Chapter 21

Purinergic receptors

Brian F. King and Geoffrey Burnstock

21.1 Introduction

The term *purinergic receptor* (or *purinoceptor*) was first introduced to describe classes of membrane receptors that, when activated by either neurally released ATP (*P2 purinoceptor*) or its breakdown product adenosine (*P1 purinoceptor*), mediated relaxation of gut smooth muscle (Burnstock 1972, 1978). P2 purinoceptors were further divided into five broad phenotypes (P2X, P2Y, P2Z, P2U, and P2T) according to pharmacological profile and tissue distribution (Burnstock and Kennedy 1985; Gordon 1986; O'Connor *et al.* 1991; Dubyak 1991). Thereafter, they were reorganized into families of metabotropic ATP receptors (P2Y, P2U, and P2T) and ionotropic ATP receptors (P2X and P2Z) (Dubyak and El-Moatassim 1993), later redefined as extended P2Y and P2X families (Abbracchio and Burnstock 1994).

In the early 1990s, cDNAs were isolated for three heptahelical proteins—called $P2Y_1$, $P2Y_2$, and $P2Y_3$—with structural similarities to the rhodopsin GPCR template. At first, these three GPCRs were believed to correspond to the P2Y, P2U, and P2T receptors. However, the complexity of the P2Y receptor family was underestimated. At least 15, possibly 16, heptahelical proteins have been associated with the P2Y receptor family (King *et al.* 2001, see Table 21.1). Multiple expression of P2Y receptors is considered the norm in all tissues (Ralevic and Burnstock 1998) and mixtures of P2 purinoceptors have been reported in central neurones (Chessell *et al.* 1997) and glia (King *et al.* 1996). The situation is compounded by P2Y protein dimerization to generate receptor assemblies with subtly distinct pharmacological properties from their constituent components (Filippov *et al.* 2000). Also, the range of naturally occurring nucleotides capable of stimulating P2Y receptors has extended beyond ATP and its immediate breakdown products (Jacobson *et al.* 2000).

21.2 Molecular characterization

Cloning and heterologous expression of each of the three first P2Y receptors in oocytes, resulted in increased Ca^{2+}-mobilization following activation by ATP (Lustig *et al.* 1993; Webb *et al.* 1993; Barnard *et al.* 1994). These three GPCRs were assimilated into the $P2Y_{1–n}$ receptor family, as opposed to the cloned ionotropic ATP receptors that now form the $P2X_{1–n}$ receptor family (Abbracchio and Burnstock 1994). The $P2Y_1$ receptor was cloned from chick brain and its agonist potency profile (2-MeSATP $>$ ATP $\geq$ ADP) approximated the native P2Y phenotype (Webb *et al.* 1993). The $P2Y_2$ receptor was cloned from murine neuroblastoma (NG108-15) cells and its agonist profile (ATP $\approx$ UTP $>$ ATPγS) approximated the native P2U phenotype (Lustig *et al.* 1993). The $P2Y_3$ receptor was cloned from chick brain and

its preference for ADP over ATP appeared to approximate the P2T phenotype (Barnard *et al.* 1994). Thus, a seemingly perfect correspondence was established between these recombinant P2Y receptors and subtypes of metabotropic ATP receptors in mammalian tissues. However, since that time, many other GPCRs associated with the P2Y receptor family have been identified and now include $P2Y_{1–12}$, turkey p2y, skate p2y, and the human UDP-glucose receptor. A dinucleotide receptor, $P2Y_{Ap4A}$ or its older name P2D, is anticipated but has not yet been cloned (Fredholm *et al.* 1997). The fifteen cloned receptor proteins are 328–532 amino acids in length and represent some of the shortest GPCRs found in mammalian cells. They possess seven hydrophobic regions, forming the transmembrane spanning regions TM1-VII, which lie between an extracellular N-terminus (21–51 residues in length) and a cytosolic C-terminus (16–217 residues in length) possessing multiple consensus motifs for phosphorylation by intracellular kinases. Alignment of the protein sequences for the TM1-VII region reveals 17–62 per cent identity (35–80 per cent similarity).

