Published in final edited form as:

Drug Discov Today. 2010 July ; 15(13-14): 570–578. doi:10.1016/j.drudis.2010.05.011.

P2Y nucleotide receptors: Promise of therapeutic applications

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Abstract

Extracellular nucleotides, such as ATP and UTP, have distinct signaling roles through a class of G protein-coupled receptors, termed P2Y. However, the receptor ligands are typically charged molecules of low bioavailability and stability in vivo. Recent progress in the development of selective agonists and antagonists for P2Y receptors and study of knockout mice have led to new drug concepts based on these receptors. The rapidly accelerating progress in this field has already resulted in drug candidates for cystic fibrosis, dry eye disease, and thrombosis. On the horizon are novel treatments of cardiovascular diseases, inflammatory diseases, and neurodegeneration.

Keywords

G protein-coupled receptors; nucleotides; purines; pyrimidines; signaling

I. Introduction

Although nucleotides, such as ATP **1** and UTP **2**, are mainly intracellular, they can be released in the extracellular fluids by various mechanisms. One of them is cell damage: both necrotic and apoptotic cells release ATP and other nucleotides that thus constitute “danger signals” [1,2]. But they can also be released without cell lysis by specific mechanisms: exocytosis of secretory granules, vesicular transport and membrane channels, such as ABC transporters, pannexins and connexins [3,4]. Nucleotides are released by exocytosis during platelet aggregation and synaptic transmission. They are also released in response to various types of stress: mechanical stimulation (stretch, shear stress), hypoxia or pathogen invasion. Once in the extracellular fluid, nucleotides can activate two families of receptors, e.g., metabotropic P2Y receptors that are coupled to G proteins and fast P2X ion channels. The P2X receptors are more structurally restrictive than P2Y in agonist selectivity. They respond

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Teaser sentence: New opportunities for drug development are provided by the family of eight P2Y nucleotide receptors that regulate functions of the cardiovascular, immune, and nervous systems.

principally to ATP as the active ligand, while the P2Y receptors are activated by a group of five or more naturally occurring nucleotides, including **1**, **2**, ADP **3**, UDP **4**, and UDP-glucose **5**.

The P2Y family is composed of eight members encoded by distinct genes, that can be subdivided into two groups based on their coupling to specific G proteins [5]. The P2Y₁, P2Y₂, P2Y₄, P2Y₆, and P2Y₁₁ receptors couple to G_q, to activate PLC β , and the P2Y₁₂, P2Y₁₃, and P2Y₁₄ receptors couple to G_i to inhibit adenylyl cyclase (Table 1). The P2Y₁₁ receptor has the unique property to couple through both G_q and G_s. It is also the only P2Y receptor of which the coding sequence is interrupted by an intron [6]. Comparisons of the structural characteristics and functionally important amino acid residues within the family have been explored using mutagenesis and modeling [7–10]. Specific conserved cationic residues that interact with the negatively charged phosphate groups have been identified [10]; they differ between the two subfamilies of P2Y receptors mentioned earlier [11].

The specificity of nucleotides for the various P2Y receptors is presented in Table 1. Naturally occurring dinucleotide phosphates activate various P2Y receptors, such as the endothelium-derived vasoconstrictive factor Ap₄U **6** (pEC₅₀ 5.32, 6.46, and 5.84 at P2Y₁, P2Y₂, and P2Y₄ receptors, respectively) [12,13,36]. Since many cells express multiple P2Y receptor subtypes, as well as receptors of adenosine, a metabolic product of the adenine nucleotides, there is a complex and time-dependent signaling process at the cell surface. Indeed a large family of ectonucleotidase enzymes hydrolyzes the native nucleotides, and, thus, there is a chronological progression of longer phosphate chains leading to shorter phosphate chains and to the nucleosides [5].

