ABSTRACT

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Title of diploma thesis:

Site-directed mutagenesis of the human histamine H_4 receptor: The role of Arg-341 in the interaction with cyanoguanidine-type H_4R agonists

The human histamine H_4 receptor (hH_4R) was discovered in 2000. The H_4R is supposed to be involved in immunological processes and is considered a potential drug target, e. g., for the treatment of inflammatory diseases. Cloning and expression of the hH_4R inspired to the search for selective agonists and antagonists. Recently, UR-PI376 (2-cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-[(2-phenylthio)ethyl]guanidine) was identified within a series of cyanoguanidines as a highly potent and subtype-selective H_4R agonist with pronounced preference for the human over the murine H_4R (mH_4R). According to molecular modelling studies, the cyanoguanidine moiety of UR-PI376 forms charge-assisted hydrogen bonds with Arg-341 of the hH_4R, suggesting this amino acid brings about selectivity for the hH_4R over the hH_3R and is the reason for the preference of UR-PI376 for hH_4R over mH_4R as well.
To elucidate the role of this amino acid in the interaction with cyanoguanidines, three mutants were generated: Arg-341 was replaced by serine, glutamate or alanine, respectively, using overlap-extension PCR. Compared to the hH_4R, in the H_4Rs of rat and mouse, Arg-341 is replaced with Ser, whereas Glu is the corresponding amino acid in both, the canine H_4R and the human histamine H_3 receptor (hH_3R). The mutant H_4Rs were co-expressed with $G\alpha_{i2}$ and $G\beta_1\gamma_2$ in Sf9 insect cells and characterized in functional studies. The potencies and efficacies of histamine and UR-PI376 (agonists) and the antagonistic activity of thioperamide were determined in steady-state GTPase activity assays. However, relevant differences between the wildtype and the mutant hH_4Rs were not observed. Obviously, Arg-341 can be eliminated to account for the H_4R subtype selectivity of UR-PI376 as well as for species selectivity.