ENGINEERING OF PURINE RECEPTORS AND THEIR LIGANDS

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Extracellular adenosine, purine nucleotides (such as ATP and ADP), and pyrimidine nucleotides (such as UTP, UDP, and UDP-glucose) act as signaling molecules by activating G protein-coupled adenosine and P2Y nucleotide receptors. We have designed selective ligands for these ubiquitous receptors to explore their pharmacological function under both normal and pathophysiological conditions. Selective agonists for the A_3 adenosine receptor (AR) are sought as anti-inflammatory, cardioprotective, cerebroprotective, and anticancer agents. We designed and synthesized novel A_3ARs agonists containing a conformationally constrained ribose substitution, i.e. a North (N)-methanocarba (bicyclo[3.1.0]hexane) ring system, which maintains a conformation that is highly preferred by the A_3AR. Allosteric enhancers of the A_3AR have also been identified. A_3AR antagonists are efficacious in reducing intraocular pressure in glaucoma models. The problems of species dependence of affinity as A_3AR antagonists (i.e., many of the nonnucleoside antagonists are effective in humans but not rats) have been overcome with nucleoside antagonists that are truncated or otherwise impaired in flexibility or H-bonding ability at the 5′-uronamide group. Platelets express a proaggregatory $P2Y_1$ receptor and an antiaggregatory A_{2A}AR. We recently reported the first nanocarriers with covalently conjugated functionalized congeners of AR ligands, which activate the A_{2A}AR to display antithrombotic activity (1). The multivalent conjugation to polyamidoamine (PAMAM) dendrimers alters the pharmacological properties and provides targeting options. The bisphosphate nucleotide MRS2500 is a potent $P2Y_1$ receptor antagonist (Ki 0.79 nM) and the first one demonstrated to be suitable for use *in vivo* (2). This antagonist contains a (N)-methanocarba ring system in place of the ribose moiety, which maintains a preferred conformation for both $P2Y_1$ agonists and antagonists. Activation of the $P2Y_6$ receptor in microglial cells induces phagocytosis (3). Thus, this receptor might be a target for neurodegenerative diseases. The conformational requirements of the ribose moiety of UDP in binding to the $P2Y_6$ receptor are unlike those of the $P2Y_1$ receptor. UDP analogues locked in the South (S) envelope conformation, predicted by receptor docking to be $P2Y_6$-preferred, were synthesized and found to be more potent than the native ribose-containing nucleotide at this subtype. Another potential means of using the protective effects of AR activation was achieved through receptor engineering. We have introduced the approach of neoceptors, intended for eventual delivery by tissue-targeted vectors for gene therapy, in which the putative agonist binding site is redesigned to accept only agonist molecules altered in a complementary fashion. We are exploring this approach conceptually with tailor-made agonist ligands (de novo designed neoligands that are selective for the neoceptor and not the native receptor) in combination with receptor mutagenesis. Complete orthogonality in a neoceptor/neoligand pair has been achieved for the H272E mutant A_3AR.

1. Kim, Y., et al., Bioconjugate Chem., 2008, 19:406.

2. Hechler, B., et al., J. Pharm. Exp. Therap., 2006, 316:556.

3. Koizumi, S., et al., Nature, 2007, 446:1091.