II. New ligand probes for P2Y receptors

As new clinical targets are revealed, there is an intense ongoing effort to design selective agonist and antagonist ligands for the P2Y receptors, both as pharmacological tools and as potential therapeutic agents (Table 2) [11,15]. Many selective ligand probes, both agonists and antagonists of the P2Y receptors, are now available. Nevertheless, much more work is needed, and some subtypes, such as the P2Y₄ receptor, are entirely lacking such selective ligands. Detailed structure-activity relationships (SARs) have been constructed for P2Y₁ and P2Y₁₂ receptors. Nucleotide agonists selective for P2Y₁, P2Y₂, P2Y₆, and P2Y₁₄ receptors and nucleotide antagonists selective for P2Y₁ and P2Y₁₂ receptors have been described [16–19]. Because of the difficulty of synthesizing and purifying nucleotide analogues and applying them in pharmacological models, in which stability and bioavailability might be limited, there is a quest for selective non-nucleotide antagonists, as already reported for all subtypes except P2Y₄ receptors [5,20–27]. The screening of chemically diverse compound libraries has resulted in competitive P2Y₁₂ receptor antagonists that are being tested as potential antithrombotic agents.

Few selective radioligands are available for the P2Y receptors. Previously, various radioactive nucleotides have been proposed for use as P2Y receptor radioligands, but currently only the P2Y₁ and P2Y₁₂ receptors have viable radioligands. Thus, improved and more versatile radioligands and other affinity probes, such as fluorescent probes, are still needed.

Some caution must be added in the use of the current selective ligand probes of the P2Y receptors. First of all, there can be cross-reactivity with other P2Y receptors or with P2X receptors, i.e. some of these probes are not altogether selective. There can also be interactions with nonreceptor proteins, such as G proteins. Second, the antagonists might be metabolized to other active species, such as the conversion of 5'-triphosphates to 5'-diphosphates, which could activate other subtypes. Third, P2 receptor agonists and

antagonists are known to inhibit ectonucleotidases, which can introduce artifactual results by disturbing the balance of extracellular nucleotides.

At the P2Y₁ receptor, 2-MeSADP **8** (Figure 1) has been used widely for activation, but this compound also activates the P2Y₁₂ and P2Y₁₃ receptors. 2-MeSADP is preferred as a P2Y₁ receptor agonist over 2-MeSATP **9**, which can also activate P2X₁ (pEC₅₀ 7.27), P2X₃ (pEC₅₀ 6.46), and other receptors. Subsequent generations of ligands are more definitive for elucidating the action of the P2Y₁ receptor. Notably, the 2'-deoxy N⁶-methyl derivative MRS2179 **12** (Figure 2) is a prototypical selective P2Y₁ antagonist (pK_B 6.99, throughout this review – values at the human subtypes are given unless noted) [28], which was based on the discovery of receptor antagonism by various naturally occurring bisphosphate nucleotides, such as A3P5P [29].

The favored ribose-ring conformation for each of the subtypes of the P2Y₁-like family has been established using conformationally-restricted (i.e. rigid) ribose equivalents. The later generation of synthetic bisphosphate antagonists incorporates a rigid substitute for the normally flexible ribose ring. MRS2279 **25** (pK_B 8.10) and MRS2500 **26** are generally useful as selective, high affinity antagonists of the P2Y₁ receptor in various species [30,31]. The presence of the (N)-methanocarba ring in these nucleotide analogues both enhances receptor affinity and improves stability toward nucleotidases. Antagonists of the P2Y₁ receptor of moderate affinity may also be derived from acyclic nucleotides (bisphosphates and bisphosphonates), such as MRS2496 **27** [32]. When the cyclic ribose-like ring is intact, either agonism or antagonism might result, while in the acyclic series only antagonism has been observed.

The same conformational constraint of the ribose moiety that enhances antagonist action favors the potency and selectivity in nucleotide agonists. Evidently, the two series bind to the same site on the receptor in a similar mode [32]. The (N)-methanocarba analogue of 2-MeSADP, MRS2365 **10**, is a selective, high affinity agonist of the P2Y₁ receptor [16]. Another means of improving hydrolytic stability is the introduction of a borano group in the phosphate moiety of P2Y receptor agonists; compound **11** was equipotent to 2-MeSADP at the P2Y₁ receptor [33].

