Title Page

UDP-sugars as extracellular signaling molecules: cellular and physiological consequences of P2Y$_{14}$ receptor activation

Eduardo R. Lazarowski and T. Kendall Harden

Departments of Medicine (ERL) and Pharmacology (TKH), University of North Carolina School of

Medicine, Chapel Hill, NC 27599

Running title: UDP-sugars as extracellular signaling molecules

Address correspondence to:

T. Kendall Harden, Ph.D.

Department of Pharmacology

University of North Carolina School of Medicine

Chapel Hill, NC 27599

Email: tkh@med.unc.edu

Phone: 919-966-4816

FAX: 919-966-5640

24 text pages

2 tables

2 figures

Abstract: 63 words

Manuscript text: 6136 words

Abbreviations: ENPP, ectonucleotide pyrophosphatase; HSC, hematopoietic stem cell; GPCR, G protein-coupled receptor; PMN, polymorphonuclear neutrophils; $P2Y_{14}R$; $P2Y_{14}$ receptor; PPTN, 4-((piperidin-4-yl)-phenyl)-7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid

Abstract

UDP-sugars, which are indispensable for protein glycosylation reactions in cellular secretory

pathways, also act as important extracellular signaling molecules. We discuss here the broadly

expressed P2Y$_{14}$ receptor (P2Y$_{14}$R), a G protein-coupled receptor (GPCR) targeted by UDP-sugars, and the

increasingly diverse set of physiological responses discovered recently functioning downstream of this

receptor in many epithelia as well as in immune, inflammatory, and other cells.

1. Introduction

UDP-sugars are key components of glycosylation reactions in the lumen of the endoplasmic reticulum (ER) and Golgi apparatus, and also are important extracellular signaling molecules acting as potent agonists of the broadly expressed $P2Y_{14}R$ (Harden et al., 2010). The $P2Y_{14}R$ is activated by UDP-glucose, UDP-galactose, UDP-N-acetylglucosamine, and other UDP-sugars. UDP also acts as an agonist on this receptor, but ATP, UTP, or other naturally occurring di- or triphosphate nucleotides have no $P2Y_{14}R$ activity (Chambers et al., 2000;Harden et al., 2010;Carter et al., 2009). The $P2Y_{14}R$ is a subtype in the eight member family of nucleotide-activated P2Y receptors, which are all G protein-coupled receptors (GPCR) (Abbracchio et al., 2006;von Kugelgen and Harden, 2011;Ralevic and Burnstock, 1998). These receptors can be sub-classified based on sequence and cell signaling response similarities into two subgroups, the $P2Y_{12}$ receptor-like and the $P2Y_1$ receptor-like subgroups. The $P2Y_{14}R$ is a member of the $P2Y_{12}$ receptor-like subgroup, which also includes the ADP-activated $P2Y_{12}$ and $P2Y_{13}$ receptors. An orphan GPCR, GPR87, with high homology to the $P2Y_{14}R$ also exists in the $P2Y_{12}$-like subfamily of receptors. These GPCR share >40 percent primary sequence identity, and all three couple to the Gi family of heterotrimeric G proteins. They exhibit much lower sequence identity (<25%) with the $P2Y_1$ receptor-like subgroup, which includes the ADP-activated $P2Y_1$ receptor, the UTP/ATP-activated $P2Y_2$ receptor, the UTP-activated $P2Y_4$ receptor, the UDP-activated $P2Y_6$ receptor and the ATP-activated $P2Y_{11}$ receptor. All members of the $P2Y_1$ receptor-like subgroup couple to heterotrimeric proteins of the Gq family and activate phospholipase C.

2. Mechanisms of release and metabolism of nucleotide sugars

The initial characterization of the $P2Y_{14}R$ as a UDP-sugar-activated receptor of widespread tissue distribution (Chambers et al., 2000;Moore et al., 2003;Skelton et al., 2003;Lee et al., 2003), suggested that nucleotide sugars are released from cells in a regulated manner to accomplish autocrine/paracrine signaling functions. Furthermore, functional studies overexpressing recombinant $P2Y_{14}R$ in null cell lines

4

revealed the presence of endogenous agonists in extracellular solutions. Using a chimeric Gα-subunit

(Gα$_{q/i}$) that couples Gi-linked GPCR to phospholipase C-β, robust P2Y$_{14}$R-dependent inositol phosphate

production was shown to occur in COS-7 cells in the absence of added agonist. That is, expression of the

P2Y$_{14}$R resulted in a large increase in basal [^{3}H]inositol phosphate levels that were further elevated by

the addition of UDP-glucose to the medium (Lazarowski et al., 2003). The elevated levels of [^{3}H]inositol

phosphates observed in the absence of exogenous agonists were markedly reduced by addition to the

medium of *Crotalus adamentus* phosphodiesterase, which hydrolyzes nucleotides and nucleotide

sugars; apyrase, which hydrolyzes nucleotides but not nucleotide sugars, had no effect (Lazarowski et

al., 2003). Using high sensitive radioenzymatic/high performance liquid chromatography-based

methodology, UDP-glucose, UDP-*N*-acetylglucosamine, and UDP-galactose were identified and

quantified in extracellular solutions (Lazarowski, 2010;Sesma et al., 2009;Lazarowski et al., 2003).

Actual concentrations of UDP-sugars in the physiological extracellular milieu are not known due

to difficulties in sampling the thin cell surface liquid without causing cell damage and uncontrolled

nucleotide release. When assessed together in diluted culture medium (300 µl/cm^2 culture surface),

steady state concentration values of UDP-glucose and UDP-*N*-acetylglucosamine on various airway

epithelial cell types were similar (both in the 5-10 nM range) (Sesma et al., 2009) and, as discussed

below, they increased considerably in response to environmental challenges, likely attaining micromolar

levels at the surface of stressed cells.

UDP-sugars are synthesized in the cytosol and translocated into the lumen of the ER and Golgi

apparatus where they serve as donor substrates of glycosylation reactions (Berninsone and Hirschberg,

1998;Abeijon and Hirschberg, 1992). Vesicular UDP-sugars cannot be transported back to the cytosol

and thus, are likely delivered as co-cargo with exported glycoconjugates (**Fig. 1**). Several lines of

evidence support this hypothesis. Gene deletion of Yea4, a major UDP-*N*-acetylglucosamine transporter

in *S. cerevisiae* resulted in impaired release of this nucleotide sugar and was associated with reduced

production of chitin, an acetylglucosamine-rich component of the yeast wall. Complementation of *yea4*-deficient cells with *yea4* restored UDP-*N*-acetylglucosamine release and chitin formation (Sesma et al., 2009). Similarly, overexpression of the human UDP-*N*-acetylglucosamine transporter HFRC1/SLC35D2 in bronchial epithelial cells resulted in enhanced release of UDP-*N*-acetylglucosamine and enhanced cell surface expression of acetylglucosamine -containing glycans (Sesma et al., 2009). Studies with mucin-producing goblet epithelial cells indicated that UDP-glucose release was associated with mucin secretion. For example: (i) goblet-like Calu-3 cells exhibited Ca^{2+}-regulated exocytosis of mucin granules, which was accompanied by enhanced release of UDP-glucose (Kreda et al., 2007); and (ii) both mucin secretion and UDP-glucose levels increased concurrently in human bronchial epithelial cell cultures that were induced to develop goblet (mucous) cell metaplasia (Okada et al., 2011). Lastly, 1321N1 human astrocytoma cells exhibit both constitutive and protease-activated receptor 1-promoted release of UDP-glucose, which were Ca^{2+}-dependent and markedly reduced by brefeldin A_1, a fungal metabolite that disrupts retrograde movements along the secretory pathway (Kreda et al., 2008).

Collectively, these observations suggest that the secretory pathway is a major contributor to the cellular release of UDP-sugars (**Fig. 1**). However, non-vesicular mechanisms that contribute to the cellular release of ATP in many cell types also could be involved in the release of UDP-sugars. For example, caspase 3-mediated proteolytic irreversible activation of plasma membrane pannexin 1 channels resulted in robust release of cytosolic ATP, as well as ADP, AMP, and UTP, from apoptotic lymphocytes (Elliott et al., 2009;Chekeni et al., 2010;Boyd-Tressler et al., 2014). These data suggest that the pannexin 1 pore exhibits no specificity toward nucleotide species. Thus, it is conceivable that, at least in apoptotic cells, cytosolic UDP-glucose is released together with adenine and uracil nucleotides via pannexin 1.

