ABSTRACT

Crystallization of the zebrafish P2X4 receptor in both open and closed states revealed conformational differences in the ectodomain structures, including the dorsal fin and left flipper domains. The role of these domains in forming of ATP-binding pocket and receptor function was investigated by using alanine scanning mutagenesis of the R203-L214 (dorsal fin) and the D280-N293 (left flipper) sequences of the rat P2X4 receptor and by examination of the responsiveness to ATP and orthosteric analog agonists 2-(methylthio)adenosine 5’-triphosphate, adenosine 5'-(γ-thio)triphosphate, 2’(3’-O-(4-benzoylbenzoyl)adenosine 5’-triphosphate, and α,β-methyleneadenosine 5’-triphosphate. ATP potency/efficacy was reduced in 15 out of 26 alanine mutants. The R203A, N204A, and N293A mutants were essentially non-functional, but receptor function was restored by ivermectin, an allosteric modulator. The I205A, T210A, L214A, P290A, G291A, and Y292A mutants exhibited significant changes in the responsiveness to orthosteric analog agonists. In contrast, the responsiveness of L206A, N208A, D280A, T281A, R282A, and H286A mutants to analog agonists was comparable to that of the wild type receptor. These experiments, together with homology modeling, indicate that residues of the first group located in the upper part of the dorsal fin and left flipper domains, contribute to the organization of the ATP binding pocket and to the initiation of signal transmission towards residues of the second group located in the lower part of both domains. The R203 and N204 residues, deeply buried in the protein, may integrate the output signal from these two domains towards the gate. In addition, the left flipper residues predominantly account for the control of transition of channels from an open to a desensitized state. Strong conformation changes occur during transformation from closed to open state also in the extracellular vestibule. Native residues of Y54A, Q55A, F324A and G325A mutants, that could not be restore by ivermectin, have been identified as important for correct vestibule structure and function. Finally, new kinetic (Markov state) model of P2X4 receptor was developed and indicates that the ivermectin-dependent transition from open to dilated state is coupled to receptor sensitization, which rescues the receptor from desensitization and subsequent internalization.