Pflugers Arch - Eur J Physiol (2006) 452: 552–562
DOI 10.1007/s00424-006-0069-2

INVITED REVIEW

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P2 receptors: intracellular signaling

Received: 4 March 2006 / Accepted: 6 March 2006 / Published online: 4 April 2006

Abstract P2 receptors for extracellular nucleotides are divided into two categories: the ion channel receptors (P2X) and the G-protein-coupled receptors (P2Y). For the P2X receptors, signal transduction appears to be relatively simple. Upon activation by extracellular ATP, a channel comprised of P2X receptor subunits opens and allows cations to move across the plasma membrane, resulting in changes in the electrical potential of the cell that, in turn, propagates a signal. This regulated flux of ions across the plasma membrane has important signaling functions, especially in impulse propagation in the nervous system and in muscle contractility. In addition, P2X receptor activation causes the accumulation of calcium ions in the cytoplasm, which is responsible for activating numerous signaling molecules. For the P2Y receptors, signal transduction is more complex. Intracellular signaling cascades are the main routes of communication between G-protein-coupled receptors and regulatory targets within the cell. These signaling cascades operate mainly by the sequential activation or deactivation of heterotrimeric and monomeric G proteins, phospholipases, protein kinases, adenylyl and guanylyl cyclases, and phosphodiesterases that regulate many cellular processes, including proliferation, differentiation, apoptosis, metabolism, secretion, and cell migration. In addition, there are numerous ion channels, cell adhesion molecules and receptor tyrosine kinases that are modulated by P2Y receptors and operate to transmit an extracellular signal to an intracellular response. These intracellular signaling pathways and their regulation by P2 receptors are discussed in this review.

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Keywords G-protein-coupled receptors · Ion channels · Signal transduction · Purinergic receptors · G proteins · ATP receptors

P2X receptors

P2X receptors are ATP-gated ion channels that mediate sodium influx, potassium efflux and, to varying extents, calcium influx, leading to depolarization of the cell membrane [10, 37, 92]. Membrane depolarization subsequently activates voltage-gated calcium channels, thus causing accumulation of calcium ions (Ca^{2+}) in the cytoplasm. Currently, seven human P2X receptor subunits ($P2X_{1-7}$) have been cloned [46, 47, 72, 79, 104, 128, 129] and display 30–50% sequence identity at the protein level. The predicted structure of the P2X subunits is a transmembrane protein with two membrane spanning domains (TM1 and TM2) that are involved in gating the ion channel and lining the ion pore, respectively [18]. The TM regions are separated by a large hydrophilic extracellular loop containing several conserved amino acid residues, including ten cysteines that form a series of disulphide bridges, 13 glycines and 2–6 asparagines that may serve as N-linked glycosylation sites [13, 92, 103, 129]. The extracellular loop also contains an ATP-binding site and a hydrophobic H5 region that may be important for regulation of the channel by magnesium, zinc, copper and other cations [18]. The intracellular N and C termini contain consensus phosphorylation sites for protein kinases (PKA and PKC) that have been shown to be involved in modulating $P2X_2$ receptor activity [12, 23].

Functional P2X receptor ion channels are now thought to consist of three subunits [18]. At the present time, six homomers ($P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_7$) and three heteromers ($P2X_2/P2X_3$, $P2X_4/P2X_6$, $P2X_1/P2X_5$) have been functionally characterized (reviewed by [94]). The desensitization and permeability properties, as well as the agonist and antagonist specificities of the various homomeric and heteromeric P2X receptors, have been thoroughly described [93] and will not be discussed further in this review.

P2X receptors are widely distributed, and functional responses have been demonstrated in neurons, glial cells, bone, muscle, endothelium, epithelium, and hematopoietic cells [18, 93]. It is well-documented that in the central and peripheral nervous systems P2X receptors carry out many important functions, such as fast synaptic transmission [9, 17, 38], neurotransmitter release [65], and the generation of pain signals [27, 36, 124]. Studies have suggested that P2X receptors may play a role in the pathophysiology of Parkinson's disease, Alzheimer's disease, and multiple sclerosis. For example, in a transgenic mouse model of Alzheimer's disease (Tg2576) the $P2X_7$ receptor is upregulated in microglial cells and astrocytes around amyloid plaques, as compared to age-matched controls [99]. Furthermore, activation of $P2X_7$ receptors in primary rat microglial cells causes the release of reactive oxygen species, such as superoxide $\left(O_2^-\right)$. This effect was suppressed by PPADS and oxidized ATP, $P2X_7$ receptor antagonists, and by inhibitors of p38 and PI3K, but not MEK1/2 [99], demonstrating the ability of P2X receptors to activate signaling pathways also coupled to P2Y receptors. Activation of $P2X_7$ receptors in BV-2 microglial cells enhances the effect of interferon-γ on the upregulation of iNOS and the production of NO [49], suggesting that $P2X_7$ receptors promote glial cell activation associated with the pathology of neurodegenerative diseases.