The human $P2Y_1$ receptor is found on chromosome 3q25 (Ayyanathan *et al.* 1996). $P2Y_1$ orthologues (89–98 per cent identical over TMI-VII) have been cloned from brain tissue of cow, chick, and turkey. Transcripts for mouse and rat orthologues are also present in nervous tissue. Recently, a $P2Y_1$-deficient mouse model has been generated with a phenotype showing decreased platelet aggregation and increased bleeding (Fabre *et al.* 1999; Leon *et al.* 1999). $P2Y_2$ orthologues (94 per cent identical) have been cloned from canine, mouse, and rat tissues. Rat $P2Y_2$ was cloned from a pituitary cDNA library (Chen *et al.* 1996), although $P2Y_2$ transcripts are more commonly associated with epithelial cell lines than brain derived cell lines. It should be borne in mind that the anterior pituitary is derived from epithelial tissue, and this may explain why $P2Y_2$ was found in the rat pituitary. The human $P2Y_2$ receptor gene located on chromosome 11q13.5–14.1, lies adjacent to the $P2Y_6$ gene at 11q13.3–13.5 (Pidlaoan *et al.* 1997). In addition, a $P2Y_2$-deficient mouse model has been generated which suggests this receptor is critical in regulating airway epithelial ion transport but not ion transport in non-respiratory epithelia (Homolya *et al.* 1999). The human $P2Y_2$ receptor protein shows polymorphism at position 334 (an arginine–cysteine transition), due to a replication error at nucleotide 1000 (thymine for cytosine), although this mutation does not significantly alter functionality (Janssens *et al.* 1999).

The human $P2Y_4$ gene encodes a receptor stimulated by UTP and antagonized by ATP (Kennedy *et al.* 2000), and is located in region q13 of chromosome X (Nguyen *et al.* 1995). By contrast, both ATP and UTP stimulate the mouse and rat $P2Y_4$ receptors (Bogdanov *et al.* 1998; Webb *et al.* 1998; Lazarowski *et al.* 2001). Rat $P2Y_4$ was cloned from brain (Webb *et al.* 1998) and heart (Bogdanov *et al.* 1998). The human $P2Y_4$ protein is 89 per cent identical to mouse and rat orthologues, whilst the two rodent $P2Y_4$ receptors are 95 per cent identical (over TMI-VII). The structural basis for the divergent pharmacology between human and rodent $P2Y_4$ receptors has not been elucidated. Agonist-activated human $P2Y_4$ receptors are rapidly internalized by a phosphorylation process involving serine-333 and serine-334 on the C-terminus (Brinson and Harden 2001).

The open reading frame for the $P2Y_5$ receptor is contained in intron 17 of the human retinoblastoma susceptibility gene, a tumour-suppressing gene located on chromosome 13q14.12–14.2 (Herzog *et al.* 1996). Opinions vary on whether the $P2Y_5$ receptor is a functional or orphan GPCR. The chick orthologue (83 per cent identical over TM1-VII) avidly binds [^{35}S]-dATPαS (Webb *et al.* 1996*b*), but turkey $P2Y_5$ does not respond functionally to either dATPαS or other nucleotides (Li *et al.* 1997). The human $P2Y_5$ receptor, when expressed in oocytes, is weakly stimulated by ATP and slowly activates the PLC_β/Ca^{2+}

Table 21.1 Purinergic receptors

	$P2Y_1$	**$P2Y_2$**	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{11}$**	**$P2Y_{12}$**
Alternative names	P_{2Y}	P_{2U} or nucleotide	Uridine nucleotide or pyrimidinoceptor	Uridine nucleotide	—	$P2Y_{ADP}$, P_{2T}
Structural Information (Accession no.)	h 373 aa (U42029) r 373 aa (U22830) m 373 aa (U22829)	h 377 aa (U07225) r 374 aa (U09402) m 373 aa (L14751)	h 365 aa (U40223) r 361 aa (Y14705) m 361aa (AF277752)	h 328 aa (X97058) r 328 aa (Q63371) m 328aa (AF298899)	h 371 aa (AF030335)	h 342 aa (AF313449) r 341 aa (AF313450)
Gene location (human)	3q25	11q13.5–14.1	Xq13	11q13.3–13.5	19p31	3q24–25
Selective agonists	2-MeSADP, 2-MeSATP, ADPβS	Up_4U or UTPγS	Up_3U or UTP	UDPβS or UDP	AR-C67085	2-MeSADP,
Selective antagonist	MRS 2179 MRS 2279	—	—	—	—	C1330-7 AR-C67085
Radioligands	[^{35}S]-dATPαS	—	—	—	—	[^{3}H]-ADP
G protein coupling	G_q/G_{11}	$G_q/G_{11}G_i$	$G_q/G_{11}G_i$	$G_q/G_{11}G_i$	G_q/G_s	G_i/G_o

