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P2 Receptors in Health and Disease

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Introduction

P2 receptors are expressed in a broad range of tissues and play important roles in many physiological and pathological processes. The main activating ligands *in vivo* are extracellular ATP, ADP, UTP, and UDP. Sources of these extracellular nucleotides include degranulating macrophages, excitatory neurons, injured cells, and cells undergoing mechanical or oxidative stress. P2 receptors are subdivided into two distinct categories, the metabotropic G protein-coupled (P2Y) receptors and the ionotropic ligand-gated channel (P2X) receptors. To date, 7 P2X and 8 P2Y receptor subtypes have been identified by molecular cloning and pharmacological characterization, leading to a greater understanding of the diversity of signal transduction pathways coupled to P2X and P2Y receptors in different systems. In addition, responses coupled to the activation of different P2X and P2Y receptors

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Abbreviations: 2MeSADP, 2-methylthio-ADP; 2MeSATP, 2-methylthio-ATP; ADP, adenosine 5'-diphosphate; AMP, adenosine 5'-monophosphate; ATP, adenosine 5'-triphosphate; ATPγS, adenosine 5'-triphosphate gamma thiol; BzATP, 2'- and 3'-O-(4-benzoylbenzoyl)-ATP; α,β-meATP, α,β-methylene-adenosine 5'-triphosphate; $[Ca^{2+}]_i$, cytoplasmic calcium ion concentration; cAMP, cyclic AMP; CFTR, cystic fibrosis transmembrane conductance regulator; CNS, central nervous system; COX-2, cyclooxygenase-2; DAG, diacylglycerol; EGF, epidermal growth factor; EMP-2, epithelial membrane protein-2; ERK, extracellular-signal regulated kinase; GPCR, G protein-coupled receptor; HEK293, human embryonic kidney 293 cells; IL-1β, interleukin-1β; iNOS, inducible nitric oxide synthase; IP_3, inositol-1,4,5-trisphosphate; MAPK, mitogen-activated protein kinase; MoDC, monocyte-derived dendritic cells; NF-κB, nuclear factor κB; NO, nitric oxide; oATP, oxidized ATP; PDEC, pancreatic duct epithelial cells; PKC, protein kinase C; PPADS, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid; PAGE, polyacrylamide gel electrophoresis; TNFα, tumour necrosis factor-α; UDP, uridine 5'-diphosphate; UTP, uridine 5'-triphosphate; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells; YO-PRO, quinolinium,4-[(3-methyl-2-(3H)-benzoxazolylidene)methyl]-1-[3-(triethylammonio)-propyl]di-iodide.

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have now been associated with a variety of pathological and physiological processes, including inflammation, platelet aggregation, bone resorption, pain, neurodegeneration, neurotransmission, wound healing, vascular tone, carcinogenesis, apoptosis, and angiogenesis. These findings suggest that P2X and P2Y receptors may be promising pharmacological targets in the treatment of diseases, including atherosclerosis, cystic fibrosis, Alzheimer's disease, Parkinson's disease, and autoimmune diseases.

P2X receptors

P2X receptors are ATP-gated ion channels that were first cloned from excitable cells and then found in various tissues and cell types, such as neurons, glial cells, smooth muscle cells, endothelia, and epithelia (Dubyak and el-Moatassim, 1993; North, 1996). These receptors mediate fast membrane depolarization and increased transmembrane permeability of cations (Na^+, K^+, and Ca^{2+}). Since the isolation of the cDNA for the first P2X receptor in 1994, seven P2X receptor subunits have been cloned and named, $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$, and $P2X_7$ (see *Table 9.1*).

MOLECULAR STRUCTURE OF P2X RECEPTORS

Hydrophobicity plots of all P2X receptors predict a structure for a transmembrane protein with two membrane-spanning domains and N- and C-termini projecting into the cytoplasm (see *Figure 9.1*). The predicted P2X receptor proteins have 397 to 471 amino acids (except for the $P2X_7$ receptor that is comprised of 595 amino acids) with their two hydrophobic transmembrane domains separated by a large hydrophilic extracellular loop. The extracellular domain of the seven P2X receptor subtypes is enriched in conserved amino acid residues, including 10 cysteines, 13 glycines, and 2–6 asparagines that may serve as N-linked glycosylation sites (Brake *et al.*, 1994; Valera *et al.*, 1994; North, 1996; Ralevic and Burnstock, 1998). Disulfide bonds in the extracellular loops of P2X receptors likely form structural constraints necessary for the coupling of ATP binding to ion channel activity (Newbolt *et al.*, 1998; Jiang *et al.*, 2000; Ennion and Evans, 2002). Individual subunits of P2X receptors do not form ion channels as monomers but rather as oligomers (both homo- and hetero-multimers). Six homo-multimers ($P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_7$) and three hetero-multimers ($P2X_2/P2X_3$, $P2X_4/P2X_6$, $P2X_1/P2X_5$) have been functionally characterized (reviewed by North and Surprenant, 2000; see *Table 9.1*). The exception is the $P2X_7$ receptor, which only forms homo-multimers (Torres *et al.*, 1999). Although the subunit stoichiometry of P2X oligomers is unclear, $P2X_1/P2X_3$ hetero-multimers expressed in oocytes have been isolated as trimers under non-denaturing conditions (Nicke *et al.*, 1998). It was reported also that $P2X_7$ receptors exist as monomers in brain glia and/or astrocytes (Kim *et al.*, 2001b). Further research is necessary to define the functional monomeric and oligomeric forms of P2X receptors.

$P2X_7$ receptors are distinct among P2X receptors in that they can form both cationic channels and non-selective pores that allow the passage of small, normally membrane-impermeable molecules (Erb *et al.*, 1990; Surprenant *et al.*, 1996; Virginio *et al.*, 1999), including nucleotides, quinolinium,4-[(3-methyl-2-(3H)-benzoxazolylidene)methyl]-1-[3-(triethylammonio)propyl]di-iodide (YO-PRO), and