Although the detailed signaling mechanisms have not been established for most P2X receptor subtypes, it is well-known that cytoplasmic Ca^{2+} triggers a variety of intracellular events, in part, through activation of MAPKs, PKC, and calmodulin. For example, ERK1/2 and p38 are activated by the P2X7 receptor in astrocytes, and activation of these kinases is required for upregulation of monocyte chemoattractant protein 1 [97]. ERK1 and/or ERK2 also are/is activated by P2X1 receptors in platelets [96], P2X2 receptors in PC12 cells [119], and P2X7 receptors in T cells [15], HEK293 cells [4], macrophages [2], and mast cells [16]. ERK activation by the platelet P2X1 receptor was shown to occur through a PKC- and Ca^{2+}-dependent pathway [96], while ERK activation by the astrocyte P2X7 receptor requires PI3K and Src kinase activity, as well as PKC activity and cytoplasmic Ca^{2+} [50].

In addition, cytoplasmic Ca^{2+} is generally important for neurotransmitter release via a calmodulin-dependent process. In this signaling cascade, Ca^{2+} activates calmodulin, which then activates protein kinase II. Protein kinase II phosphorylates synapsin on the surface of synaptic vesicles, causing the vesicles to be released from the actin cytoskeleton so that they can fuse to active zones at the presynaptic terminal and release their neurotransmitter contents in the synapse [76]. Although the role of calmodulin in P2X receptor-mediated neurotransmitter release has not been established, it has been demonstrated that P2X receptor-mediated glutamate release from neurons is completely blocked by cadmium, a broad-spectrum calcium channel blocker [65].

Studies of the $P2X_7$ receptor subtype currently provide the most information about intracellular signaling pathways activated by P2X receptors (Fig. 1). $P2X_7$ receptors are distinct among P2X receptors in that they can form both cation channels and, after prolonged activation in some cell types, non-selective pores that allow passage of larger molecules, including nucleotides and fluorescent dyes [40, 118, 134]. $P2X_7$ receptors have an intracellular C-terminal tail of 235 amino acids that is about 150 amino acids longer than the other P2X receptor subunits. It is believed that the long C-terminal tail of the $P2X_7$ receptor regulates receptor function as well as cellular localization. For example, the $P2X_7$ receptor C terminus interacts with epithelial membrane protein-2 (EMP-2), which is involved in cell blebbing [145]. A $P^{451}L$ substitution in the C-terminal tail region of the $P2X_7$ receptor prevents ATP-induced cell death [73]. Truncation of the $P2X_7$ receptor at F^{581} (i.e., removing 14 amino acid residues at the C terminus) completely abolishes pore formation measured by ethidium$^+$ uptake, whereas, truncation at P^{582} does not significantly affect pore formation [115]. A double mutation in the C-terminal region ($R^{578}E$, $K^{579}E$) causes defective $P2X_7$ receptor transport to the plasma membrane in HEK293 cells [35]. Similarly, an $I^{568}N$ substitution results in decreased $P2X_7$ receptor expression on the cell surface [143]. Proteomic analysis has identified 11 proteins that associate with the rat $P2X_7$ receptor, including laminin α3, integrin β2, β-actin, α-actinin, MAGuK, phosphatidylinositol-4 kinase, receptor protein tyrosine phosphatase-β and three heat shock proteins (HSP70, HSP71 and HSP90) [66], suggesting that the $P2X_7$ receptor is part of a multi-protein complex that facilitates communication between the extracellular matrix, the actin cytoskeleton, and intracellular signaling cascades. Furthermore, HSP90 has been shown to act as a negative regulator of $P2X_7$ receptor activity by stimulating tyrosine phosphorylation of the receptor [1].

Activation of $P2X_7$ receptor signaling pathways can have proinflammatory effects by stimulating the synthesis and secretion of cytokines. For example, activation of $P2X_7$ receptors in BAC1 murine macrophages causes IL-1β release that occurs independent of RhoA activation [131] but requires cytoplasmic Ca^{2+} [56]. In mast cells, $P2X_7$ receptor activation causes increased expression of IL-4, IL-6, IL-13 and tumor necrosis factor-α (TNF-α) [16]. In macrophages, $P2X_7$ receptor activation enhances the increase in mRNA levels for inducible NOS (iNOS) and TNF-α caused by lipopolysaccharides (LPS) [63, 122, 123]. Sequence analysis of the iNOS gene promoter has identified an element that potentially binds to NF-κB, a transcription factor activated by $P2X_7$ receptors in macrophages [2] and osteoclasts [67] but deactivated by $P2X_7$ receptors in mast cells [15]. Other transcription factors and signaling molecules activated by $P2X_7$ receptors include $p56^{lck}$ and $p56^{lck}$-mediated activation of AP-1, ERK, and JNK in human T cells [15], JAK2, STAT6, and caspase-3 and caspase-8 in several mast cell lines [16], and RhoA and the Rho-effector kinase (ROCK) in BAC1 murine macrophages and HEK cells expressing the recombinant $P2X_7$ receptor [131].

