A HOMOLOGY MODEL OF THE CLOSED STATE OF THE 5-HT_{3A} RECEPTOR

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The Cys-loop family of pentameric ligand-gated ion channels, of which the 5-HT_3 and nicotinic acetylcholine (nACh) receptors are members, share many structural and functional characteristics. Each receptor subunit comprises an extracellular ligand-binding domain (ECD), a transmembrane region forming the channel pore and a large intracellular loop. This study describes, for the first time, the generation of a model of the closed state of the full-length 5-HT_3 receptor, based on homology to the recent cryo-electron microscopy structure of the nACh receptor (Unwin, 2005).

Amino acid sequences of the mouse 5-HT_{3A} receptor subunit and the *Torpedo marmorata* nACh receptor subunits were aligned using ClustalX. Ten models of the 5-HT_{3A} receptor were generated using MODELLER (Sali *et al*., 1993) based on the coordinates of the nACh receptor (PDB ID: 2BG9). The four lowest-energy models were subjected to Ramachandran plot analysis and the model showing the lowest proportion of phi/psi angles in the disallowed region was selected for further study. Surface electrostatic potential calculations were performed with Swiss-PdbViewer.

The model reveals important structural features of each aspect of receptor function. The structure of the ECD differs from that in an open state model (Reeves *et al*., 2003) notably by the positioning of binding loop C which is seen to project away from the core of the pentamer and the ligand binding site in the closed state. The binding site is therefore more accessible to ligands in this state. The β1-β2 loop of the ECD straddles the M2-M3 loop forming contacts which may transmit agonist-induced conformational change to the pore, however K81 of the β1-β2 loop is solvent-accessible. The corresponding residue in the nACh receptor (αV46) resides in a hydrophobic pocket formed by transmembrane domain residues and is proposed to propagate rotations to open the pore by virtue of this position. The model also shows a narrowing of the pore at conserved hydrophobic residues in M2, as previously predicted (Reeves *et al*., 2001). Furthermore, the intracellular vestibule formed by the M3-M4 loops displays a less negative electrostatic potential than that of the nACh receptor. This may be why the ion charge selectivity of the 5-HT_3 receptor can be more easily reversed (Thompson *et al*., 2003). The model also shows that arginine residues known to affect ion conductance (Kelley *et al*., 2003) line openings in this vestibule thought to be part of the cation conduction pathway.

The model thus provides structural explanations for many published observations of receptor function and will be a useful tool in the design and interpretation of future experiments.

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