

Published in final edited form as:

*Acta Physiol (Oxf)*. 2010 June ; 199(2): 149–160. doi:10.1111/j.1748-1716.2010.02116.x.

## Signalling and pharmacological properties of the P2Y<sub>14</sub> receptor

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### Abstract

The P2Y<sub>14</sub> receptor is a relatively broadly expressed G protein-coupled receptor that is prominently associated with immune and inflammatory cells as well as with many epithelia. This receptor historically was thought to be activated selectively by UDP-glucose and other UDP-sugars. However, UDP is also a very potent agonist of this receptor, and may prove to be one of its most important cognate activators.

### Keywords

Gi; immunological response; nucleotide; nucleotide sugar; P2Y receptor; UDP

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The concept that adenine and uridine nucleotides and nucleotide sugars function as extracellular signalling molecules has expanded markedly in the past decade. At least 15 nucleotide-activated cell surface receptors exist in mammals, and remarkably broad and varied physiological responses occur downstream of nucleotide receptor activation (Ralevic & Burnstock 1998, Leipziger 2003, Burnstock 2007). The significance of extracellular nucleotides/nucleotide sugars as signalling molecules is also underscored by the ubiquitous distribution of several large classes of ectoenzymes that catalyse nucleotide breakdown and interconversion (Zimmermann 2000, Lazarowski *et al.* 2003a). Purinergic signalling was initially proposed on the basis of smooth muscle responses to autonomic nerve stimulation that were not blocked by adrenergic receptor antagonists (Burnstock 1972). However, observation of responses to nucleotides in the brain and in essentially all peripheral tissues, including those not significantly innervated by the autonomic nervous system, indicates that extracellular nucleotides arising from both neuronal and non-neuronal sources underlie many important physiological processes.

Two distinct families of cell surface receptors mediate the actions of extracellular nucleotides. P2X receptors are ligand-gated ion channels that conduct extracellular cations in response to adenosine triphosphate (ATP) (Khakh & North 2006). Seven receptors (P2X<sub>1</sub>–P2X<sub>7</sub>) comprise this family found largely, but not exclusively, on neurones and other excitatory tissues. P2Y receptors (P2Y-R) are a group of eight molecularly defined G protein-coupled receptors (GPCR) that exist both in the central and autonomic nervous systems as well as on most non-excitatory cells (Abbracchio *et al.* 2006).

## The P2Y<sub>1</sub> receptor subfamily

The P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub> and P2Y<sub>11</sub> receptors (hereafter referred to as P2Y<sub>1</sub>-R, P2Y<sub>2</sub>-R, P2Y<sub>4</sub>-R, P2Y<sub>6</sub>-R and P2Y<sub>11</sub>-R) were the first molecularly identified P2Y-R (Harden *et al.* 1998). Non-consecutive numbering of these receptors occurred due to premature and erroneous designation of six other GPCR that were species homologues of unambiguously identified P2Y-R or proved to be members of other GPCR families. The P2Y<sub>1</sub>-R, P2Y<sub>2</sub>-R, P2Y<sub>4</sub>-R and P2Y<sub>6</sub>-R activate Gq/phospholipase C-β and can be grouped into a P2Y<sub>1</sub> receptor subfamily of P2Y-R that share 35–52% primary sequence identity (Fig. 1). The Gq- (and Gs-) linked P2Y<sub>11</sub>-R is also considered a member of this subfamily although it exhibits only 28–30% identity to the four other P2Y<sub>1</sub> receptor subfamily members and is not present in rodents. In contrast to the P2X receptors, which are all activated by ATP [but not adenosine diphosphate (ADP)], the sparsely expressed P2Y<sub>11</sub>-R is the only P2Y-R that is activated selectively by ATP (Communi *et al.* 1997, Qi *et al.* 2001). The P2Y<sub>1</sub>-R is selectively activated by ADP (Palmer *et al.* 1998, Waldo & Harden 2004), and the P2Y<sub>2</sub>-R is activated equipotently by UTP and ATP (Lustig *et al.* 1993, Nicholas *et al.* 1996). The human P2Y<sub>4</sub>-R is selectively activated by UTP, whereas rodent P2Y<sub>4</sub>-R are equipotently activated by UTP and ATP (Communi *et al.* 1995, Nicholas *et al.* 1996). The P2Y<sub>6</sub>-R is selectively activated by UDP (Chang *et al.* 1995, Nicholas *et al.* 1996). Receptors of the P2Y<sub>1</sub> receptor subfamily regulate a broad range of physiological responses (Ralevic & Burnstock 1998, Leipziger 2003, Abbracchio *et al.* 2006, Burnstock 2007) and are particularly important in brain (P2Y<sub>1</sub>-R and P2Y<sub>2</sub>-R), in the autonomic nervous system (most or all of these P2Y-R), in epithelia of the airway, gastrointestinal tract, kidney, eye and other tissues (P2Y<sub>2</sub>-R, P2Y<sub>4</sub>-R and P2Y<sub>6</sub>-R), in vascular tissue (most or all of these P2Y-R), in platelets (P2Y<sub>1</sub>-R) and in immune and inflammatory cells (P2Y<sub>2</sub>-R and P2Y<sub>6</sub>-R).

## The P2Y<sub>12</sub> receptor subfamily

The P2Y<sub>12</sub>-R, P2Y<sub>13</sub>-R and P2Y<sub>14</sub>-R exhibit 45–50% homology and comprise a second group of P2Y-R, the so-called P2Y<sub>12</sub> receptor subfamily of nucleotide/nucleotide sugar-activated receptors (Fig. 1). These GPCR have little sequence identity (20–25%) with members of the P2Y<sub>1</sub> receptor subfamily. The P2Y<sub>12</sub> receptor (P2Y<sub>12</sub>-R) accounts for ADP-promoted inhibition of adenylyl cyclase in platelets (Cooper & Rodbell 1979, Hollopeter *et al.* 2001), and promotion of aggregation by ADP requires simultaneous activation of this Gi-coupled receptor and the Gq-coupled P2Y<sub>1</sub>-R (Abbracchio *et al.* 2006, Gachet 2006). The P2Y<sub>13</sub> receptor (P2Y<sub>13</sub>-R) is a third ADP-activated P2Y-R that like the P2Y<sub>12</sub>-R is Gi-, but not Gq-, coupled (Communi *et al.* 2001). The P2Y<sub>14</sub> receptor (P2Y<sub>14</sub>-R) was identified initially as a receptor activated by UDP-glucose and other nucleotide sugars (Chambers *et al.* 2000). As discussed in more detail below, UDP recently was shown to potentially activate both rodent and human P2Y<sub>14</sub>-R (Fricks *et al.* 2008, Carter *et al.* 2009).

In addition to its important presence in platelets, the P2Y<sub>12</sub>-R is widely distributed in brain (Hollopeter *et al.* 2001) and mediates cell migration responses of microglia during inflammation (Haynes *et al.* 2006). The ADP-activated P2Y<sub>13</sub>-R is also expressed in brain and is present in high amounts in spleen (Communi *et al.* 2001). The P2Y<sub>14</sub>-R is expressed at relatively high levels in brain and placenta, in neutrophils, lymphocytes and megakaryocytic cells, in epithelial cells from various tissues and in gastrointestinal smooth muscle (Chambers *et al.* 2000, Abbracchio *et al.* 2006). Although the platelet P2Y<sub>12</sub>-R receptor has been broadly studied, much less is known about the signalling properties and physiological roles of the P2Y<sub>13</sub>-R and P2Y<sub>14</sub>-R. Recent progress accrued in understanding of the P2Y<sub>14</sub>-R is the subject of this review.

## The P2Y<sub>14</sub>-R

Chambers and his colleagues (Chambers *et al.* 2000, Freeman *et al.* 2001) expressed a number of orphan GPCR in yeast strains engineered to respond to agonist activation with increased signal from a pheromone receptor-induced reporter gene, and tested the potential activation of these GPCR by a library of biologically active small molecules. This screen led to the discovery that the orphan GPCR, KIAA0001 (subsequently referred to as the P2Y<sub>14</sub>-R), is activated by UDP-glucose. UDP-galactose, UDP-glucuronic acid and UDP-*N*-acetylglucosamine were also activators of KIAA0001 in the yeast test system whereas a series of nucleoside diphosphates and triphosphates and ADP-, CDP- and GDP-sugars were not.

The P2Y<sub>14</sub>-R was both transiently and stably expressed with the promiscuous G $\alpha$ -subunit G $\alpha$ <sub>16</sub> in human embryonic kidney-293 (HEK293) cells, and Ca<sup>2+</sup> responses were quantified in response to potential agonists (Chambers *et al.* 2000). Again, UDP-sugars were potent agonists of the receptor with a relative order of potency of UDP-glucose > UDP-galactose > UDP-glucuronic acid > UDP-*N*-acetylglucosamine observed. In contrast, CDP-glucose was inactive at concentrations as high as 10  $\mu$ M.

