ABSTRACT

Extracellular adenosine-5´-triphosphate (ATP), released from damaged cells or coreleased as a cotransmitter from synaptic vesicles, acts on its plasma membrane receptors termed purinergic. Purinergic P2X receptors are ATP-gated cation channels. To date seven P2X isoforms designated $P2X_{1-7}$ have been cloned that are organized as trimeric homomers or heteromers. All P2X subunits share a similar structure consisting of a large extracellular loop, two transmembrane domains and intracellular N- and C- termini. An additional structural feature is conserved aminoacids, these include ten conserved cysteine residues in the extracellular loop. All ectodomain cysteines form disulfide bonds which are organized in two areas: three disulfide bridges are localized in the N-termini half and two in the C-termini half at P2X receptor. ATP binding pocket is apparently localized between two neighbouring subunits. The aim of this Diploma Thesis was to examine the relevance of ectodomain cysteine residue and/or disulfide bonds for the expression, function and ATP binding properties of the P2X receptor. All ten, one by one, ectodomain cysteines were substituted by alanines and ATP-induced currents was recorded in HEK293 cells expressing wild-type $P2X_4$ receptor and its mutants. Low responsible or nonfunctional mutants (C126A, C149A, C217A, C227A, C270A) were estimated using ivermectin that potentiates maximum current amplitude and prolongs time of deactivation. Results obtained on mutations C116A, C126A, C149A and C165A that disruption of one from by them forming disulfide bonds affects ATP-binding pocket. The third cysteine bridge (Cys^{132}-Cys^{159}) seems to be dispensable. Fourth cysteine bridge (Cys^{217}-Cys^{227}) is very important for $P2X_4$ receptor function. Gating or signal conducting is under control of Cys^{261} and Cys^{270}.

SUBJECT WORDS

molecular biology, polymerase chain reaction, cell line, passage, transfection, elektrofyziology, patch clamp, confocal mikroskopy

KEY WORDS

HEK293 cell, purinergic receptor, $P2X_4$ receptor, extracellular adenosin-5´-triphosphate, ivermectin, channel binding, channel gating, ATP-binding site, green fluorescent protein, mutagenesis, EC_{50}