It is worth noting that, in most cases where regulated nucleotide release was assessed, rates of UDP-glucose release were markedly lower than ATP release rates, whereas steady state levels of UDP-

glucose were often higher than ATP levels. Differences in rates of extracellular metabolism account for this apparent discrepancy. UDP-sugars are not metabolized by ecto-ATPase (ecto-nucleoside triphospho diphosphohydrolase; NTPDase) activities that are abundantly expressed on the surface of most cell types (Zimmermann et al., 2012). Instead, ecto-nucleotide pyrophosphatases (ENPPs) break down UDP-sugars, according to the following reaction:

UDP-sugar → UMP + sugar-1P

ENPPs also metabolize nucleoside triphosphates (to nucleoside monophosphates + pyrophosphate) as well as nicotine adenine dinucleotide, ADP-ribose, and dinucleoside polyphosphates. ENPP expression has been reported in several tissues (Zimmermann et al., 2012), but only a few reports have examined its possible action in extracellular metabolism of UDP-sugars. UDP-[^{3}H]glucose added as a radiotracer to confluent cultures of 1321N1 human astrocytoma cells was metabolized at rates that were much lower than ATP hydrolysis rates (Lazarowski et al., 2003). UDP-[^{3}H]glucose remained unchanged after a 1h incubation with purified human neutrophils (Sesma et al., 2012) or primary cultures of human airway epithelial cells (Lazarowski, unpublished), whereas [^{3}H]ATP was completely hydrolyzed.

The high stability of UDP-glucose on airway epithelia and potentially other tissues exhibiting regulated release of UDP-sugars provides a mechanism by which these molecules could accumulate in extracellular environments at pathophysiologically relevant levels. For example, UDP-glucose and UDP-N-acetylglucosamine concentrations in diluted bronchioalveolar lavages from patients with chronic lung obstruction and inflammation reached the 200-400 nM range. As discussed further below, these observations suggest a link between local UDP-glucose release and inflammation.

3. Targeting of the P2Y$_{14}$R by native agonists and high affinity molecular probes

The work of Chambers and colleagues (Chambers et al., 2000) led to realization that the orphan receptor GPR105, now P2Y$_{14}$R, is activated by nucleotide sugars with a relative potency order of UDP-

glucose > UDP-galactose > UDP-glucuronic acid > UDP-N-acetylglucosamine, and all of these molecules

exhibited potencies in the 0.1-1.0 μM range of concentrations. One potential caveat from this and

additional early studies was that they were accomplished using overexpressed G protein α-subunits:

either with $G\alpha_{16}$, which mediates coupling of essentially all GPCR to phospholipase C-β isozymes or with

the engineered protein, $G\alpha_{q/i}$, which selectively couples Gi-linked GPCR to activation of phospholipase C-

β (Freeman et al., 2001;Chambers et al., 2000;Lazarowski et al., 2003;Fricks et al., 2008). Nonetheless,

results from work studying the agonist selectivity of the $P2Y_{14}R$ in cell systems in which it coupled

through native Gi heterotrimers to promote inhibition of adenylyl cyclase confirmed the nucleotide

sugar selectivity established by Chambers and coworkers; that is, UDP-glucose is the most potent

agonist, but the activities of other nucleotide sugars are within an order of magnitude (Fricks et al.,

2009).

The studies of Carter and coworkers (Carter et al., 2009) examining $P2Y_{14}R$-dependent activities

measured downstream of natively expressed Gi also revealed that UDP is a very potent agonist of the

$P2Y_{14}R$. Indeed, this nucleotide was five-fold more potent than UDP-glucose in studies with several

different cell lines. Although direct comparisons have not been published, it is unlikely that the

concentration of extracellular UDP approaches that of UDP-sugars under most physiological and

pathological conditions. UDP generated in the ER/Golgi as a product of glycosylation reactions is

predicted to be either degraded to UMP by Golgi resident UDPase or released with UDP-sugars via

vesicle exocytosis; UDP also is generated extracellularly from the hydrolysis of released UTP. However,

cell surface NTPDases rapidly metabolize UDP but not UDP-sugars. Thus, we discuss the $P2Y_{14}R$ below as

a nucleotide sugar-activated GPCR with the realization that UDP may also play an important, albeit not

yet clearly established, role in its physiological regulation.

Although nucleotide sugars are very potent P2Y$_{14}$R agonists that are present at receptor-activating concentrations in extracellular medium, the existence of other physiological agonists of this receptor cannot be entirely ruled out. The realization that at least one orphan GPR87 with high (~47%) homology to the P2Y$_{14}$R exists in the P2Y$_{12}$-like subclass is not inconsistent with this possibility.

Both synthetic agonists and high affinity competitive antagonists have been developed that selectively target the P2Y$_{14}$R **(Table 1)**. For example, 2-thio-UDP-glucose (MRS2690) exhibits 30-fold greater potency for the P2Y$_{14}$R than does UDP-glucose (Ko et al., 2009;Ko et al., 2007). Several analogues of UDP also have been developed that exhibit high potency at the P2Y$_{14}$R (Das et al., 2010). These include α,β-difluoromethylene-UDP (MRS2802) and α,β-methylene-2-thio-UDP (MRS2905). The importance of these molecules lies in their selectivity for activation of the P2Y$_{14}$R at concentrations that are inactive at the UDP-activated P2Y$_6$ receptor as well as at other P2Y receptors (Das et al., 2010;Carter et al., 2009).

Black and coworkers applied high-throughput screens to identify dihydropyridopyrimidine (Guay et al., 2011) and naphthoic acid (Gauthier et al., 2011) compounds as potential P2Y$_{14}$R antagonists. Analogs of each type of molecule subsequently were synthesized. Whereas dihydropyridopyrimidine derivatives appeared to be noncompetitive antagonists of the P2Y$_{14}$R, the napthoic acid derivatives inhibited [^{3}H]UDP binding suggesting antagonism of the orthosteric ligand binding site of the receptor (Robichaud et al., 2011;Gauthier et al., 2011). Problems with high affinity binding to serum proteins observed with several of these napthoic acid analogs were partially circumvented in the analog PPTN [4-((piperidin-4-yl)-phenyl)-7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid] that retained activity as a P2Y$_{14}$R antagonist. A prodrug based on PPTN also was developed to increase bioavailability. Barrett et al (2013) illustrated that PPTN is indeed a high affinity (K$_B$ ~400 pM) competitive antagonist of the P2Y$_{14}$R that exhibits no activity at the other seven P2Y receptors at concentrations as high as 10 μM.

PPTN was applied subsequently to selectively block P2Y$_{14}$R-dependent responses. Thus, UDP-glucose-promoted chemotaxis of human neutrophils (Barrett et al., 2013), P2Y$_{14}$R agonist-promoted release of β-hexosaminidase from human LAD2 mast cells (Gao et al., 2013), and UDP-glucose-promoted contractile effects on porcine vascular smooth muscle (Alsaqati et al., 2014) all were antagonized by low concentrations of PPTN. Several other potential P2Y$_{14}$R inhibitors have been reported by Hamel and co-workers (Hamel et al., 2011).

Jacobson and colleagues examined a series of chain-elongated alkynyl or amino derivatives of PPTN with the goal of generating a fluorescent probe for the P2Y$_{14}$R (Kiselev et al., 2014). Many of these retained activity at the P2Y$_{14}$R, and an Alexa Fluor 488 (AF488)-derivatized version (MRS4174) of one of these exhibited remarkably high (Ki = 80 pM) binding activity. This molecule selectively labelled the P2Y$_{14}$R in live cells as quantified by flow cytometry.

4. P2Y$_{14}$R-mediated cell signaling responses

The idea that the P2Y$_{14}$R is Gi-coupled was initially suggested by the observation that UDP-glucose stimulated [^{35}S]GTPγS binding in membranes from P2Y$_{14}$R-expressing HEK293 cells, which was inhibited by pre-treatment of cells with pertussis toxin (Chambers et al., 2000), and as discussed above, this notion was further supported by studies using chimeric Gα$_{q/i}$ proteins that translate Gi signaling into phospholipase Cβ activation (Freeman et al., 2001;Fricks et al., 2008;Lazarowski et al., 2003). Fricks and coworkers (Fricks et al., 2009) showed that the human P2Y$_{14}$R indeed couples through natively occurring Gi **(Fig. 2)** and that its activation results in robust inhibition of the classical effector of Gi, adenylyl cyclase.