Expression profile	Cerebral cortex, cerebellum, hippocampus, caudate nucleus, putamen, corpus callosum, midbrain, subthalamic nuclei, DRG, astrocytes, placenta, heart, muscle, prostate,intestine, platelets, bone, pancreas	Pituitary, heart, blood vessels, lung, kidney, placenta, skeletal muscle, endocrine tissue, bone, astrocytes	Brain, placenta, heart, epithelium, pancreas, smooth muscle, kidney, intestine, liver	Kidney, lung, spleen, thymus, placenta, heart, bone, smooth muscle, epithelium, intestine	Brain, spleen, placenta, intestine, smooth muscle, granulocytes	Brain, platelets
Physiological function	Astrogliosis, transmitter release, bone resorption	Astrogliosis, transmitter release, mucus escalation, airway hydration	Glial growth, epithelial growth, SM growth	Lymphocytic maturation	Granulocyte differentiation	Haemostasis
Knockout phenotype	Resistance to thromboembolism, defective platelet aggregation	Reduced nucleotide induced airway epithelial ion transport	—	—	—	Bleeding disorder, defective platelet aggregation
Disease relevance	Stroke, epilepsy, neurodegeneration, thromboembolism, cardiovascular disease, osteoporosis	Cystic fibrosis, chronic bronchitis, dry eye	Proliferative disorders of the kidney epithelia	Inflammatory bowel disease	Neutropenia, leukaemia	Thromboembolism, cardiovascular disease

pathway (King and Townsend-Nicholson 2000). Human $P2Y_5$ seems to couple inefficiently to G_q isoforms and its role in nucleotide signalling is unclear.

The chick $P2Y_3$ receptor is proposed to be an orthologue of mammalian $P2Y_6$ receptors—on the basis of similarity in protein sequence (78 per cent over TMI-VII) and a shared pharmacological profile (Li *et al.* 1998). The latter receptor gene is located on human chromosome 11q13.3–13.5, lying adjacent to the $P2Y_2$ gene (q13.5–14.1; Pidlaoan *et al.* 1997; Somers *et al.* 1997). Three forms of human $P2Y_6$ cDNA were found by RT-PCR amplification (Maier *et al.* 1997). Two forms contain the coding region for $P2Y_6$ but possess different 5′-untranslated regions, probably the consequence of alternative gene splicing. The third cDNA appears to be a pseudogene and shows a frame shift in the coding region that cannot be translated into protein. $P2Y_6$ transcripts for coding cDNAs were found in a series of brain-derived cell lines (Maier *et al.* 1997). $P2Y_6$ orthologues (88 per cent identical over TMI-VII) have been cloned from rat and mouse.

The human $P2Y_{11}$ gene is located on chromosome 19p31 (Suarez-Heurta *et al.* 2000), where an intron interrupts the coding sequence at the 5′-end and separates the first 3 codons from the remainder of the coding region (Communi *et al.* 1997). The $P2Y_{11}$ and SSF1 genes on chromosome 19 can undergo intergenic splicing to create a fusion protein (Communi *et al.* 2001). This SSF1-$P2Y_{11}$ protein is functionally indistinguishable from human $P2Y_{11}$ itself. A canine $P2Y_{11}$ receptor has been cloned from a kidney epithelial cell line (Zambon *et al.* 2001).

$P2Y_{12}$ is structurally related to the UDP-glucose receptor (49 per cent identical) but distinct from $P2Y_1$ (22 per cent identical) and is located on chromosome 3q24–25, adjacent to genes for the UDP-glucose and $P2Y_1$ receptors (Hollopeter *et al.* 2001). Both $P2Y_1$ and $P2Y_{12}$ are present in human blood platelets where they play a key role in haemostasis. A mutated $P2Y_{12}$ receptor arises through a two base pair deletion at the coding region for residue 240, a transcriptional frame shift and premature truncation of the protein (Hollopeter *et al.* 2001). The truncated $P2Y_{12}$ protein is non-functional and, in one patient, was associated with a mild bleeding disorder. $P2Y_{12}$ transcripts were also found in rat glioma cells and rat brain.

