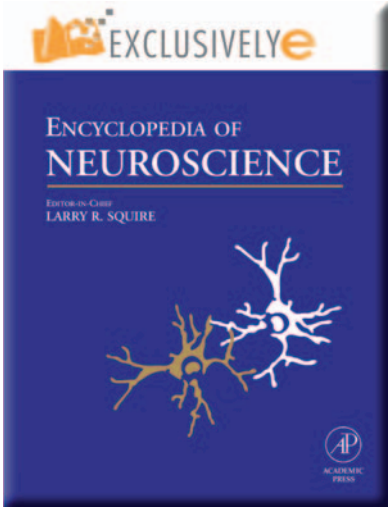

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Purines and Purinoceptors: Molecular Biology Overview

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Early Studies

A seminal paper describing the potent actions of adenine compounds was published by Drury and Szent-Györgyi in 1929. Many years later, ATP was proposed as the transmitter responsible for nonadrenergic, noncholinergic transmission in the gut and bladder, and the term 'purinergic' was introduced by Burnstock in 1972. Early resistance to this concept appeared to stem from the fact that ATP was recognized first for its important intracellular roles in many biochemical processes, and the intuitive feeling was that such a ubiquitous and simple compound was unlikely to be utilized as an extracellular messenger, although powerful extracellular enzymes involved in its breakdown were known to be present.

Implicit in the concept of purinergic neurotransmission was the existence of postjunctional purinergic receptors, and the potent actions of extracellular ATP on many different cell types also implicated membrane receptors. Purinergic receptors were first defined in 1976, and 2 years later a basis for distinguishing two types of purinoceptor, identified as P1 and P2 (for adenosine and ATP/ADP, respectively), was proposed. At about the same time, two subtypes of the P1 (adenosine) receptor were recognized, but it was not until 1985 that a pharmacological basis for distinguishing two types of P2 receptor (P2X and P2Y) was made. In 1993, the first G-protein-coupled P2 receptors were cloned and a year later two ion-gated receptors were cloned, and in 1994 Abbracchio and Burnstock, on the basis of molecular structure and transduction mechanisms, proposed that purinoceptors should belong to two major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G-protein-coupled purinoceptors. This nomenclature has been widely adopted and currently seven P2X subtypes and eight P2Y receptor subtypes are recognized, including receptors that are sensitive to pyrimidines as well as purines.

It is widely recognized that purinergic signaling is a primitive system involved in many nonneuronal as well as neuronal mechanisms, including exocrine and endocrine secretion, immune responses, inflammation, pain, platelet aggregation, and endothelial-mediated vasodilatation. Cell proliferation, differentiation, and death that occur in development and regeneration are also mediated by purinergic receptors.

P1 Receptors

Four different P1 receptor subtypes, A_1, A_{2A}, A_{2B}, and A_3, have been cloned. All are G-protein-coupled receptors (GPCRs). At 318 amino acids in length, the A_3 subtype is the shortest, while A_{2A} is the longest (412 residues). Their N-termini are relatively short (7–13 residues in length), as are their C-termini (32–120 residues). In the transmembrane domains (TMI–TMVII), human adenosine receptors share 39–61% sequence identity with each other and 11–18% identity with P2Y receptors. Each of the four human P1 receptor genes contains an intron within the coding region, located immediately after the end of the third transmembrane domain (see Figure 1(a)).

P1 receptors couple principally to adenylate cyclase. A_1 and A_3 are negatively coupled to adenylate cyclase through the $G_{i/o}$ protein α subunits, whereas A_{2A} and A_{2B} are positively coupled to adenylate cyclase through G_s. The human A_{2B} receptor has also been observed to couple through $G_{q/11}$ to regulate phospholipase C activity, and the A_3 receptor may interact directly with G_s.

A number of P1 subtype-selective agonists and antagonists have been identified (see Table 1). Alteration or opening of the ribose ring drastically reduces affinity. The hydroxyl group at the 2′ position is needed for both affinity and activity. The most selective agonist for the A_1 subtype is 2-chloro-N^6-cyclopentyladenosine (CCPA). CGS 21680 is the most selective A_{2A} agonist; NECA is the most potent A_{2B} receptor agonist. 2-Cl-IB-MECA is 11-fold selective for the human A_3 receptor and about 1400-fold selective for the rat A_3 receptor. In general, methylxanthines such as caffeine and theophylline are weak P1 receptor antagonists. DPCPX (8-cyclopentyl-1,3-dipropylxanthine) is an A_1 receptor antagonist with subnanomolar affinity. The most selective A_{2B} receptor antagonist is MRS1754. MRE3008-F20 is the most selective human A_3 receptor antagonist. The diverse physiological effects mediated by the different P1 receptor subtypes, particularly modulation of the cardiovascular, immune, and central nervous systems, have been confirmed by transgenic knockout mice for A_1, A_{2A}, and A_3 receptors. In contrast to knockout studies, overexpression of either A_1 or A_3 subtypes in transgenic mice has a cardioprotective effect.