Gi heterotrimers are expressed at high levels in plasma membranes compared to other Gα-subunits, and largely but not entirely, signal through release of Gβγ-subunits from the heterotrimer (Smrcka, 2008). Thus, Gβγ-activated effectors can be expected to be activated downstream of activated

P2Y$_{14}$R, although data directly illustrating responses from most of these are not available. Activation of MAP kinase signaling pathways predictably occurs downstream of Gi-coupled GPCR (Crespo et al., 1994), and therefore, it is not surprising that P2Y$_{14}$R-dependent phosphorylation of MAP kinases also is well-established. UDP-glucose promoted ERK1/2 phosphorylation in P2Y$_{14}$R-expressing HEK293 cells with a potency similar to that observed in the same cells for inhibition of adenylyl cyclase and by a mechanism that was blocked by pertussis toxin pretreatment (Fricks et al., 2009). Differentiation of undifferentiated HL-60 promyelocytic leukemia cells results in expression of P2Y$_{14}$R mRNA (Fricks et al., 2009), and whereas effects of UDP-glucose were not observed in undifferentiated cells, ERK1/2 phosphorylation occurred in response to UDP-glucose in differentiated HL-60 cells. Again, pertussis toxin pretreatment blocked UDP-glucose-promoted ERK activation. Activation of ERK also was reported for UDP-glucose-treated human neutrophils (Scrivens and Dickenson, 2006).

Sesma and coworkers showed that UDP-glucose promotes activation of Rho and chemotaxis of human neutrophils, and the latter was blocked by inhibitors of Rho kinase. Activation of Rho and chemotaxis also occurred in differentiated but not wild-type HL-60 cells. These responses were absent after pretreatment of differentiated HL-60 cells with pertussis toxin and were also inhibited by pharmacological inhibitors of phosphatidylinositol (PtdIns)-3-kinase or Rho kinase. PtdIns-3-kinase is a well-established effector of Gβγ (Stephens et al., 1994), and this work suggests that neutrophils respond to P2Y$_{14}$R activation in a pathway that involves Gβγ-dependent activation of PtdIns-3-kinase, plasma membrane recruitment of an exchange factor for Rho, production of GTP-Rho, actin cytoskeleton rearrangement, and enhanced neutrophil shape change and locomotion. Additional effectors of Gβγ **(Fig. 2)** that may also function downstream of activated P2Y$_{14}$R include K^{+} channels (GIRK), GPRC kinase 2 and 3, phospholipase C β2 and β3, SNAP-25, and P-Rex 1 Rac GEF (Smrcka, 2008).

5. Physiological and pathophysiological consequences of P2Y$_{14}$ receptor activation

While several rigorous studies indicate a role of $P2Y_{14}R$ in UDP-sugar-promoted physiological

responses, unambiguous evidence of the involvement of the $P2Y_{14}R$ in cellular functions often has been

difficult to assimilate. For example, 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 had no effect (Brautigam et al., 2008). As pointed out by the

authors, these results likely reflected an effect due to a contaminant of UDP-glucose solutions and were

independent of $P2Y_{14}R$ activation (Brautigam et al., 2008). Scrivens and Dickenson reported that UDP-

glucose promoted inhibition of cyclic AMP formation in forskolin-stimulated human neuroblastoma SH-

SY5Y cells, but the authors noted that RT-PCR analysis showed no $P2Y_{14}R$ mRNA expression in these cells

(Scrivens and Dickenson, 2005b). In another report, intraperitoneal administration of high

concentrations of UDP-glucose to wild type mice resulted in an increase in gastric emptying, but this

effect was retained in $P2Y_{14}R$ knockout mice (Bassil et al., 2009). Thus, caution should be taken in

interpreting studies of UDP-sugar-evoked responses that are not validated by data with receptor gene

deletion or knockdown, or by selective pharmacological antagonism. Conversely, weak or no responses

to exogenous UDP-glucose in tissues known to express $P2Y_{14}R$ mRNA could be due to receptor

desensitization or elevated baselines caused by cellular UDP-sugar release, low receptor protein

abundance, robust ENPP activity, or a combination of these factors. Caution also should be taken when

analyzing results from $P2Y_{14}R$-targetted mice, as epigenetic changes may contribute to phenotypes not

necessarily associated with $P2Y_{14}R$ lack of function. Further, antisera purportedly generated against

$P2Y_{14}R$ have been used to assess receptor protein expression in various tissues, but strict validation of

these antibodies has not been provided. Bearing in mind these concerns, evidence supporting

physiological/pathophysiological roles for the $P2Y_{14}R$ is discussed below.

Immune/inflammatory cells

12

P2Y$_{14}$R transcripts are robustly expressed in leukocytes and other immune/inflammatory cells and are up-regulated in rat brain after immunological challenge with lipopolysaccharide (Moore et al., 2003) suggesting that the P2Y$_{14}$R plays an important role in immune/inflammatory responses.

Monocytes and macrophages. Macrophages play important roles in both innate and adaptive immunity and are critical effector cells in pro-inflammatory processes. The release of pro-inflammatory cytokines from macrophages in obesity is associated with insulin receptor phosphorylation and insulin resistance (Olefsky and Glass, 2010), and a recent study suggests contribution of the P2Y$_{14}$R to this process (Xu et al., 2012). Specifically: (i) wild-type mice developed severe glucose intolerance when fed a high fat diet (HFD), whereas P2Y$_{14}$R knockout mice were protected from HFD-induced glucose intolerance; (ii) P2Y$_{14}$R-deficient mice exhibited improved systemic insulin sensitivity following HFD and enhanced insulin action in adipose tissue, liver and skeletal muscles; (iii) following HFD, P2Y$_{14}$R knockout mice exhibited a reduced number of macrophages in the liver compared to wild type animals; (iv) P2Y$_{14}$R-deficient monocytes showed reduced migration to the liver of HFD-fed wild type mice; (v) plasma levels of UDP-glucose were 4-fold elevated in HFD-fed animals compared to controls; (vi) UDP-glucose-promoted migration of bone marrow-derived macrophages (BMDM) *in vitro* was reduced with P2Y$_{14}$R knockout-derived BMDM; and (vii) transplantation of P2Y$_{14}$R-deficient bone marrow cells re-established insulin sensitivity in HFD-fed wild type mice. Taken together, these results suggest that UDP-glucose released from liver cells in obese states, possibly via hepatocellular apoptosis (Wang et al., 2008), acts as a chemoattractant to recruit monocytes/macrophages, leading to liver inflammation and insulin resistance.

Neutrophils. Polymorphonuclear neutrophils (PMN) constitute the first line of defense to eradicate bacterial infection. Circulating PMNs are among the peripheral human cell types that most abundantly expresses P2Y$_{14}$R mRNA (Moore et al., 2003), but initial attempts to identify P2Y$_{14}$R-promoted responses in neutrophils were inconclusive. UDP-glucose promoted a modest inhibition of

13

forskolin-stimulated cyclic AMP formation in neutrophils, but other UDP-sugars had no effect (Scrivens

and Dickenson, 2006). UDP-glucose also evoked a modest increase in ERK phosphorylation in these cells,

but this response was observed only at high micromolar concentrations and was not mimicked by other

UDP-sugars; neutrophil elastase secretion was not enhanced by UDP-glucose (Scrivens and Dickenson,

2006). Lastly, neutrophils exhibited no migration response towards UDP-glucose when assessed via the

classic transwell filter staining method (Arase et al., 2009).

Subsequent studies have provided more compelling evidence for functional expression of $P2Y_{14}R$

in neutrophils. As discussed above, expression of native $P2Y_{14}R$ is induced during differentiation of

neutrophil-like HL-60 human myeloid leukemia cells, and UDP-glucose and other UDP-sugars promoted

activation of MAP kinase and Rho signaling in neutrophil-differentiated (but not in undifferentiated)

HL60 cells in a pertussis toxin-sensitive manner (Fricks et al., 2009;Sesma et al., 2012). UDP-glucose-

promoted migration of differentiated HL60 cells was observed using a modified Boyden chamber under

conditions that quantified chemotaxis of fluorescently labeled cells with high sensitivity. UDP-glucose

also promoted Rho kinase-dependent chemotaxis of freshly isolated human neutrophils (Sesma et al.,

2012), and this response was abolished when the $P2Y_{14}R$ antagonist PPTN was added to the PMN

suspension (Barrett et al., 2013).

Observation of $P2Y_{14}R$-dependent PMN migration and potentially additional neutrophil

functions could be relevant to chronic inflammatory lung diseases associated with elevated levels of

extracellular UDP-sugars in the lung (Sesma et al., 2009). As discussed further below, an additional

mechanism by which UDP-sugars may evoke PMN recruitment in the lung involves activation of $P2Y_{14}R$

on epithelial cells and release of IL-8 (Muller et al., 2005).

