Abstract

Purinergic signaling represents a primitive signaling system that employs widespread receptors which serve many different tissues. The purinergic receptor family consists of two different subgroups, the nucleoside (P1) and nucleotide (P2) receptors. The P2 receptors are further divided into ionotropic (P2X) and metabotropic (P2Y) receptors and till date seven P2X subtypes and eight human P2Y receptor subtypes have been discovered. Among the P2Y receptor subtypes, the $P2Y_1$ and $P2Y_{11}$ receptor are closely related. Agonists acting at the $P2Y_{11}$ receptor are also capable of activating the $P2Y_1$ receptor whereas the $P2Y_1$ receptor already responds to very low concentrations of these nucleotides. Therefore, it is important to identify mechanisms, which can regulate activation of one or the other receptor in tissues or cells where both are expressed. Pharmacological intervention provides one alternative to regulate the activation of individual P2Y receptors. For this, chemically modified agonists have to be studied to characterize agonist preferences of different receptor subtypes.

The focus of the present study was to elucidate differences in the ligand recognition between the less explored $P2Y_{11}$ receptor and the more widely studied $P2Y_1$ receptor. Therefore, we first investigated the diastereoselectivity of the $P2Y_{11}$ receptor stably expressed as GFP fusion protein in 1321N1 cells. These cells lack any functional expression of endogenous P2 receptors. Different diastereoisomers were tested at the stably expressed $P2Y_{11}$ receptor. These isomers show substitution of one of the non-bridging oxygen atoms of P_α by borane or sulfur (ATP-α-B/S). Thereby, a new chiral centre in the ATP molecule is introduced. In summary, a diastereoselective activity of ATP-α-B and ATP-α-S diastereoisomers with a preference for the (B) isomers was found at the $P2Y_{11}$ receptor. This diastereoselectivity is opposite to that of the $P2Y_1$ receptor which prefers the (A) isomers of these compounds. This shows that both receptors prefer different diastereoisomers of the chiral ATP analogues, in spite of being close homologues in the P2Y receptor family, sharing >50% of the amino acid residues involved in ligand recognition. These findings add to the understanding of the structural and conformational determinants of nucleotides which activate different P2Y receptors. The difference in diastereoselectivity allows a more detailed insight into the structure-activity relationships of these P2Y receptors.

Such a detailed insight can be realized by studying the binding mode of nucleotides at the P2Y receptors using mutagenesis analysis in combination with molecular modeling. As a next step in the thesis study, we analyzed amino acid residues putatively involved in ligand recognition at the $P2Y_{11}$ receptor.

During the course of this study, it became obvious that the nucleotide binding pockets of the $P2Y_1$ and $P2Y_{11}$ receptors are very similar, as expected. Three cationic amino acid residues in the TM regions of the $P2Y_{11}$ receptor (Arg106, Arg268, Arg307) coordinate the phosphate chain of a bound ATP molecule. Interaction with the adenine ring via the aromatic amino acid Phe109 seems to be a minor interaction. A glutamate (Glu186) in the EL2 of the $P2Y_{11}$ receptor also proved significant for ligand recognition. Moreover, further analysis of the mutant receptors gave the first indications about the molecular determinants of the stereoselective preference of ATP derivatives at the $P2Y_{11}$ receptor. Upon mutation of the arginine residue Arg268 in TM6 the preference of the (B)-isomers of ATP-α-S analogues at the receptor was lost. This finding is of considerable importance, particularly for the development of subtype-specific agonists or antagonists at the $P2Y_{11}$ receptor as potentially attractive drug candidates.

The use of such drugs for therapeutic intervention requires information on the desensitization of the $P2Y_{11}$ receptor induced by agonists. Therefore, we studied the agonist-induced endocytosis of the receptor in living cells. To our surprise we found that the internalization of the $P2Y_{11}$ receptor was clearly dependent on the presence of the $P2Y_1$ receptor. Further experiments using pulldown and immunoprecipitation techniques showed that both receptors associate physically in HEK293 cells to form a hetero-oligomer. These hetero-oligomers showed a distinct pharmacology. The specific $P2Y_{11}$ receptor antagonist had no affinity at the $P2Y_1$-$P2Y_{11}$ receptor hetero-oligomer whereas the $P2Y_1$ receptor antagonist MRS2179 could still interfere with signaling. The importance of GPCR oligomerization becomes apparent when the spatial organization of purinergic receptors and signaling are considered. The receptor oligomerization might be an advantage for the cell to respond to extracellular nucleotides in a well-regulated manner.

Taken together, the present thesis study has provided new insights into the structural determinants of ligands acting at the $P2Y_{11}$ receptor and insights into the ligand binding mode of the receptor. The study gives also convincing evidence for the existence of P2Y receptor hetero-oligomers.