Table 9.1. Cloned P2 receptor subtypes and pharmacology

Receptor subtypes	Species	GenBank Accession Number	Agonists	Antagonists
$P2X_1$	human[2,4], rat[1], mouse[3]	NM_002558	ATP, α,β-meATP > BzATP[1,2]	suramin, PPADS, oATP, NF023, TNP-ATP[1,2,5,6]
$P2X_2$	rat[7-9]	NM_053565 (*rattus norvegicus*)	ATP > BzATP > α,β-meATP[7]	suramin, PPADS, oATP, NF023, TNP-ATP[1,5,6]
$P2X_3$	human[10], rat[12,13]	NM_002559	ATP, α,β-meATP[12-14]	suramin, PPADS, NF023, TNP-ATP[12,13]
$P2X_4$	human[11], rat[15-19]	NM_002560, NM_175567, NM_175568	ATP > α,β-meATP > BzATP[11,15-19]	NF023, TNP-ATP[6,11,15-17,19]
$P2X_5$	rat[20]	NM_080780 (*rattus norvegicus*)	ATP > α,β-meATP > BzATP[14,20,21]	suramin, PPADS[22]
$P2X_6$	human (predicted), rat[15,20]	XM_496501	–	–
$P2X_7$	human[21], rat[22], mouse[24,25]	NM_002562, NM_177427	BzATP > ATP > α,β-meATP[22,23,25,28]	suramin, PPADS, oATP, TNP-ATP[5,22,25,26,30]
$P2X_2/P2X_3$	–	–	ATP, α,β-meATP[13,31]	NF023, PPADS, TNP-ATP[6,13]
$P2X_1/P2X_5$	–	–	ATP > α,β-meATP[27, 29]	TNP-ATP[27, 29]
$P2X_4/P2X_6$	–	–	ATP, α,β-meATP[29]	no selective antagonist[29]
$P2Y_1$	human, rat, mouse[32,33]	NM_002563	2MeSADP > ADP > ATP[32,34]	A3P5PS, PPADS, suramin[35-37]
$P2Y_2$	human, rat, mouse, porcine[38-41]	NM_176072, NM_002564,NM_176071	UTP > = ATP > 5BrUTP[42]	suramin[43]
$P2Y_4$	human, rat, mouse[44-47]	NM_002565	UTP > ATP[47-48]	PPADS[43]
$P2Y_6$	human, rat, mouse[45,49-50]	NM_176797, NM_176876	UDP > UTP > ADP[50]	PPADS, suramin[51]
$P2Y_{11}$	human[52]	NM_002566	ATPγS > BzATP > ATP[53]	suramin[53]
$P2Y_{12}$	human, rat, mouse[54,55]	NM_022788, NM_176876	2MeSATP = 2MeSADP > ADP[55]	suramin[63], AR-C69931MX[56]
$P2Y_{13}$	human, rat, mouse[57-59]	NM_176894	2MeSATP = 2MeSADP > ADP > ATP[59-60]	AR-C69931MX, PPADS, suramin[60]
$P2Y_{14}$	human, rat, mouse[61-63]	NM_014879	UDP-glucose > UDP-galactose[62]	not defined

Valera *et al.*, 1994[1], 1995[2], 1996[3]; Longhurst *et al.*, 1996[4]; Evans *et al.*, 1995[5]; Soto *et al.*, 1999[6]; Brake *et al.*, 1994[7]; Brandle *et al.*, 1997[8]; Simon *et al.*, 1997[9]; Garcia-Guzman *et al.*, 1997a[10], 1997b[11]; Chen *et al.*, 1995[12]; Lewis *et al.*, 1995[13]; Garcia-Guzman *et al.*, 1996[14]; Soto *et al.*, 1996[15]; Séguéla *et al.*, 1996[16]; Bo *et al.*, 1995[17]; Buell *et al.*, 1996[18]; Wang *et al.*, 1996[19]; Collo *et al.*, 1996[20]; Lê *et al.*, 1997[21]; Surprenant, 1996[22]; Rassendren *et al.*, 1997[23]; Nuttle *et al.*, 1993[24]; Chessell *et al.*,1998a[25], 1998b[26]; Lê *et al.*, 1999[27]; Pizzo *et al.*, 1991[28]; Lê *et al.*, 1998[29]; Virginio *et al.*, 1997[30]; Khakh *et al.*, 1999[31]; Ayyanathan *et al.*, 1996[32]; Tokuyama *et al.*, 1995[33]; Palmer *et al.*, 1998[34]; Boyer *et al.*, 1996[35]; Lambrecht *et al.*, 1992[36]; Lambrecht, 1996[37]; Chen *et al.*, 1996[38]; Lustig *et al.*, 1993[39]; Parr *et al.*, 1994[40]; Shen *et al.*, 2004[41]; Lazarowski *et al.*, 1995[42]; Charlton *et al.*, 1996[43]; Bogdanov *et al.*, 1998[44]; Lazarowski *et al.*, 2001[45]; Nguyen *et al.*, 1995[46]; Communi *et al.*, 1995[47], 1996a[48]; Chang *et al.*, 1995[49]; Communi *et al.*, 1996b[50]; Robaye *et al.*, 1997[51]; Communi *et al.*, 1997[52], 1999[53]; Hollopeter *et al.*, 2001[54]; Zhang *et al.*, 2001[55]; Ingall *et al.*, 1999[56]; Communi *et al.*, 2001[57]; Fumagalli *et al.*, 2004[58]; Zhang *et al.*, 2002[59]; Marteau *et al.*, 2003[60]; Abbracchio *et al.*, 2003[61]; Chambers *et al.*, 2000[62]; Freeman *et al.*, 2001[63].

ethidium$^+$. Repeated or prolonged activation of $P2X_7$ receptors promotes the transition from ion channel to non-selective pore and it was proposed that this transition is the result of successive oligomerization of $P2X_7$ receptor subunits (Cockcroft and Gomperts, 1979; di Virgilio, 1995; Surprenant, 1996; Collo *et al.*, 1997; di Virgilio *et al.*, 1998). $P2X_7$ receptors have an intracellular C-terminal tail of 235 amino acids that is at least 120 amino acids longer than any other P2X receptor subunit. It is believed that the long C-terminal tail of the $P2X_7$ receptor regulates receptor function and cellular localization (Denlinger *et al.*, 2003; Wiley *et al.*, 2003; le Stunff *et al.*, 2004). Large-scale proteomic analysis has identified a number of putative protein:protein interactions involving the C-terminal tail of $P2X_7$ receptors (Kim *et al.*, 2001a). Importantly, $P2X_7$ receptors interact via their C-terminus with epithelial membrane protein-2 (EMP-2), which is involved in membrane blebbing (Wilson *et al.*, 2002). Studies have identified $P2X_7$ receptor polymorphisms of the C-terminal tail that impair receptor function and trafficking (Gu *et al.*, 2001; Wiley *et al.*, 2003; le Stunff *et al.*, 2004; Sluyter *et al.*, 2004). Furthermore, C-terminal truncation of the $P2X_7$ receptor (595 residues) at amino acid Phe^{581} completely abolished pore formation measured by ethidium$^+$ uptake, while truncation at amino acid Pro^{582} did not affect ethidium$^+$ uptake significantly. Therefore, $P2X_7$ receptor function is dependent on the integrity of the distal C-terminal tail. Truncations at positions between amino acids 551 and 581 inhibited the cell surface expression of the $P2X_7$ receptor (Smart *et al.*, 2003).