Membranes were also prepared from HEK293 cells stably expressing the human P2Y<sub>14</sub>-R, and potential agonist-promoted binding of [<sup>35</sup>S]GTP $\gamma$ S to endogenous G-proteins was quantified (Chambers *et al.* 2000). UDP-glucose stimulated [<sup>35</sup>S]GTP $\gamma$ S binding with an EC<sub>50</sub> of 234 nm, and this effect was blocked by pre-treatment of cells with pertussis toxin, suggesting that the human P2Y<sub>14</sub>-R couples to G $\alpha$ -subunits of the Gi family of heterotrimeric G proteins.

Fricks *et al.* (2008) examined the pharmacological selectivity of the human P2Y<sub>14</sub>-R in COS-7 cells transiently co-expressing a chimeric G protein G $\alpha$ <sub>q/i</sub> in which the last five amino acids at the carboxyl terminus of G $\alpha$ <sub>q</sub> were substituted with those of G $\alpha$ <sub>i</sub>. This chimeric G $\alpha$  subunit is activated by GPCRs that couple to the Gi family of G-proteins and directs signals through downstream effectors of G $\alpha$ <sub>q</sub> such as phospholipase C- $\beta$  (Coward *et al.* 1999). The activities of UDP-sugars determined in this test system were similar to those previously reported by Chambers and colleagues. That is, [<sup>3</sup>H]inositol phosphate accumulation was stimulated with a relative order of potency of UDP-glucose > UDP-galactose > UDP-glucuronic acid > UDP-*N*-acetylglucosamine.

## Release of nucleotide sugars

Whereas expression of the human P2Y<sub>14</sub>-R or the G $\alpha$ <sub>q/i</sub> chimera alone in COS-7 cells had no effect, co-expression of the hP2Y<sub>14</sub>-R with G $\alpha$ <sub>q/i</sub> resulted in increased [<sup>3</sup>H]inositol phosphate accumulation in the absence of added agonist (Lazarowski *et al.* 2003b, Fricks *et al.* 2008). Application of an assay that provides direct quantification of UDP-glucose mass revealed that UDP-glucose is basally released from a broad range of cell types (Lazarowski *et al.* 2003b) and led to the hypothesis that P2Y<sub>14</sub>-R-dependent increases in [<sup>3</sup>H]inositol phosphate accumulation in the transfected cell system occurred as a consequence of autocrine release of an activating agonist(s). Co-expression of the UDP-sugar-inactivating ecto-enzyme, ectonucleotide pyrophosphatase-1, allowed direct testing of this idea. Thus, expression of this ecto-phosphatase with the human P2Y<sub>14</sub>-R and G $\alpha$ <sub>q/i</sub> resulted in a decrease in basal [<sup>3</sup>H]inositol phosphate levels that corresponded with a decrease in extracellular UDP-glucose levels (Fricks *et al.* 2008).

Realization that UDP-glucose is a naturally occurring extracellular signalling molecule also prompted studies focused on the properties and potential mechanism of regulated release of nucleotide sugars. The hypothesis that UDP-glucose is released in a regulated manner was

first examined using 1321N1 human astrocytoma cells, one of the few cell models exhibiting enhanced release of ATP in response to activation of a GPCR (Joseph *et al.* 2003). Activation of an endogenous protease-activated receptor (PAR) of 1321N1 cells by thrombin resulted in rapid and robust release of ATP, which was accompanied by enhanced UDP-glucose release (Kreda *et al.* 2008). As was observed with ATP release, PAR-stimulated release of UDP-glucose was abolished by the intracellular  $\text{Ca}^{2+}$  chelator BAPTA and the Golgi-disrupting agent brefeldin A. Release also was attenuated after disruption of the cytoskeleton with cytochalasin D, suggesting that nucleotide release from these cells occurs via a vesicular mechanism. This conclusion was also in agreement with studies illustrating that airway epithelial goblet cells displayed enhanced release of UDP-glucose and ATP concomitantly with  $\text{Ca}^{2+}$ -regulated secretion of mucins (Kreda *et al.* 2007).

The hypothesis that extracellular UDP-sugars originate in a vesicular compartment is supported by the fact that these molecules play important roles during glycosylation reactions within the secretory pathway. That is, UDP-sugars are synthesized in the cytosol and transported into the lumen of the endoplasmic reticulum (ER) and Golgi apparatus to serve as sugar donors for glycosyltransferase-catalysed reactions on exportable glycoconjugates. UDP-sugar concentrations in the ER/Golgi are up to 20-fold higher than cytosolic levels (Hirschberg *et al.* 1998). As ER/Golgi UDP-sugars do not diffuse back to the cytosol, they potentially are cargo molecules released from cells during export of glycoconjugates.

Unambiguous proof of a Golgi contribution to extracellular UDP-sugars was illustrated by a tight correlation between Golgi expression of a Golgi-resident UDP-sugar transporter and the release of the cognate UDP-sugar substrate (Sesma *et al.* 2009). As the ER/Golgi UDP-glucose transporter has not been identified at the molecular level, Sesma and co-workers chose an alternative system, i.e. the ER/Golgi-resident UDP-*N*-acetylglucosamine transporter, for their studies. Deletion of *Yea4*, a gene encoding the ER-resident UDP-*N*-acetylglucosamine transporter (*YEA4*) in yeast, resulted in impaired release of this nucleotide sugar. Conversely, overexpression of *YEA4* in *Yea4*<sup>-/-</sup> cells restored UDP-*N*-acetylglucosamine release. In addition, overexpression of HFRC1, a human UDP-*N*-acetylglucosamine transporter, in airway epithelial cells, resulted in enhanced release of UDP-*N*-acetylglucosamine from these cells. Quantification of mass of UDP-*N*-acetylglucosamine revealed that this P2Y<sub>14</sub>-R agonist accumulated with UDP-glucose in submicromolar concentrations in lung secretions from patients with inflammatory lung diseases (Sesma *et al.* 2009). This observation together with the expression of the P2Y<sub>14</sub>-R in inflammatory cells strongly suggests a patho-physiological relevance of nucleotide sugar-activated cell signalling responses.

Release of UDP-sugars from cells under various physiological conditions is not restricted to UDP-glucose and UDP-*N*-acetylglucosamine. Development of an assay that allows quantification of another P2Y<sub>14</sub>-R agonist, UDP-galactose, with subnanomolar sensitivity (Lazarowski 2010) revealed that UDP-galactose is present in the conditioned medium of various cell models, including physiologically relevant primary cultures of human bronchial epithelial cells. Like UDP-glucose, enhanced release of UDP-galactose was observed in thrombin-stimulated 1321N1 cells. Given the likelihood that release of the activating P2Y<sub>14</sub>-R ligand may differ according to cell type, UDP-galactose predictably is an important autocrine/paracrine regulator of P2Y<sub>14</sub>-R activity.

## P2Y<sub>14</sub>-R-dependent activation of native cell signalling pathways

Agonist-promoted activation of Gi and consequential inhibition of adenylyl cyclase is thought to be a primary signalling response of the members of the P2Y<sub>12</sub>-R subfamily of

P2Y receptors. However, initial studies of the P2Y<sub>14</sub>-R largely relied on overexpression of G $\alpha$ -subunits, e.g. G $\alpha$ <sub>16</sub> or the G $\alpha$ <sub>q/i</sub> chimera, that engineer coupling of Gi-coupled receptors to activation of phospholipase C (Chambers *et al.* 2000, Freeman *et al.* 2001, Fricks *et al.* 2008). Several recent studies of the P2Y<sub>14</sub>-R have taken advantage of stable cell lines that allow direct quantification of native downstream signalling pathways.

The human P2Y<sub>14</sub>-R was stably expressed in HEK293 cells using a retrovirus expression system (Fricks *et al.* 2009). UDP-glucose promoted a concentration-dependent inhibition of forskolin-stimulated accumulation of cyclic AMP in these cells with an EC<sub>50</sub> value of 82 nM, and a maximal inhibition of forskolin-stimulated cyclic AMP accumulation of up to 80% was observed. Pre-incubation of P2Y<sub>14</sub>-HEK293 cells with pertussis toxin resulted in complete loss of UDP-glucose-dependent inhibition of cyclic AMP accumulation, indicating that the P2Y<sub>14</sub>-R signals through G $\alpha$ -subunits of the Gi family. UDP-galactose, UDP-glucuronic acid and UDP-N-acetylglucosamine also promoted inhibition of cyclic AMP accumulation in P2Y<sub>14</sub>-HEK cells, and the maximal inhibition observed with these UDP-sugars was similar to that observed with UDP-glucose. The potencies of UDP-galactose and UDP-glucuronic acid were similar to that of UDP-glucose, while UDP-N-acetylglucosamine exhibited an approx. 10-fold higher EC<sub>50</sub>.

