Structure of Purinergic $P2Y_{12}$ receptors and some aspects of theirbiochemistry

Estructura de los receptores purinérgicos de P2Y12 y algunos aspectos de su bioquímica

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Abstract

The goal of this study is to develop a more rigorous understanding of the $P2Y_{12}$ receptor first described in blood platelets where it plays a central role in the complex processes of activation and aggregation. $P2Y_{12}$ receptors are 7-membrane-spanning proteins coupled to G proteins, which are activated by nucleotides, extracellular signaling molecules that are released from damaged cells or secreted via non-lytic mechanisms during inflammatory, ischemic, and hypoxic conditions. It is the drug targets for inhibition of platelet aggregation. We paid attention to structure and properties that provide essential insights for the development of improved $P2Y_{12}$R ligands and allosteric modulators as drug candidates.

Keywords: $P2Y_{12}$ receptor, G-protein coupled receptors (GPCP), platelets, inhibitors of the $P2Y_{12}$ receptor.

Resumen

El objetivo de este estudio es desarrollar una comprensión más rigurosa del receptor $P2Y_{12}$ descrito por primera vez en las plaquetas de la sangre, donde desempeña un papel central en los complejos procesos de activación y agregación. El receptor $P2Y_{12}$ es un receptor de siete dominios transmembranales acoplado a proteína G, que se activa mediante nucleótidos que se liberan de las células dañadas o se secretan a través de mecanismos no líticos durante condiciones inflamatorias, isquémicas e hipóxicas. Son los blancos farmacológicos para la inhibición de la agregación plaquetaria. Prestamos atención a la estructura y las propiedades que proporcionan información esencial para el desarrollo de ligandos $P2Y_{12}$R mejorados y moduladores alostéricos como candidatos a fármacos.

Palabras clave: Receptor $P2Y_{12}$, receptores acoplados a proteínas G (GPCP), plaquetas, inhibidores del receptor $P2Y_{12}$.

Introduction

In recent decades over 800 G-protein coupled receptors (GPCP) have been identified in human cells. Human GPCRs are divided into four subfamilies: rhodopsin-like receptors (class A), secretin and adhesion receptors (class B), glutamate receptors (class C), and frizzled/taste2 receptors (class F), based on their structural similarities and properties. These receptors are activated by the extracellular stimuli, including, ions, neurotransmitters, lipids, chemokines, and hormones, and then couple to G proteins and initiate signaling networks, resulting in a broad range of biochemical processes[1]. New methods and technologies in membrane protein engineering and crystallization have been developed to facilitate GPCR structure determination which allowed solving the structure of the P2Y12 receptor.

Structure of $P2Y_{12}$ receptor

There have been several studies in the literature reporting about cell types that can express it. It is present in macrophages[2], endothelial cells, platelets, glial cells, and vascular smooth muscle cells[3], in yet unspecified leukocytes[4], on several immune cells including dendritic cells[5], in osteoclasts[6] and breast cancer cell lines[7]. It was also shown that P2Y12 is not expressed by human endothelial cells of cerebral, aortic, or cardiac origin[8]. Still, the expression and function of $P2Y_{12}$ in other cell types remain poorly investigated. $P2Y_{12}$ is expressed on the plasma membrane of the platelet with about 400 copies per cell[9]. There are published studies describing various radioligands that have been used to characterize and quantify the platelet $P2Y_{12}$ receptor. Thus, the number of $P2Y_{12}$ receptors was measured in intact platelets and mem-

brane preparations by using [^{3}H]PSB-0413 selective $P2Y_{12}$ that is bound to 425 ± 50 sites/platelet[10].

The receptor contains 342 amino acid residues, including 4 extracellular Cys residues at positions 17, 97, 175, and 270: Cys 97 and Cys 175, which are linked by a disulfide bridge and are important for receptor expression; 2 potential N-linked glycosylation sites at its extracellular amino-terminus may modulate its activity[11]. $P2Y_{12}$ exists as homo-oligomers situated in lipid rafts and they are disrupted into nonfunctional dimers and monomers[12]. In 2014, Zhang et al. reported the 2.6 Å resolution crystal structure of human $P2Y_{12}R$ in complex with a non-nucleotide reversible antagonist ethyl 6-(4-((benzyl-sulfonyl)carbamoyl)piperidine-1-yl)-5-cyano-2-methyl nicotinate (AZD1283) and also identified three structures of $P2Y_{12}R$ in complex with an antagonist AZD1283 and two agonists 2-methylthio-adenosine-5′-diphosphate (2MeSADP, a close analog of endogenous agonist ADP) and derivative 2-methylthio-adenosine-5′-triphosphate (2MeSATP)[13,14].