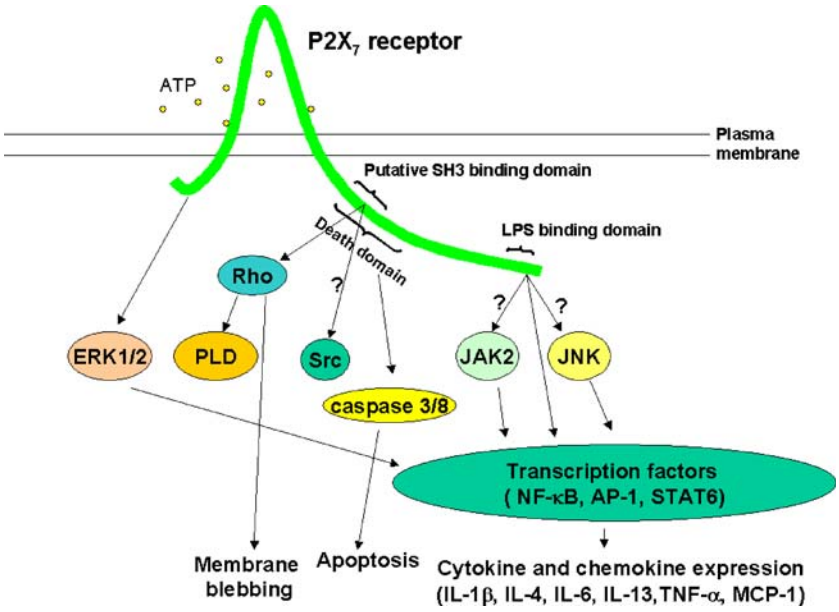

Fig. 1 Intracellular signaling pathways activated by the $P2X_7$ receptor. The $P2X_7$ receptor is a homomeric cation channel. In this diagram, an individual $P2X_7$ receptor subunit is shown (total length 595 amino acids). Activation of $P2X_7$ receptors by ATP results in Na^+ and Ca^{2+} influx, K^+ efflux, membrane depolarization and formation of a non-selective 4-nm pore (not depicted), as well as membrane blebbing, inflammatory cytokine, and chemokine expression and cell apoptosis. N-terminal deletion of the $P2X_7$ receptor impairs ERK1/2 activation, whereas, C-terminal deletion impairs Ca^{2+} influx [4]. A putative Src homology (SH3)-binding domain is located within residues 441–460, suggesting the involvement of the C terminus in regulating phospholipase D (PLD) activity by interacting with SH3 proteins that control Rho or other small G proteins [34]. Residues 436–531 also contain a conserved death domain, which may contribute to $P2X_7$ receptor-mediated caspase activity and apoptosis [34]. An LPS-binding domain was identified between residues 573 and 590, which controls trafficking of the $P2X_7$ receptor and, therefore, modulates receptor availability and activity during an inflammatory response [34, 35]. *ERK* extracellular-signal regulated kinase, *IL-1β, -4, -6, -13* interleukin subtypes, *JAK2* Janus kinase 2, *JNK* c-Jun N-terminal kinase, *LPS* lipopolysaccharide, *MCP-1* monocyte chemoattractant protein-1, *NF-κB* nuclear factor κB, *STAT6* signal transducer and activator of transcription 6, *TNF-α* tumor necrosis factor-α

P2Y receptors

P2Y receptors are G-protein-coupled receptors (GPCRs) that are activated by purine and/or pyrimidine nucleotides. Currently, eight P2Y receptor subtypes ($P2Y_{1, 2, 4, 6, 11, 12, 13, \text{ and } 14}$) have been cloned [5, 21, 28, 29, 31, 32, 60, 91, 98] and display 19–55% sequence identity at the protein level. The predicted structure of P2Y receptors includes an extracellular N terminus containing several potential N-linked glycosylation sites, seven transmembrane spanning regions that assist in forming the ligand binding pocket, and an intracellular C terminus that contains several consensus binding/phosphorylation sites for protein kinases. Like other members of the GPCR superfamily, P2Y receptor stimulation leads to activation of heterotrimeric G proteins and their dissociation into α and βγ subunits that can then interact with a variety of effector proteins. Individual P2Y receptor subtypes have been linked to one or more of the four subfamilies of heterotrimeric G proteins (G_s, $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$) (Table 1).