The capacity of the P2Y<sub>14</sub>-R to couple to adenylyl cyclase also was examined in other cell backgrounds. For example, C6 rat glioma cells have proved useful for the study of several Gi-coupled receptors (Schachter *et al.* 1997, Thomas *et al.* 2000, Castillo *et al.* 2007), and therefore, the human P2Y<sub>14</sub>-R was stably expressed in these cells using recombinant pLXSN virus harbouring the human P2Y<sub>14</sub>-R coding sequence (Fricks *et al.* 2009). Again, robust inhibitory effects were observed with UDP-glucose in P2Y<sub>14</sub>-R-expressing C6 cells but not in empty vector-infected cells. An EC<sub>50</sub> value of 107 nM was determined and up to 90% inhibition of forskolin-stimulated cyclic AMP accumulation occurred in the presence of a maximally effective concentration of UDP-glucose. The action of UDP-glucose in P2Y<sub>14</sub>-R-C6 cells was completely blocked by pre-treatment with pertussis toxin, and other UDP-sugars also promoted inhibition of cyclic AMP accumulation with relative potencies similar to that observed in the P2Y<sub>14</sub>-R-HEK293 cell line. Unambiguous demonstration of coupling of the P2Y<sub>14</sub>-R to adenylyl cyclase was established by illustrating that GTP-dependent inhibition of adenylyl cyclase activity by UDP-glucose occurred in plasma membrane-enriched fractions prepared from P2Y<sub>14</sub>-C6 glioma cells. This UDP-glucose-promoted response was not observed in cell-free preparations from P2Y<sub>14</sub>-R-C6 cells pre-treated with pertussis toxin.

Krzeminski *et al.* (2008) observed a second messenger response to a high concentration of UDP-glucose in a C6 glioma cell line in the absence of recombinant P2Y<sub>14</sub>-R expression. No statistically significant effect on cyclic AMP accumulation was observed, but a robust Ca<sup>2+</sup> response to 100  $\mu$ M UDP-glucose was reported that was blocked completely by pre-treatment of cells with pertussis toxin. Neither the agonist selectivity nor the concentration dependence of UDP-glucose for producing this effect was reported.

## MAP kinase signalling

Mitogen-activated protein (MAP) kinase signalling pathways often are stimulated by activation of receptors that signal through Gi, and therefore, the capacity of the P2Y<sub>14</sub>-R to promote phosphorylation of MAP kinases also has been examined. Whereas no effect was observed in mock-infected cells, UDP-glucose-dependent extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation occurred in P2Y<sub>14</sub>-R-HEK293 cells (Fricks *et al.* 2009). The effects of UDP-glucose on ERK1/2 phosphorylation were concentration dependent, and the EC<sub>50</sub> (30 nM) observed was similar to that obtained in studies of



inhibition of cyclic AMP accumulation in these cells. As was observed in studies of adenylyl cyclase, P2Y<sub>14</sub>-R-regulated ERK1/2 phosphorylation was not observed in cells pre-incubated with pertussis toxin. The time course for UDP-glucose-dependent activation of ERK1/2 differed from that occurring as a consequence of activation of PAR2, which is natively expressed in HEK293 cells (Amadesi *et al.* 2004, Dai *et al.* 2007). Thus, incubation of cells with a PAR2 agonist resulted in marked phosphorylation of ERK1/2 within 5 min, and phosphorylation quickly diminished thereafter. In contrast, UDP-glucose-dependent activation of ERK1/2 was not maximal until 15 min of incubation and was retained for at least 30 min.

## Signalling responses of P2Y<sub>14</sub>-R endogenously expressed in differentiated HL-60 cells

Whereas P2Y<sub>14</sub>-R mRNA was not detected in undifferentiated HL-60 promyeloleukaemia cells, differentiation of these cells with 1.3% DMSO to a neutrophil-like cell resulted in a marked increase in expression of P2Y<sub>14</sub>-R mRNA (Fricks *et al.* 2009). Similarly, no effect of UDP-glucose was observed in wild-type cells, but a time-dependent activation of ERK1/2 occurred in the presence of UDP-glucose after differentiation of the cells. This effect was apparent within 5 min and peaked within 30 min. In contrast to the time course of phosphorylation in response to UDP-glucose, cells treated with formyl-Met-Leu-Phe, an agonist of the formyl peptide receptor natively expressed in HL-60 cells (Boulay *et al.* 1990, Klinker *et al.* 1996), exhibited a robust ERK1/2 activation at 5 min that quickly diminished thereafter. Neither UDP-glucose- nor formyl-Met-Leu-Phe-dependent ERK1/2 activation was observed in cells pre-incubated with pertussis toxin, consistent with the idea that ERK1/2 activation in differentiated HL-60 cells occurs through a mechanism involving Gi. Consistent with the results observed after stable expression of recombinant P2Y<sub>14</sub>-R and after up-regulation P2Y<sub>14</sub>-R in HL-60 cells, Scrivens & Dickenson (2006) reported that UDP-glucose promotes ERK activation in isolated human neutrophils.

Activation of the P2Y<sub>14</sub>-R or formyl peptide receptor in differentiated HL-60 cells apparently does not result in regulation of adenylyl cyclase activity. That is, incubation of these cells with either UDP-glucose or formyl-Met-Leu-Phe had no effect on cyclic AMP accumulation promoted by forskolin, by amthamine (an agonist of the Gs-coupled H<sub>2</sub> histamine receptor) or by forskolin and amthamine in combination (Fricks *et al.* 2009).

## UDP is a cognate agonist of the P2Y<sub>14</sub>-R

Ault & Broach (2006) used a yeast model system for directed evolution of the P2Y<sub>14</sub>-R with the goal of identifying mutant P2Y<sub>14</sub>-R with differential agonist sensitivities. Studies performed using one of these mutant receptors revealed that UDP antagonized UDP-glucose-promoted receptor activation in a concentration-dependent manner. On the basis of these results, Fricks *et al.* (2008) hypothesized that the P2Y<sub>14</sub>-R binding pocket may not be restricted to interaction with nucleotide sugars alone, and that UDP and/or other nucleotides potentially are natural ligands at the wild-type P2Y<sub>14</sub>-R. To test this idea, the human P2Y<sub>14</sub>-R and G $\alpha_{q/i}$  were transiently coexpressed in COS-7 cells, and inositol lipid hydrolysis was quantified. Tests of potential activity of UDP revealed a parallel rightward shift of the UDP-glucose concentration–effect curves in the presence of the diphosphate, and Schild analysis confirmed that the antagonism produced by UDP was competitive. Subsequent studies revealed that UDP acts as a partial agonist in this test system with human receptors (Carter *et al.* 2009). That is, competitive antagonist activity was observed at low cellular levels of the human P2Y<sub>14</sub>-R, whereas UDP exhibited agonist activity at higher receptor densities. The rat P2Y<sub>14</sub>-R exhibits approx. 80% amino acid sequence identity to the human P2Y<sub>14</sub>-R and was shown to exhibit a similar UDP-sugar selectivity to that of the human P2Y<sub>14</sub>-R. Co-

expression of the rat P2Y<sub>14</sub>-R with G $\alpha_{q/i}$  in COS-7 cells also revealed that UDP is a cognate ligand of this receptor orthologue (Fricks *et al.* 2008). However, in contrast to the results observed with the human receptor in this engineered test system, UDP was a potent full agonist at the rat receptor.

More recent studies have focused on comparison of the actions of UDP vs. UDP-sugars in cells in which P2Y<sub>14</sub>-R-promoted signalling was quantified by measuring natural downstream signalling pathways. For example, UDP caused a concentration-dependent inhibition of forskolin-stimulated cyclic AMP accumulation in P2Y<sub>14</sub>-HEK293, P2Y<sub>14</sub>-R-C6 glioma and P2Y<sub>14</sub>-R-chinese hamster ovary (CHO) cells, but no effect of UDP was observed in wild-type HEK293, C6 or CHO cells (Carter *et al.* 2009). The maximal inhibition observed with UDP was similar to that seen with UDP-glucose, and the potency of UDP was approximately fivefold greater than that of UDP-glucose in the three different P2Y<sub>14</sub>-R stable cell lines. As was previously observed with UDP-glucose (Fricks *et al.* 2009), UDP had no effect on cyclic AMP accumulation in P2Y<sub>14</sub>-HEK293, P2Y<sub>14</sub>-R-C6 or P2Y<sub>14</sub>-R-CHO cells that were pre-incubated with pertussis toxin. UDP apparently is the only naturally occurring nucleoside diphosphate that activates the human P2Y<sub>14</sub>-R as only modest activities were observed with CDP, GDP and ADP at concentrations greater than 100-fold higher than the concentrations of UDP necessary to produce a maximal response through this receptor (Carter *et al.* 2009).

The capacity of UDP to function as a P2Y<sub>14</sub>-R agonist was also examined by quantification of phosphorylation of ERK1/2. No effect of UDP on MAP kinase phosphorylation was observed in wild-type HEK293 cells. In contrast, robust UDP-dependent stimulation of ERK1/2 phosphorylation was observed in P2Y<sub>14</sub>-HEK293 cells (Carter *et al.* 2009). The EC<sub>50</sub> for this effect was similar to that for UDP-dependent inhibition of adenylyl cyclase activity, and the actions of UDP were blocked by pre-treatment with pertussis toxin. Thus, as was observed in measurements of cyclic AMP accumulation, quantification of MAP kinase signalling also reveals that UDP and UDP-glucose exhibit similar agonist activities at the P2Y<sub>14</sub>-R.