Mast cells. Mast cells are bone marrow-derived immune effector cells that play important roles

in allergic diseases such as bronchial asthma, rhinitis, anaphylaxis, and urticaria. IgE (Fc)-stimulated mast

cells release histamine, proteases, and other hydrolytic activities from secretory granules and generate

eicosanoid mediators that promote production and secretion of pro-inflammatory cytokines and

chemokines. Whether the P2Y$_{14}$R is expressed in primary mast cells is not known, but studies with mast

cell-like cell lines suggest that the P2Y$_{14}$R is a mediator of degranulation in these cells. P2Y$_{14}$R mRNA was

abundantly present in RBL-2H3 rat mast cells, and UDP-glucose as well as the potent P2Y$_{14}$R agonist, 2-

thio-UDP-glucose, enhanced the release of β-hexosaminidase, an indicator of granule secretion (Gao et

al., 2010). Hexosaminidase secretion was reduced by knocking down the P2Y$_{14}$R via siRNA. Similar

results were reported using the human bone marrow-derived mast cell line LAD2, and incubation of

these cells with PPTN (10 μM) resulted in reduced UDP-sugar-potentiated C3a-promoted hexaminidase

secretion (Gao et al., 2013).

Dendritic cells. P2Y$_{14}$R mRNA was detected at high levels in immature human monocyte-derived

dendritic cells (MDDC) that mature and migrate to sites of inflammation (Skelton et al., 2003). Exposure

of immature MDDC to UDP-glucose resulted in transient mobilization of Ca^{2+} and increased expression

of CD86, suggesting that the P2Y$_{14}$R regulates DC maturation (Skelton et al., 2003).

Lymphocytes. T-lymphocytes express P2Y$_{14}$R transcripts (Moore et al., 2003), and UDP-glucose

was reported to cause a modest decrease in cyclic AMP levels in these cells as well as to inhibit T-cell

proliferation in response to anti-CD3 antibody or interleukin 2 (Scrivens and Dickenson, 2005a).

Hematopoietic stem cells

The P2Y$_{14}$R was reported to be selectively expressed in sub-populations of primitive bone

marrow hematopoietic stem cells (HSC), suggesting a role for this receptor in bone marrow cell

localization/compartmentalization (Lee et al., 2003). Subsequent studies illustrated that subcutaneous

administration of UDP-glucose to mouse resulted in enhanced mobilization of HSC into the peripheral

blood and spleen (Kook et al., 2013a). In a separate study (Kook et al., 2013b) these authors reported

that subcutaneous administration of UDP-glucose to *in utero* irradiated pregnant mice attenuated the

effect of irradiation on the growth and mortality rates of the offspring. Whether these effects of UDP-glucose reflected a $P2Y_{14}R$-dependent mechanism is not known. Strikingly, prenatal irradiation of $P2Y_{14}R$ knockout mice resulted in enhanced growth and survival rates compared with wild-type mice (Kook et al., 2013b).

In a more recent study, this group showed that HSC from $P2Y_{14}R$ knockout mice exhibit increased senescence, a state of permanent irreversible growth arrest in response to aging, radiation, chemotherapy, and other environmental stresses (Cho et al., 2014). Enhanced senescence was associated with increased ROS, elevated p16INK4a expression, and reduced hyper-phosphorylation of the retinoblastoma protein pRb, an inducer of cell senescence. Treatment of wild type cells with pertussis toxin recapitulated the phenotype observed in $P2Y_{14}R$-deficient mice. Furthermore, primitive HSC lacking $P2Y_{14}R$ exhibited impaired ability to restore hematopoiesis in irradiated mice. These observations suggest that the $P2Y_{14}R$ on stem/progenitor hematopoietic cells promotes regenerative responses after injury (Cho et al., 2014;Garrison and Rossi, 2014).

Osteoclast differentiation

Osteoclasts are multinucleated cells that develop from the fusion of bone marrow-derived monocyte/macrophage (BMM) progenitors; receptor activator of NF-κB (RANK) ligand (RANKL) is a key regulator of osteoclast differentiation and function (Teitelbaum, 2000). Using tartrate-resistant acid phosphatase as an osteoclast gene expression marker, Lee and coworkers showed that the $P2Y_{14}R$ is selectively transcribed during RANKL-induced osteoclastogenesis (Lee et al., 2013). $P2Y_{14}R$ siRNA markedly decreased $P2Y_{14}R$ protein expression and caused >50% reduction of RANKL-induced osteoclast formation, suggesting that $P2Y_{14}R$ expression is required for the osteoclast differentiation program. This hypothesis was further supported by the observation that RANKL-induced osteoclast formation is markedly potentiated by co-incubating BMM precursors with UDP-glucose, UDP-galactose, UDP-*N*-

acetylglucosamine, or UDP-glucuronic acid (Lee et al., 2013). In line with this work, the above-mentioned study of Kook and colleagues indicated that UDP-glucose-stimulated hematopoietic stem cell mobilization was associated with enhanced bone marrow RANKL expression and enhanced osteoclastogenesis (Kook et al., 2013a).

Glia and astrocytes

Initial reports indicated broad expression of $P2Y_{14}R$ mRNA in various areas of the brain (Chambers et al., 2000), and strong immunohistochemical staining of brain astrocytes and glia with an affinity-purified $P2Y_{14}R$ antiserum was subsequently reported (Moore et al., 2003). Cultured rat primary astrocytes and murine microglia N9 cells express $P2Y_{14}R$ mRNA, and addition of 100 μM UDP-glucose to rat astrocytes resulted in transient elevation of intracellular calcium (Fumagalli et al., 2003;Bianco et al., 2005). More recently, Kinoshita et al (2013) reported that UDP-glucose-promoted Ca^{2+} signaling was reduced by ~50% in astrocytes transfected with $P2Y_{14}R$ siRNA, although the extent of $P2Y_{14}R$ suppression was not reported. In the absence of exogenous agonists, $P2Y_{14}R$ siRNA-transfected cells displayed increased release of tumor necrosis factor-α (TNFα) and expression of matrix metalloprotease-9 (MMP-9), suggesting that astrocyte remodeling events are suppressed by constitutively active $P2Y_{14}R$ (Kinoshita et al., 2013).

Innate mucosal immunity

Female reproductive tract epithelia: $P2Y_{14}R$ mRNA is highly expressed in the surface epithelium of the female reproductive tract, and immuno-histochemical studies also were consistent with prominent expression of this receptor in epithelium but not stromal tissues (Arase et al., 2009). Similarly, UDP-glucose caused a concentration-dependent increase in interleukin-8 (IL-8) secretion in primary cultures of human glandular cells but had no effect on stromal cells (Arase et al., 2009). Ishikawa endometrial epithelial cells also express $P2Y_{14}R$, and UDP-glucose caused an increase in IL-8

production and secretion in these cells, which was reduced by pre-treatment of cells with pertussis toxin and by siRNA-mediated knockdown of the $P2Y_{14}R$. Conditioned medium from UDP-glucose-stimulated Ishikawa cells exhibited neutrophil chemoattractant activity, which was blocked by a neutralizing anti-IL-8 antibody (Arase et al., 2009).

Incubation of mouse uterine tissues with 10 nM UDP-glucose resulted in enhanced expression of two functional homologues of IL-8, MIP-2 and KC (Arase et al., 2009). Surprisingly, the effect of UDP-glucose was not observed at higher (1000 nM) concentrations, and UDP-N-acetylglucosamine had no effect. Direct injection of UDP-glucose (but not saline) into one horn of the mouse uterus in vivo induced MIP-2 and KC protein. 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 ~5-fold increase in the number of neutrophils present in the endometrium after 48h, which was reduced by sonoporation of $P2Y_{14}R$ siRNA into the mouse uterus (Arase et al., 2009). LPS also induced leukocytosis in mouse uterus, and this effect was attenuated by $P2Y_{14}R$ siRNA.

Arase and coworkers also noted that LPS induced up-regulation of $P2Y_{14}R$ in Ishikawa cells as well as in mouse uterus, which is consistent with the observation of $P2Y_{14}R$ up-regulation in the human endometrium during inflammation. For example, message for the $P2Y_{14}R$ as well as for IL-1β, IL-8 and TNF-α all were upregulated in endometria from patients with pelvic inflammatory disease (Arase et al., 2009). Reports of $P2Y_{14}R$ mRNA up-regulation in mouse uterus after estradiol treatment for 7 days (Crabtree et al., 2008;Crabtree et al., 2006) also suggest that $P2Y_{14}R$ expression may be regulated by circulating hormone levels.

In sum, these studies strongly suggest that the $P2Y_{14}R$ promotes innate mucosal immunity in the female reproductive tract by inducing IL-8.