The ability of individual P2Y receptor subtypes to couple to specific G proteins was initially inferred from indirect evidence [measurement of increases in inositol phosphate, cytoplasmic Ca^{2+}, or cyclic AMP levels, and determination of sensitivity to the $G_{i/o}$ protein inhibitor pertussis toxin (PTX)]. Direct evidence for G protein coupling to P2Y receptors was recently obtained in vesicles reconstituted with $P2Y_1$ and either $G\alpha_q\beta_1\gamma_2$ or $G\alpha_{11}\beta_1\gamma_2$ by demonstrating that the $P2Y_1$ receptor agonist ADP can induce GTP hydrolysis [135]. It also has been demonstrated that $P2Y_{12}$ receptors can couple to $G\alpha_{i2}$ more effectively than to $G\alpha_{i1}$ and $G\alpha_{i3}$, but not to $G\alpha_o$ or $G\alpha_q$ [11]. Other evidences support the notion that individual P2Y receptors can couple to functionally distinct G proteins. For example, activation of phospholipase C (PLC) by the $P2Y_2$ receptor in HEL cells is inhibited by $G\alpha_{16}$ antisense oligonucleotide, and also by PTX [8], suggesting the ability of the $P2Y_2$ receptor to couple to G proteins in the $G_{q/11}$ and $G_{i/o}$ subfamilies. Similarly, $P2Y_2$ receptors have been shown to couple to PLCβ$_1$ via $G\alpha_{q/11}$ and to PLCβ$_3$ via $G\alpha_{i3}\beta_1\gamma_2$-derived βγ subunits in gastric smooth muscle cells [89].

Other studies have shown that P2Y receptors can couple to the activation of both heterotrimeric and monomeric G proteins. For example, the $P2Y_2$ receptor can activate $G_{q/11}$, G_o and G_{12} as well as Rac and RhoA (see Table 1). Furthermore, Rac activation by the $P2Y_2$ receptor is dependent on G_o activity [6], whereas, RhoA activation is dependent on G_{12} activity [Z. Liao, unpublished data]. Activation of PI3K by the $P2Y_{12}$ receptor was found to be dependent on $G\alpha_i$ and also RhoA and Rho kinase activities [117]. The PTX insensitivity of $P2Y_{12}$ receptors in platelets, may suggest that ADP-induced platelet aggrega-

Table 1 P2Y receptor subtypes and G-protein coupling

Receptor	Agonist (human)	G protein	Main effector molecules	References
$P2Y_1$	ADP	$G_{q/11}$	PLC (+), Ca^{2+} release	[69, 114]
$P2Y_2$	ATP, UTP	$G_{q/11}$	PLC (+), Ca^{2+} release	[6, 39, 71, 137]
	ATP, UTP	G_o	PLC (+), Ca^{2+} release	
			Rac (+)	
	ATP, UTP	G_{12}	RhoA (+)	(Z. Liao, unpublished data)
$P2Y_4$	UTP	$G_{q/11}$	PLC (+), Ca^{2+} release	[30, 43, 91]
	UTP	G_o	PLC (+), Ca^{2+} release	
$P2Y_6$	UDP	$G_{q/11}$	PLC (+), Ca^{2+} release	[31, 105]
$P2Y_{11}$	ATP	$G_{q/11}$	PLC (+), Ca^{2+} release	[29, 102, 141]
	ATP	G_s	AC (+), increased cAMP	
	UTP	G_o	PLC-independent Ca^{2+} release	
$P2Y_{12}$	ADP	G_i	AC (−), decreased cAMP	[60, 70, 117, 151]
	ADP	$G_{12/13}$?	RhoA (+)	
$P2Y_{13}$	ADP	$G_{i/o}$	AC (−), decreased cAMP PLC (+), Ca^{2+} release	[28, 152]
$P2Y_{14}$	UDP-glucose	$G_{i/o}$	PLC (+), Ca^{2+} release	[21]

tion is regulated by the $G\alpha_{12/13}$ subfamily, as $G\alpha_{13}$ was recently reported to control platelet aggregation induced by thrombin, thromboxane A(2), and collagen [87]. Coupling of an individual P2Y receptor subtype to multiple G proteins and signaling pathways may suggest that receptor activation can lead to the induction of more than one conformational state that enables associations with different Gα subunits. This may explain how $P2Y_{11}$ receptor activation by ATP can stimulate adenylyl cyclase leading to a rise in cAMP and also can activate PLC to induce the formation of IP_3 and the mobilization of intracellular Ca^{2+}, whereas, activation of the $P2Y_{11}$ receptor by UTP mobilizes intracellular Ca^{2+} without increasing IP_3 or cAMP levels [141]. Variations in the ADP/2-methylthioADP concentration ratio can affect the extent of $P2Y_{13}$ receptor coupling to G_{16} or G_i protein and, at high ADP concentrations, $P2Y_{13}$ receptors can couple to G_s, suggesting the existence of distinct receptor conformations dependent on the structure and concentration of the receptor ligand [81].

