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Mini Review

John Daly Lecture: Structure-guided Drug Design for Adenosine and P2Y Receptors☆

Kenneth A. Jacobson *, Zhan-Guo Gao, Silvia Paoletta, Evgeny Kiselev, Saibal Chakraborty, P. Suresh Jayasekara, Ramachandran Balasubramanian, Dilip K. Tosh

Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892 USA

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ABSTRACT

We establish structure activity relationships of extracellular nucleosides and nucleotides at G protein-coupled receptors (GPCRs), e.g. adenosine receptors (ARs) and P2Y receptors (P2YRs), respectively. We synthesize selective agents for use as pharmacological probes and potential therapeutic agents (e.g. A_3AR agonists for neuropathic pain). Detailed structural information derived from the X-ray crystallographic structures within these families enables the design of novel ligands, guides modification of known agonists and antagonists, and helps predict polypharmacology. Structures were recently reported for the $P2Y_{12}$ receptor ($P2Y_{12}R$), an anti-thrombotic target. Comparison of agonist-bound and antagonist-bound $P2Y_{12}R$ indicates unprecedented structural plasticity in the outer portions of the transmembrane (TM) domains and the extracellular loops. Nonphosphate-containing ligands of the P2YRs, such as the selective $P2Y_{14}R$ antagonist PPTN, are desired for bioavailability and increased stability. Also, A_{2A}AR structures are effectively applied to homology modeling of closely related A_1AR and A_3AR, which are not yet crystallized. Conformational constraint of normally flexible ribose with bicyclic analogues increased the ligand selectivity. Comparison of rigid A_3AR agonist congeners allows the exploration of interaction of specific regions of the nucleoside analogues with the target and off-target GPCRs, such as biogenic amine receptors. Molecular modeling predicts plasticity of the A_3AR at TM2 to accommodate highly rigidified ligands. Novel fluorescent derivatives of high affinity GPCR ligands are useful tool compounds for characterization of receptors and their oligomeric assemblies. Fluorescent probes are useful for characterization of GPCRs in living cells by flow cytometry and other methods. Thus, 3D knowledge of receptor binding and activation facilitates drug discovery.

Contents

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* Corresponding author at: NIDDK, NIH, Bldg. 8A, Rm. B1A-19, Bethesda, MD 20892-0810, USA. Tel.: +1 301 496 9024.
E-mail address: kajacobs@helix.nih.gov (K.A. Jacobson).

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1. Introduction

A vast biology is associated with action at the G protein-coupled adenosine receptors (ARs) and P2Y receptors and at ionotropic P2X receptors, which is modulated by all of the nucleoside and nucleotide processing enzymes and transporters. This extensive signaling system is qualified to be considered part of the 'purinome' [1], a term already applied in the context of the >3200 proteins that utilize purine cofactors, including intracellular kinases [2], as well as to describe the actions of extracellular and intracellular purines (and pyrimidines) in this collection of related receptors and enzymes.

The release of ATP, UTP and other nucleotides by various routes from cells results in a temporal sequence of activation of these three families of cell surface receptors [3]. The receptors activated initially are the fast P2X ion channels (ATP-responsive trimeric channels composed of seven distinct subunits) and some of the metabotropic P2Y receptors (i.e. $P2Y_2$, $P2Y_4$ and $P2Y_{11}$Rs that respond fully to nucleoside 5′-triphosphates and $P2Y_{14}$R that responds to UDP-sugars). Upon the sequential action of ectonucleotidases CD39 (ectonucleoside triphosphate diphosphohydrolase 1, ENTPD1) and CD73 (ecto-5′-nucleotidase, 5′-NT) [4], different sets of receptors are activated (i.e. $P2Y_1$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$Rs that respond to nucleoside 5′-diphosphates), followed by the activation of four AR subtypes (A_1, A_{2A}, A_{2B} and A_3ARs), which are not appreciably activated by any endogenous nucleotides. Naturally occurring dinucleotides, such as Up_4A, are also known to activate various P2YRs [5].