The skate p2y receptor was cloned from liver, but also found in brain, and is 69 per cent similar to human $P2Y_1$ (Dranoff *et al.* 2000). This chordate p2y receptor was claimed to be the most primitive form of the $P2Y_1$ receptor. In contrast, the turkey p2y receptor was cloned from a cDNA library from whole blood, but the physiological role of this receptor is unknown. This avian p2y receptor inhibits cAMP levels via the G_i/AC pathway, as well as stimulating IP_3 production via the G_q/PLC_β pathway (Boyer *et al.* 2000). The receptor is structurally related to the *Xenopus* $p2y_8$ receptor (67 per cent identical, 79 per cent similar over TMI-VII). Both tp2y and $xp2y_8$ receptors are stimulated by most naturally occurring nucleoside triphosphates (ATP, CTP, GTP, ITP, UTP) and couple strongly to G_q/PLC_β (Bogdanov *et al.* 1997; Boyer *et al.* 2000). Expression of amphibian $xp2y_8$ receptor is confined to the neural plate of developing *Xenopus* embryos and closely related to periods of neurogenesis (Bogdanov *et al.* 1997). $P2Y_9$ (or $P2Y_5$-like) and $P2Y_{10}$ receptors are considered to be orphan GPCRs (Janssens *et al.* 1997; Ralevic and Burnstock 1998). Human $P2Y_7$ receptor was wrongly identified as a nucleotide receptor (Akbar *et al.* 1996). This P2Y-like GPCR, located on chromosome 14q11.2–q12 (Owman *et al.* 1996), has been reclassified as a chemoattractant leucotriene B_4 (LTB_4) receptor (Yokomizo *et al.* 1997). The P2Y-like protein (fb1) cloned from human foetal hippocampus is located on chromosome 2q21 (Blasius *et al.* 1998) and joins an extended family of orphan GPCRs with low sequence homology to functional

P2Y receptors (Marchese *et al.* 1999). Finally, the human UDP-glucose receptor which is probably the most unusual of the known P2Y-like receptors recognizes UDP-glucose and UDP-galactose as its natural ligands, but not UTP or UDP and related mononucleotides (Chambers *et al.* 2000). This receptor is 49 per cent identical to $P2Y_{12}$ and, like the latter, can couple to pertussis toxin sensitive G_i protein (Chambers *et al.* 2000). The physiological role of the UDP-glucose receptor is unknown, but transcripts are spread widely throughout the neuraxis. The UDP-glucose receptor is also structurally related to $P2Y_5$, $P2Y_9$, and $P2Y_{10}$ receptors.

21.3 Cellular and subcellular localization

Presently, only six P2Y receptors $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, and $P2Y_{12}$ are accepted as clearly defined, distinct, nucleotide receptors in the P2Y receptor family. The UDP-glucose receptor fulfils many criteria for acceptance, but the physiological role for UDP-glucose signalling is mainly unexplored. The remainder of this review will concentrate on the six, accepted, P2Y receptors.

The $P2Y_1$ receptor was isolated first from chick brain, in which exceedingly high levels of $P2Y_1$ receptor expression are observed (37 pmol mg^{-1} protein for [^{35}S]-dATPαS binding (K_d, 9 nM); *cf.* 1–2 pmol mg^{-1} for muscimol binding at $GABA_A$) (Webb *et al.* 1993, 1994). Mammalian orthologues were later cloned from corpus callosum, as well as from endothelial cells, insulinoma cells, and placenta. $P2Y_1$-like immunoreactivity is located throughout the human, rat, and bovine neuraxis and concentrated in neuronal cells in cerebral and cerebellar cortex, hippocampus, caudate nucleus, putamen, subthalamic nucleus, and midbrain (Moore *et al.* 2000; Moran-Jimenez and Matute 2000). $P2Y_1$ receptor transcripts are present in rat brain cortical astrocytes (Webb *et al.* 1996*a*) and $P2Y_1$-like immunoreactivity also observed in rat and bovine brain astrocytes (Moran-Jimenez and Matute 2000). [^{35}S]-dATPαS binding was observed throughout the rat neuraxis at putative $P2Y_1$ receptors (Simon *et al.* 1997). The related compound, [^{35}S]-ATPαS, binds with high affinity (K_d, 10.5 nM) to $P2Y_1$-like receptors in rat cortex synaptosomes (Schäfer and Reiser 1999). Outside the brain, $P2Y_1$ transcripts have been found in mammalian placenta, heart, blood vessels, skeletal muscle, pancreas, blood platelets, and leucocytes, prostate, ovary, small and large intestine, and in some large DRG neurones (Ralevic and Burnstock 1998; King *et al.* 2001). $P2Y_1$ transcripts are abundant in developing limb buds, mesonephros, brain, somites, and facial primordia in the chick embryo (Meyer *et al.* 1999). At the subcellular level, a $P2Y_1$-GFP construct expressed in HEK 293 cells was identified in plasmalemma, endoplasmic reticulum, Golgi and microsomal fractions but was absent from nuclear and mitochondrial fractions (Vöhringer *et al.* 2000).