Agonist-induced activation of GPCRs also initiates receptor desensitization, which diminishes GPCR responsiveness, leading to receptor internalization. Agonist-dependent GPCR desensitization is generally mediated by a family of GPCR kinases (GRK 1-7), which phosphorylate residues in intracellular domains of the receptor and, in some instances, promote the binding of β-arrestins that assist in receptor internalization to clathrin coated pits. Although not all P2 receptors exhibit agonist-induced desensitization, the $P2Y_2$ receptor undergoes agonist-induced desensitization in several cell types [26, 48, 95, 106, 130, 144]. Receptor mutagenesis studies indicate that deletion of structural motifs or point mutations (e.g., $S^{243}A$, $T^{344}A$, and $S^{356}A$) in the $P2Y_2$ receptor C-terminal tail that are putative phosphorylation sites for GRKs diminishes agonist-induced desensitization and internalization of the $P2Y_2$ receptor [45, 48]. Arrestins do not appear to play a role in $P2Y_1$ or $P2Y_2$ receptor internalization [88], although β-arrestin-2 is involved in internalization of the $P2X_7$ receptor [42], suggesting distinct mechanisms of desensitization exist for P2Y and P2X receptor subtypes. Also, siRNA designed to suppress the function of specific GRKs was used to demonstrate that $P2Y_{12}$ receptor desensitization requires GRK2 and GRK6 activity [58]. In contrast, $P2Y_1$ receptor desensitization is largely dependent on PKC activity [58].

Over the last decade, the classical mechanism of GPCR signaling as a linear and sequential pathway has been modified. Many studies have revealed that cross-talk exists between different GPCRs and their downstream effectors as well as between GPCRs and other signaling proteins, such as ion channels, integrins, and receptor and non-receptor tyrosine kinases. For example, the $P2Y_1$ receptor contains a PDZ binding domain (DTSL) in its C-terminal tail that interacts directly with the Na^+/H^+-exchanger regulatory factor to control Na^+/H^+ exchange [57]. Similarly, an RRSE-QXK/RSE motif that is present in $P2Y_{1,\ 2,\ 6,\ 11}$ receptors is required for the modulation of a voltage-gated ion channel that mediates a transient inward current in *Xenopus* oocytes [74]. $P2Y_1$ receptor activation also causes PKC-dependent phosphorylation of the capsaicin receptor (a VR1 cation channel), shifts the capsaicin concentration–response curve twofold to the left, and decreases the threshold for capsaicin receptor activation by heat from 42 to 35°C [121]. Thus, cross-talk between the $P2Y_1$ receptor and the capsaicin receptor may represent a novel mechanism for the perception of pain induced by P2 receptors.

Cross-talk between P2X receptor ion channels and P2Y GPCRs has also been demonstrated. In *Xenopus* oocytes expressing the recombinant human $P2X_1$ receptor, a transient inward current occurs in response to ATP [133]. This response undergoes desensitization (i.e., current flow decreases upon prolonged or repeated exposure to ATP); however, coexpression and activation of either the $P2Y_1$ or $P2Y_2$ receptor (stimulated by ADP or UTP, respectively) was found to inhibit $P2X_1$ receptor desensitization. The mechanism of P2Y receptor-mediated inhibition of $P2X_1$

receptor desensitization does not appear to involve direct phosphorylation of the $P2X_1$ receptor but does involve protein kinase activity, perhaps mediated by an accessory protein [133]. $P2X_1$ receptors can also modulate P2Y receptor activity. In megakaryocytes, $P2X_1$ receptor activation with α,β-meATP causes a rapid and transient Ca^{2+} influx, whereas, $P2Y_1$ receptor activation with ADP causes a slower, larger, and more sustained increase in cytoplasmic Ca^{2+} [132]. Co-application of the two agonists, however, accelerates the Ca^{2+} response and potentiates the peak amplitude, suggesting that the $P2X_1$ receptor may have a priming role in the activation of $P2Y_1$ receptors during platelet stimulation. The $P2Y_{12}$ receptor, through activation of PI3K, has also been shown to play a synergistic role in both $P2Y_1$ and $P2X_1$-receptor-dependent currents in megakaryocytes [120].

In addition to modulation of ion channel activity, P2Y receptors can modulate the activity of receptor tyrosine kinases. For example, studies have identified SH3-binding domains (PXXPs) in the C-terminal tail of the $P2Y_2$ receptor that are necessary for this GPCR to bind and activate the non-receptor tyrosine kinase Src and for Src-dependent transactivation of several receptor tyrosine kinases, including the epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) [77]. In human vascular smooth muscle cells, UTP or UDP alone had no effect on cell proliferation, but these nucleotides significantly reduced the proliferative response to PDGF [140]. Interestingly, ATP potentiated the proliferative response elicited by PDGF. The mechanism underlying these excitatory and inhibitory effects of P2Y receptor activation is not known, and more than one P2Y receptor subtype may contribute, but the authors demonstrated clearly that changes in intracellular Ca^{2+} levels and ERK1/2 activity were not involved.