The screening of structurally diverse chemical libraries by the pharmaceutical industry has led to nonnucleotide antagonists of the P2Y₁ receptor [20,34,35]. Compound **28** is a selective antagonist that displays a K_i value of 90 nM at the human P2Y₁ receptor and oral bioavailability in rats with a t_{1/2} of 2.8 h. A tetrahydro-4-quinolinamine derivative **29** inhibited the P2Y₁ receptor effects and platelet aggregation (pIC₅₀ 6.30) [35].

At the P2Y₂ and P2Y₄ receptors, both of which are activated by UTP, there is a need for more definitive agonists and antagonists. UTP-γ-S **12** (Figure 1) has been used as a more stable activator of these P2Y subtypes than UTP. However, this compound suffers from chemical instability. Dinucleoside tetraphosphates have the preferred phosphate chain length for activation of the P2Y₂ and P2Y₄ subtypes. Several INS compounds have been introduced to clinical trials as P2Y₂ receptor agonists, e.g., Up₄U **14** (INS365, Diquafosol, pEC₅₀ 7.00) and Up₄dC **15** (INS37217, Denufosol, pEC₅₀ 6.66), but these agonists are nonselective compared to the P2Y₄ receptor [36]. By virtue of being dinucleotides, they are more stable to enzymatic hydrolysis than nucleoside triphosphates. A selective P2Y₂ agonist, the 5'-triphosphate derivative MRS2698 **16** is 300-fold selective in comparison to the P2Y₄ receptor [17]. Alternative nucleobases have been shown to be acceptable in P2Y₂ receptor agonists, such as the benzothiazole derivative **17**, which is twice as potent as UTP at the P2Y₂ receptor [37]. Definitive antagonists of the P2Y₂ receptor are not available. AR-C126313 **30** and its higher-molecular-weight analogue AR-C118925 **31** [21] were reported

to selectively antagonize the P2Y₂ receptor, however it appears that these compounds are only micromolar in affinity (Figure 2). The uracil phosphonate derivative **32** antagonized the P2Y₂ receptor with an pIC₅₀ of 4.04 [38]. The large polyanionic molecules Reactive Blue 2 (structure not shown) and suramin **34** are often used as slightly selective antagonists of the P2Y₂ and P2Y₄ receptors, respectively. Truly selective agonists and antagonists for the P2Y₄ receptor are needed to distinguish this subtype pharmacologically from the P2Y₂ receptor, which is also activated by UTP. The agonist 2'-azido-2'-deoxy-UTP displayed slight P2Y₄ selectivity [39].

UDP activates both the P2Y₆ and P2Y₁₄ receptors [91], however it is to be cautioned that addition of UDP to tissue can generate UTP through the action of nucleoside diphosphokinase (NDPK) [41]. Thus, artifactual results might be obtained using UDP alone in pharmacological studies if multiple P2Y subtypes are present. UDP-β-S **18** is a more stable activator of these P2Y subtypes than UDP. The SAR of nucleotide derivatives in activating the P2Y₆ receptor has been explored [42,43]. Other UDP derivatives, e.g. **20** and **21**, are selective P2Y₆ agonists. Molecular modeling predicted that the South (S) conformation of the ribose ring is the P2Y₆-preferred conformation, which was then confirmed by synthesis of a rigid methanocarpa analogue of UDP **20** [44,90]. Dinucleotides have been explored as P2Y₆ receptor ligands with diuridine triphosphates such as INS48823 **19** (pEC₅₀ 6.90) having the preferred phosphate chain length [18]. The only P2Y₆ receptor antagonist class reported includes MRS2578 **33** and its related chemically reactive diisothiocyanate derivatives, which have a spectrum of P2Y receptor interactions, and suffer from aqueous instability and hydrophobicity [22].