$P2Y_2$ receptors were first isolated from a murine neuroblastoma *x* glioma hybrid cell line (Lustig *et al.* 1993) and later cloned from alveolar cells, bone, epithelial, endothelial, and pituitary cells. $P2Y_2$ receptor transcripts are found in mammalian heart and vasculature, lung, kidney, osteoblasts, placenta, skeletal muscle, and endocrine tissues (King *et al.* 2001). On a functional level, $P2Y_2$-like receptors are found in astrocytes, chromaffin cells, epithelia, endothelia, fibroblasts, glia, hepatocytes, keratinocytes, leucocytes, myocytes, pituitary cells, and tumour cells (Ralevic and Burnstock 1998). $P2Y_2$ receptors tagged with Haemagglutinin A (HA) showed a punctate distribution on the surface of 1321N1 cells and, after agonist activation, $P2Y_2$-HA immunoreactivity was randomly internalized into the cytosolic pool (Sromek and Harden 1998).

Less information on the distribution of the remainder of the P2Y receptors exists. $P2Y_4$ receptors were cloned from human placenta (Communi *et al.* 1995) and later isolated from mammalian brain, epithelial cells, heart, and pancreas. $P2Y_4$ transcripts are also found in astrocytes, epithelial lining of hollow organs, kidney, leucocytes, and vascular smooth muscle (King *et al.* 2001). $P2Y_4$-like receptors were identified functionally in the jejunal lining in $P2Y_2$R-deficient mice (Cressman *et al.* 1999). The $P2Y_6$ receptor was cloned first from rat aortic smooth muscle (Chang *et al.* 1995) and later from human placenta and T-lymphocytes. $P2Y_6$ transcripts are found in mammalian bone, epithelia, heart, kidney, leucocytes, lung, spleen, and thymus (King *et al.* 2001). A $P2Y_6$-like receptor was identified functionally in the epithelial lining of gallbladder and trachea in $P2Y_2$ receptor-deficient mice (Cressman *et al.* 1999) and a $P2Y_6$-like receptor has also been reported in human nasal epithelial cells (Lazarowski *et al.* 1997). The $P2Y_{11}$ receptor was cloned from human placenta and its transcripts are found in human spleen, intestine, and granulocytes (HL-60 cells) (Communi *et al.* 1997). The $P2Y_{11}$ receptor approximates the native P2Y receptor in HL-60 cells (Conigrave *et al.* 1998; Communi *et al.* 1999), and appears to be directly involved in the differentiation of human granulocytes into neutrophils (Communi *et al.* 2000). Transcripts for the canine $P2Y_{11}$ receptor are more widespread and also found in brain (Zambon *et al.* 2001). $P2Y_{12}$ receptors were cloned from rat and human blood platelets and its transcripts are abundant in platelets but also present in brain (Hollopeter *et al.* 2001). $P2Y_{12}$ receptor transcripts were found in rat C6-2B glioma cells. The $P2Y_{12}$ receptor is pharmacologically similar to the human platelet P2T receptor and the endogenous P2Y receptor of C6-2B glioma cells (Boyer *et al.* 1993; Hollopeter *et al.* 2001).

21.4 Pharmacology

The pharmacology of the P2Y receptors is complex and involves a wide range of purine- and pyrimidine-based, mononucleotidic and dinucleotidic, compounds (King *et al.* 2001). Many ligands are naturally occurring, but synthetic nucleotides are now available to test against P2Y receptor subtypes.

21.4.1 Agonists

Human $P2Y_1$ receptors are activated fully by ADP and the naturally occurring dinucleotide, Ap_4A. ATP can act either as a full agonist, partial agonist, or antagonist depending on receptor reserve (King *et al.* 2001). Other mononucleotides (e.g. CTP, GTP, ITP, UTP and their immediate breakdown products) are inactive. The synthetic alkylthio-ATP derivatives are potent agonists (e.g. 2-MeSATP, 2-MeSADP, 2-HT-ATP, PAPET-ATP), as are phosphorothioate ATP derivatives (e.g. ATPγS and ADPβS), while methylene phosphonate-ATP derivatives (α,β-meATP and β,γ-meATP) are inert (Jacobson *et al.* 2000). At recombinant $P2Y_1$ receptors, dATPαS is either a weak agonist (Simon *et al.* 1995) or an antagonist (King and Townsend-Nicholson 2000), whereas at $P2Y_1$-like receptors in rat brain, ATPαS and ATP are equipotent agonists (Schäfer and Reiser 1999). Human $P2Y_2$ receptors are activated equally by ATP and UTP, as well as by the dinucleotides Ap_4A and Up_4U (King *et al.* 2001; Pendergast *et al.* 2001). The phosphorothioate derivatives ATPγS and UTPγS are potent stimulants, but other major classes of synthetic nucleotides are not. Human $P2Y_4$ receptors are activated by UTP and Up_3U (Pendergast *et al.* 2001), while CTP, GTP, ITP, and Ap_4A are considered to be weak agonists (Jacobson *et al.* 2000; King *et al.* 2001). In contrast, rat and mouse $P2Y_4$ receptors