Cross-talk between integrins and P2Y receptors has been shown to enable receptor coupling to specific G proteins involved in cell migration. For example, the $P2Y_2$ receptor contains a consensus integrin-binding motif (RGD) in its first extracellular loop that facilitates interaction with $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins [39]. This interaction is necessary for G_o but not G_q-mediated Ca^{2+} release, and, recently, it was demonstrated that α_V integrin expression is required for the $P2Y_2$ receptor to induce cell migration by enabling activation of G_o, Rac, and Vav2, a RacGEF [6]. Further studies are needed to determine how integrins control G protein activation by the $P2Y_2$ receptor. It is known, however, that integrins regulate many processes, including proliferation, differentiation, apoptosis, and cell migration. Integrins belong to a family of cell adhesion molecules that bind to and are activated by extracellular matrix proteins. Upon activation, many types of integrins will cluster together and recruit a host of cytoskeletal and cytoplasmic proteins into specialized adhesive structures called focal adhesions. These focal adhesion complexes not only serve as a physical link between the extracellular and intracellular matrix, but also are important sites of signal transduction for integrins and many other types of receptors [52].

Receptor cross-talk can produce a synergistic or more than additive change in the level of second messengers. It has been shown that co-activation of G_i-coupled receptors and $G_{q/11}$-coupled P2Y receptors induces a synergistic rise in intracellular IP_3 and Ca^{2+} levels [51, 83, 109, 139] and release of AA [41, 109], as compared to activation of $G_{q/11}$-coupled P2Y receptors alone. Also, pre-stimulation of $P2Y_1$ or $P2Y_2$ receptors in HEK293 cells enables the subsequent activation of G_i-coupled CXCR2 chemokine receptors or G_s-coupled β-adrenergic receptors expressed in the same cells to dramatically increase intracellular Ca^{2+} levels, a response not normally regulated by these G_i- and G_s-coupled receptors [139]. $G_{q/11}$-coupled P2Y receptors stimulate PLC activity that generates DAG, an endogenous activator of PKC, which, in turn, can activate phospholipase A2 and generate AA from membrane phospholipids. Although activation of G_i-coupled m2, α_2, and D_2 receptors in CHO-K1 cells does not induce AA release, these receptor activities can enhance the effect of P2Y receptor activation on AA generation [41].

GPCRs have been shown to form homodimers as well as heterodimers, either with subtypes of the same receptor family or with receptors of other GPCR families. Currently, the only example of dimerization involving P2Y receptors that has been well-characterized is the interaction between the $P2Y_1$ and adenosine A_1 receptors [150]. In HEK293 cells, coexpressed rat $P2Y_1$ and adenosine A_1 receptors could be co-immunoprecipitated from whole-cell membrane lysates, indicating that they form a heteromeric complex. Coexpression of the $P2Y_1$ receptor did not alter surface expression of the A_1 receptor, but it did inhibit the binding of radiolabeled A_1 agonists and antagonists in membrane preparations. This change in ligand binding affinity was not seen with a mixture of membranes from cells expressing each receptor individually. Additionally, the binding of an A_1 agonist was displaced by the $P2Y_1$ agonist ADPβS or the $P2Y_1$ antagonist MRS2179 in cotransfected cells, but not in cells expressing the A_1 receptor only. These data indicate formation of a heteromeric receptor complex that is capable of demonstrating cross-talk at the level of ligand binding.

Furthermore, the G_i-coupled A_1 receptor regulates the inhibition of adenylyl cyclase, thereby decreasing intracellular levels of cAMP, whereas, $G_{q/11}$-coupled $P2Y_1$ receptors do not couple directly to adenylyl cyclase. However, when co-expressed with A1 receptors, $P2Y_1$ receptor activation by ADPβS can inhibit cAMP production, an effect that is prevented by the A_1 receptor antagonist DPCPX, or the G_i protein inhibitor PTX. These results demonstrate that interactions between these two different GPCRs confer signal transduction properties that are not mediated by the individual GPCR subtype alone. This cross-talk between A1 and $P2Y_1$ receptors appears to be unidirectional, as A1 receptor activation by CPA does not potentiate the effects of $P2Y_1$ receptor activation on inositol phosphate generation [150].

Signal transduction mediated by the $P2Y_2$ receptor subtype has been extensively investigated (Fig. 2). $P2Y_2$ receptors due to their $G\alpha_{q/11}$-dependent coupling to PLCβ

increase the IP_3-mediated release of Ca^{2+} from intracellular stores and the DAG-induced activation of PKC that, in turn, increases the synthesis and/or release of AA, prostaglandins, and nitric oxide (NO) [78, 100, 101, 138, 146–148]. In primary murine astrocytes, $P2Y_2$ receptors mediate the activation of both calcium-dependent and calcium-independent PKCs and ERK1/2, leading to the activation of cytosolic phospholipase A_2 and the production of AA [148], the precursor of eicosanoids, prostaglandins, and leukotrienes [7]. Activation of $P2Y_2$ receptors in isolated UTP- or ATP-perfused rat hearts induces pronounced vasodilatation [53], consistent with the role of $P2Y_2$ receptors in relaxation of smooth muscle through the endothelium-dependent release of NO and the prostaglandin prostacyclin [78, 100, 101]. $P2Y_2$ receptor expression in smooth muscle cells is upregulated by agents that mediate inflammation, including interleukin (IL)-1β, interferon-γ, and TNF-α [61, 62], and $P2Y_2$ receptor upregulation has been shown to promote nucleotide-induced activation of PKC, cyclooxygenase (regulates prostaglandin synthesis) and MAPKs [68, 111, 127].