18

Lung epithelial cells. P2Y$_{14}$R mRNA is expressed in primary cultures of human alveolar epithelial

type II cells, as well as in an human adenocarcinoma alveolar epithelial cell line (A549 cells) and

immortalized human bronchial epithelial BEAS-2B cells (Muller et al., 2005). UDP-glucose dose-

dependently evoked Ca^{2+} mobilization and IL-8 secretion in A549 and BEAS-2 cells, and pertussis toxin

abolished these effects. Alveolar type II cells are located at the boundary between the alveolar airspace

and the interstitium, and addition of UDP-glucose to primary alveolar type II cells resulted in enhanced

IL-8 secretion, suggesting that the P2Y$_{14}$R on type II cells senses UDP-sugars accumulating in the alveolar

space, potentially triggering neutrophil recruitment via IL-8 secretion (Muller et al., 2005).

Gastrointestinal functions

Message for the P2Y$_{14}$R is widely expressed in the rodent gastrointestinal tract (Chambers et al.,

2000) with the highest levels observed in the forestomache (Bassil et al., 2009). UDP-glucose and UDP-

galactose promoted concentration-dependent increases in baseline muscle tension in isolated

forestomache longitudinal muscle preparations (Bassil et al., 2009). The effects of UDP-glucose were lost

in tissue isolated from the *p2y14* knockout mouse (generated by interrupting the open reading frame of

the P2Y$_{14}$R with an IRES-LacZ-polyA reporter expression cassette), and strong β-galactosidase staining

was observed in the muscularis externa of the forestomach of these mice (Bassil et al., 2009). P2Y$_{14}$R-

deficient mice exhibited no apparent alteration in gastric clearance of a nutrient meal (Bassil et al.,

2009). Interestingly, using a similarly generated P2Y$_{14}$R-deficient mouse model, Meister and colleagues

reported that robust β-galactosidase activity was observed in subsets of smooth muscle cells from the

ileum to the colon, and excretion of orally administrated dextran blue in feces was delayed in P2Y$_{14}$R

knockout mice compared to wild type mice (Meister et al., 2014). These observations suggest that

P2Y$_{14}$R function is associated with meal passage throughout the entire gastrointestinal tract.

Endocrine pancreas

Meister and coworkers also found high levels of P2Y$_{14}$R mRNA in the pancreas of wild type mice, with robust β-galactosidase expression in the islets as well as exocrine pancreas of P2Y$_{14}$R targeted mice; the cell type in which the receptor is expressed was not identified (Meister et al., 2014). Insulin immunostaining of pancreatic tissue was similar in both genotypes, but glucose-promoted insulin secretion in perfused islets was markedly reduced in P2Y$_{14}$R-deficient mice. P2Y$_{14}$R knockout mice kept under a western type diet (rich in fat and cholesterol) for 12 weeks exhibited reduced serum insulin levels in response to glucose administration compared to wild type animals. Perfusion of wild type islets with UDP-glucose promoted no insulin secretion, suggesting that the P2Y$_{14}$R regulates functions that are necessary, but not sufficient to promote insulin granule exocytosis. Transcriptome analysis of pancreatic islets indicated that P2Y$_{14}$R deletion resulted in down regulation of >300 genes, including the glucose transporter Slc2a2 and several components of the Ca^{2+}-triggered and GPCR-modulated insulin release pathways (Meister et al., 2014). In addition, >400 transcripts were upregulated in these preparations. Notably, no differences in insulin sensitivity were observed between western diet-fed wild type and P2Y$_{14}$R-deficient mice (Meister et al., 2014), an observation that is in apparent conflict with those described by Xu and colleagues with HFD-fed mice (Xu et al., 2012). The discrepancy may reflect different contributions of liver inflammation to insulin sensitivity between the two mouse models.

Vascular and airway tone

Vascular tone. P2Y$_{14}$R transcripts are localized in middle meningeal and rat aortic smooth muscle cells (Haanes and Edvinsson, 2014b;Govindan et al., 2010). Meister and collaborators found strong β-galactosidase staining in smooth muscle cells of venous blood vessels as well as in a few arteries such as lung arteries and CNS *circulus arteriosus* Willisii. P2Y$_{14}$R-targeted mice exhibited a mild increase in blood pressure and heart rates (Meister et al., 2014).

Haanes & Edvinsson (2014a) reported that UDP-glucose promoted contraction of mouse coronary arteries, and this effect was reduced by pertussis toxin. High micromolar concentrations of

UDP-glucose were used to induce contraction of freshly excised arteries, but UDP-glucose potency

increased considerably in coronary arteries that were maintained in culture medium for 24h; all these

responses were reproduced with arteries isolated from $P2Y_2$ receptor knockout mice, ruling out the

possibility that traces of ATP or UTP in UDP-glucose solutions accounted for the observed results

(Haanes and Edvinsson, 2014a). The enhanced potency of UDP-glucose effects on pre-cultured coronary

arteries correlated with an enhanced immunohistochemistry staining and increased intensity of a 70 kDa

band on Western blots assessed with a commercial antibody against $P2Y_{14}R$. Results similar to those

with coronary arteries were observed with basilar arteries, except that in the latter, treatment with

pertussis toxin resulted in UDP-glucose-evoked relaxation rather than contraction (Haanes and

Edvinsson, 2014a). The availability of $P2Y_{14}R$ antagonists and $P2Y_{14}R$ knockout mouse models should

now provide tools to more conclusively define the involvement of the $P2Y_{14}R$ in UDP-glucose-promoted

contraction of coronary and basilar arteries.

Alsaqati and coworkers reported that UDP-glucose (1-1000 μM) and the synthetic $P2Y_{14}R$ agonist

MRS2690 (0.1-30 μM) elicited vasoconstriction of porcine pancreatic arteries, and the $P2Y_{14}R$ antagonist

PPTN (10 μM) inhibited these responses (Alsaqati et al., 2014). In contrast, UTP-promoted contraction

was not affected by PPTN. UDP-sugar-promoted contraction was attenuated by removal of the

endothelium as well as by antagonists of endothelin ET_A and thromboxane/PG receptors and by

inhibitors of intracellular signaling (e.g., Ca^{2+}, Rho kinase). UDP-glucose (1 mM) and MRS2690 (10 μM)

promoted a decrease of cyclic AMP formation in pancreatic arterial rings co-stimulated with forskolin

and the prostaglandin analogue U46619. By promoting endothelium-dependent artery contraction in

the pancreas, the $P2Y_{14}R$ may influence pancreatic functions by regulating blood flow into the pancreas.

Airway tone. In addition to vascular and gastrointestinal smooth muscles, Meister and

coworkers observed $P2Y_{14}R$ reporter expression (β-galactosidase) in smooth muscle cells of bronchioles.

In line with this $P2Y_{14}R$ expression pattern, reduced lung resistance and increased dynamic compliance

21

in response to methacholine was observed in P2Y$_{14}$R knockout mice (Meister et al., 2014). These observations may be relevant to altered airway responsiveness associated with obstructive lung diseases.

6. Conclusions

Fifteen years have elapsed since the initial characterization of the product of the orphan gene GPR105 (KIAA0001) as P2Y$_{14}$R, a Gi-coupled receptor potently activated by UDP-sugars (Chambers et al., 2000). The abundant message expression of the human P2Y$_{14}$R in adipose tissue, stomach, intestine, some brain regions, skeletal muscles, spleen, lung, and heart, suggests that released UDP-sugars fulfill important extracellular signaling roles in these tissues. Realization that mRNA for the GPR105 rat orthologue in brain and spleen is regulated by immunologic challenge and that the P2Y$_{14}$R mRNA is also prominently expressed in peripheral inflammatory cells further suggested a role for this receptor in immunity and inflammation (Chambers et al., 2000;Moore et al., 2003).

Utilizing P2Y$_{14}$R gene deletion/down-regulation approaches as well as newly developed highly selective P2Y$_{14}$R agonists and antagonists, considerable progress was achieved during recent years to further support and expand those early observations. The work of Arase et al (2009) successfully targeting P2Y$_{14}$-R expression by introducing siRNA in mouse uterus *in vivo* revealed a mechanism relevant to mucosal inflammation, i.e., UDP-glucose-promoted P2Y$_{14}$R-mediated IL-8 release and neutrophil recruitment in the uterus. Studies by Sesma and co-workers (2012) and Barrett and co-workers (2013) support an additional scenario for UDP-sugar-promoted neutrophil inflammation, i.e., direct activation of the neutrophil P2Y$_{14}$R.