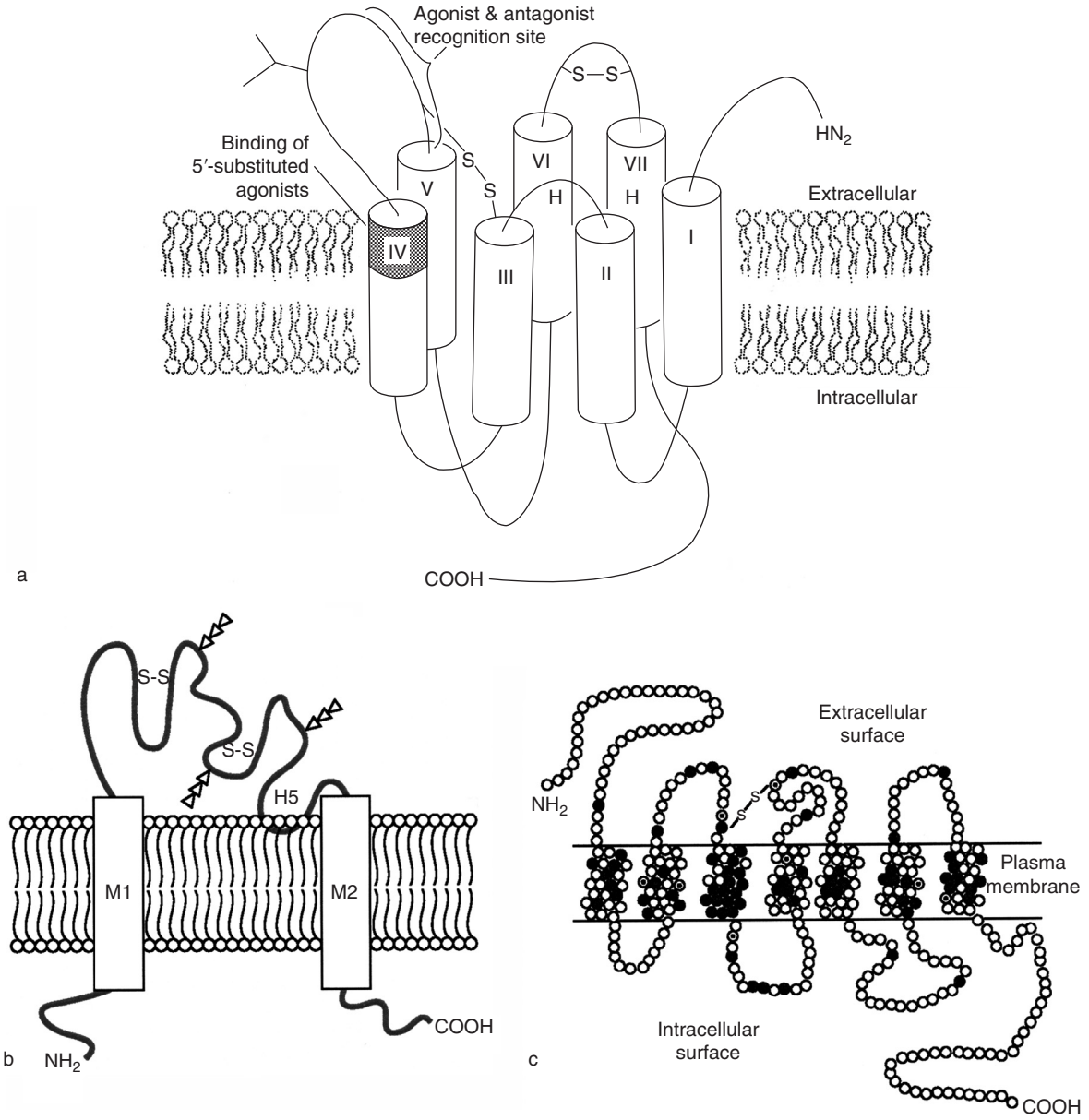

Figure 1 Membrane receptors for extracellular ATP and adenosine. The P1 family of receptors for extracellular adenosine comprises G-protein-coupled receptors signaling by inhibiting or activating adenylate cyclase (a). The P2 family of receptors binds extracellular ATP or ADP, and comprises two types of receptors (P2X and P2Y). The P2X family receptors are ligand-gated ion channels (b), and the P2Y family members are GPCRs (c). (a) Reproduced from Ralevic V and Burnstock G (1998) Receptors for purines and pyrimidines. *Pharmacological Reviews* 50: 413–492, with permission from the American Society for Pharmacology and Experimental Therapeutics. (b) Reprinted by permission from Macmillan Publishers Ltd: [Nature] (Brake AJ, Wagenbach MJ, and Julius D (1994) New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature* 371: 519–523), copyright (1994). (c) Adapted from Barnard EA, Burnstock G, and Webb TE (1994) G protein-coupled receptors for ATP and other nucleotides: A new receptor family. *Trends in Pharmacological Sciences* 15: 67–70, with permission from Elsevier.

P2X Receptors

Molecular Structure

The first cDNAs encoding P2X receptor subunits were isolated in 1994. Members of the family of ionotropic $P2X_{1-7}$ receptors show a subunit topology of intracellular N- and C-termini possessing consensus binding motifs for protein kinases; two transmembrane-spanning regions (TM1 and TM2), the first involved with channel gating and the second lining the ion pore; a large extracellular loop, with ten conserved cysteine residues forming a series of disulfide bridges; a hydrophobic H5 region close to the pore vestibule, for possible receptor/channel modulation by cations; and an ATP-binding site, which may involve regions of the extracellular loop adjacent

Table 1 Characteristics of purine-mediated receptors

Receptor	*Main distribution*	*Agonists*	*Antagonists*	*Transduction mechanisms*
P1 (adenosine)				
A_1	Brain, spinal cord, testis, heart, autonomic nerve terminals	CCPA, CPA, S-ENBA	DPCPX, N-0840, MRS1754	$G_{i/o}$ ↓cAMP
A_{2A}	Brain, heart, lungs, spleen	CGS 21680, HENECA	KF17837, SCH58261, ZM241385	G_S ↑cAMP
A_{2B}	Large intestine, bladder	NECA (nonselective)	Enprofylline, MRE2029-F20, MRS1754, MRS1706	G_S ↑cAMP
A_3	Lung, liver, brain, testis, heart	IB-MECA, 2-Cl-IB-MECA, DBXRM, VT160	MRS1220, L-268605, MRS1191, MRS1523, VUF8504	$G_{i/o}$ $G_{q/11}$ ↓cAMP ↑IP_3