The UDP-activated P2Y<sub>6</sub>-R is natively expressed at relatively high levels in HL-60 cells (Carter *et al.* 2009). Thus, these cells could not be utilized in a simple fashion to determine whether up-regulation of the P2Y<sub>14</sub>-R after DMSO-induced differentiation results in the appearance of responses to UDP-glucose as well as to UDP. Quantification of inhibition of cyclic AMP accumulation potentially would allow resolution of the effects of UDP on the Gi-coupled P2Y<sub>14</sub>-R vs. the Gq-coupled P2Y<sub>6</sub>-R, but as described earlier, no P2Y<sub>14</sub>-R- or formyl peptide receptor-promoted inhibition of adenylyl cyclase is observed in wild-type or differentiated HL-60 cells (Fricks *et al.* 2009). However, these limitations were pharmacologically circumvented by identifying analogues of UDP that activate the P2Y<sub>14</sub>-R at concentrations that exhibit little if any activity at the P2Y<sub>6</sub>-R. Thus, difluoro- $\alpha,\beta$ -methylene-UDP (MRS2802) and UDP- $\beta$ -propylester (MRS2907) caused concentration-dependent inhibition of forskolin-stimulated cyclic AMP accumulation in P2Y<sub>14</sub>-HEK293 cells over a range of concentrations that caused little or no inositol phosphate response in 1321N1 human astrocytoma cells stably expressing the human P2Y<sub>6</sub>-R (Carter *et al.* 2009). Neither difluoro- $\alpha,\beta$ -methylene-UDP nor UDP- $\beta$ -propylester promoted ERK1/2 phosphorylation in wild-type HL-60 cells which express the P2Y<sub>6</sub>-R but do not express the P2Y<sub>14</sub>-R. However, following differentiation of HL-60 cells with DMSO (and upregulation of the P2Y<sub>14</sub>-R), both agonists promoted MAP kinase signalling responses that were similar to that observed with UDP-glucose. These results are consistent with the idea that UDP analogues, and by deduction UDP itself, are potent agonists of the P2Y<sub>14</sub>-R in a native environment.

## Drug studies

A broad series of structure activity studies were carried out with the goals of delineating the P2Y<sub>14</sub>-R pharmacophore, developing highly selective P2Y<sub>14</sub>-R agonists, and ultimately developing competitive antagonists that selectively block this signalling protein. These studies are only briefly summarized here, and the reader is referred to several published studies (Ko *et al.* 2007, 2009, Das *et al.* 2010) for more detailed considerations of the medicinal chemistry and associated molecular modelling directed at the P2Y<sub>14</sub>-R. Nearly all modifications of the uracil or ribose moieties of UDP-glucose abolished activity, suggesting that the binding pocket of the P2Y<sub>14</sub>-R is among the least permissive for ligand modification among the P2Y receptors. However, 2-thiouracil modification of UDP-glucose increased potency by sevenfold. Molecular modelling of the human P2Y<sub>14</sub>-R based on the structure of rhodopsin was applied to hypothesize potential interactions within the ligand binding site, and this model accurately predicted that replacement of the glucose moiety with other sugars, e.g. UDP-fructose, UDP-mannose and UDP-inositol, would result in P2Y<sub>14</sub>-R molecules exhibiting significant agonist activity (Ko *et al.* 2009). Activity was also retained after substitution of hydroxyl groups of glucose with fluorine and by chain extension.

The observation that UDP is a potent agonist of the P2Y<sub>14</sub>-R (Carter *et al.* 2009) together with structure activity analyses of molecules at the P2Y<sub>6</sub>-R (Besada *et al.* 2006, Ko *et al.* 2008) led to the synthesis of UDP analogues containing  $\beta$ -phosphate substitution that might favour activation of the P2Y<sub>14</sub>-R (Carter *et al.* 2009, Das *et al.* 2010). Although the glucose moiety of UDP-glucose was suggested to interact with multiple H-bonding and/or charged residues within the putative binding site of the P2Y<sub>14</sub>-R, its deletion or substitution with smaller phosphoester groups was tolerated at this receptor. Simple alkyl esters of UDP displayed highly potent agonist activity at the P2Y<sub>14</sub>-R. The effects of these modifications to preserve and enhance potency at the P2Y<sub>14</sub>-R were additive with the previously identified potency-enhancing effect of 2-thio substitution of the uracil moiety, and several of these molecules achieved low or subnanomolar potency. Moreover, a number of these UDP analogues exhibit high selectivity for activation of the P2Y<sub>14</sub>-R over the P2Y<sub>6</sub>-R. For example, 2-thiouracil derivatives displayed selectivity for activation of the human P2Y<sub>14</sub>-R vs. the P2Y<sub>6</sub>-R, and a 2-thio-substituted  $\beta$ -methyl ester of UDP is a highly potent ( $EC_{50} = 56$  nM) and selective P2Y<sub>14</sub>-R agonist. Interestingly, UDP analogues with phosphate chains containing  $\alpha,\beta$ -methylene or difluoromethylene groups were well tolerated at the P2Y<sub>14</sub>-R, and  $\alpha,\beta$ -methylene-2-thio-UDP exhibited subnanomolar potency.

Das *et al.* (2009) synthesized polymeric macromolecular nanostructures incorporating UDP-glucose with the ultimate idea of applying these dendrimers as nanocarriers for drug delivery and other applications. Uridine-5'-diphosphoglucuronic acid and its ethylenediamine adduct proved to be suitable functionalized congeners for coupling to several generations of dendrimers, and for example, a conjugate containing 20 bound nucleotide moieties was 100-fold more potent than UDP-glucose. Molecular modelling suggests that the nucleotide-substituted branches of this conjugate could extend far beyond the dimensions of the receptor and be available for multivalent docking to receptor aggregates. Indeed, larger dendrimer carriers with greater loading resulted in subnanomolar potency at the P2Y<sub>14</sub>-R.

## P2Y<sub>14</sub>-R in immune/inflammatory responses

Nucleotides and nucleotide sugars are released from cells as a consequence of cell damage, stress, bacterial infection and various other deleterious stimuli. Similarly, message for many if not all of the P2Y-R has been reported to be present in multiple inflammatory cell types, including mast cells, dendritic cells, monocytes, neutrophils and eosinophils. Although P2Y-R-regulated signalling is almost certainly important in a panoply of events associated with



immune responses, it has been difficult to associate unambiguously the presence of a given extracellular agonist with the functional presence of its target P2Y-R(s) mediating a physiologically relevant immune or inflammatory response. However, several rigorous studies suggest a role of, for example, the ATP/UTP-activated P2Y<sub>2</sub>-R and the A3 adenosine receptor in chemotactic responses of human neutrophils (Chen *et al.* 2006) and the P2Y<sub>2</sub>-R in 'find-me' signalling associated with phagocytic clearance of apoptotic cells (Elliott *et al.* 2009).

While the physiological role(s) of the P2Y<sub>14</sub>-R in immunological responses remains to be clearly defined, the high expression levels of this receptor often encountered in leucocytes and other cells involved in immune responses support a potential role for the P2Y<sub>14</sub>-R in immune system homeostasis. P2Y<sub>14</sub>-R mRNA was markedly up-regulated in rat brain after immunological challenge with lipopolysaccharide (LPS) (Moore *et al.* 2003), and P2Y<sub>14</sub>-R mRNA was detected at high levels in immature human dendritic cells that mature and migrate to sites of inflammation in response to insult or injury (Skelton *et al.* 2003). The P2Y<sub>14</sub>-R was selectively expressed on bone marrow haematopoietic cells where it was proposed to mediate chemotaxis (Lee *et al.* 2003). Non-neuronal spinal cord cells express high levels of the P2Y<sub>14</sub>-R (Kobayashi *et al.* 2006), as do synoviocytes from human patients suffering from rheumatoid arthritis (Caporali *et al.* 2008). Similarly, UDP-glucose has been reported to stimulate Ca<sup>2+</sup> accumulation in a variety of cells related to immune or inflammatory responses including astrocytes, monocyte-derived dendritic cells, microglia and several cells of epithelial origin. Scrivens and Dickenson also reported P2Y<sub>14</sub>-R expression in rodent T-lymphocytes (Scrivens & Dickenson 2005) and human neutrophils (Scrivens & Dickenson 2006). UDP-glucose was reported to cause a modest decrease in cyclic AMP levels, although this effect was not observed with other UDP-sugars (Scrivens & Dickenson 2005, 2006). All four UDP-sugars partially inhibited IL-2- and anti-CD3-induced cell proliferation of T-lymphocytes. A caution has been introduced by Brautigam *et al.* (2008) for studies of inflammatory and other effects potentially regulated by the P2Y<sub>14</sub>-R. That is, although commercial sources of UDP-glucose induced inflammatory effects in N9 microglia, this action was only observed with bacterially derived but not synthetic UDP-glucose, and other nucleotide sugars such as UDP-galactose also had no effect.

The P2Y<sub>14</sub>-R is expressed in RBL-2H3 mast cells (Gao *et al.* 2010). UDP-glucose promoted a concentration-dependent increase in Ca<sup>2+</sup> mobilization in these cells, which was blocked by pre-treatment with pertussis toxin. MAP kinase signalling pathways were also activated by UDP-glucose, and UDP-glucose-promoted phosphorylation of ERK1/2 was pertussis toxin-sensitive. Moreover, both UDP-glucose and the P2Y<sub>14</sub>-R-selective agonist MRS2690 caused concentration-dependent increases in β-hexosaminidase release from RBL-2H3 cells. This effect was blocked by pertussis toxin and decreased by P2Y<sub>14</sub>-R-specific siRNA.