PHARMACOLOGICAL PROFILES OF P2X RECEPTORS

The pharmacological characteristics of individual P2X receptor subtypes were determined in studies using recombinant P2X receptors expressed in heterologous cell lines. Activation of P2X receptors can be detected by monitoring increases in the intracellular calcium ion concentration, $[Ca^{2+}]_i$, due to calcium uptake, which usually occurs within a few milliseconds of ATP stimulation.

The kinetics and sensitivity of P2X receptors to nucleotide agonists and antagonists varies among P2X receptor subtypes (see *Table 9.1*). $P2X_1$ receptors are activated by α,β-meATP or ATP and rapidly desensitize, whereas $P2X_2$ receptors are not activated by α,β-meATP and do not undergo agonist-induced desensitization (Brake *et al.*, 1994; Valera *et al.*, 1994). $P2X_3$ receptors can be activated by ATP and α,β-meATP (less than 1 μM), similar to $P2X_1$ receptors. However, Ap3A (diadenosine triphosphate) preferentially activates $P2X_3$ rather than $P2X_1$ (Wildman *et al.*, 1999). $P2X_3$ can be discriminated from $P2X_1$ also by using a selective inhibitor NF023 that is 40 times more potent at $P2X_1$ (Soto *et al.*, 1999). $P2X_4$ and $P2X_5$ receptors are activated by ATP, but are usually insensitive to α,β-meATP (Bo *et al.*, 1995; Séguéla *et al.*, 1996). In contrast, $P2X_6$ receptors were unable to form currents in response to ATP when expressed in oocytes (Collo *et al.*, 1996). $P2X_7$ receptors are activated by 2'- and 3'-O-(4-benzoylbenzoyl)-ATP (BzATP) more effectively than ATP (Gonzalez *et al.*, 1989; Erb *et al.*, 1990; el-Moatassim and Dubyak, 1992; McMillian *et al.*, 1993; Nuttle *et al.*, 1993; Soltoff *et al.*, 1993). Oxidized ATP is a potent irreversible antagonist of $P2X_7$ receptors when it is preincubated with cells for 1 or 2 hours; however, it blocks $P2X_1$ and $P2X_2$ receptor activities at similar concentrations (Evans *et al.*, 1995).

It was first assumed that the homo-multimers mentioned above are the main functional forms of P2X receptors *in vivo*. In addition to measuring the expression of different P2X receptor subtypes by reverse transcriptase PCR and immunoblotting with specific P2X receptor antibodies, the identity of endogenously expressed P2X receptors was usually determined by comparison to the properties of recombinant P2X receptors expressed in heterologous cell systems. However, it is not always straightforward to compare the properties of recombinant P2X receptors with those of endogenous receptors, in part due to the co-expression of multiple P2 receptor subtypes in some tissue and cell types. For example, $P2X_{1,2,3,4}$ receptors are expressed in rat sensory neurons. The P2X receptors in sensory neurons mediate ATP-induced currents that also are activated by α,β-meATP but do not desensitize, which is distinctive from the properties of any homomeric recombinant P2X receptor subtype (Lewis *et al.*, 1995). However, this pharmacological profile is mimicked by co-expression of $P2X_2$ and $P2X_3$ receptor cDNAs in HEK293 cells (Lewis *et al.*, 1995). It was found that these unique properties were due to the assembly of $P2X_2/P2X_3$ receptor hetero-multimers (Lewis *et al.*, 1995), which was supported by the immunofluorescence co-localization and co-immunoprecipitation of these two receptor subunits (Torres *et al.*, 1999).

The discovery of $P2X_2/P2X_3$ receptor hetero-multimers in neurons provided significant insight into the composition of P2X receptors in other tissues and cell types. Although multiple P2X receptor subtypes are expressed in many cell types, not all subunit combinations are tolerated. To date, three hetero-multimers of P2X receptors, $P2X_2/P2X_3$, $P2X_1/P2X_5$, and $P2X_4/P2X_6$, have been functionally characterized in heterologous expression systems (Lewis *et al.*, 1995; Radford *et al.*, 1997; Lê *et al.*, 1998, 1999; Torres *et al.*, 1998; Khakh *et al.*, 1999), although other combinations have been detected by co-immunoprecipitation analysis, with the exception of $P2X_7$ receptors (Torres *et al.*, 1999), $P2X_2/P2X_3$ hetero-multimers having distinct pharmacology as compared to $P2X_2$ and $P2X_3$ homo-multimers. $P2X_2$ homo-multimers are not activated by α,β-meATP, whereas $P2X_3$ homo-multimers are desensitized by α,β-meATP within milliseconds. However, $P2X_2/P2X_3$ hetero-multimers are activated by α,β-meATP and do not desensitize rapidly (Bianchi *et al.*, 1999). Similarly, $P2X_1$ receptors are rapidly desensitized by α,β-meATP, and $P2X_5$ receptors are not activated by α,β-meATP, but $P2X_1/P2X_5$ hetero-multimers undergo sustained activation by α,β-meATP (Torres *et al.*, 1998, Lê *et al.*, 1999). $P2X_4/P2X_6$ hetero-multimers have been reported in oocytes where α,β-meATP is about 12% more potent than ATP, measured as maximal current elicited. For $P2X_4$ homo-multimers, this value is about 7% (Lê *et al.*, 1998).

SIGNALLING PATHWAYS ACTIVATED BY P2X RECEPTORS

P2X receptors are relatively low-affinity receptors that are activated by high concentrations of ATP (EC_{50} for ATP: 1–100 μM). Activation of P2X receptors by extracellular ATP or other agonists opens an ion channel that can mediate the influx of sodium as well as calcium, and the efflux of potassium, leading to depolarization of the cell membrane (Bean, 1992; Dubyak and el-Moatassim, 1993; North, 1996). Membrane depolarization subsequently activates voltage-gated calcium channels. The modulation of intracellular cation homeostasis activates various intracellular

signalling molecules, including MAPK, ERK1/2, and p38. Although the detailed signalling mechanisms for P2X receptors have not been established, it seems that these receptors are involved in a broad range of functions. For example, stimulation of $P2X_7$ receptors can activate p38 and Rho kinase, both of which are required for $P2X_7$ receptor-mediated actin cytoskeletal rearrangements and membrane blebbing in HEK293 cells, responses that were insensitive to the MEK1/2 inhibitor, U0126 (Morelli *et al.*, 2003; Verhoef *et al.*, 2003; Pfeiffer *et al.*, 2004).