Potent agonists at the P2Y₁₁ receptor are ATP-γ-S **13** (Figure 1, pEC₅₀ 4.87) and the P2Y₁₂ antagonist 2-propylthio-β,γ-dichloromethylene-ATP **41** (AR-C67085, Figure 2, pEC₅₀ 5.05) [45]. Thus, **41** must be used with caution in pharmacological studies in which both P2Y₁₁ and P2Y₁₂ subtypes might be present because of pharmacological ambiguity of an agonist/antagonist, respectively. A potent P2Y₁₁ receptor antagonist NF157 **35** (pK_i value of 7.35) derived from nonselective P2 antagonist suramin **34** [46] also antagonizes the P2X₁, P2X₂, and P2X₃ receptors. Related derivatives **36** and **37** were found to activate and antagonize the P2Y₁₁ receptor, respectively [91].

The medicinal chemistry of the P2Y₁₂ receptor has been extensively explored. The thienopyridines, such as clopidogrel **38** (Figure 2), were serendipitously identified as inhibitors of platelet aggregation by ADP 20 years before the cloning and identification of their target, the P2Y₁₂ receptor. They act as liver-activated prodrugs whose active metabolites are irreversible inhibitors of the P2Y₁₂ receptor [47]. Thiol **39** is reported to be the active metabolite of clopidogrel [57]. It binds covalently to cysteine 97 in the first extracellular loop of P2Y₁₂ receptor. This results in the breakdown of P2Y₁₂ oligomers into monomers or dimers and their partitioning out of lipid rafts [48].

A drug development program by Astra Zeneca to design P2Y₁₂ receptor antagonists has introduced numerous directly-acting P2Y₁₂ receptor antagonists. The observation that ATP acts as an antagonist at this ADP-activated subtype has enabled the introduction of various 5'-triphosphate analogues as selective receptors probes, and one of them, AR-C69931MX **42** (Cangrelor), has been tested clinically as antithrombotic agent. Curiously, the requirement of having a 5'-triphosphate group in adenine nucleotides that serve as P2Y₁₂ receptor antagonists can be circumvented. One of the products of this effort is ticagrelor **43** (AZD6140), an uncharged nucleoside derivative with a high affinity at the P2Y₁₂ receptor that is currently in clinical trials [49–51].

The search for selective non-nucleotide antagonists of the P2Y₁₂ receptor, for potential use as antithrombotic agents, is continuing. The fact that the phosphate groups may be eliminated entirely, at least for the P2Y₁₂ receptor, has fueled this effort. Library screening has aided in this effort leading to other molecules with promise as competitive P2Y₁₂ receptor antagonists, e.g. piperazinyl glutamate pyridine derivative **44** (pEC₅₀ 7.82) [89], tricyclic benzothiazolo[2,3-c]thiadiazine derivative CT50547 **45** (pEC₅₀ 6.74) [52], BX677 **46** [53]. The highly potent P2Y₁₂ receptor antagonist PSB-0739 **47** is an analogue of the known antagonist Reactive blue 2 [8].

The agonist potency at the P2Y₁₃ receptor is ADP > 2-MeSADP > ATP. A selective P2Y₁₃ receptor antagonist MRS2211 **49** is a derivative of PPADS **48**, a nonselective P2Y and P2X receptor antagonist derived from pyridoxal phosphate [26]. MRS2211 has the disadvantage of containing a phosphate ester group and also an aryl diazo linkage, both of which are subject to instability in tissue systems.