ATP released from stressed cells acts as a find-me signal to recruit macrophages at the site of injury (Chekeni et al., 2010;Elliott et al., 2009), and the work by Xu and colleagues (2012) suggests a

22

similar role for UDP-glucose. That is, HFD-induced macrophage inflammation in the liver was attenuated in P2Y$_{14}$R-targetted mice. Moreover, blood levels of UDP-glucose were elevated in HFD-fed mice and UDP-glucose promoted chemotaxis of wild type mice, but not P2Y$_{14}$-deficient monocytes.

In addition to promoting recruitment of circulating neutrophils and monocytes, UDP-glucose induced the mobilization and enhanced re-location to extra-medullary organs of a subset of bone marrow stem cells (Kook et al., 2013a). P2Y$_{14}$R-mediated hematopoietic stem cell mobilization provides a potential tool to improve the efficiency and outcome of hematopoietic peripheral stem cell transplantation. Furthermore, primitive hematopoietic stem cells lacking P2Y$_{14}$R exhibited impaired ability to restore hematopoiesis in irradiated mice, suggesting that the P2Y$_{14}$R on progenitor stem cells promotes regenerative responses after injury (Cho et al., 2014).

P2Y$_{14}$R-targetted mice have been useful to identify functions of this receptor that are associated with gastrointestinal and digestive functions: (i) UDP-glucose-promoted baseline muscle tension in isolated forestomache longitudinal muscle preparations was abolished in P2Y$_{14}$ knockout mice (Bassil et al., 2009); (ii) P2Y$_{14}$R knockout mice exhibited delayed excretion of orally administrated dextran blue in feces (Meister et al., 2014), suggesting that the P2Y$_{14}$R regulates meal passage thought the gastrointestinal tract; and (iii) P2Y$_{14}$R knockout mice exhibited impaired glucose tolerance and deficient insulin secretion in response to glucose (Meister et al., 2014).

Realization that the P2Y$_{14}$R is abundantly expressed in subsets of smooth muscle cells of the vasculature and airways and that P2Y$_{14}$R-deficient mice exhibited increased blood pressure and heart rates as well as reduced lung resistance and increased dynamic compliance in response to methacholine (Meister et al., 2014) suggest important additional areas of potential pharmacological intervention.

Indeed, potent and selective $P2Y_{14}R$ antagonists (e.g., PPTN) are now available (Kiselev et al.,

2014) and one of them, PPTN, significantly reduced UDP-glucose-promoted neutrophil chemotaxis, mast

cell degranulation, and pancreatic artery contraction. This suggests novel strategies for the treatment of

inflammation and, potentially, other disorders associated with the increasing number of $P2Y_{14}R$-

regulated functions recently identified (**Table 2**).

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Lazarowski, Harden.

References

Abbracchio MP, Burnstock G, Boeynaems J M, Barnard E A, Boyer J L, Kennedy C, Knight G E, Fumagalli M, Gachet C, Jacobson K A and Weisman G A (2006) International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* **58**:281-341.

Abeijon C and Hirschberg C B (1992) Topography of glycosylation reactions in the endoplasmic reticulum. *Trends Biochem Sci* **17**:32-36.

Alsaqati M, Latif M L, Chan S L and Ralevic V (2014) Novel vasocontractile role of the P2Y(1)(4) receptor: characterization of its signalling in porcine isolated pancreatic arteries. *Br J Pharmacol* **171**:701-713.

Arase T, Uchida H, Kajitani T, Ono M, Tamaki K, Oda H, Nishikawa S, Kagami M, Nagashima T, Masuda H, Asada H, Yoshimura Y and Maruyama T (2009) The UDP-glucose receptor P2RY$_{14}$ triggers innate mucosal immunity in the female reproductive tract by inducing IL-8. *J Immunol* **182**:7074-7084.

Barrett MO, Sesma J I, Ball C B, Jayasekara P S, Jacobson K A, Lazarowski E R and Harden T K (2013) A selective high-affinity antagonist of the P2Y$_{14}$ receptor inhibits UDP-glucose-stimulated chemotaxis of human neutrophils. *Mol Pharmacol* **84**:41-49.

Bassil AK, Bourdu S, Townson K A, Wheeldon A, Jarvie E M, Zebda N, Abuin A, Grau E, Livi G P, Punter L, Latcham J, Grimes A M, Hurp D P, Downham K M, Sanger G J, Winchester W J, Morrison A D and Moore G B (2009) UDP-glucose modulates gastric function through P2Y$_{14}$ receptor-dependent and -independent mechanisms. *Am J Physiol Gastrointest Liver Physiol* **296**:G923-G930.

Berninsone P and Hirschberg C B (1998) Nucleotide sugars, nucleotide sulfate, and ATP transporters of the endoplasmic reticulum and Golgi apparatus. *Ann N Y Acad Sci* **842**:91-99.

Bianco F, Fumagalli M, Pravettoni E, D'Ambrosi N, Volonte C, Matteoli M, Abbracchio M P and Verderio C (2005) Pathophysiological roles of extracellular nucleotides in glial cells: differential expression of purinergic receptors in resting and activated microglia. *Brain Res Brain Res Rev* **48**:144-156.

Boyd-Tressler A, Penuela S, Laird D W and Dubyak G R (2014) Chemotherapeutic drugs induce ATP release via caspase-gated pannexin-1 channels and a caspase/pannexin-1-independent mechanism. *J Biol Chem* **289**:27246-27263.

Brautigam VM, Dubyak G R, Crain J M and Watters J J (2008) The inflammatory effects of UDP-glucose in N9 microglia are not mediated by P2Y$_{14}$ receptor activation. *Purinergic Signal* **4**:73-78.

Carter RL, Fricks I P, Barrett M O, Burianek L E, Zhou Y, Ko H, Das A, Jacobson K A, Lazarowski E R and Harden T K (2009) Quantification of Gi-mediated inhibition of adenylyl cyclase activity reveals that UDP is a potent agonist of the human P2Y$_{14}$ receptor. *Mol Pharmacol* **76**:1341-1348.

Chambers JK, Macdonald L E, Sarau H M, Ames R S, Freeman K, Foley J J, Zhu Y, McLaughlin M M, Murdock P, McMillan L, Trill J, Swift A, Aiyar N, Taylor P, Vawter L, Naheed S, Szekeres P, Hervieu G, Scott C, Watson J M, Murphy A J, Duzic E, Klein C, Bergsma D J, Wilson S and Livi G P (2000) A G protein-coupled receptor for UDP-glucose. *J Biol Chem* **275**:10767-10771.

Chekeni FB, Elliott M R, Sandilos J K, Walk S F, Kinchen J M, Lazarowski E R, Armstrong A J, Penuela S, Laird D W, Salvesen G S, Isakson B E, Bayliss D A and Ravichandran K S (2010) Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. *Nature* **467**:863-867.

Cho J, Yusuf R, Kook S, Attar E, Lee D, Park B, Cheng T, Scadden D T and Lee B C (2014) Purinergic P2Y(1)(4) receptor modulates stress-induced hematopoietic stem/progenitor cell senescence. *J Clin Invest* **124**:3159-3171.

Crabtree JS, Peano B J, Zhang X, Komm B S, Winneker R C and Harris H A (2008) Activity of three selective estrogen receptor modulators on hormone-dependent responses in the mouse uterus and mammary gland. *Mol Cell Endocrinol* **287**:40-46.

Crabtree JS, Zhang X, Peano B J, Zhang Z, Winneker R C and Harris H A (2006) Development of a mouse model of mammary gland versus uterus tissue selectivity using estrogen- and progesterone-regulated gene markers. *J Steroid Biochem Mol Biol* **101**:11-21.

Crespo P, Xu N, Simonds W F and Gutkind J S (1994) Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits. *Nature* **369**:418-420.

Das A, Ko H, Burianek L E, Barrett M O, Harden T K and Jacobson K A (2010) Human P2Y(14) receptor agonists: truncation of the hexose moiety of uridine-5'-diphosphoglucose and its replacement with alkyl and aryl groups. *J Med Chem* **53**:471-480.

Elliott MR, Chekeni F B, Trampont P C, Lazarowski E R, Kadl A, Walk S F, Park D, Woodson R I, Ostankovich M, Sharma P, Lysiak J J, Harden T K, Leitinger N and Ravichandran K S (2009) Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* **461**:282-286.

Freeman K, Tsui P, Moore D, Emson P C, Vawter L, Naheed S, Lane P, Bawagan H, Herrity N, Murphy K, Sarau H M, Ames R S, Wilson S, Livi G P and Chambers J K (2001) Cloning, pharmacology, and tissue distribution of G-protein-coupled receptor GPR105 (KIAA0001) rodent orthologs. *Genomics* **78**:124-128.

