Abstract

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Title of diploma thesis: Role of two lysine amino acid residues in transmembrane regions in agonist recognition of the human $P2Y_{12}$ receptor

Human $P2Y_{12}$ receptor expressed on platelets plays a key role in their physiological function. It importantly participates in maintenance of normal haemostasis and is often targeted by antiplatelet medication, such as clopidogrel. Replacement of amino acid residues responsible for ADP binding, which is a naturally occurring negatively charged agonist, can lead to impaired ligand-receptor interaction. Aim of this work was to study the role of two lysine amino acid residues in the position 173 and 174 of the P2Y12 receptor protein and subsequent changes in 2-methylthio-ADP binding. Two positively charged lysine amino acid residues were substituted with neutral residue alanine. Receptor expression in Chinese hamster ovary was analyzed by immunofluorescence staining and laser scanning confocal microscopy. Subsequently, the wild-type receptor and three mutant variants (K173A, K174A and K173A/K174A) were examined by means of 2-methylthio-ADP-induced responses in two reporter gene assays: the cAMP-directed luciferase expression in the presence of forskolin and the serum response element dependent luciferase expression. None of IC_{50} and EC_{50} values exceeded nanomolar range and neither of mutant constructs showed properties different from the wild-type receptor. Although the mutation Lys174Ala doesn't seem to have any effect on receptor function, recently published data of Lys174Glu are suggesting the disruption ADP binding site of receptor. Future research could provide more information on this mutant construct. Antagonist binding studies on K173A, K174A and K173A/K174A mutant constructs are yet to be conducted.