## P2Y<sub>14</sub>-R in epithelial cells

The P2Y<sub>14</sub>-R is broadly expressed in epithelial tissues. For example, high levels are observed in various epithelium of the mouse including endometrium, oviduct, uterine cervix, vagina, bronchiolar epithelial cells, alveoli pneumocytes, mucosal epithelial cells of the convoluted tubules and enterocytes (Arase *et al.* 2009). The P2Y<sub>14</sub>-R is expressed in alveolar epithelial type II cells, as well as in several human airway epithelial cell lines (Muller *et al.* 2005). Consistent with the presence of this receptor, UDP-glucose caused a concentration-dependent increase in Ca<sup>2+</sup> accumulation in both A549 and BEAS-2B human airway epithelial cells. This effect was blocked by pre-treatment of cells with pertussis toxin implicating Gi and most likely a Gβγ-dependent activation of a phospholipase C-β isozyme in this signalling response. Respiratory epithelium is known to produce chemokines that are neutrophil chemoattractants, and UDP-glucose caused a concentration-dependent

stimulation of IL-8 production by A549, BEAS-2B and AEC-II cells. This stimulatory effect of UDP-glucose also was inhibited by pre-treatment of cells with pertussis toxin. In contrast, no effect of UDP-glucose was observed on other chemokines including IP-10, MIG and I-TAC.

Message for the P2Y<sub>14</sub>-R is highly expressed in the surface epithelium of the human and mouse female reproductive tract (Arase *et al.* 2009). Immunohisto-chemical studies with P2Y<sub>14</sub>-R-specific antisera were also consistent with prominent expression of this receptor in the epithelium and the lack of receptor expression in stromal tissues. Previous studies suggesting a role of the P2Y<sub>14</sub>-R in immune responses (Lee *et al.* 2003, Skelton *et al.* 2003, Muller *et al.* 2005, Scrivens & Dickenson 2005) prompted Arase *et al.* (2009) to quantify the expression of the P2Y<sub>14</sub>-R in endometria from patients with pelvic inflammatory disease. Message for the P2Y<sub>14</sub>-R as well as for IL-1 $\beta$ , IL-8 and TNF- $\alpha$  all were up-regulated. Moreover, UDP-glucose caused a concentration-dependent increase in IL-8 secretion in primary cultures of human glandular cells but had no effect on cultured stromal cells (Arase *et al.* 2009). Ishikawa endometrial epithelial cells also express the P2Y<sub>14</sub>-R, and UDP-glucose caused a concentration-dependent increase in IL-8 message and secretion of IL-8 protein. In contrast, other proinflammatory cytokines including IL-1 $\beta$ , IL-6 and TNF- $\alpha$  were not upregulated by UDP-glucose. A direct role of the P2Y<sub>14</sub>-R in stimulating IL-8 expression was supported by two lines of investigation. First, pre-treatment of Ishikawa cells with pertussis toxin completely blocked the effect of UDP-glucose. Second, specific knockdown of the P2Y<sub>14</sub>-R using gene-specific siRNA resulted in marked attenuation in the capacity of UDP-glucose to induce IL-8 release.

As IL-8 induces neutrophil chemotaxis, Arase *et al.* (2009) studied the capacity of UDP-glucose-activated Ishikawa cells to promote neutrophil chemotaxis in transwell chamber assays. Whereas UDP-glucose had no effect on chemotaxis in the absence of Ishikawa cells, it increased transmigration activity in the presence of these cells. A role of IL-8 in this response was suggested by the observation that a neutralizing anti-IL-8 antibody blocked the Ishikawa cell-dependent effect of UDP-glucose on neutrophil chemotaxis.

UDP-glucose also induced expression of two functional homologues of IL-8, MIP-2 and KC, in cultured mouse uterine tissue (Arase *et al.* 2009). Surprisingly, the effect of UDP-glucose was observed at 10 nm but not at 1000 nm, and UDP-*N*-acetylglucosamine, which is an agonist (albeit less potent than UDP-glucose) of recombinant murine and human P2Y<sub>14</sub>-R, had no effect. Although these results were inconsistent with the pharmacological selectivity expected of a physiologically relevant P2Y<sub>14</sub>-R, direct injection of UDP-glucose into one horn of the mouse uterus *in vivo* induced MIP-2 and KC protein but injection of saline in the opposite horn had no effect. Importantly, induction of these two chemokines was restricted to the endometrial luminal and glandular cells as was observed with IL-8 in the human endometrium. Finally, administration of UDP-glucose to mouse uterus resulted in a time-dependent increase in the number of neutrophils present in the endometrium.

To more directly examine the role of the P2Y<sub>14</sub>-R, expression of this receptor was reduced by sonoporation of P2Y<sub>14</sub>-R siRNA into one of the horns of the mouse uterus (Arase *et al.* 2009). Immunoblots indicated that a 30% reduction in receptor protein was effected, and a similar decrease in the capacity of UDP-glucose to promote neutrophil recruitment also occurred. LPS induced up-regulation of P2Y<sub>14</sub>-R in Ishikawa cells as well as after administration to mouse uterus. Interestingly, LPS also induced leucocytosis in mouse uterus, and this effect was attenuated by P2Y<sub>14</sub>-R siRNA. These results also are consistent with the observation that the P2Y<sub>14</sub>-R is up-regulated in the human endometrium during inflammation. Reports of P2Y<sub>14</sub>-R mRNA upregulation in mouse uterus after estradiol

treatment for 7 days (Crabtree *et al.* 2006, 2008) also suggest that P2Y<sub>14</sub>-R expression may be regulated by circulating hormone levels.

## P2Y<sub>14</sub>-R in the gastrointestinal tract

Message for the P2Y<sub>14</sub>-R is widely expressed in the rodent gastrointestinal tract with the highest levels observed in the forestomach (Chambers *et al.* 2000, Moore *et al.* 2003, Bassil *et al.* 2009). Both UDP-glucose and UDP-galactose promoted concentration-dependent increases in baseline muscle tension in isolated stomach forestomach preparations (Bassil *et al.* 2009). In contrast, the effects of UDP-glucose were lost in tissue isolated from P2Y<sub>14</sub>-R(-/-) mice. Interestingly, administration of a high concentration of UDP-glucose *in vivo* resulted in an increase in gastric emptying in wild-type mice, and this effect was retained in P2Y<sub>14</sub>-R knock-out mice. Whether these results reflect a P2Y<sub>14</sub>-R-independent effect of UDP-glucose or an effect due to a contaminant of UDP-glucose purified from microbially derived material as has been demonstrated in studies of UDP-glucose-stimulated responses in microglia remains to be established.

## Concluding ideas

The P2Y<sub>14</sub>-R is unique among the P2Y-R extant because of its regulation by nucleotide sugars. Indeed, its rather potent activation by four different naturally occurring nucleotide sugars suggests that the identity of its principal activator may change in a tissue-specific manner. Nucleotide sugars are subject to breakdown by the ecto-nucleotide pyrophosphatases that also inactivate the nucleotide agonists of the other seven known P2Y-R. In contrast, they are not good substrates for the ecto-nucleotidases that inactivate the extracellular nucleotide agonists. Thus, the kinetics of metabolism of, for example, UDP-glucose vs. ATP markedly differ (Lazarowski *et al.* 2003b), and therefore, the kinetics of activation of the P2Y<sub>14</sub>-R are almost certainly to differ from that of any co-expressed P2Y-R.

The P2Y<sub>14</sub>-R has been historically considered a nucleotide sugar-activated receptor, but the recent discovery (Carter *et al.* 2009) that UDP is a potent agonist adds a new twist to P2Y<sub>14</sub>-R-dependent signalling. UDP is fivefold more potent than UDP-glucose and other sugars at the P2Y<sub>14</sub>-R, which suggests but does not prove that it may be the most important agonist of the receptor under certain physiological conditions. Moreover, realization that the P2Y<sub>6</sub>-R is selectively activated by UDP and is co-expressed with the P2Y<sub>14</sub>-R in many tissues suggests that these two receptors may be activated by UDP in a coordinated manner to produce certain physiological responses (Fig. 2). Activation of the P2Y<sub>6</sub>-R results in Gq-dependent activation of phospholipase C and inositol lipid signalling whereas the P2Y<sub>14</sub>-R activates the Gi-regulated pathways discussed above. Perhaps co-activation of these two receptors results in synergistic responses analogous to that underlying ADP-promoted platelet aggregation, which occurs through P2Y<sub>1</sub>-R-dependent activation of Gq and P2Y<sub>12</sub>-R-mediated activation of Gi (Gachet 2006).