are activated equally by UTP and ATP. Human $P2Y_6$ receptors are activated by UDP and, to a lesser extent, ADP with all other nucleoside triphosphates being very weak agonists (Communi *et al.* 1996*b*). Of the synthetic compounds, UDPβS and Up_3U are both potent agonists at $P2Y_6$ receptors (Malmsjo *et al.* 2000; Pendergast *et al.* 2001). Rat and mouse orthologues show a similar pharmacological profile to human $P2Y_6$ (Filippov *et al.* 1999; Lazarowski *et al.* 2001). The human $P2Y_{11}$ receptor is activated by ATP and ADP (Communi *et al.* 1999) and the synthetic nucleotides BzATP, deoxyATP, 2-MeSATP, and AR-C67085 (2-propyl-D-β, γ-dichloromethylene-ATP) are also all potent agonists (Communi *et al.* 1999; Qi *et al.* 2001). In contrast, some synthetic nucleotides—ADPβS, ATPγS, and A3P5PS—are partial agonists, although, under some circumstances, they can also act as antagonists (Communi *et al.* 1999). Human $P2Y_{12}$ receptors are activated by ADP, 2-MeSADP and to a lesser extent by ATPγS (Hollopeter *et al.* 2001).

21.4.2 Antagonists

Some synthetic adenosine 3′, 5′-bisphosphate derivatives are potent antagonists (e.g. MRS 2179 and MRS 2279) (Nandanan *et al.* 2000) at $P2Y_1$ receptors and the classical P2 receptor antagonists (PPADS, Reactive blue-2 and suramin) also inhibit $P2Y_1$ receptor activity (King *et al.* 2001). Suramin is also the one and only weak antagonist of $P2Y_2$ receptors. For human $P2Y_4$, ATP is reported to be the most potent competitive antagonist (K_b, 0.7 μM; Kennedy *et al.* 2000). Weak antagonist activity has also been reported for PPADS, suramin and Reactive blue-2 at $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors (Robaye *et al.* 1997; Bogdanov *et al.* 1998; Lazarowski *et al.* 2001; King *et al.* 2001;). Human $P2Y_{12}$ is antagonized by 2-MeSAMP (Hollopeter *et al.* 2001) and C13307 (Scarborough *et al.* 2001), while the native form of the receptor is potently blocked by ARC67085 (Humphries *et al.* 1995). $P2Y_1$ antagonists, in the form of adenosine 3′, 5′-bisphosphate derivatives are inert at human $P2Y_{12}$.

21.5 Signal transduction and receptor modulation

Most of the recombinant P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$) couple via the G_q/PLC_β pathway to cause IP_3 production, Ca^{2+}-mobilization and activation of Ca^{2+}-dependent reporter currents in heterologous expression systems (King *et al.* 2001). When expressed in cultured sympathetic neurones, some P2Y receptors inhibit native Ca^{2+} and K^+ currents by a direct action on ion channels by G protein catalytic and regulatory subunits (Filippov *et al.* 1997, 1998, 1999, 2000). Endogenous metabotropic P2 receptors affect a much wider range of intracellular signalling pathways and utilize PLC_β, PLD, PLA_2, AC, MEP/MAP kinases and Rho-dependent kinase, as well as coupling directly to some ion channels. The narrow selectivity of recombinant P2Y subtypes may only reflect the limited availability of signalling pathways in expression systems used so far.

A native $P2Y_1$-like receptor, in the clonal line (B10) of rat brain capillary endothelial cells, appeared to couple negatively to adenylate cyclase and inhibit cAMP levels through a PTX-sensitive G protein (Webb *et al.* 1996). The possibility that recombinant $rP2Y_1$ receptors might affect cAMP production was investigated by expression into 1321N1 and C6 rat glioma cells that, respectively, utilize G_q/PLC_β and G_i/AC signalling mechanisms (Schachter *et al.* 1997). Experiments showed that rat $P2Y_1$ receptors selected only the G_q/PLC_β pathway in 1321N1 cells, and not the G_i/AC pathway in C6 glioma cells. Although B10 cells possess $P2Y_1$ transcripts, it was later shown that B10 cells also possessed a $P2Y_{12}$-like receptor that

could activate the G_i/AC pathway and help explain earlier results (Simon *et al.* 2001). The available evidence suggests that known species orthologues of $P2Y_1$ couple primarily to the G_q/PLC_β pathway. The skate p2y receptor, considered to be the most primitive form of $P2Y_1$, is the only GPCR in skate liver to signal via the G_q/PLC_β pathway (Dranoff *et al.* 2000). Apart from the G_q/PLC_β signalling, $P2Y_1$ receptors directly inhibit N-type Ca^{2+}-currents in rat sympathetic neurones (Filippov *et al.* 2000).