The SAR of analogues of the native ligand UDP-glucose **5** at the P2Y₁₄ receptor was recently systematically explored [19]. The P2Y₁₄ receptor appears to be the most structurally restrictive member of the P2Y family, at least with respect to modification of the nucleobase, ribose, and phosphate moieties of agonist ligands. The glucose moiety of UDPG, however, may be modified. Other naturally occurring UDP-sugars activate this receptor less potently, such as UDP-galactose (EC₅₀ 0.67 μM) and UDP-*N*-acetylglucosamine (EC₅₀ 4.38 μM). The 2-thio analogue MRS2690 **22** (Figure 1) is a 6-fold more potent agonist for P2Y₁₄ receptor and, unlike UDPG, is inactive at the P2Y₂ receptor. Recently, α,β-difluoromethylene-UDP **23** (MRS2802), which is inactive at the P2Y₆ receptor, was found to fully activate the human P2Y₁₄ receptor [90]. Therefore, the glucose moiety is amenable to modification and is not required for activation of the P2Y₁₄ receptor. Non-nucleotide antagonists of the P2Y₁₄ receptor **48** and **49** have been reported with pIC₅₀ values of 8.66 and 8.40, respectively [27].

In conclusion, most of the ligands of P2Y receptors identified so far are polyanionic molecules that do not readily cross the cell membrane. This raises a major problem of bioavailability. However this lack of systemic action can be an asset in case of topical applications, f.i. spray or eye drops. The hydrolysis of nucleotide compounds by ectonucleotidases constitutes another limiting factor partially overcome by the development of more stable dinucleotide compounds. Orally-active uncharged antagonists, e.g. thienopyridines and ticagrelor, are available only for the P2Y₁₂ receptor.

III. Current and potential therapeutic application of P2Y agonists and antagonists

The most highly developed therapeutic application of P2Y receptor ligands is that of P2Y₁₂ antagonists for thrombosis [14]. Indeed the only P2Y ligands currently in pharmaceutical use are the thienopyridine compounds: ticlopidine (Ticlid) and clopidogrel (Plavix) have been on the market for years. Plavix is a blockbuster with sales of roughly \$8 billion in 2008. Prasugrel (Effient) was recently approved by the FDA and the EMA for the prevention of clots in patients undergoing percutaneous coronary intervention. It has the advantage that its transformation into an active metabolite is more rapid and less variable than that of clopidogrel [54,55]. Other P2Y₁₂ antagonists acting in a reversible way are under development. Ticagrelor (Brilinta), an orally active reversible antagonist of P2Y₁₂, was compared to clopidogrel in the PLATO study and showed a greater efficacy in reducing cardiovascular death [51]: Astra Zeneca has submitted a new drug application to the FDA in November 2009. Cangrelor is a reversible P2Y₁₂ antagonist that must be administered by i.v. perfusion, since it is a charged molecule with a very short half-life. Two recent phase III

studies in percutaneous coronary intervention failed to demonstrate a benefit and this program was discontinued [56,57]. A phase II study in coronary artery bypass graft surgery has been initiated. Elinogrel (PRT128), a tricyclic benzothiazolo[2,3-c]thiadiazine, is another reversible P2Y₁₂ antagonist, that can be used orally or i.v. [88]. It is currently in phase II and as part of an agreement with Portola Pharmaceuticals, Novartis will be responsible for its phase III development.

P2Y₂ receptor agonists are in phase III clinical trials for the treatment of cystic fibrosis and dry eye disease. This strategy developed by Inspire Pharmaceuticals is based on the observation that activation of P2Y₂ receptors on epithelial cells, in the airways and the eye (conjunctiva, cornea), stimulates the secretion of chloride via outwardly-rectifying chloride channels, leading to mucus hydration and surface lubrication [58,59]. This action is independent from CFTR (Cystic Fibrosis Transmembrane Regulator) and can thus bypass the CFTR defect in cystic fibrosis. Furthermore P2Y₂ activation inhibits sodium absorption and stimulates the other components of the mucociliary escalator – ciliary beating and mucus secretion [58]. A New Drug Application for diquafosol (Prolacria) eye drops in the treatment of dry eye disease has been submitted to the FDA that requested a new phase III trial, which was initiated in 2009. In this placebo-controlled trial including 450 patients, the primary efficacy endpoint was the clearing of the fluorescein staining of the central region of the cornea after a 6 weeks treatment. Inspire recently announced on its web site that this endpoint was not met. However, diquafosol was recently approved in Japan for the same indication. A first phase III trial of denufosal in cystic fibrosis (TIGER-1) has demonstrated a statistically significant increase in FEV₁ (Forced Expiratory Volume 1 sec) in patients inhaling denufosal for 24 weeks. A second phase III trial (TIGER-2), comparing denufosal to placebo in 450 patients over 48 weeks, was initiated recently.