It is known that extracellular loops of receptors play important roles in shaping the entrance to the ligand-binding pockets. In 2016, Mengjie and Beili[15] described that two ligand-binding pockets exist in the receptor. The agonist 2MeSADP and the antagonist AZD1283 occupy one of the pockets, while the other one remains available, suggesting that $P2Y_{12}R$ may simultaneously bind to two different ligands. It was suggested that the endogenous ligand ADP can also bind to this allosteric site and serves as an inhibitor of the receptor[15].

The 7TM regions of GPCRs play important roles in signal transduction. The overall fold of the $P2Y_{12}R$ structure consists of a canonical seven-transmembrane bundle of α-helices and a carboxy-terminal helix VIII that is parallel to the membrane bilayer[16].

Another study indicates the involvement of Arg256, Tyr259, and, possibly, H253 (transmembrane region TM6) amino acid residues in the function of the human $P2Y_{12}$. Arg256 appears to play a role in the recognition of nucleotide agonists and the non-nucleotide antagonist reactive blue-2, but no role in the recognition of the nucleotide antagonist cangrelor[17].

Deficiency of $P2Y_{12}$ in the platelets

Genetic variations in GPCR genes can disrupt receptor function in a wide variety of human genetic diseases, including platelet bleeding disorders[18]. Some studies have primarily concentrated on $P2Y_{12}$ congenital deficiency results in bleeding disorders characterized by a platelet impaired response to ADP. Deficiencies of $P2Y_{12}$ are associated with nucleotide deletions in the open-reading frame, frameshifts, and early truncation of the protein, or with a nucleotide substitution in the transduction initiation codon[19].

In 2016, Li et al. studied the methylation of the $P2Y_{12}$ promoter of patients with ischemic cerebrovascular disease that is associated with higher platelet reactivity and increased risk of ischemic events. Hypermethylation of promoter DNA is related to transcriptional silencing of gene expression resulting in decreased protein activity. Methylation analysis of peripheral blood samples might be a novel molecular marker to help early identification of patients at high risk for clinical ischemic events[20,21]. The study by Su et al., reported the association of methylation levels of $P2Y_{12}$ promoter DNA and the risk of clopidogrel resistance in coronary artery disease patients[22]. Polymorphisms of the $P2Y_{12}$ receptor have been proposed to be associated with an increased risk of cardiovascular disease[23].

Transduction of the $P2Y_{12}R$ signal

As it is known purinergic signaling can regulate hemostasis, thrombosis, and inflammation through the co-stimulation of various cell types, including platelets, leukocytes, endothelial, and vascular smooth muscle cells[24]. Adenine nucleotide mediated cell activation is an important mechanism in the biochemical steps of hemostasis (including thrombosis), and it involves $P2Y_{12}$ receptor [25,26].

ADP binding to the $P2Y_{12}$ receptor causes a conformational change in the receptor allowing it to act as a guanine-nucleotide factor and activate the membrane-associated heterotrimeric G-protein of the Gi-family. The active GTP-bound Gαi-subunit binds to adenylate cyclase leading to decrease cAMP synthesis. Gi triggers the phosphatidylinositol 3-kinase (PI3-kinase) pathway, which has substrate phosphatidylinositol 4,5-bisphosphate ($PI4,5P_2$)[27]. The enzyme PI3-kinase consists of a catalytic subunit associated with a regulatory subunit [28]. By this enzyme, $PI4,5P_2$ is converted to phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3) at the plasma membrane. It leads to increase calcium level and the activation of the kinase Akt leading to inducing downstream of the Von Willibald Factor receptor, glycoprotein Ib-IX-V, the collagen receptor glycoprotein VI and inhibition of Rap1GAP RASA3[29,30,31]. Battram et al., characterized the RAP1GAP RASA3 as a major PI(3,4,5)P3-binder and PI3-kinase regulated protein in human platelets. These changes initiate platelet aggregation by altering the ligand-binding properties of the glycoprotein IIb/IIIa[32]. On the final stage of platelet activation IIb-IIIa receptor binds to fibrin and ensures platelet aggregation resulting in the formation of a thrombus[33,53].