Studies have indicated that $P2Y_2$ receptor-mediated ERK1/2 activation in rat-1 fibroblasts and PC12 cells is dependent on transactivation of EGFR via a Src/Pyk2-dependent pathway [116]. In addition to ERK1/2, $P2Y_2$ receptor activation can induce the phosphorylation of the stress-activated kinases JNK and p38 [49]. $P2Y_2$ receptor activation also induces p38- and ERK1/2-dependent phosphorylation of the cAMP responsive element binding (CREB) protein and upregulation of genes that regulate cell survival in human astrocytoma cells (i.e., Bcl-2 and Bcl-xL) and genes that regulate neurite outgrowth in PC-12 cells [22]. Human neutrophil $P2Y_2$ receptors have been shown to regulate neutrophil degranulation induced by fibrinogen, independent of AA metabolites [84], and $P2Y_2$ receptors have been suggested to play a role in the wound-healing process [19, 54, 55].

Activation of $P2Y_2$ receptors also causes proliferation and/or migration of human epidermal keratinocytes, lung

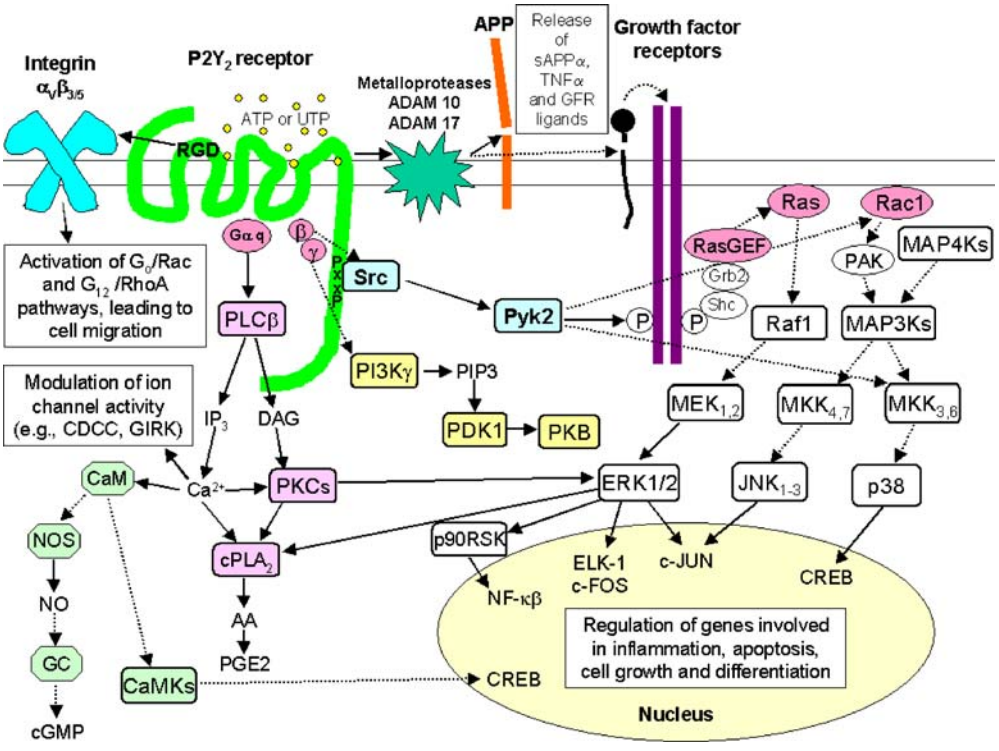

Fig. 2 Intracellular signaling pathways activated by the $P2Y_2$ receptor. Responses to GPCR activation in different cell types vary, partially due to the cell type specific expression of effector proteins and to cross-talk occurring between various signaling pathways [75]. This diagram represents a comprehensive account of signaling events mediated by the $P2Y_2$ receptor. *Arrows with solid lines* indicate established responses mediated by the $P2Y_2$ receptor, whereas, *dashed lines* indicate established responses for other receptor signaling systems that have not yet been elucidated for $P2Y_2$ receptors. *AA* arachidonic acid, *ADAM* a disintegrin and metalloproteinase, *APP* amyloid precursor protein, *CaM* calmodulin, *CaMK* calmodulin-dependent protein kinase, *CDCC* calcium-dependent chloride channel, *cGMP* cyclic GMP, *$cPLA_2$* cytosolic phospholipase A2, *CREB* cAMP response element-binding protein, *DAG* diacylglycerol, *ELK-1* ETS-domain transcription factor, *ERK* extracellular signal-regulated protein kinase, *GC* guanylyl cyclase, *GIRK* G protein-activated inward rectifier, *GFR* growth factor receptor, *Grb2* growth factor receptor bound protein 2, *IP_3* inositol-1,4,5-triphosphate, *JNK* c-Jun N-terminal kinase, *MAP4K* MAPK kinase kinase kinase, *MAP3K* MAPK kinase kinase, *MKK* MAPK kinase, *MEK* mitogen/extracellular signal protein kinase, *NO* nitric oxide, *NOS* nitric oxide synthase, *p38* mitogen-activated protein-serine kinase p38, *p90RSK* p90 ribosomal S6 kinases, *PAK* p21-activated serine kinase, *PGE2* prostaglandin E2, *PI3K* phosphatidylinositol 3-kinase, *PIP_3* phosphatidyl-3,4,5-triphosphate, *PKB* protein kinase B, *PKC* protein kinase C, *PLCγ* phospholipase Cγ, *PXXP* proline-rich Src homology 3 domain, *Pyk2* proline-rich tyrosine kinase, *RGD* Arg-Gly-Asp integrin binding domain, *sAPPα* α-secretase-dependent amyloid precursor protein, *Rho, Rac, Ras, Raf* monomeric (small) G proteins, *Shc* Src-homology collagen protein, *TNF-α* tumor necrosis factor-α