The $P2Y_2$ receptor appears to couple mainly to the G_q/PLC_β pathway, although 35 per cent of the evoked Ca^{2+}-signal is inhibited by PTX (Erb *et al.* 1993; Parr *et al.* 1994). PLC_β activation, via G_α of PTX-insensitive G_q and $G_{\beta,\gamma}$ complex of PTX-sensitive G_i, could account for the $P2Y_2$-induced Ca^{2+}-signal (Lustig *et al.* 1996). In *Xenopus* oocytes, $P2Y_2$ receptors couple directly to co-expressed K^+ channels of the Kir 3.0 subfamily via PTX-sensitive $G_{i/o}$ proteins (Mosbacher *et al.* 1998). In sympathetic neurons, $P2Y_2$ receptors inhibit N-type Ca^{2+}-currents via a PTX-sensitive mechanism (Filippov *et al.* 1997, 1998). A native $P2Y_2$-like receptor, in canine MDCK-D1 epithelial cells, was reported to couple indirectly to G_s/AC through an indomethacin-sensitive pathway (Zambon *et al.* 2000). Dual signalling also occurs with the human $P2Y_4$ receptor, since PTX limits the Ca^{2+} signal by 60 per cent in the first 30 s of agonist activation but fails to inhibit Ca_i^{2+} levels following prolonged (>300 s) agonist activation (Communi *et al.* 1996*a*). By contrast, $P2Y_6$ receptor signalling via Ca^{2+}-mobilisation was reported to be PTX-insensitive (Chang *et al.* 1995; Robaye *et al.* 1997). However, $P2Y_6$ receptors inhibit N-type Ca^{2+}-currents via a PTX-sensitive mechanism in sympathetic neurons (Filippov *et al.* 1999).

The $P2Y_{11}$ receptor couples strongly to the G_q/PLC_β pathway, but also activates the G_s/AC pathway (Communi *et al.* 1997, 1999; Qi *et al.* 2001). It was reported that inositol hydrolysis and Ca^{2+}-mobilisation, via the G_q/PLC_β pathway, could potentiate cAMP production via the G_s/AC pathway in 1321N1 and CHO-K1 cells (Qi *et al.* 2001). This potentiating effect may help explain differences in agonist potencies when a range of nucleotides was tested against the two signalling pathways (Communi *et al.* 1999; Qi *et al.* 2001). The signalling and pharmacological properties of $P2Y_{11}$ mirror the endogenous P2Y receptor in HL-60 cells (Conigrave *et al.* 1998; Suh *et al.* 2000).

$P2Y_1$ receptors couple to the G_i/AC pathway to inhibit cAMP production, an effect blunted by PTX (Hollopeter *et al.* 2001). While human $P2Y_{12}$ receptors directly inhibit cAMP production in CHO cells, receptor activation was otherwise assessed in oocytes by $G_{\beta,\gamma}$ subunit stimulation of Kir 3.1 and 3.4 ion channels co-expressed with $P2Y_{12}$ (Hollopeter *et al.* 2001).

21.6 Physiology and disease relevance

The multiplicity of P2Y receptor subtypes and ubiquitous presence in all human tissues indicates nucleotidic signalling is important in the major physiological systems. The role of purines and pyrimidines in the pathophysiology of disease, has been broadly described in a number of reviews over the last five years (Burnstock 1997, 2002; Abbracchio and Burnstock 1998; Burnstock and Williams 2000; Williams and Jarvis 2000; Boeynaems *et al.* 2001).