P2X				
$P2X_1$	Smooth muscle, platelets, cerebellum, dorsal horn spinal neurons	α,β-meATP = ATP = 2-MeSATP (rapid desensitization), L-β,γ-meATP	TNP-ATP, IP_5I, NF023, NF449	Intrinsic cation channel (Ca^{2+} and Na^+)
$P2X_2$	Smooth muscle, CNS, retina, chromaffin cells, autonomic and sensory ganglia	ATP ≥ ATPγS ≥ 2-MeSATP ≫ α,β-meATP (pH + zinc sensitive)	Suramin, isoPPADS, RB2, NF770	Intrinsic ion channel (particularly Ca^{2+})
$P2X_3$	Sensory neurons, NTS, some sympathetic neurons	2-MeSATP ≥ ATP ≥ α,β-meATP ≥ Ap_4A (rapid desensitization)	TNP-ATP, PPADS, A317491, NF110	Intrinsic cation channel
$P2X_4$	CNS, testis, colon	ATP ≫ α,β-meATP, CTP, ivermectin	TNP-ATP (weak), BBG (weak)	Intrinsic ion channel (especially Ca^{2+})
$P2X_5$	Proliferating cells in skin, gut, bladder, thymus, spinal cord	ATP ≫ α,β-meATP, ATPγS	Suramin, PPADS, BBG	Intrinsic ion channel
$P2X_6$	CNS, motor neurons in spinal cord	(Does not function as homomultimer)		Intrinsic ion channel
$P2X_7$	Apoptotic cells in, for example, immune cells, pancreas, skin	BzATP > ATP ≥ 2-MeSATP ≫ α,β-meATP	KN62, KN04, MRS2427, Coomassie brilliant blue G	Intrinsic cation channel and a large pore with prolonged activation
P2Y				
$P2Y_1$	Epithelial and endothelial cells, platelets, immune cells, osteoclasts	2-MeSADP = ADPβS > 2-MeSATP = ADP > ATP, MRS2365	MRS2179, MRS2500, MRS2279, PIT	G_q/G_{11}; PLC-β activation
$P2Y_2$	Immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts	UTP = ATP, UTPγS, INS37217	Suramin > RB2, AR-C126313	G_q/G_{11} and possibly G_i; PLC-β activation
$P2Y_4$	Endothelial cells	UTP ≥ ATP, UTPγS	RB2 > suramin	G_q/G_{11} and possibly G_i; PLC-β activation
$P2Y_6$	Some epithelial cells, placenta, T cells, thymus	UDP > UTP ≫ ATP, UDPβS	MRS2578	G_q/G_{11}; PLC-β activation
$P2Y_{11}$	Spleen, intestine, granulocytes	AR-C67085MX > BzATP ≥ ATPγS > ATP	Suramin > RB2, NF157, 5′-AMPS	G_q/G_{11} and G_S; PLC-β activation
$P2Y_{12}$	Platelets, glial cells	2-MeSADP ≥ ADP ≫ ATP	CT50547, AR-C69931MX, INS49266, AZD6140, PSB0413, ARL66096, 2-MeSAMP	$G_{i/o}$; inhibition of adenylate cyclase
$P2Y_{13}$	Spleen, brain, lymph nodes, bone marrow	ADP = 2-MeSADP ≫ ATP and 2-MeSATP	MRS2211, 2-MeSAMP	$G_{i/o}$
$P2Y_{14}$	Placenta, adipose tissue, stomach, intestine, discrete brain regions	UDP glucose = UDP-galactose		G_q/G_{11}