Activation of P2X receptor signalling pathways can have proinflammatory effects in the central nervous system through stimulation of the synthesis and secretion of cytokines. For example, activation of $P2X_7$ receptors in BAC1 murine macrophages causes IL-1β release, independent of RhoA activation and formation of membrane blebs (Verhoef *et al.*, 2003). In macrophages treated with lipopolysaccharides, activation of $P2X_7$ receptors results in the elevation of the levels of mRNA for inducible NOS (iNOS) and tumour necrosis factor-α (TNF-α) (Tonetti *et al.*, 1994, 1995; Hu *et al.*, 1998). Sequence analysis of the iNOS gene promoter has identified an element that potentially binds NF-κB, a transcription factor known to be activated via $P2X_7$ receptors in a variety of systems (Budagian *et al.*, 2003; Aga *et al.*, 2004; Korcok *et al.*, 2004). Other transcription factors that could be activated by P2X receptor signalling pathways include AP-1 and Egr-1 (Gerasimovskaya *et al.*, 2002; Budagian *et al.*, 2003). Further work is required to define the specific P2X receptor signal transduction pathways that lead to transcriptional activation in different cells and tissues.

PHYSIOLOGICAL RESPONSES TO P2X RECEPTOR ACTIVATION

In the nervous system, nucleotides are released from neurons in response to excitation or injury. It is well documented that various P2X receptor subtypes are expressed in the brain and the peripheral nervous system (Evans *et al.*, 1992; Harms *et al.*, 1992; Silinsky *et al.*, 1992; Tschöpl *et al.*, 1992; Chen *et al.*, 1994; Hiruma and Bourque, 1995; Nabekura *et al.*, 1995). These receptors may carry out important functions in the nervous system, such as mediating fast synaptic transmission in dorsal horn neurons (Bardoni *et al.*, 1997), causing release of proinflammatory factors (Majid *et al.*, 1992, 1993; Nakazawa and Inoue, 1992), and generation of pain signals (reviewed by Ralevic and Burnstock, 1998). Studies have suggested that P2X receptors may play a role in the pathophysiology of Parkinson's disease, Alzheimer's disease, and multiple sclerosis, although the relationship of specific P2X receptor subtypes has not been firmly established. In a transgenic mouse model of Alzheimer's disease (Tg2576), $P2X_7$ receptor protein is up-regulated in microglial cells and astrocytes around amyloid plaques, as compared to age-matched controls (Parvathenani *et al.*, 2003). Activation of $P2X_7$ receptors by ATP or BzATP in primary rat microglial cells causes release of reactive oxygen species, such as superoxide (O_2^-). This effect was suppressed by PPADS and oxidized ATP, $P2X_7$ receptor antagonists, and by inhibitors of p38 and phosphatidylinositol 3-kinase, but not by inhibitors of MAPK (Parvathenani *et al.*, 2003). Activation of $P2X_7$ receptors in BV-2 microgial cells enhanced the effect of interferon-γ on the up-regulation of iNOS and production of NO (Gendron *et al.*, 2003), suggesting that $P2X_7$ receptors can promote glial cell activation associated with the pathology of

neurodegenerative diseases. The C-terminal tail of $P2X_7$ receptors may play an important role in neuronal apoptosis. Substitution of proline 451 with leucine in the C-terminal domain of the $P2X_7$ receptor prevented ATP-induced cell death (le Stunff *et al.*, 2004). R578E/K579E double mutations in the C-terminal region also caused defective $P2X_7$ receptor transport to the cell membrane in HEK293 cells (Denlinger *et al.*, 2003). Similarly, I568N polymorphism in the C-terminus resulted in decreased $P2X_7$ receptor expression on the cell surface (Wiley *et al.*, 2003).

There is evidence also that P2X receptors play key roles in cardiovascular function. P2X receptors ($P2X_4$ and $P2X_5$) are abundantly expressed in vascular endothelial cells of the umbilical vein, aorta, and pulmonary artery (Schwiebert *et al.*, 2002). In vascular smooth muscle cells, P2X receptors ($P2X_1$ and $P2X_4$) mediate vasoconstriction resulting from extracellular ATP release from nerves and aggregating platelets, which serves to counteract the effects of P2Y receptor-mediated vasodilation (see below; Phillips *et al.*, 1998). In human umbilical vein endothelial cells (HUVEC), P2X receptors mediate the release of ATP (Schwiebert *et al.*, 2002). Furthermore, treatment of HUVEC with $P2X_4$ antisense oligonucleotide significantly reduced ATP-induced calcium influx (Yamamoto *et al.*, 2000), suggesting that the $P2X_4$ receptor is the main functional P2X receptor in these cells. In chronic shear stress, $P2X_4$ receptor mRNA is down-regulated by a mechanism involving transcription factor Sp1, providing a means to prevent the deleterious effects of prolonged $P2X_4$ receptor activation (Yamamoto *et al.*, 2000, 2003).

In addition to vascular cells, P2X receptors ($P2X_1$ and $P2X_7$) also regulate functions of blood-derived cells. Activation of $P2X_7$ receptors on monocytes/macrophages augments release of proinflammatory cytokines, such as LPS-dependent production of NO and expression of iNOS (Tonetti *et al.*, 1994, 1995; Denlinger *et al.*, 1996; Hu *et al.*, 1998) that cause immune cell activation, an early step in atherosclerotic lesion development. The P2X receptor antagonists, oATP and PPADS, have been shown to inhibit the effects of ATP on NO release, and the activation of NF-κB and MAPK in macrophages. However, oATP and PPADS had no effect on UTP-stimulated MAPK activation (Hu *et al.*, 1998). It has been reported that $P2X_7$ receptor activation can affect the shedding of cell adhesion molecules, such as L-selectin and CD23 on B lymphocytes, a characteristic of chronic lymphocytic leukaemia (Gu *et al.*, 1998).

P2Y receptors

P2Y receptors for purine and pyrimidine nucleotides belong to the superfamily of G protein-coupled receptors (GPCR) comprised of seven transmembrane domains (see *Figure 9.1*). Currently, eight mammalian P2Y receptor subtypes have been cloned and characterized in heterologous expression systems: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors (see *Table 9.1*). Human P2Y receptors consist of 328($P2Y_6$) to 377($P2Y_2$) amino acids with glycosylation sites in the extracellular N-terminal domains.