The P2Y<sub>14</sub>-R is relatively broadly expressed and its prominent presence in immune and epithelial cells is consistent with an increasingly broad literature suggesting an important physiological role(s) for the receptor in these and other tissues. Selective and potent P2Y<sub>14</sub>-R agonists were identified recently (Das *et al.* 2010). These now can be applied to physiological studies of the receptor, but a P2Y<sub>14</sub>-R selective competitive antagonist is also greatly needed. A P2Y<sub>14</sub>-R-knockout mouse is now available (Bassil *et al.* 2009), and the work of Arase *et al.* (2009) successfully targeting P2Y<sub>14</sub>-R expression by introducing siRNA in an epithelial cell line as well as in mouse uterus also introduces useful technology to the field. The purinergic signalling field has travelled a long and exciting road since Geoff Burnstock's ground-breaking work four decades ago. Encouraging results from a rather

broad range of recent studies on the P2Y<sub>14</sub>-R suggest that investigations focused on nucleotide sugar/UDP-dependent signalling will continue to add both new concepts and physiological understanding to the field.

## Acknowledgments

Research from the authors' laboratories was supported by USPHS grants GM38213 and HL34322.

## References

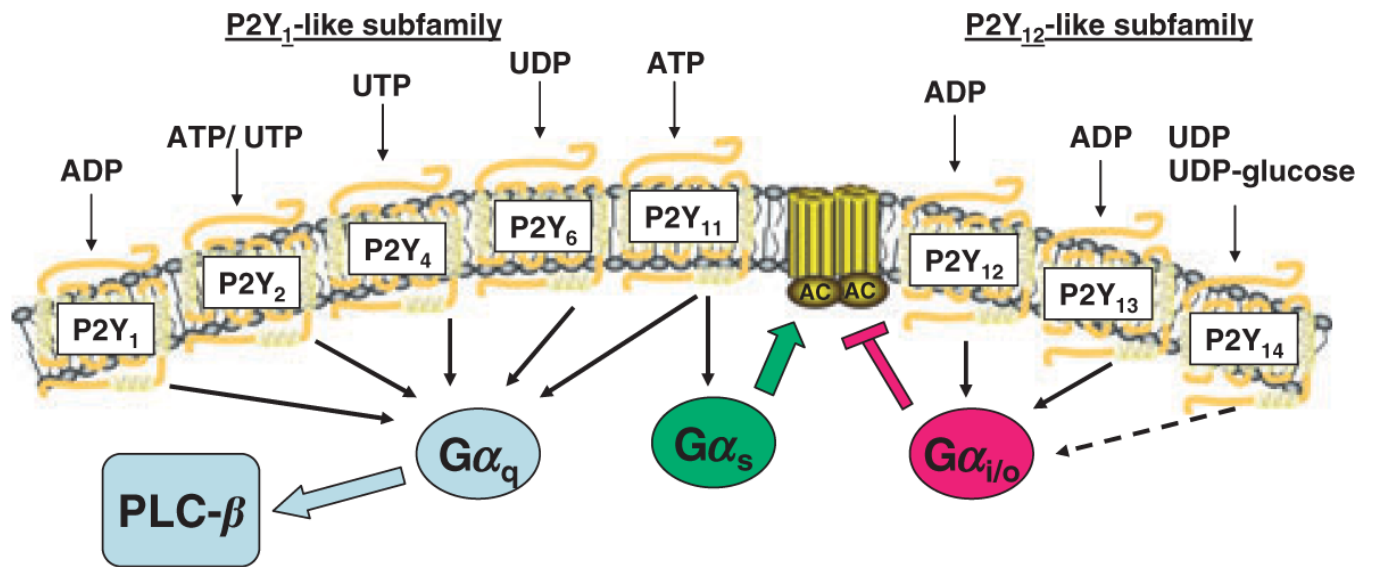
- Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA, Weisman GA. International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* 2006;58:281–341. [PubMed: 16968944]
- Amadesi S, Nie J, Vergnolle N, Cottrell GS, Grady EF, Trevisani M, Manni C, Geppetti P, McRoberts JA, Ennes H, Davis JB, Mayer EA, Bunnett NW. Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. *J Neurosci* 2004;24:4300–4312. [PubMed: 15128844]
- Arase T, Uchida H, Kajitani T, Ono M, Tamaki K, Oda H, Nishikawa S, Kagami M, Nagashima T, Masuda H, Asada H, Yoshimura Y, Maruyama T. The UDP-glucose receptor P2RY14 triggers innate mucosal immunity in the female reproductive tract by inducing IL-8. *J Immunol* 2009;182:7074–7084. [PubMed: 19454705]
- Ault AD, Broach JR. Creation of GPCR-based chemical sensors by directed evolution in yeast. *Protein Eng Des Sel* 2006;19:1–8. [PubMed: 16263727]
- Bassil AK, Bourdu S, Townson KA, Wheeldon A, Jarvie EM, Zebda N, Abuin A, Grau E, Livi GP, Punter L, et al. UDP-glucose modulates gastric function through P2Y<sub>14</sub> receptor-dependent and -independent mechanisms. *Am J Physiol Gastrointest Liver Physiol* 2009;296:G923–G930. [PubMed: 19164486]
- Besada P, Shin DH, Costanzi S, Ko H, Mathe C, Gagneron J, Gosselin G, Maddileti S, Harden TK, Jacobson KA. Structure–activity relationships of uridine 5'-diphosphate analogues at the human P2Y<sub>6</sub> receptor. *J Med Chem* 2006;49:5532–5543. [PubMed: 16942026]
- Boulay F, Tardif M, Brouchon L, Vignais P. The human N-formylpeptide receptor. Characterization of two cDNA isolates and evidence for a new subfamily of G-protein-coupled receptors. *Biochemistry* 1990;29:11123–11133. [PubMed: 2176894]
- Brautigam VM, Dubyak GR, Crain JM, Watters JJ. The inflammatory effects of UDP-glucose in N9 microglia are not mediated by P2Y<sub>14</sub> receptor activation. *Purinergic Signal* 2008;4:73–78. [PubMed: 18368535]
- Burnstock G. Purinergic nerves. *Pharmacol Rev* 1972;24:509–581. [PubMed: 4404211]
- Burnstock G. Physiology and pathophysiology of purinergic neurotransmission. *Physiol Rev* 2007;87:659–797. [PubMed: 17429044]
- Caporali F, Capecchi PL, Gamberucci A, Lazzarini PE, Pompella G, Natale M, Lorenzini S, Selvi E, Galeazzi M, Laghi Pasini F. Human rheumatoid synoviocytes express functional P2X7 receptors. *J Mol Med* 2008;86:937–949. [PubMed: 18545980]
- Carter RL, Fricks IP, Barrett MO, Burianek LE, Zhou Y, Ko H, Das A, Jacobson KA, Lazarowski ER, Harden TK. Quantification of Gi-mediated inhibition of adenylyl cyclase activity reveals that UDP is a potent agonist of the human P2Y<sub>14</sub> receptor. *Mol Pharmacol* 2009;76:1341–1348. [PubMed: 19759354]
- Castillo CA, Albasanz JL, Fernandez M, Martin M. Endogenous expression of adenosine A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub> receptors in rat C6 glioma cells. *Neurochem Res* 2007;32:1056–1070. [PubMed: 17401671]
- Chambers JK, Macdonald LE, Sarau HM, Ames RS, Freeman K, Foley JJ, Zhu Y, McLaughlin MM, Murdock P, McMillan L, et al. A G protein-coupled receptor for UDP-glucose. *J Biol Chem* 2000;275:10767–10771. [PubMed: 10753868]
- Chang K, Hanaoka K, Kumada M, Takuwa Y. Molecular cloning and functional analysis of a novel P2 nucleotide receptor. *J Biol Chem* 1995;270:26152–26158. [PubMed: 7592819]