A current challenge to medicinal chemists is to identify selective P2R agonist and antagonist ligands, which remains an unmet need for most of the P2XRs and many of the P2YRs. The effort to design purine receptor ligands is now aided by representative X-ray crystallographic structures in each of the three classes: A_{2A}AR, $P2Y_{12}$R and P2X4R [6–11]. Many more receptor structures in complex with different ligands will be needed to gain a detailed and broad knowledge of molecular recognition within these families. The high resolution G protein-coupled receptor (GPCR) structures and, to a lesser extent, homology models obtained so far have proven valuable for in silico screening campaigns [12,13]. Biophysical mapping of binding sites, lipophilic hotspots, explicit water networks and other techniques based on GPCR structures are now used for drug design [14]. Moro and coworkers validated a general pharmacophore hypothesis for the human A_{2A}AR using an external test set of 29 newly synthesized antagonists [15]. Thus, we and others have demonstrated the predictive power of GPCR homology modeling and the value of applying newly determined X-ray structures to the medicinal chemistry of purine and pyrimidine GPCRs [11–16]. However, we are only at the beginning of exploring ligand design based on GPCR X-ray structures, and we are far away from predicting selectivity and function of ligands from such models.

In general, we establish detailed structure activity relationships in the purine receptor families, in order to provide selective agents as pharmacological probes and potential therapeutic agents. Our efforts to discover novel, selective ligands for purine receptors stem initially from the guidance and inspiration of John W. Daly, Ph.D. (1933–2008), a noted medicinal chemist and pharmacologist. He was one of those who defined the existence of receptors for adenosine and the biological effects of methylxanthines, by chemical and pharmacological means [17]. He emerged from the era in which many medicinal chemists were not yet accustomed to the idea of structure activity relationship (SAR), in which different functionality on a given molecule subserves distinct roles in receptor recognition. However, very early in the development of our field, Daly applied SAR analysis to the ARs to help define three of the four receptor subtypes and introduced important ligand tools, such as A_1AR-selective N^6-cycloalkyladenosines and 8-aryl- and 8-cycloalkyl-1,3-dipropylxanthines (with postdoctoral fellows R. F. Bruns and M. T. Shamim) [18,19]. Much of our study of the SAR of P2YRs has been in collaboration with T. Kendall Harden.

2. X-ray Structures of A_{2A} and $P2Y_{12}$ Receptors With Bound Agonists and Antagonists

2.1. Molecular Recognition at Adenosine Receptor Structures

The structure of adenosine consists of two chemically and conformationally distinct moieties, each of which is associated with separate roles in the AR orthosteric binding site(s). These two moieties can be divided into message (ribose) vs. address (adenine) portions. While adenine and similar flat, hydrophobic heterocycles often behave as AR antagonists, the addition of a ribose moiety at the appropriate position (adenine-9-ribosides or xanthine-7-ribosides) can confer the ability to activate the receptor, i.e. deliver the message by complementarity with the conformation of the AR protein required to induce its activation.

In some cases, the same substituents of the N^6 and C2 positions of an isolated adenine AR antagonist maintain the same receptor subtype binding preferences that are found in riboside-bearing agonists (see A_1 and A_3AR ligands **1**–**10** in Table 1), suggesting a common mode of receptor binding. The affinity of the AR agonists is generally much greater than the corresponding adenines, because the ribose anchors and stabilizes the bound ligand. Although the AR subtype selectivity of the lone nucleobases has much commonality with the SAR of adenosine agonists, it is not identical [20–24]. For example, in a study of 2-, 6- and 8-substituted adenines, Klotz et al. [22] noted a pharmacological similarity between C2 substitution in adenosine and 8-substitution in adenine. No AR structures containing an unmodified adenine antagonist have been determined yet, but the positions of other AR antagonists in crystal structures so far indicate a similar hydrophobic binding region (either a close overlay of a 1,2,4-triazolo[1,5-*a*][1,3,5]triazine with the adenine of agonists or non-superimposed rings for xanthines) [6,25]. π–π Stacking of the nucleobase rings with a conserved Phe in extracellular loop (EL)2 and often H-bonding with a conserved Asn (6.55, using Ballesteros–Weinstein numbering [26]) are typically common to agonists and antagonists in the A_{2A}AR structures and in models of the other AR subtypes.

Because the ribose moiety constitutes the message portion of adenosine agonists, we have focused on its conformational characteristics at the ARs and also at P2X and P2YRs. From the X-ray structure of the human A_{2A}AR containing a bound nucleoside (Fig. 1A) [7], we now understand that the ribose moiety fits in a deep subpocket of the ARs, where it activates the receptor by drawing hydrophilic residues in transmembrane helices (TMs) 3 and 7 toward it, like the tightening of a belt. The ribose of adenosine agonists is coordinated by H-bonding to conserved residues Thr (3.36), Thr or Ser (7.42) and His (7.43) [7,27,28]. At the same time, the binding of ribosides is driven entropically