Other P2Y subtypes might become therapeutic targets as suggested *inter alia* by the phenotype of knockout mice, which are now available for all P2Y subtypes, except P2Y₁₁ that is not present in the murine genome. Some findings support further developments in the two current areas of drug development related to P2Y receptors – platelet inhibition and topical stimulation of fluid secretion – but others suggest potential applications in entirely different therapeutic areas.

Platelet aggregation by ADP actually requires the cooperation between two P2Y receptors: P2Y₁₂ and P2Y₁ [60]. P2Y₁ is involved in the initial platelet shape change and transient aggregation, while P2Y₁₂ is responsible for sustained aggregation and potentiation of secretion. P2Y₁^{-/-} mice show defective platelet aggregation *ex vivo*, increased bleeding time and resistance to thrombosis [61,62]. Therefore P2Y₁ antagonists might constitute a new class of antithrombotic agents [63].

The stimulatory effect of nucleotides on chloride and water secretion by epithelial cells is not restricted to the airways or the eye. It also occurs in the gut, where it involves the P2Y₄ receptor. Indeed both in jejunum and colon, the ATP/UTP induced Cl⁻ current was abolished in P2Y₄-deficient mice [64,65]. P2Y₄ agonists might thus be used to treat chronic constipation in a similar way to lubiprostone (Amitiza), that activates the CIC-2 chloride channel on the apical membrane of intestinal epithelial cells and thereby enhances intestinal fluid secretion and accelerates gastrointestinal transit [66].

Other studies suggest additional potentials of P2Y receptors as therapeutic targets especially in cardiovascular diseases, inflammatory diseases such as asthma and neurodegeneration. Multiple P2Y receptors might play a role in the development of atherosclerotic lesions, independently from their role in platelet activation. Aortic lesions were smaller in double ApoE/P2Y₁ knockout mice than in ApoE^{-/-} mice [67]. This difference was unrelated to the

role of P2Y₁ in platelet activation since it was unaffected by bone marrow transplantation from P2Y₁ wild type mice, indicating the role of P2Y₁ in non-hematopoietic-derived cells, most likely endothelial cells. The P2Y₆ receptor might also be a target since it is functionally expressed in the three cell types that play a major role in the development of atherosclerotic lesions, i.e., endothelial cells, smooth muscle cells and macrophages [68] and P2Y₆ mRNA is elevated in atherosclerotic plaques [69]. Acting on the P2Y₆ receptor, UDP might induce the expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) on arterial endothelial cells, a key step in the infiltration of circulating monocytes, stimulate the growth of smooth muscle cells and amplify the release of cytokines by macrophages. Finally the P2Y₁₃ receptor plays a role in the reverse cholesterol transport, at the level of hepatocytes. It has indeed been shown that HDL Apo A-I activates an ecto-ATPase that generates ADP from ATP on the surface of hepatocytes [70]. ADP then stimulates the endocytosis of HDL particles via the activation of P2Y₁₃ receptors, as demonstrated by the use of siRNA [71].

Multiple P2Y receptors are expressed in the heart. The P2Y₂ and P2Y₆ receptors are expressed on cardiomyocytes [72], whereas the P2Y₄ receptor is present on microvascular endothelial cells [Horckmans, Communi et al, submitted for publication]. Nucleotides are released from cardiomyocytes in response to mechanical stretch [73] or ischemia [72]. Pharmacological experiments suggest that the P2Y₂ receptor might play a role in protection of cardiomyocytes against ischemia [74], while the use of siRNA revealed that the P2Y₆ receptor plays a role in cardiac fibrosis resulting from pressure overload [73].

