no greater than the diameter of a permeating ion with its first hydration shell (Miyazawa et al., 1999), so the charge of the encircling amino acids would greatly influence both the ionic species and the rate of movement into and out of the cytoplasmic vestibule receptor (Brown et al., 1998; Lambert et al., 1989; Malone et al., 1991; Mochizuki et al., 2000; Yang, 1990; Yakel et al., 1990).

Phosphorylation of the LGIC family members has been observed within the M3-M4 loop (Swope et al., 1999) and has been observed in 5-HT_3R (Lankiewicz et al., 2000) as well as in nAChR (Moss et al., 1996; Wecker et al., 2001), $GABA_A$R (McDonald and Moss, 1994; 1997; Moss et al., 1992) and GlyRs (Ruiz-Gomez et al., 1991). Experiments on the 5-HT_3R have shown that protein kinase C and casein kinase II have a potentiating effect on the 5-HT-mediated current (Coultrap and Machu, 2002; Hubbard et al., 2000;

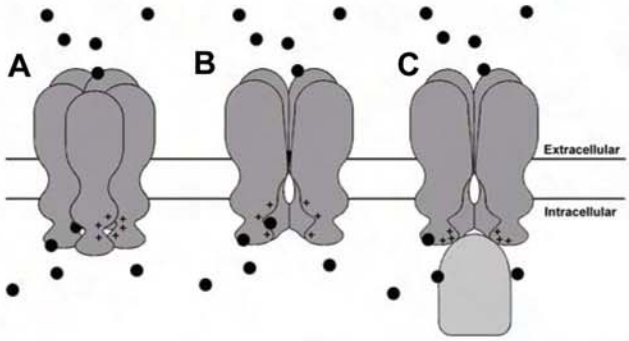

Figure 6. Schematic representation of a 5-HT_3R. Black + at the cytoplasmic side of the receptor represent charged residues in the M3-M4 loop that have been shown to influence channel conductance (Kelley et al., 2003). Three possible roles for these residues are shown. A. Charges clustered around the apertures that are located within the walls of the intracellular vestibule influence ion movements as ions enter and leave the cytoplasmic region of the channel. B. Charges line the cytoplasmic pore and influence ion movement before reaching the selectivity region further up the channel. C. Charges at the base of the receptor affect the association of auxiliary proteins.

Sun et al., 2003; Zhang et al., 1995) which may cause an increase in the single channel conductance (Van Hooft and Vijverberg, 1995). However, it has also been documented that increases in current amplitude can be accounted for by changes in the cell surface expression of the 5-HT_3R (Sun et al., 2003), a conclusion that has been drawn in other receptors of the family (Balduzzi et al., 2002; Connolly et al., 1999; Filippova et al., 2000; Kusama et al., 2000; Sun et al., 2003). Protein kinase A has been found to significantly accelerate desensitisation of the 5-HT_3R response (Yakel et al., 1991) and labelling has identified a potential target site at residue Ser^{409} (Lankiewicz et al., 2000). Differences in the effects of both protein kinases A and C have been observed in the long and short forms (see section 4) of the 5-HT_3R and may provide an explanation for the physiological role of these splice variants (Hubbard et al., 2000).

6. Conclusions

The 5-HT_3R has been widely used in the construction of chimaeric LGIC receptors which have demonstrated the modular properties of these proteins (Bouzat et al., 2004; Eiselé et al., 1993; Kriegler et al., 1999; Verbitsky et al., 2003). The transmembrane domain in particular has been shown to be representative of other Cys-loop receptors with its similar structure and function that is typified by the α-helical M2 pore-lining region. The 5-HT_3R transmembrane domain has also proved useful in probing the role of the M3-M4 loop as mutations within the amphipathic helices of this region have been shown to have a role in controlling conductance. These helices probably form the apertures in the cytoplasmic vestibule, and may control ion movement in and out of this region. This array of evidence and the ability of the 5-HT_{3A} subunit to form functional homomeric receptors highlights the convenience of the 5-HT_3R as an experimental model. As a result of its versatility, the 5-HT_3R has played an important role in our current understanding of structure function relationships in all Cys-loop proteins.

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