21.6.1 Diseases of the central nervous system

The widespread distribution and density of $P2Y_1$ receptors in the neuraxis indicates a role in central transmission. $P2Y_1$-like receptors can inhibit (von Kügelgen *et al.* 1994, 1997;

Bennett and Boarder 2000; Mendoza-Fernandez *et al.* 2000) or facilitate (Zhang *et al.* 1995) transmitter release from central neurones. These pre-synaptic effects on transmitter release could be explained by the known effector systems for $P2Y_1$ receptors—for example, a direct inhibitory action on N-type Ca^{2+}-channels to limit exocytosis (Filippov *et al.* 2000) or large Ca^{2+}-transients via the G_q/PLC_β pathway to stimulate exocytosis (Mirinov 1994; Schäfer and Reiser 1999). With regard to disease states, the presence of $P2Y_1$ and P2U-like receptor receptors on astrocytes (King *et al.* 1996) coupled to their signalling through the ERK pathway (Neary *et al.* 1998, 1999, 2000), has implicated these receptors in the mitogenic action of ATP in reactive astrogliosis. This is a hyperplastic condition associated with CNS injury in a number of conditions including trauma, stroke, epilepsy and Alzheimer's disease and multiple sclerosis. Astrocytes support the viability of neurones and $P2Y_1$ and P2U-like receptor signalling is implicated in the reparative processes following such CNS injury (Ciccarelli *et al.* 2001). It has been difficult to define a clear role for $P2Y_2$ receptors in the nervous system due to a lack of selective agonists and antagonists able to distinguish this receptor from the other P2Y subtypes showing a P2U-like phenotype (i.e. $P2Y_4$ and $P2Y_6$). The recent discovery of the di-uridine polyphosphate series, particularly Up_4U as a stable agonist of $P2Y_2$ receptors, may alter these circumstances. A P2U-like receptor has been implicated in the regulation of transmitter release from hypothalamic vasopressin neurones (Hiruma and Bourque 1995) and paravertebral sympathetic neurones (Boehm 1998).

21.6.2 **Peripheral indications**

$P2Y_1$-like receptors in rodent osteoclasts show an interesting chemosensitivity to extracellular pH (Hoebertz *et al.* 2001) and this modulatory effect of H^+ ions may have some bearing on the purinergic (P2Y- and P2X-based) control of central respiratory drive during acidosis (Ralevic *et al.* 1999). In addition, $P2Y_2$ receptors are known to stimulate the mucus escalator in lung and directly affect airway hydration (Yerxa 2001). Consequently, the utility of uridine-based nucleotides (e.g. INS365 (Up_4U)) is under investigation for chronic bronchitis and cystic fibrosis (Shaffer *et al.* 1998).

P2Y receptors are being pursued for a number of potential peripheral disease indications. For example, $P2Y_2$ receptors may have an ameliorating influence in dry eye by stimulating tear production (Pintor and Peral 2001; Yerxa 2001) and $P2Y_4$ receptors have been implicated in proliferative disorders of the kidney (Harada *et al.* 2000). Reports also suggest that modulation of $P2Y_6$ receptor activity may be implicated in nucleotide-promoted colonic damage in inflammatory bowel disease (Somers *et al.* 1998). The $P2Y_{11}$ receptor has been strongly implicated in the differentiation of HL60 cells from granulocytes to neutrophils (Communi *et al.* 2000) and it has therefore been postulated that $P2Y_{11}$ receptor-directed ligands may be important in neutropenia (a loss of neutrophils) and some forms of leukaemia (Boeynaems *et al.* 2001). Finally, the $P2Y_{12}$ receptor corresponds to the G_i-linked ADP receptor in human blood platelets (Hollopeter *et al.* 2001) and, like the $P2Y_1$ receptor, is intimately involved in the clotting response (Hourani 2001). Use of a highly selective and competitive antagonist (C13307) has been developed for the $P2Y_{12}$ receptor and should help to clarify the role of this receptor in disorders where the regulation of blood clotting may be of importance (Scarborough *et al.* 2001).

21.7 **Concluding remarks**

The understanding of purinergic GPCRs has advanced significantly in the last ten years, with the cloning of multiple P2Y receptor subtypes and isolated study of their pharmacological and signalling properties. Advances have also been made in the medical chemistry of P2Y receptors, with the beginnings of agonist and antagonist selectivity for various P2Y subtypes. Purinergic signalling in the nervous system is less than clear, but an improved armament of selective ligands is now showing benefit. The role of P2Y receptors in diseases states is slowly being revealed and clinical trials have begun in key therapeutic areas, although CNS investigations are in their infancy. The role of purinergic signalling in neural development is now being considered, as are conditions for upregulation of P2Y receptors at various stages in the life cycle. The complexity of purinergic signalling represents a major challenge, which has now been taken up by many laboratories in academia and industry.

Note: A G_i-coupled $P2Y_{13}$ receptor—activated by ADP and inhibited by PTX—has been found in brain and spleen (Communi *et al. J Biol Chem* **276**, 41 479–85, December 2001).

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