MOLECULAR STRUCTURE

Although all P2Y receptors share a seven transmembrane domain tertiary structure, the amino acid sequences, pharmacological profiles, and signal transduction

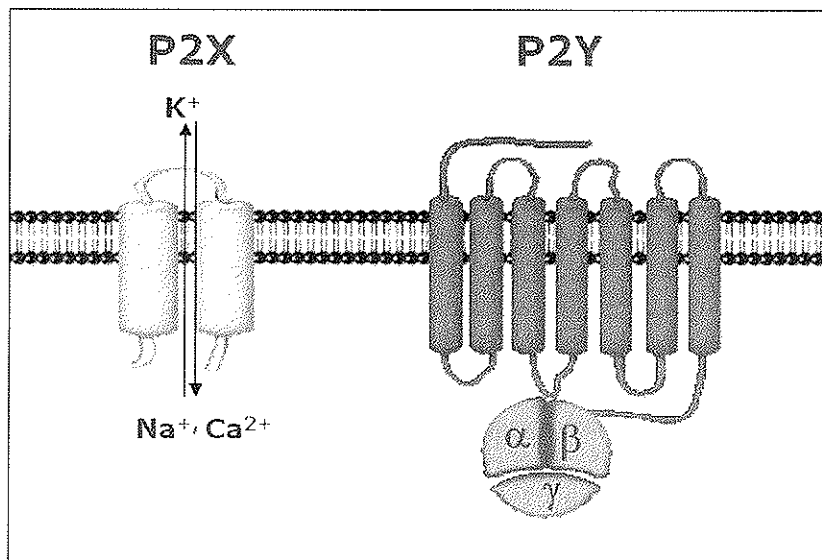

Figure 9.1. Structure of P2X and P2Y receptors.

pathways of these G protein-coupled receptors vary significantly. Using rhodopsin as a template, models of the $P2Y_1$ and $P2Y_2$ receptor ligand binding sites have been produced that indicate the presence of several positively charged amino acids in highly conserved transmembrane regions 3, 6, and 7 that could act as counterions for the binding of negatively charged nucleotide ligands (Erb *et al.*, 1995; van Rhee *et al.*, 1995). Consistent with this conclusion, site-mutagenesis of the $P2Y_2$ receptor to replace positively charged amino acid residues in transmembrane regions 6 and 7 with neutral amino acids produced receptors with dramatically altered agonist potencies, as compared to the wild-type $P2Y_2$ receptor (Erb *et al.*, 1995). These positively charged amino acids are found in domains 6 and 7 of all P2Y receptors cloned to date. Beyond these similarities in structure, P2Y receptors vary significantly in pharmacological and physiological properties and, therefore, each receptor subtype will be considered separately.

$P2Y_1$ RECEPTORS

$P2Y_1$ receptor cDNA was first cloned from chick brain (Webb *et al.*, 1993), and the human $P2Y_1$ receptor, when expressed in Jurkat cells, is activated selectively by adenine nucleotides with a rank order of agonist potency of 2MeSADP > ADP (Leon *et al.*, 1997). ATP also acts as a potent agonist in $P2Y_1$ receptor-transfected COS-7 cells (Janssens *et al.*, 1996). The human $P2Y_1$ receptor gene has been localized to region q25 of chromosome 3 (Ayyanathan *et al.*, 1996). $P2Y_1$ receptors are coupled to G protein activation, which results in IP_3 generation and the mobilization of intracellular Ca^{2+} from intracellular storage sites (Simon *et al.*, 1995).

$P2Y_1$ receptors are widely distributed in brain, placenta, prostate, heart, skeletal muscle, neuronal tissue, and platelets, but barely detectable in human liver, kidney,

stomach, bone marrow, and lymphocytes (Moore *et al.*, 2001). $P2Y_1$ receptor and $P2Y_1$-like receptor activation in smooth muscle cells and vascular endothelium regulates smooth muscle relaxation and the release of endothelium-derived relaxing factors (EDRF) and endothelium-derived hyperpolarizing factors (EDHF) (Ralevic and Burnstock, 1998).

In cultured aortic smooth muscle cells, a $P2Y_1$-like receptor has been found to mediate ATP-induced mitogenic effects via PKC, Raf-1, and MAPK, indicating a role in vascular smooth muscle cell proliferation (Yu *et al.*, 1996). Phenotypic changes in rat aortic smooth muscle cells are accompanied by $P2Y_1$ and $P2Y_2$ receptor mRNA up-regulation, whereas mRNA for the contractile $P2X_1$ receptor is down-regulated (Seye *et al.*, 1997; Erlinge *et al.*, 1998). There are abundant sources for extracellular ATP in the blood vessel wall (Yamamoto *et al.*, 2003) and ATP has been shown to stimulate mitogenesis in rat, porcine, and bovine vascular smooth muscle cells (VSMC) and in cells from human coronary arteries, aorta, and subcutaneous arteries and veins, suggesting a possible pathophysiological role of $P2Y_1$ and $P2Y_2$ receptors (see below) in the stimulation of smooth muscle cell growth associated with the development of neointimal formation in atherosclerosis (Seye *et al.*, 1997, 2002; Erlinge, 1998). $P2Y_1$ receptors expressed on platelets regulate platelet shape and aggregation by mediating ADP-induced increases in $[Ca^{2+}]_i$ (Kunapuli *et al.*, 2003a). A $P2Y_1$ receptor-null mouse model was used to demonstrate that the lack of $P2Y_1$ receptor expression leads to prolonged bleeding times and resistance to ADP-induced thromboembolism (Fabre *et al.*, 1999; Leon *et al.*, 1999), consistent with the role of this receptor in the regulation of platelet aggregation.

Both $P2Y_1$ and $P2Y_2$ receptor expression is necessary to produce maximal Ca^{2+} wave propagation in spinal astrocytes, indicating a role for co-expression of these two receptors in gliotransmission in the CNS (Gallagher and Salter, 2003). The $P2Y_1$ receptor is also expressed on osteoclasts and osteoblasts, whereupon activation causes increases in osteoclastic bone resorption, suggesting a potential role for $P2Y_1$ receptors in mediating inflammatory bone loss (Hoebertz *et al.*, 2001).

$P2Y_2$ RECEPTORS

$P2Y_2$ receptors have been cloned from human, rat, mouse, and porcine cells or tissues (Lustig *et al.*, 1993; Parr *et al.*, 1994; Chen *et al.*, 1996; Shen *et al.*, 2004). ATP and UTP are equipotent agonists of the $P2Y_2$ receptor, whereas ADP and UDP are less effective (Lazarowski *et al.*, 1995). The human $P2Y_2$ receptor gene has been mapped to q13.5–14.1 on chromosome 11 (Dasari *et al.*, 1996). $P2Y_2$ receptors couple to both $G_{i/o}$ and G_q proteins to mediate the activation of phospholipase Cβ, leading to the production of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), second messengers for calcium release from intracellular storage sites and protein kinase C (PKC) activation, respectively (Weisman *et al.*, 1998). $P2Y_2$ receptor mRNA is expressed in human skeletal muscle, heart, brain, spleen, lymphocytes, macrophages, bone marrow, and lung, with lower levels expressed in liver, stomach, and pancreas (Moore *et al.*, 2001).