Activation of P2Y12 leads to inhibition of vasodilator-stimulated phosphoprotein, which restrains either secretory or adhesive events in platelets. Vasodilator-stimulated phosphoprotein phosphorylation flow cytometry assay is used to monitor platelet responsiveness to $P2Y_{12}$ targeted antiplatelet therapy[34,35,36]. $P2Y_{12}$ Gi signaling leads positive regulation of other intracellular pathways including extracellular-signal-regulated kinase, myosin light chain kinase, and Src family kinases as well as to membrane lipid shifts toward a pro-coagulant state such as phosphatidylserine and P-selectin exposure. ADP also contributes to the release of several agonists such as TXA_2 by acting on $P2Y_{12}$[37,52]. $P2Y_{12}$ has also been shown to regulate the migration of vascular smooth muscle cells (VSMCs). In these cells, ADP via $P2Y_{12}$ $G\alpha_i$ activation inhib-

ited cAMP/PKA signaling pathway resulting in cofilin dephosphorylation, actin disassembly, and as a consequence, an increase in VSMCs motility and migration[38-40].

Gratacap et al., studied $P2Y_{12}$ supporting of thrombin generation by amplifying membrane exposition of phosphatidylserine, platelet-derived microparticle formation, and collagen-induced exposure of tissue factor. It contributes to leukocyte activation induced by surface P-selectin exposure and the formation of platelet-leukocyte aggregates[41,42].

P2Y12 receptor activation leads also to reduce platelets NO responsiveness and reinforces the production of reactive oxygen species (ROS). ROS can further activate platelets, enhance platelets-leukocytes interactions, and accelerate lipids oxidation and inflammation processes. In endothelial cells and the activation of the P2Y12 receptor decreases the intracellular cAMP concentration, with negative effects on endothelial barrier functions, promoting VSMC contraction and vasoconstriction[43].

Drugs as inhibitors of $P2Y_{12}$ receptor

$P2Y_{12}$ receptor is involved in the central pathological process in atherothrombosis of the coronary, cerebral, or peripheral arteries that can lead to coronary syndromes, stroke ischemic attack, and acute limb ischemia is platelet activation.

During last year's much more information has become available on applying inhibitors of $P2Y_{12}$ receptors in platelet inhibition. Dual-antiplatelet therapy with aspirin and a $P2Y_{12}$ receptor inhibitor is the standard treatment for patients undergoing percutaneous coronary intervention, acute myocardial infarction[44,45]. There are two main classes of oral $P2Y_{12}$ inhibitors: thienopyridines (ticlopidine, clopidogrel, and prasugrel) and non-thienopyridine (ticagrelor) agents. The availability of clopidogrel, prasugrel, ticagrelor with varying levels of potency has enabled physicians to contemplate individualized treatment regimens, which may include escalation or de-escalation of $P2Y_{12}$-inhibiting therapy. Clopidogrel and prasugrel are oral prodrugs requiring hepatic metabolism to generate an active metabolite that irreversibly inhibits the $P2Y_{12}$ receptor and it has long been the gold standard but has major pharmacological limitations such as a slow onset and long duration effect[46]. Ticagrelor is a direct-acting (no metabolism required) oral agent that reversibly inhibits the $P2Y_{12}$ receptor. Cangrelor is a direct-acting intravenous agent that reversibly inhibits the $P2Y_{12}$ receptor. Ticagrelor binds reversibly to the $P2Y_{12}$ receptor at a site that is distinct from the ADP-binding site[47,53]. Two novel P2Y receptor antagonists vicagrel and selatogrel analogs of clopidogrel with the enhanced and more efficient formation of its active metabolite[48,49]. Of the P2Y inhibitors, ticagrelor seems to be associated with a lower incidence of de *novo* cancer during follow-up comparison with prasugrel and clopidogrel, regardless of the duration of dual antiplatelet therapy[50].

Conclusion

$P2Y_{12}$-mediated nucleotide signaling is now considered to be a critical player in the development of cardiovascular diseases. Recently it was found that it has the primary role in the inflammatory response[51]. $P2Y_{12}$ is expressed in many cells and not just has a function in the platelets. In addition to its antithrombotic properties, $P2Y_{12}$ inhibitors can, therefore, be considered to have valuable pharmacological targets for inflammation. More information on the interaction of different substances with P2Y212 would help to establish a greater degree of accuracy on this matter.

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