- Chen Y, Corriden R, Inoue Y, Yip L, Hashiguchi N, Zinkernagel A, Nizet V, Insel PA, Junger WG. ATP release guides neutrophil chemotaxis via P2Y<sub>2</sub> and A<sub>3</sub> receptors. *Science* 2006;314:1792–1795. [PubMed: 17170310]
- Communi D, Piroton S, Parmentier M, Boeynaems JM. Cloning and functional expression of a human uridine nucleotide receptor. *J Biol Chem* 1995;270:30849–30852. [PubMed: 8537336]
- Communi D, Govaerts C, Parmentier M, Boeynaems JM. Cloning of a human purinergic P2Y receptor coupled to phospholipase C and adenylyl cyclase. *J Biol Chem* 1997;272:31969–31973. [PubMed: 9405388]
- Communi D, Gonzalez NS, Dethoux M, Brezillon S, Lannoy V, Parmentier M, Boeynaems JM. Identification of a novel human ADP receptor coupled to Gi. *J Biol Chem* 2001;276:41479–41485. [PubMed: 11546776]
- Cooper DM, Rodbell M. ADP is a potent inhibitor of human platelet plasma membrane adenylate cyclase. *Nature* 1979;282:517–518. [PubMed: 503232]
- Coward P, Chan SD, Wada HG, Humphries GM, Conklin BR. Chimeric G proteins allow a high-throughput signaling assay of Gi-coupled receptors. *Anal Biochem* 1999;270:242–248. [PubMed: 10334841]
- Crabtree JS, Zhang X, Peano BJ, Zhang Z, Winneker RC, Harris HA. Development of a mouse model of mammary gland versus uterus tissue selectivity using estrogen- and progesterone-regulated gene markers. *J Steroid Biochem Mol Biol* 2006;101:11–21. [PubMed: 16920353]
- Crabtree JS, Peano BJ, Zhang X, Komm BS, Winneker RC, Harris HA. Activity of three selective estrogen receptor modulators on hormone-dependent responses in the mouse uterus and mammary gland. *Mol Cell Endocrinol* 2008;287:40–46. [PubMed: 18367319]
- Dai Y, Wang S, Tominaga M, Yamamoto S, Fukuoka T, Higashi T, Kobayashi K, Obata K, Yamanaka H, Noguchi K. Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. *J Clin Invest* 2007;117:1979–1987. [PubMed: 17571167]
- Das A, Zhou Y, Ivanov AA, Carter RL, Harden TK, Jacobson KA. Enhanced potency of nucleotide-dendrimer conjugates as agonists of the P2Y<sub>14</sub> receptor: multivalent effect in G protein-coupled receptor recognition. *Bioconjug Chem* 2009;20:1650–1659.
- Das A, Ko H, Buriánek LE, Barrett MO, Harden TK, Jacobson KA. Human P2Y<sub>14</sub> receptor agonists: truncation of the hexose moiety of uridine-5'-diphosphoglucose and its replacement with alkyl and aryl groups. *J Med Chem* 2010;53:471–480. [PubMed: 19902968]
- Elliott MR, Chekení FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, Park D, Woodson RI, Ostankovich M, Sharma P, Lysiak JJ, Harden TK, Leitinger N, Ravichandran KS. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* 2009;461:282–286. [PubMed: 19741708]
- Freeman K, Tsui P, Moore D, Emson PC, Vawter L, Naheed S, Lane P, Bawagan H, Herrity N, Murphy K, Sarau HM, Ames RS, Wilson S, Livi GP, Chambers JK. Cloning, pharmacology, and tissue distribution of G-protein-coupled receptor GPR105 (KIAA0001) rodent orthologs. *Genomics* 2001;78:124–128. [PubMed: 11735218]
- Fricks IP, Maddileti S, Carter R, Lazarowski ER, Nicholas RA, Jacobson KA, Harden TK. UDP is a competitive antagonist at the human P2Y<sub>14</sub> receptor. *J Pharmacol Exp Ther* 2008;325:588–594. [PubMed: 18252808]
- Fricks IP, Carter RL, Lazarowski ER, Harden TK. Gi-dependent cell signaling responses of the human P2Y<sub>14</sub> receptor in model cell systems. *J Pharmacol Exp Ther* 2009;330:162–168. [PubMed: 19339661]
- Gachet C. Regulation of platelet functions by P2 receptors. *Annu Rev Pharmacol Toxicol* 2006;46:277–300. [PubMed: 16402906]
- Gao ZG, Ding Y, Jacobson KA. UDP-glucose acting at P2Y<sub>14</sub> receptors is a mediator of mast cell degranulation. *Biochem Pharmacol* 2010;79:873–879. [PubMed: 19896471]
- Harden, TK.; Barnard, EA.; Boeynaems, JM.; Burnstock, G.; Dubyak, GR. P2Y receptors. In: Girdlestone, D., editor. *The IUPHAR Compendium of Receptor Characterization and Classification*. IUPHAR Media; London, UK: 1998. p. 209-217.



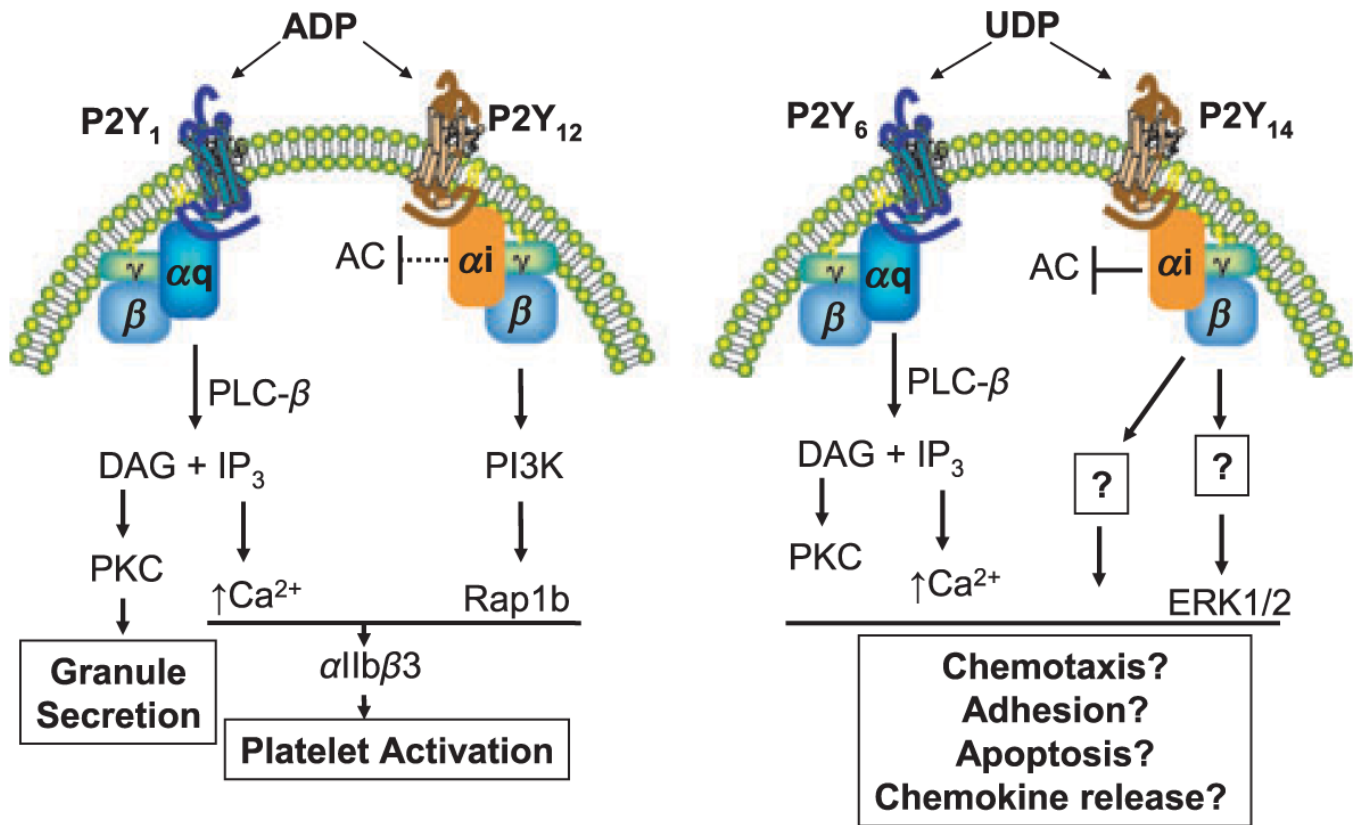
- Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D. The P2Y<sub>12</sub> receptor regulates microglial activation by extracellular nucleotides. *Nat Neurosci* 2006;9:1512–1519. [PubMed: 17115040]
- Hirschberg CB, Robbins PW, Abeijon C. Transporters of nucleotide sugars, ATP, and nucleotide sulfate in the endoplasmic reticulum and Golgi apparatus. *Annu Rev Biochem* 1998;67:49–69. [PubMed: 9759482]
- Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurden P, Nurden A, Julius D, Conley PB. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* 2001;409:202–207. [PubMed: 11196645]
- Joseph SM, Buchakjian MR, Dubyak GR. Colocalization of ATP release sites and ecto-ATPase activity at the extracellular surface of human astrocytes. *J Biol Chem* 2003;278:23331–23342. [PubMed: 12684505]
- Khakh BS, North RA. P2X receptors as cell-surface ATP sensors in health and disease. *Nature* 2006;442:527–532. [PubMed: 16885977]
- Klinker JF, Wenzel-Seifert K, Seifert R. G-protein-coupled receptors in HL-60 human leukemia cells. *Gen Pharmacol* 1996;27:33–54. [PubMed: 8742493]
- Ko H, Fricks I, Ivanov AA, Harden TK, Jacobson KA. Structure–activity relationship of uridine 5'-diphosphoglucose analogues as agonists of the human P2Y<sub>14</sub> receptor. *J Med Chem* 2007;50:2030–2039. [PubMed: 17407275]
- Ko H, Carter RL, Cosyn L, Petrelli R, de Castro S, Besada P, Zhou Y, Cappellacci L, Franchetti P, Grifantini M, Van Calenbergh S, Harden TK, Jacobson KA. Synthesis and potency of novel uracil nucleotides and derivatives as P2Y<sub>2</sub> and P2Y<sub>6</sub> receptor agonists. *Bioorg Med Chem* 2008;16:6319–6332. [PubMed: 18514530]
- Ko H, Das A, Carter RL, Fricks IP, Zhou Y, Ivanov AA, Melman A, Joshi BV, Kovac P, Hajdich J, Kirk KL, Harden TK, Jacobson KA. Molecular recognition in the P2Y<sub>14</sub> receptor: probing the structurally permissive terminal sugar moiety of uridine-5'-diphosphoglucose. *Bioorg Med Chem* 2009;17:5298–5311. [PubMed: 19502066]
- Kobayashi K, Fukuoka T, Yamanaka H, Dai Y, Obata K, Tokunaga A, Noguchi K. Neurons and glial cells differentially express P2Y receptor mRNAs in the rat dorsal root ganglion and spinal cord. *J Comp Neurol* 2006;498:443–454. [PubMed: 16874807]
- Kreda SM, Okada SF, van Heusden CA, O'Neal W, Gabriel S, Abdullah L, Davis CW, Boucher RC, Lazarowski ER. Coordinated release of nucleotides and mucin from human airway epithelial Calu-3 cells. *J Physiol* 2007;584:245–259. [PubMed: 17656429]
- Kreda SM, Seminario-Vidal L, Heusden C, Lazarowski ER. Thrombin-promoted release of UDP-glucose from human astrocytoma cells. *Br J Pharmacol* 2008;153:1528–1537. [PubMed: 18204471]
- Krzeminski P, Pomorski P, Baranska J. The P2Y<sub>14</sub> receptor activity in glioma C6 cells. *Eur J Pharmacol* 2008;594:49–54. [PubMed: 18638471]
- Lazarowski ER. Quantification of extracellular UDP-galactose. *Anal Biochem* 2010;396:23–29. [PubMed: 19699703]
- Lazarowski ER, Boucher RC, Harden TK. Mechanisms of release of nucleotides and integration of their action as P2X- and P2Y-receptor activating molecules. *Mol Pharmacol* 2003a;64:785–795. [PubMed: 14500734]
- Lazarowski ER, Shea DA, Boucher RC, Harden TK. Release of cellular UDP-glucose as a potential extracellular signaling molecule. *Mol Pharmacol* 2003b;63:1190–1197. [PubMed: 12695547]
- Lee BC, Cheng T, Adams GB, Attar EC, Miura N, Lee SB, Saito Y, Olszak I, Dombkowski D, Olson DP, Hancock J, Choi PS, Haber DA, Luster AD, Scadden DT. P2Y-like receptor, GPR105 (P2Y<sub>14</sub>), identifies and mediates chemotaxis of bone-marrow hematopoietic stem cells. *Genes Dev* 2003;17:1592–1604. [PubMed: 12842911]
- Leipzig J. Control of epithelial transport via luminal P2 receptors. *Am J Physiol Renal Physiol* 2003;284:F419–F432. [PubMed: 12556361]
- Lustig KD, Shiao AK, Brake AJ, Julius D. Expression cloning of an ATP receptor from mouse neuroblastoma cells. *Proc Natl Acad Sci USA* 1993;90:5113–5117. [PubMed: 7685114]