epithelial tumor cells, glioma cells, smooth muscle cells, endothelial cells, and primary rat astrocytes [6, 55, 107, 111, 125, 136, 142]. As mentioned above, $P2Y_2$ receptor-mediated astrocyte migration requires $\alpha_v\beta_{3/5}$ integrins to activate G_o and to initiate G_o-mediated signaling events leading to cell migration [6, 136]. In smooth muscle cells, $P2Y_2$ receptor activation induces cell cycle progression from G1 to S and M phases [80, 86]. HeLa cell proliferation in response to $P2Y_2$ receptor activation is associated with the PI3K- and ERK1/2-dependent expression of the early response protein *c*-fos [90]. Consistent with a role for $P2Y_2$ receptors in cell proliferation, $P2Y_2$ receptor mRNA expression is downregulated during cell differentiation [82].

$P2Y_2$ receptors are upregulated in response to tissue injury or stress. For example, functional $P2Y_2$ receptor activity and mRNA levels increase in several models of salivary gland stress or disease [3, 108, 126] and in blood vessels after balloon angioplasty [110] or stress induced by insertion of a vascular collar [111]. In endothelial cells, activation of the $P2Y_2$ receptor increases the expression of vascular endothelial cell adhesion molecule-1 (VCAM-1) [113], a response that promotes monocyte adhesion to endothelial cells and, thus, vascular inflammation. Upregulation of VCAM-1 is dependent on $P2Y_2$ receptor-mediated transactivation of VEGF receptor-2 (KDR/Flk-1), and can be inhibited by deletion of the SH3 binding motifs from the intracellular C-terminal domain of $P2Y_2$ receptor, demonstrating a novel mechanism whereby $P2Y_2$ receptors can cause inflammatory responses in blood vessels [112].

$P2Y_2$ receptor activation increases Cl^- secretion and inhibits Na^+ absorption in epithelial cells, which has potential relevance to the treatment of cystic fibrosis, a disease that is caused by mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR), a major epithelial anion channel [24, 25, 64, 98]. The synthetic $P2Y_2$ receptor agonist INS37217 has been employed to promote chloride and water secretion in tracheal epithelium, to increase ciliary beat frequency and mucin release in human airway epithelium [149] and to stimulate subretinal fluid reabsorption in a rabbit model of retinal detachment [85].

A $P2Y_2$ receptor knock-out mouse has been produced that is defective in nucleotide-stimulated ion secretion in airway epithelial cells, confirming a physiological role for the $P2Y_2$ receptor in the regulation of epithelial transmembrane ion transport [33]. In addition, $P2Y_2$ receptors have been shown to inhibit bone formation by osteoblasts [59], and N-type calcium currents in neurons [14, 44]. $P2Y_2$ receptors also can induce α-secretase-dependent amyloid precursor protein processing in astrocytoma cells, suggesting a neuroprotective role [20]. Collectively, studies with the $P2Y_2$ receptor and its signaling pathways have elucidated potential pharmacological targets in atherosclerosis, inflammation, cystic fibrosis, osteoporosis, cancer, and neurodegenerative disorders.

Summary

The realization that ion channels and GPCRs participate in multi-protein complexes with signaling molecules and other receptors, as well as with cytoskeletal, tight junctional, and extracellular matrix proteins should revolutionize our understanding of the classical signaling pathways coupled to P2 receptors. These connections not only influence intracellular signaling but also allow an individual P2 receptor subtype to respond differently, depending on the repertoire of proteins expressed in a particular cell type.

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