P2Y receptors are involved at various steps in the inflammatory process. ATP released from neutrophils amplifies their attraction by chemotactic signals [75] and its release from apoptotic cells constitutes a “find-me signal” for monocytes/macrophages [2]. These actions are abrogated in leukocytes from P2Y₂^{-/-} mice. Nucleotides upregulate the expression on endothelial cells of VCAM-1, that plays a crucial role in the tissue infiltration of eosinophils and monocytes. This action is P2Y₂ receptor-mediated in coronary arteries [76], but P2Y₄ and P2Y₆ receptors might also be involved in other vascular beds. Nucleotides also stimulate the release of various cytokines and chemokines. For instance, UTP stimulates the release of CCL20 from human nasal epithelial cells [77], and UDP amplifies the release of IL-8 from human monocytes via the autocrine activation of the P2Y₆ receptor [78]. P2Y receptors are also involved in adaptive immunity. In particular ATP induces via the P2Y₁₁ receptor the semi-maturation of human monocyte-derived dendritic cells, characterized by an upregulation of co-stimulatory molecules and the inhibition of IL-12 secretion, resulting in an enhanced ability to induce Th2 differentiation of T lymphocytes [79,80]. These various mechanisms of action might play a role in asthma and indeed allergen challenge causes an acute accumulation of ATP in the airways of asthmatic patients and mice with experimental asthma [1]. Neutralizing this increase in ATP by the ATP-hydrolyzing enzyme apyrase reduced airway inflammation in sensitized mice. Furthermore ATP derived from commensal bacteria stimulates the differentiation of Th17 cells in the intestinal lamina propria [81], likely via the P2Y₁₁ receptor and an increase of cAMP in dendritic cells [82]. These data suggest that antagonists of P2Y₂ and/or P2Y₆ and/or P2Y₁₁ receptor might be beneficial in asthma and inflammatory bowel disease.

Microglia from P2Y₁₂^{-/-} mice are unable to polarize, migrate or extend processes towards ADP, and in vivo they showed decreased directional branch extension towards sites of laser-induced cortical damage [83]. Independently from this chemotactic action of ADP, UDP stimulates the uptake of microspheres by rat microglia and this action was blocked by an antisense oligonucleotide targeting the P2Y₆ receptor [84]. These complementary actions of ADP, a find-me signal, and UDP, an eat-me signal, involving a cooperation between P2Y₁₂ and P2Y₆, might be beneficial in neurodegenerative conditions such as Alzheimer's disease, via an increased clearance of amyloid- β deposits. However, microglia can be a double-edged

sword and they play a key role in neuropathic pain resulting from nerve injury. Tactile allodynia after nerve injury, a hallmark of neuropathic pain, was decreased in P2Y₁₂^{-/-} mice [85,86]. These data suggest that a P2Y₁₂ antagonist might be effective in neuropathic pain, while a P2Y₆ agonist could be beneficial in Alzheimer's disease.

IV. Conclusions

Probing the SAR of the P2Y receptor family by medicinal chemical approaches is an ongoing process. Selective agonists for P2Y₁, P2Y₂, and P2Y₆ receptors have been reported, but there are not yet any selective agonists for the P2Y₄, P2Y₁₁, and P2Y₁₃ receptors. Among the methods used recently to achieve selectivity of nucleotides for P2Y receptor subtypes is the conformational locking of the ribose moiety. Selective antagonists for P2Y₁, P2Y₂, P2Y₆, P2Y₁₁, P2Y₁₂, and P2Y₁₃ receptors are also known, but antagonists that are truly selective for the P2Y₄ and P2Y₁₄ receptors are still needed. For antagonist development, screening of chemically diverse compound libraries has begun to yield new lead compounds for the development of P2Y₁ receptor antagonists and directly acting P2Y₁₂ receptor antagonists, both of which are sought for antithrombotic activity. The discovery of new, selective P2Y receptor agonists and antagonists holds promise of providing new opportunities for therapeutics.