Nucleotides are released during vascular injury from activated platelets and damaged cells (Schwiebert and Kishore, 2001; Oike *et al.*, 2004). Released ATP and UTP can activate $P2Y_2$ receptors in human neutrophils to induce fibrinogen-

dependent degranulation independent of arachidonic acid metabolites (Meshki *et al.*, 2004). Activation of $P2Y_2$ receptors in human keratinocytes, along with other P2 receptors, has been postulated to play a role in the wound healing process (Burrell *et al.*, 2003; Greig *et al.*, 2003a,b).

Functional analysis revealed that activation of $P2Y_2$ receptors in isolated UTP- or ATP-perfused rat hearts induced pronounced vasodilation of blood vessels (Godecke *et al.*, 1996), consistent with earlier studies indicating that $P2Y_2$ receptor activation induces relaxation of smooth muscle through the endothelium-dependent release of NO and prostacyclin (Lustig *et al.*, 1992; Pearson *et al.*, 1992a,b). Up-regulation of $P2Y_2$ receptors occurs in response to balloon angioplasty (Seye *et al.*, 1997) and causes intimal hyperplasia in collared rabbit carotid arteries due to the stimulation of smooth muscle cell proliferation and migration (Seye *et al.*, 2002), suggesting an important role for $P2Y_2$ receptors in the initiation of atherosclerotic lesion formation. Up-regulation of the $P2Y_2$ receptor was also observed in short-term culture (3 h to 6 days) of normal rat submandibular gland cells (Turner *et al.*, 1997), and in activated mouse thymocytes (Koshiba *et al.*, 1997). $P2Y_2$ receptor activation also increases the synthesis of pro-inflammatory mediators, such as prostaglandin E2 in rat astrocytes (Xu *et al.*, 2003) and the expression of vascular cell adhesion molecule-1 (VCAM-1) that mediates the adherence of monocytes to vascular endothelium (Seye *et al.*, 2003), leading to their penetration into the blood vessel wall to promote arterial inflammation associated with cardiovascular disease (Seye *et al.*, 2002). Similarly, $P2Y_2$ receptor-mediated release of arachidonic acid and activation of type 2 cyclooxygenase (COX-2) in astrocytes mediate inflammation and reactive astrogliosis associated with neurodegenerative diseases, including Alzheimer's disease (Brambilla *et al.*, 1999; Brambilla and Abbracchio, 2001).

Recent studies have revealed that a Src homology-3 (SH3) binding domain in the C-terminal tail of the $P2Y_2$ receptor promotes the nucleotide-induced association of Src with the $P2Y_2$ receptor, leading to the transactivation of growth factor receptors, such as the EGF and VEGF receptors (Liu *et al.*, 2004; see *Figure 9.2*), the pathway that causes up-regulation of VCAM-1 in UTP-treated endothelial cells (Seye *et al.*, 2004). Deletion of the SH3 binding domain inhibits $P2Y_2$ receptor-mediated transactivation of the EGF and VEGF receptors and nucleotide-induced up-regulation of VCAM-1 (Liu *et al.*, 2004; Seye *et al.*, 2004). In addition, an integrin-binding domain (Arg-Gly-Asp) in the first extracellular loop of the $P2Y_2$ receptor mediates its association with $\alpha_v\beta_3$ integrins, enabling the coupling of the $P2Y_2$ receptor to G_o but not G_q proteins (Erb *et al.*, 2001; see *Figure 9.2*). This novel $P2Y_2$ receptor/$\alpha_v\beta_3$ integrin interaction was found to regulate UTP-induced actin cytoskeletal rearrangements and increased cell migration (unpublished data; see *Figure 9.2*). Since leukocyte infiltration and migration are key processes involved in atherosclerosis, these findings suggest that $P2Y_2$ receptors represent a novel target for reducing arterial inflammation associated with cardiovascular disease.

Activation of the $P2Y_2$ receptor also increases epithelial cell Cl^- secretion and inhibits Na^+ absorption, an effect that has been explored for its potential therapeutic application in the treatment of cystic fibrosis, a dehydrating disease characterized by defective Cl^- secretion due to genetic mutations in CFTR in airway epithelium (Clarke and Boucher, 1992; Parr *et al.*, 1994; Kellerman *et al.*, 2002). The role of the $P2Y_2$ receptor in regulating ion secretion in airway epithelial cells has been

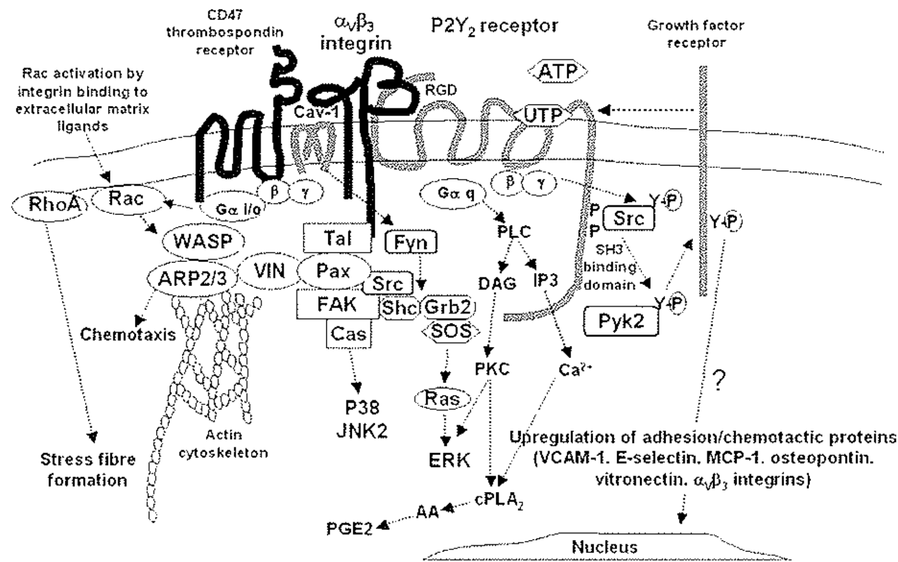

Figure 9.2. Protein–protein interactions and signalling pathways linked to the P2Y$_2$ receptor. The P2Y$_2$ receptor contains an RGD integrin-binding domain that interacts with integrins ($\alpha_V\beta_3$ and $\alpha_V\beta_5$) that are associated with the integrin-associated thrombospondin receptor (CD47). The RGD domain is necessary for P2Y$_2$ receptor-mediated chemotaxis, actin stress fibre formation, and coupling to signalling pathways involving Go and RhoA GTPases. Two consensus SH3 binding sites (PXXP) in the C-terminal tail of the P2Y$_2$ receptor bind directly to Src upon receptor activation and mediate Src-dependent co-localization and transactivation of growth factor receptors that are required for upregulation of cell adhesion/chemotactic proteins (e.g. VCAM-1). Key abbreviations used in this diagram are defined in the list of abbreviations.

confirmed in P2Y$_2$ receptor knock-out mice (Cressman *et al.*, 1999). A selective P2Y$_2$ receptor agonist, INS27217, also has been shown to increase chloride and water secretion in tracheal epithelium, cilia beat frequency, and mucin release from human airway epithelium, suggesting a potential treatment for dry eye disease and cystic fibrosis (Yerxa *et al.*, 2002). In contrast to these therapeutic effects, P2Y$_2$ receptors in osteoblasts function as negative modulators of bone remodelling by blocking bone formation by osteoblasts (Hoebertz *et al.*, 2002). Thus, depending upon the tissue, P2Y$_2$ receptor expression and activation can promote deleterious (e.g. endothelium; smooth muscle; glia; bone) or beneficial (e.g. epithelium) responses. Clearly, more research is required to fully understand the role of P2Y$_2$ receptors in diseased tissues.

