ABSTRACT

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Title of diploma thesis: Role of disulphide bonds in hA_{2A} subtype adenosine receptor

The adenosine A_{2A} receptor belongs to the G protein coupled receptor family (GPCR). GPCRs are the targets of almost 40% of the drugs on the market. GPCRs are characterized by seven transmembrane helices, which are linked by three extracellular and three intracellular loops (ECL and ICL). The structure of the receptor has been revealed by crystallography, hence we know that ECL1 and ECL2 are connected by several disulphide bonds. The ECL2 is believed to be involved in ligand binding and recognition.

In order to understand the relevance of those disulphide bonds involved in this process, four adenosine A_{2A} receptor mutants were generated by one-site direct mutagenesis, in which the cysteine residues were replaced with serine residues (C146S, C159S, C166S and C146S-C159S). These receptor mutants were expressed in the mammalian cell line, CHO K1(Chinese Hamster Ovary) and the receptor expression was tested with ELISA (Enzyme-linked immunosorbent assay). The determination of ligand binding has been carried out by radioligand competition binding studies. Several adenosine receptor agonists (NECA, CGS-21680, PSB-826 and BAY60-6583) were tested against the radioligand [^{3}H]CGS-21680. Only the C146S receptor mutant was tested against [^{3}H]MSX-2. The function of the receptors was analyzed by cAMP accumulation assays using adenosine receptor agonists, including the endogenous agonist adenosine.

Data presented in this paper confirmed the important role of disulphide bonds for the agonist binding and recognition process. Some of the receptor mutants showed significant differences in ligand binding and function compared to the human A_{2A} adenosine receptor wildtype. In conclusion, disulphide bonds have a great importance in the ECL2.