Acknowledgments

We thank the Intramural Research Program of the NIH, National Institute of Diabetes and Digestive and Kidney Diseases, as well as the Fonds de la Recherche Scientifique Médicale (FRSM), the Communauté Française de Belgique (Action de Recherche Concertée) and the Walloon Region (Programme d'Excellence CIBLES).

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Figure 1.
Nucleotides derivatives that have been useful as agonists in the study of the P2Y receptors.

Figure 2A

Figure 2B

Figure 2.

Nucleotides and nonnucleotides that have been useful antagonists in the study of P2Y receptors. A) Antagonists of the Gq-coupled P2Y₁-like subfamily. B) Antagonists of the Gi-coupled P2Y₁₂-like subfamily.

Table 1

Properties of P2Y receptors.^a

Group	Receptor	Chromosome (human)	Native agonist (human, pEC ₅₀)	Selective agonist (pEC ₅₀)	Selective antagonist (pIC ₅₀)	G protein
A	P2Y ₁	3q24-25	ADP (5.09)	MRS2365 (9.40)	MRS2500 (9.02), MRS2179 (6.48)	G _q
	P2Y ₂	11q13.5	UTP (8.10), ATP (7.07)	MRS2698 (8.10), MRS2768 (5.72)	PSB-716 (5.01), AR-C126313 (6)	G _q (+ G _i)
	P2Y ₄	Xq13	UTP (5.60) ^b	c	c	G _q (+ G _i)
	P2Y ₆	11q13.5	UDP (6.52) ^d	PSB-0474 (7.15), 5-iodo-UDP (7.83)	MRS2578 (7.43) [non-competitive]	G _q
B	P2Y ₁₁	19p31	ATP (4.77)	NF546 (6.27)	NF340 (7.14)	G _q + G _s
	P2Y ₁₂	3q21-25	ADP (7.22)	c	AZD6140 (7.90), AR-C6931MX (9.40), PSB-0739 (9.8)	G _i
	P2Y ₁₃	3q24-25	ADP (7.94)	c	MRS2211 (5.97)	G _i
	P2Y ₁₄	3q24-25	UDP-glucose (6.45), UDP (6.80)	MRS2690 (7.31), MRS2802 (7.20)	e	G _i

^aThe missing numbers in the classification represent either nonmammalian orthologs or receptors having some sequence homology to P2Y receptors, but for which there is no functional evidence of responsiveness to nucleotides.

^bThe pharmacology of some P2Y receptors exhibits species differences: whereas the human P2Y₄ is a UTP receptor, the rat and mouse P2Y₄ receptors are activated equipotently by ATP and UTP.

^cSelective ligands not yet available. Other useful nonselective agonists include (pEC₅₀): INS365 (7.00 at P2Y₂), 2'-azido-2'-deoxyUTP (7.14 at P2Y₄), INS48823 (6.90 at P2Y₆), AR-C67085 (5.05 at P2Y₁₁), 2-MeSADP (7.85 at P2Y₁₂ and P2Y₁₃). Other useful nonselective antagonists include (pIC₅₀): PPADS (<5.0 at P2Y₄), 2-MeSAMP (4.00 at P2Y₁₂).

^dUTP is also an agonist of the P2Y₆ receptor [68,87].

^eNonnucleotide antagonists have been reported [27].

Table 2

New P2Y ligands currently in clinical development

Molecule	Mechanism	Administration	Pathology	Phase	Company
Ticagrelor	P2Y ₁₂ antagonist	oral	Acute coronary syndrome	NDA submitted	AstraZeneca
Cangrelor	P2Y ₁₂ antagonist	iv	CABG	II	The Medicines Co.
Elinogrel	P2Y ₁₂ antagonist	oral or iv	PCI	II	Portola/Novartis
Diquafosol	P2Y ₂ agonist	local	Dry eye disease	III	Inspire
Denufosol	P2Y ₂ agonist	local	Cystic fibrosis	III	Inspire

PCI: percutaneous coronary intervention

CABG: coronary artery bypass graft