Fricks IP, Carter R L, Lazarowski E R and Harden T K (2009) Gi-dependent cell signaling responses of the human P2Y$_{14}$-receptor in model cell systems. *J Pharmacol Exp Ther* **330**:162-168.

Fricks IP, Maddileti S, Carter R L, Lazarowski E R, Nicholas R A, Jacobson K A and Harden T K (2008) UDP is a competitive antagonist at the human P2Y$_{14}$ receptor. *J Pharmacol Exp Ther* **325**:588-594.

Fumagalli M, Brambilla R, D'Ambrosi N, Volonte C, Matteoli M, Verderio C and Abbracchio M P (2003) Nucleotide-mediated calcium signaling in rat cortical astrocytes: Role of P2X and P2Y receptors. *Glia* **43**:218-230.

Gao ZG, Wei Q, Jayasekara M P and Jacobson K A (2013) The role of P2Y(14) and other P2Y receptors in degranulation of human LAD2 mast cells. *Purinergic Signal* **9**:31-40.

Garrison BS and Rossi D J (2014) Loss of P2Y(1)(4) results in an arresting response to hematological stress. *J Clin Invest* **124**:2846-2848.

Gauthier JY, Belley M, Deschenes D, Fournier J F, Gagne S, Gareau Y, Hamel M, Henault M, Hyjazie H, Kargman S, Lavallee G, Levesque J F, Li L, Mamane Y, Mancini J, Morin N, Mulrooney E, Robichaud J, Therien M, Tranmer G, Wang Z, Wu J and Black W C (2011) The identification of 4,7-disubstituted naphthoic acid derivatives as UDP-competitive antagonists of P2Y$_{14}$. *Bioorg Med Chem Lett* **21**:2836-2839.

Govindan S, Taylor E J and Taylor C W (2010) Ca(2+) signalling by P2Y receptors in cultured rat aortic smooth muscle cells. *Br J Pharmacol* **160**:1953-1962.

Guay D, Beaulieu C, Belley M, Crane S N, DeLuca J, Gareau Y, Hamel M, Henault M, Hyjazie H, Kargman S, Chan C C, Xu L, Gordon R, Li L, Mamane Y, Morin N, Mancini J, Therien M, Tranmer G, Truong V L, Wang Z and Black W C (2011) Synthesis and SAR of pyrimidine-based, non-nucleotide P2Y$_{14}$ receptor antagonists. *Bioorg Med Chem Lett* **21**:2832-2835.

Haanes KA and Edvinsson L (2014a) Characterization of the contractile P2Y$_{14}$ receptor in mouse coronary and cerebral arteries. *FEBS Lett* **588**:2936-2943.

Haanes KA and Edvinsson L (2014b) Expression and characterization of purinergic receptors in rat middle meningeal artery-potential role in migraine. *PLoS One* **9**:e108782.

Hamel M, Henault M, Hyjazie H, Morin N, Bayly C, Skorey K, Therien A G, Mancini J, Brideau C and Kargman S (2011) Discovery of novel P2Y$_{14}$ agonist and antagonist using conventional and nonconventional methods. *J Biomol Screen* **16**:1098-1105.

Harden TK, Sesma J I, Fricks I P and Lazarowski E R (2010) Signalling and pharmacological properties of the P2Y$_{14}$ receptor. *Acta Physiol (Oxf)* **199**:149-160.

Kinoshita M, Nasu-Tada K, Fujishita K, Sato K and Koizumi S (2013) Secretion of matrix metalloproteinase-9 from astrocytes by inhibition of tonic P2Y$_{14}$-receptor-mediated signal(s). *Cell Mol Neurobiol* **33**:47-58.

Kiselev E, Barrett M O, Katritch V, Paoletta S, Weitzer C D, Brown K A, Hammes E, Yin A L, Zhao Q, Stevens R C, Harden T K and Jacobson K A (2014) Exploring a 2-naphthoic acid template for the structure-based design of P2Y$_{14}$ receptor antagonist molecular probes. *ACS Chem Biol* **9**:2833-2842.

Ko H, Das A, Carter R L, Fricks I P, Zhou Y, Ivanov A A, Melman A, Joshi B V, Kovac P, Hajduch J, Kirk K L, Harden T K and Jacobson K A (2009) Molecular recognition in the P2Y(14) receptor: Probing the structurally permissive terminal sugar moiety of uridine-5'-diphosphoglucose. *Bioorg Med Chem* **17**:5298-5311.

Ko H, Fricks I, Ivanov A A, Harden T K and Jacobson K A (2007) Structure-activity relationship of uridine 5'-diphosphoglucose analogues as agonists of the human P2Y$_{14}$ receptor. *J Med Chem* **50**:2030-2039.

Kook S, Cho J, Lee S B and Lee B C (2013a) The nucleotide sugar UDP-glucose mobilizes long-term repopulating primitive hematopoietic cells. *J Clin Invest* **123**:3420-3435.

Kook SH, Cho J S, Morrison A, Wiener E, Lee S B, Scadden D and Lee B C (2013b) The purinergic P2Y$_{14}$ receptor axis is a molecular determinant for organism survival under in utero radiation toxicity. *Cell Death Dis* **4**:e703.

Kreda SM, Okada S F, van Heusden C A, O'Neal W, Gabriel S, Abdullah L, Davis C W, Boucher R C and Lazarowski E R (2007) Coordinated release of nucleotides and mucin from human airway epithelial Calu-3 cells. *J Physiol* **584**:245-259.

Kreda SM, Seminario-Vidal L, Heusden C and Lazarowski E R (2008) Thrombin-promoted release of UDP-glucose from human astrocytoma cells. *Br J Pharmacol* **153**:1528-1537.

Lazarowski ER (2010) Quantification of extracellular UDP-galactose. *Anal Biochem* **396**:23-29.

Lazarowski ER, Shea D A, Boucher R C and Harden T K (2003) Release of cellular UDP-glucose as a potential extracellular signaling molecule. *Mol Pharmacol* **63**:1190-1197.

Lee BC, Cheng T, Adams G B, Attar E C, Miura N, Lee S B, Saito Y, Olszak I, Dombkowski D, Olson D P, Hancock J, Choi P S, Haber D A, Luster A D and Scadden D T (2003) P2Y-like receptor, GPR105 (P2Y$_{14}$), identifies and mediates chemotaxis of bone-marrow hematopoietic stem cells. *Genes Dev* **17**:1592-1604.

Lee SA, Park J H and Lee S Y (2013) Selective induction of P2Y$_{14}$ receptor by RANKL promotes osteoclast formation. *Mol Cells* **36**:273-277.

Meister J, Le D D, Ricken A, Burkhardt R, Thiery J, Pfannkuche H, Polte T, Grosse J, Schoneberg T and Schulz A (2014) The G Protein-coupled Receptor P2Y$_{14}$ Influences Insulin Release and Smooth Muscle Function in Mice. *J Biol Chem* **289**:23353-23366.

Moore DJ, Murdock P R, Watson J M, Faull R L, Waldvogel H J, Szekeres P G, Wilson S, Freeman K B and Emson P C (2003) 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* **118**:10-23.

Muller T, Bayer H, Myrtek D, Ferrari D, Sorichter S, Ziegenhagen M W, Zissel G, Virchow Jr J C, Luttmann W, Norgauer J, Di Virgilio F and Idzko M (2005) The P2Y$_{14}$ Receptor of Airway Epithelial Cells: Coupling to Intracellular Ca^{2+} and IL-8 Secretion. *Am J Respir Cell Mol Biol* **33**:601-609.

Okada SF, Zhang L, Kreda S M, Abdullah L H, Davis C W, Pickles R J, Lazarowski E R and Boucher R C (2011) Coupled Nucleotide and Mucin Hypersecretion from Goblet Cell Metaplastic Human Airway Epithelium. *Am J Respir Cell Mol Biol* **45**:253-260.

Olefsky JM and Glass C K (2010) Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* **72**:219-246.

Ralevic V and Burnstock G (1998) Receptors for purines and pyrimidines. *Pharmacol Rev* **50**:413-492.

Robichaud J, Fournier J F, Gagne S, Gauthier J Y, Hamel M, Han Y, Henault M, Kargman S, Levesque J F, Mamane Y, Mancini J, Morin N, Mulrooney E, Wu J and Black W C (2011) Applying the pro-drug approach to afford highly bioavailable antagonists of P2Y(14). *Bioorg Med Chem Lett* **21**:4366-4368.