$P2Y_4$ RECEPTORS

$P2Y_4$ receptors have been cloned from human, rat, and mouse (Communi *et al.*, 1995; Nguyen *et al.*, 1995; Bogdanov *et al.*, 1998; Lazarowski *et al.*, 2001). The human $P2Y_4$ receptor is selective for UTP in human cells and is not activated by nucleotide diphosphates. In some cases, the human $P2Y_4$ receptor is partially activated by ATP (Herold *et al.*, 2004). The human $P2Y_4$ receptor gene has been localized to region q13 of the X chromosome (Nguyen *et al.*, 1995). Functional studies indicate that the human $P2Y_4$ receptor can couple to G_i during the initial stage of activation (within 30 sec) and to $G_{q/11}$ at later stages (Communi *et al.*, 1996a). Two serine residues located in the C-terminal tail of $P2Y_4$ receptors play a role in receptor phosphorylation, desensitization, and internalization (Brinson and Harden, 2001). High levels of $P2Y_4$ receptor mRNA have been detected in human intestine, pituitary, and brain, with lower levels expressed in liver and bone marrow (Moore *et al.*, 2001).

$P2Y_4$ receptors play physiological roles in the cardiovascular system and in epithelial ion secretion. $P2Y_4$ receptors have been shown to regulate mitogenesis in rat aortic smooth muscle cells (Harper *et al.*, 1998). In addition, $P2Y_4$ receptors mediate UTP-induced contraction of rabbit basilar arteries (Miyagi and Zhang, 2004). Recently, it was shown that $P2Y_4$ receptor knock-out mice lost the ability of UTP and ATP to induce jejunal epithelium Cl^- secretion (Robaye *et al.*, 2003), suggesting a physiological role similar to that of the $P2Y_2$ receptor. Potassium secretion by vestibular dark cell epithelium is also controlled by apical $P2Y_4$ receptors (Marcus and Scofield, 2001). In the nervous system, it was found that $P2Y_4$ receptors are coupled to N-type calcium channels, as well as M-type potassium channels in rat sympathetic neurons (Filippov *et al.*, 2003).

$P2Y_6$ RECEPTORS

The UDP-selective $P2Y_6$ receptor has been cloned from human, rat, and mouse (Chang *et al.*, 1995; Communi *et al.*, 1996b; Lazarowski *et al.*, 2001). The human $P2Y_6$ receptor gene has been localized to chromosomal region 11q13.5 (Somers *et al.*, 1997). In 1321N1 astrocytoma cells stably expressing the recombinant $P2Y_6$ receptor, UDP-induced IP_3 formation was pertussis toxin-insensitive, suggesting that the $P2Y_6$ receptor couples to $G_{q/11}$ and not to $G_{i/o}$ proteins (Robaye *et al.*, 1997). Activation of the $P2Y_6$ receptor also leads to accumulation of cAMP, possibly via UDP-mediated generation of prostaglandins (Kottgen *et al.*, 2003).

$P2Y_6$ receptor mRNA was detected in human spleen, placenta, kidney, lung, intestine, adipose tissue, bone, heart, and brain (Moore *et al.*, 2001). In the vasculature, $P2Y_6$ receptors are present in human cerebral arteries and play a prominent role in their contraction, suggesting a new therapeutic target for the treatment of cerebral vasospasm (Malmsjo *et al.*, 2003). In rat aortic smooth muscle cells, UDP stimulates mitogenesis via $P2Y_6$ receptors and UDP-induced cell growth is coordinately regulated by phospholipase C, a tyrosine kinase pathway, eicosanoids, and protein kinase A, responses that are related to the development of cardiovascular disease (Hou *et al.*, 2002). In addition, activation of transfected $P2Y_6$ receptors in 1321N1 astrocytoma cells protected the cells from tumour necrosis factor-induced apoptosis via the protein kinase C pathway, but not from death induced by oxidative or

chemical ischaemia (Kim *et al.*, 2003). The mechanism by which $P2Y_6$ receptors deliver an anti-apoptotic signal while promoting mitogenesis may involve the ability of $P2Y_6$ receptors to transactivate growth factor receptors that are able to inhibit apoptosis and promote survival.

Basolateral $P2Y_6$ receptors in colonic epithelial cells have been found to mediate NaCl secretion and activation of CFTR via a cAMP-dependent pathway (Kottgen *et al.*, 2003). These results suggest that activation of $P2Y_6$ receptors, along with $P2Y_2$ receptors, may be beneficial in the treatment of cystic fibrosis.

$P2Y_{11}$ RECEPTORS

$P2Y_{11}$ receptors have been cloned from human placental cDNA and mapped to chromosome region 19p13.2 (Communi *et al.*, 1997). ATP and ATPγS can activate $P2Y_{11}$ receptors expressed in 1321N1 astrocytoma cells or in CHO-K1 cells, leading to PLC-dependent accumulation of IP_3, and an increase in cAMP levels (Qi *et al.*, 2001), with less potency for promoting cAMP than IP_3 accumulation. UTP has been shown recently to be an equipotent agonist to ATP for $P2Y_{11}$ receptor-stimulated increases in $[Ca^{2+}]_i$ in 1321N1 cells expressing human $P2Y_{11}$ receptors (White *et al.*, 2003).

$P2Y_{11}$ receptor mRNA has been detected in human brain, spleen, lymphocytes, intestine, and other tissues (Moore *et al.*, 2001). ATP released at sites of inflammation has been suggested to induce maturation of human monocyte-derived dendritic cells (MoDC) via $P2Y_{11}$ receptor-mediated activation of adenylyl cyclase and protein kinase A (Wilkin *et al.*, 2001). $P2Y_{11}$ receptor activation also inhibited MoDC migration (Schnurr *et al.*, 2003). Correlated with MoDC maturation, $P2Y_{11}$ receptor expression is down-regulated in mature MoDC, whereupon ATP becomes a less effective inhibitor of cell migration (Schnurr *et al.*, 2003). These findings suggest a new $P2Y_{11}$ receptor-based strategy to increase the migration of local dendritic cells from sites of inflammation to activate T cells and trigger an inflammatory response. Together with $P2Y_2$ receptors, $P2Y_{11}$ receptors have been found to activate Ca^{2+}-dependent Cl^- channels in pancreatic duct epithelial cells (PDEC) (Nguyen *et al.*, 2001).