- Moore DJ, Murdock PR, Watson JM, Faull RL, Waldvogel HJ, Szekeres PG, Wilson S, Freeman KB, Emson PC. GPR105, a novel Gi/o-coupled UDP-glucose receptor expressed on brain glia and peripheral immune cells, is regulated by immunologic challenge: possible role in neuroimmune function. *Brain Res Mol Brain Res* 2003;118:10–23. [PubMed: 14559350]
- Muller T, Bayer H, Myrtek D, Ferrari D, Sorichter S, Ziegenhagen MW, Zissel G, Virchow JC Jr, Luttmann W, Norgauer J, Di Virgilio F, Idzko M. The P2Y<sub>14</sub> receptor of airway epithelial cells: coupling to intracellular Ca<sup>2+</sup> and IL-8 secretion. *Am J Respir Cell Mol Biol* 2005;33:601–609. [PubMed: 16109883]
- Nicholas RA, Watt WC, Lazarowski ER, Li Q, Harden K. Uridine nucleotide selectivity of three phospholipase C-activating P2 receptors: identification of a UDP-selective, a UTP-selective, and an ATP- and UTP-specific receptor. *Mol Pharmacol* 1996;50:224–229. [PubMed: 8700127]
- Palmer RK, Boyer JL, Schachter JB, Nicholas RA, Harden TK. Agonist action of adenosine triphosphates at the human P2Y<sub>1</sub> receptor. *Mol Pharmacol* 1998;54:1118–1123. [PubMed: 9855642]
- Qi AD, Kennedy C, Harden TK, Nicholas RA. Differential coupling of the human P2Y<sub>11</sub> receptor to phospholipase C and adenylyl cyclase. *Br J Pharmacol* 2001;132:318–326. [PubMed: 11156592]
- Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev* 1998;50:413–492. [PubMed: 9755289]
- Schachter JB, Boyer JL, Li Q, Nicholas RA, Harden TK. Fidelity in functional coupling of the rat P2Y<sub>1</sub> receptor to phospholipase C. *Br J Pharmacol* 1997;122:1021–1024. [PubMed: 9401764]
- Scrivens M, Dickenson JM. Functional expression of the P2Y<sub>14</sub> receptor in murine T-lymphocytes. *Br J Pharmacol* 2005;146:435–444. [PubMed: 15997228]
- Scrivens M, Dickenson JM. Functional expression of the P2Y<sub>14</sub> receptor in human neutrophils. *Eur J Pharmacol* 2006;543:166–173. [PubMed: 16820147]
- Sesma JI, Esther CR Jr, Kreda SM, Jones L, O'Neal W, Nishihara S, Nicholas RA, Lazarowski ER. Endoplasmic reticulum/golgi nucleotide sugar transporters contribute to the cellular release of UDP-sugar signaling molecules. *J Biol Chem* 2009;284:12572–12583. [PubMed: 19276090]
- Skelton L, Cooper M, Murphy M, Platt A. Human immature monocyte-derived dendritic cells express the G protein-coupled receptor GPR105 (KIAA0001, P2Y<sub>14</sub>) and increase intracellular calcium in response to its agonist, uridine diphosphoglucose. *J Immunol* 2003;171:1941–1949. [PubMed: 12902497]
- Thomas EA, Matli JR, Hu JL, Carson MJ, Sutcliffe JG. Pertussis toxin treatment prevents 5-HT(5a) receptor-mediated inhibition of cyclic AMP accumulation in rat C6 glioma cells. *J Neurosci Res* 2000;61:75–81. [PubMed: 10861802]
- Waldo GL, Harden TK. Agonist binding and Gq-stimulating activities of the purified human P2Y<sub>1</sub> receptor. *Mol Pharmacol* 2004;65:426–436. [PubMed: 14742685]
- Zimmermann H. Extracellular metabolism of ATP and other nucleotides. *Naunyn Schmiedebergs Arch Pharmacol* 2000;362:299–309. [PubMed: 11111825]



**Figure 1.**

The P2Y receptors. The eight mammalian P2Y receptors and their cognate agonists are indicated. The five members of the P2Y<sub>1</sub>-like subfamily all couple to heterotrimeric G-proteins of the G $\alpha_q$  family and activate phospholipase C- $\beta$  (PLC- $\beta$ ) isozymes. The ATP-activated P2Y<sub>11</sub> receptor also couples to G $\alpha_s$  resulting in activation of adenylyl cyclase (AC). The three members of the P2Y<sub>12</sub>-like subfamily couple to heterotrimeric G proteins of the G $\alpha_{i/o}$  family and inhibit adenylyl cyclase (AC) activity.



**Figure 2.**

Hypothetical model of synergistic signalling mediated through simultaneous UDP-dependent activation of the P2Y<sub>14</sub>-R and P2Y<sub>6</sub>-R. *Left panel:* The well-established model (Gachet 2006) for ADP-induced aggregation of platelets is illustrated. ADP dually activates the P2Y<sub>1</sub>-R and P2Y<sub>12</sub>-R. Activation of the P2Y<sub>1</sub>-R results in activation of phospholipase C-β (PLC-β) resulting in hydrolysis of PtdIns(4,5)P<sub>2</sub> to the second messenger Ins(1,4,5)P<sub>3</sub> (IP<sub>3</sub>), which mobilizes intracellular Ca<sup>2+</sup>, and diacylglycerol (DAG), which activates protein kinase C. Activation of the P2Y<sub>12</sub>-R results in activation of Gi. Adenylyl cyclase (AC) is inhibited and phosphoinositide-3-kinase (PI3K) is activated. Mobilization of Ca<sup>2+</sup> downstream of activation of the P2Y<sub>1</sub>-R and activation of the GTPase Rap1b downstream of activation of the P2Y<sub>12</sub>-R result in activation of the integrin αIIbβ<sub>3</sub>. *Right panel:* Activation of the P2Y<sub>6</sub>-R results in activation of PLC-β isozymes and inositol lipid hydrolysis, whereas UDP-dependent activation of the P2Y<sub>14</sub>-R results in activation of Gi, inhibition of adenylyl cyclase (AC) and activation of MAP kinase isozymes such as ERK1/2. The P2Y<sub>6</sub>-R and P2Y<sub>14</sub>-R often are co-expressed, and simultaneous activation of these two P2Y-R by UDP is hypothesized to synergize resulting in promotion of a variety of cellular responses.