Scrivens M and Dickenson J M (2005a) Functional expression of the P2Y(14) receptor in murine T-lymphocytes. *Br J Pharmacol* **146**:435-444.

Scrivens M and Dickenson J M (2005b) Pharmacological effects mediated by UDP-glucose that are independent of P2Y$_{14}$ receptor expression. *Pharmacol Res* **51**:533-538.

Scrivens M and Dickenson J M (2006) Functional expression of the P2Y(14) receptor in human neutrophils. *Eur J Pharmacol* **543**:166-173.

Sesma JI, Esther C R, Jr., Kreda S M, Jones L, O'Neal W, Nishihara S, Nicholas R A and Lazarowski E R (2009) ER/golgi nucelotide sugar transporters contribute to the cellular release of UDP-sugar signaling molecules. *J Biol Chem* **284**:12572-12583.

Sesma JI, Kreda S M, Steinckwich-Besancon N, Dang H, Garcia-Mata R, Harden T K and Lazarowski E R (2012) The UDP-sugar-sensing P2Y$_{14}$ receptor promotes Rho-mediated signaling and chemotaxis in human neutrophils. *Am J Physiol Cell Physiol* **303**:C490-C498.

Skelton L, Cooper M, Murphy M and Platt A (2003) Human immature monocyte-derived dendritic cells express the G protein-coupled receptor GPR105 (KIAA0001, P2Y$_{14}$) and increase intracellular calcium in response to its agonist, uridine diphosphoglucose. *J Immunol* **171**:1941-1949.

Smrcka AV (2008) G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. *Cell Mol Life Sci* **65**:2191-2214.

Stephens L, Smrcka A, Cooke F T, Jackson T R, Sternweis P C and Hawkins P T (1994) A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by G protein beta gamma subunits. *Cell* **77**:83-93.

Teitelbaum SL (2000) Bone resorption by osteoclasts. *Science* **289**:1504-1508.

von Kugelgen I and Harden T K (2011) Molecular pharmacology, physiology, and structure of the P2Y receptors. *Adv Pharmacol* **61**:373-415.

Wang Y, Ausman L M, Russell R M, Greenberg A S and Wang X D (2008) Increased apoptosis in high-fat diet-induced nonalcoholic steatohepatitis in rats is associated with c-Jun NH2-terminal kinase activation and elevated proapoptotic Bax. *J Nutr* **138**:1866-1871.

Xu J, Morinaga H, Oh D, Li P, Chen A, Talukdar S, Lazarowski E, Olefsky J M and Kim J J (2012) GPR105 Ablation Prevents Inflammation and Improves Insulin Sensitivity in Mice with Diet-Induced Obesity. *J Immunol* **189**:1992-1999.

Zimmermann H, Zebisch M and Strater N (2012) Cellular function and molecular structure of ecto-nucleotidases. *Purinergic Signal* **8**:437-502.

Footnotes

Figure Legends

Figure 1. ***Pathways for UDP-sugar release***. UDP-glucose (UDPG) and other UDP-sugars are synthesized in the cytosol and enter the secretory pathway via ER/Golgi-resident SLC35 transporters, using UMP as antiporter substrates. ER/Golgi UDP-sugars are used for glycosylation yielding UDP as a reaction product, and UDP is metabolized to UMP. (i) Residual UDP-sugars in the ER/Golgi lumen are released as cargo via the constitutive secretory pathway. (ii) Unlike ATP and UTP, UDP-sugars are not predicted to be substrates of the vesicular nucleotide transporter VNUT, but Golgi-derived vesicles are a source of UDP-sugars in specialized granules susceptible to Ca^{2+}-regulated exocytosis. Not shown: Golgi UDP is released with UDP-sugars. Whether pannexin 1 mediates the release of cytosolic UDP-sugars is not known. PPi, pyrophosphate; G-1P, glucose-1P

Figure 2. ***Signaling pathways associated with $P2Y_{14}R$ activation***. The $P2Y_{14}R$ couples to Gi, thus promoting Gαi- and Gβγ-mediated inhibition of adenylyl cyclases 1, 3, 5, 6, 8, and 9. Additional direct and indirect effectors of Gβγ include K^+ channels (GIRK), phosphatidylinositol 3-kinase-γ, GPCR kinase 2 and 3, and phospholipase C β2 and β3, P-rex1 Rac GEF, and SNAP-25. Rho, MAP kinases, SNAP, and potentially other effectors not listed represent examples of signaling proteins that are also activated downstream of Gβγ.

Table 1. *Agonists and antagonists of the P2YR*. EC_{50} values are from experiments measuring [a] inhibition of cyclic AMP accumulation in P2Y$_{14}$R-expressing CHO cells (Carter et al, 2009), [b] inositol phosphate accumulation in Cos-7 cells expressing the human P2Y$_{14}$R and $G\alpha_{q/i}$ (Ko et al, 2007), and [c] inhibition of cyclic AMP accumulation in P2Y$_{14}$R-expressing HEK293 cells (Das et al, 2009). [d] K_B for 4-((piperidin-4-yl)-phenyl)-7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid (PPTN) was obtained in Schild analyses measuring antagonism of UDP-glucose-promoted inhibition of cyclic AMP accumulation in P2Y$_{14}$R-expressing CHO cells (Barrett et al, 2013).

	Molecule	Potency
Natural agonists[a]	UDP-glucose	EC_{50} = 132 nM
	UDP	EC_{50} = 33 nM
Selective synthetic agonists[b,c]	2-thio-UDP-glucose (MRS2690)	EC_{50} = 49 nM
	α,β-methylene-2-thio-UDP (MRS2905)	EC_{50} = 0.92 nM
Selective competitive antagonist[d]	PPTN	K_B = 400 pM

Table 2. *Proposed physiological effects of P2Y$_{14}$R activation*

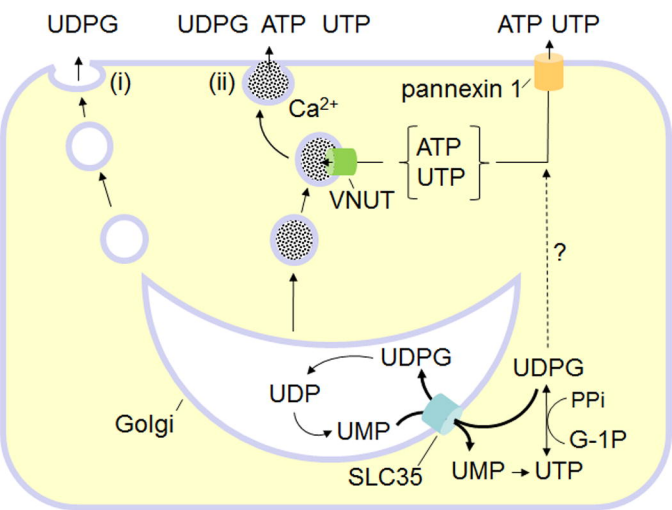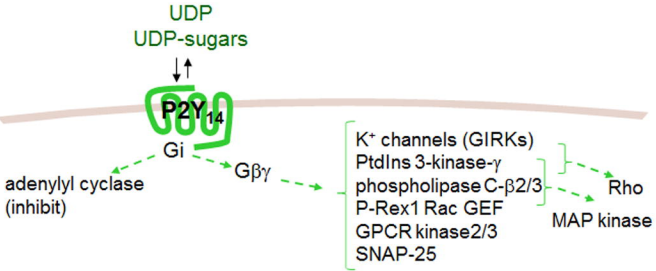| P2Y$_{14}$R-associated responses	References
Regulation of dendritic cell maturation	Skelton et al., 2003
Inhibition of T-lymphocyte proliferation	Scrivens and Dickenson, 2005a
Recruitment of monocytes and macrophages in inflammation	Xu et al., 2012
Stimulation of neutrophil chemotaxis	Sesma et al., 2012; Barrett et al 2013
Enhancement of degranulation of mast cells	Gao et al., 2010; Gao et al., 2013
Promotion of hematopoietic regenerative responses	Cho et al., 2014
Regulation of osteoclast differentiation	Lee et al., 2013; Kook et al., 2013a
Regulation of astrocyte remodeling	Kinoshita et al., 2013
Stimulation of innate mucosal immunity in the female reproductive tract	Arase et al., 2009
Promotion of lung inflammation	Muller et al., 2005
Regulation of gastrointestinal muscle contraction and meal passage	Bassil et al., 2009; Meister et al., 2014
Regulation of insulin secretion	Meister et al., 2014
Regulation of smooth muscle contraction in some vascular beds	Meister et al., 2014; Haanes and Edvinsson, 2014a; Alsaqati et al. 2014

Figure 1

Figure 2