$P2Y_{12}$ RECEPTORS

$P2Y_{12}$ receptors have been cloned from human, rat, and mouse, and the human $P2Y_{12}$ receptor gene has been mapped to region 24–25 of chromosome 3 (Hollopeter *et al.*, 2001; Zhang *et al.*, 2001). $P2Y_{12}$ receptor activation by ADP inhibits forskolin-stimulated adenylyl cyclase activity, indicating coupling of the receptor to G_i protein (Zhang *et al.*, 2001). $P2Y_{12}$ receptor mRNA was detected in human brain, spinal cord, and platelets (Zhang *et al.*, 2001). $P2Y_{12}$ receptor activation occurs in response to ADP secreted from platelets stimulated with agonists, such as thrombin, thromboxane, and collagen (Kunapuli *et al.*, 2003b). ADP is an agonist of both $P2Y_1$ and $P2Y_{12}$ receptors, which play coordinate roles in the regulation of platelet aggregation (Kunapuli *et al.*, 2003b).

A frame-shift mutation in the $P2Y_{12}$ receptor gene may be responsible for a mild bleeding disorder (Hollopeter *et al.*, 2001). The role for $P2Y_{12}$ receptors in arterial

thrombogenesis was confirmed in $P2Y_{12}$ receptor knock-out mice that exhibited impaired platelet adhesion to von Willebrand factor (vWF), deceased platelet activation indicated by less fibrinogen-binding, delayed thrombus growth, and unstable thrombi in injured arteries (Andre *et al.*, 2003). Antagonists for $P2Y_{12}$ receptors have been developed as clinical antithrombotic agents. One family of these antagonists, the AR-C compounds, analogues of ATP, have been used extensively to identify physiological functions related to $P2Y_{12}$ receptor activities. For example, rabbits treated with AR-C69931MX, a $P2Y_{12}$ and $P2Y_{13}$ receptor antagonist, showed decreased embolus and thrombus formation but not thrombus stability (van Gestel *et al.*, 2003). In a canine coronary electrolytic injury thrombosis model, AR-C69931MX inhibited ADP-mediated platelet aggregation and thrombosis, resulting in prolonged reperfusion time and decreased reocclusion (Wang *et al.*, 2003).

$P2Y_{13}$ RECEPTORS

$P2Y_{13}$ receptors for ATP and ADP have been cloned from human, rat, and mouse (Communi *et al.*, 2001; Zhang *et al.*, 2002; Fumagalli *et al.*, 2004). The human $P2Y_{13}$ receptor has been localized to region 24 of chromosome 3 (Communi *et al.*, 2001). The human $P2Y_{13}$ receptor is coupled to G_i protein and has an agonist potency profile similar to that of the $P2Y_{12}$ receptor (Zhang *et al.*, 2002). In 1321N1 cells, the presence of overexpressed $G\alpha_{16}$ protein was related to $P2Y_{13}$ receptor-mediated activation of phospholipase C, leading to IP_3 accumulation (Communi *et al.*, 2001). However, this $P2Y_{13}$ receptor-mediated phosphoinositide pathway was inhibited by pertussis toxin, suggesting a synergism between $G\alpha_{16}$ and G_i proteins (Communi *et al.*, 2001).

Human $P2Y_{13}$ receptor mRNA is expressed in spleen and brain, with lower expression levels in placenta, lung, liver, spinal cord, thymus, small intestine, uterus, stomach, testis, fetal brain, and adrenal gland (Communi *et al.*, 2001). Recent studies indicate that activation of $P2Y_{13}$ receptors, and probably $P2Y_{12}$ receptors, promotes inhibition of noradrenaline release in the prostatic portion of rat vas deferens (Queiroz *et al.*, 2003), possibly via the inhibition of Ca^{2+} channels (Wirkner *et al.*, 2004). ADP-induced activation of ERK1 is inhibited by the $P2Y_{12}$ and $P2Y_{13}$ receptor antagonist AR-C69931MX in human monocyte-derived dendritic cells, resulting in the adenine nucleotide-induced inhibition of inflammatory cytokine production (Marteau *et al.*, 2003).

$P2Y_{14}$ RECEPTORS

$P2Y_{14}$ receptors have been cloned from human, rat, and mouse, and the human receptor gene has been mapped to region 24–25 of chromosome 3, the same region as the $P2Y_{12}$ receptor (Chambers *et al.*, 2000; Freeman *et al.*, 2001; Abbracchio *et al.*, 2003). UDP-glucose, and some other UDP-sugars, are potent agonists of $P2Y_{14}$ receptors. $P2Y_{14}$ receptors expressed in HEK-293 cells couple to $G_{i/o}$, but not to G_s or $G_{q/11}$ proteins (Moore *et al.*, 2003). Human $P2Y_{14}$ receptor mRNA is widely distributed with predominant expression in placenta, adipose tissue, stomach, and intestine, and lower expression levels in brain, spleen, lung, and heart (Chambers *et al.*, 2000).

$P2Y_{14}$ receptors mediate bone marrow haematopoietic stem cell chemotaxis induced by UDP-sugars derived from the bone marrow microenvironment, suggesting a role for $P2Y_{14}$ receptor activity in responses to injury, in which stem cells provide the basic level of repair (Lee *et al.*, 2003). UDP-glucose has been shown to increase $[Ca^{2+}]_i$ in immature human monocyte-derived dendritic cells but not in mature dendritic cells, suggesting a role for this receptor in dendritic cell maturation (Skelton *et al.*, 2003). The $P2Y_{14}$ receptor is also expressed in the rat brain where its mRNA can be up-regulated by immunologic challenge with lipopolysaccharide, suggesting an important role for $P2Y_{14}$ receptors in UDP-glucose induced responses relating to neuroimmune function (Moore *et al.*, 2003).

Summary

In the past ten years since the discovery and cloning of members of the P2 receptor family, rapid progress has been made in the field regarding the function and pharmacology of different P2 receptor subtypes. This research resulted in identifying these receptors as important players in the pathology of atherosclerosis, cystic fibrosis, neurodegenerative and autoimmune diseases, among other disorders. The signalling mechanisms whereby P2 receptors mediate pathogenesis are not clear in most cases. Future studies in this field will focus on the integration of signalling pathways coupled to P2 receptors and the generation of specific agonists/antagonists for each receptor subtype to provide strategies for the treatment of a variety of diseases.

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