Abstract

Purinergic P2X receptors are membrane ion channels activated by extracellular ATP. There are seven isoforms of mammalian P2X receptors designated as P2X1-7, which according to their structure represent a specific family of ligand gated ionic channels, with extraordinary structural/functional properties. The P2X receptor consists of three subunits and each subunit has two transmembrane domains. Crystalographic data demonstrate that ionic channel pore is situated between the second transmembrane domains. Crystal structure of P2X4 receptor from the zebrafish (*Danio rerio*) is available in both open and closed state of the channel and the exact structure of ATP binding site is solved. The aim of this thesis was to study the structure-function relationships in a model of recombinant P2X4 receptor of the rat. By employing the point mutagenesis and electrophysiological recording, the functional importance of conserved cysteine residues in the ectodomain and amino acid residues which form the extracellular vestibule was investigated. All ten cysteins were substituted one by one with alanine or threonine and ATP-induced currents were measured from HEK293T cells expressing wild type (WT) and mutated P2X4 receptors. The results indicate that C116A, C126A, C149A and C165A mutations disrupt two disulfide bonds (C116-C126 and C149-C165A) which are needed for the integrity of ATP binding site. The third disulfide bond (C132-C159) is found as unimportant. The fourth (C217-C227) and the fifth (C261-C270) disulfide bonds are supposed to be important for either coupling of ligand binding and channel gating or channel gating itself. Considering the close proximity of C217-C227 to the extracellular vestibule which forms the entrance for ions, the residues V47-V61 and F324-N338 forming the lateral portals of vestibule were also investigated. Alanine mutations at positions F324, G325, V49, Y54 and Q55 have yielded a non-functional receptor indicating that these residues are essential for receptor function. It has been shown that V49 residue is important for expression of the channel on the cell surface. The substitution of Y54 to any other aromatic residue (Y54W and Y54F) resulted in restoring the receptor function, unlike non-aromatic residues (Y54L) which points out the importance of aromatic residue at this position. Furthermore, the Y54A and Y54C receptor function was partially rescued by ivermectin, a positive allosteric modulator of P2X4 receptor, suggesting a rightward shift in the potency of ATP to activate the receptor. In the case of Q55 residue, no substitution restored the receptor function; the only rescue was made by treating Q55E with ivermectin. The F324L, F324Y, and F324W mutations also rescued receptor function partially or completely, ivermectin action on channel gating was preserved in all mutants, and changes in ATP responsiveness correlated with the hydrophobicity and side chain volume of the substituent. The G325P mutant had a normal response to ATP, suggesting that G325 is a flexible hinge. A topological analysis revealed that the G325 and F324 residues disrupt an ectodomain β-sheet upon ATP binding. These results indicate multiple roles of the extracellular vestibule amino acid residues in the P2X4 receptor function.