BBG, brilliant blue green; BzATP, 2′- and 3′-*O*-(4-benzoyl-benzoyl)-ATP; cAMP, cyclic AMP; CCPA, chlorocyclopentyl adenosine; CPA, cyclopentyl adenosine; CTP, cytosine triphosphate; IP_3, inosine triphosphate; IP_5I, diinosine pentaphosphate; 2-MeSADP, 2-methylthio-ADP; 2-MeSATP, 2-methylthio-ATP; NECA, 5'-*N*-ethylcarboxamido adenosine; NTS, nucleus tractus solitarius; PLC, phospholipase C; RB2, reactive blue 2.

Adapted and reproduced from Burnstock G (2003) Introduction: ATP and its metabolites as potent extracellular agonists. *Current Topics in Membranes* 54: 1–27, with permission from Elsevier.

Table 2 Potential coassembly of P2X receptor subunits[a]

	$P2X_1$	$P2X_2$	$P2X_3$	$P2X_4$	$P2X_5$	$P2X_6$	$P2X_7$
$P2X_1$	+	+	+		+	+	
$P2X_2$		+	+		+	+	
$P2X_3$			+		+		
$P2X_4$				+	+	+	
$P2X_5$					+	+	
$P2X_6$							
$P2X_7$							+

[a]P2X receptor subunits carrying either one of two epitope tag units were expressed in pairs of HEK293 cells. +, Subunits immunoprecipitated with antibody to one epitope could be detected with an antibody to the second epitope.
Reproduced from Torres GE, Egan TM, and Voigt MM (1999) Hetero-oligomeric assembly of P2X receptor subunits. Specificities exist with regard to possible partners. *Journal of Biological Chemistry* 274: 6653–6659, with permission from the American Society for Biochemistry and Molecular Biology.

Table 3 Chromosomal localization of human P2X receptors[a]

Subunit	*Chromosome*	*Accession number*
$P2X_1$	17p13.2	X83688
$P2X_2$		AF190826
$P2X_3$	11q12	Y07683
$P2X_4$	12q24.31	Y07684
$P2X_5$	17p13.3	AF016709
$P2X_6$	22q11	AB002059
$P2X_7$	12q24.31	Y09561

[a]Accession numbers are those for the original submission of cDNA sequences. Chromosomal localizations are from human genome databases. $P2X_2$ chromosomal location is not yet determined. The mouse gene is located on chromosome 5, in a region that is syntenic with the extreme end of the long arm of human chromosome 12 (some 6 MB from the $P2X_4$ and $P2X_7$ genes).
Reproduced from North RA (2002) Molecular physiology of P2X receptors. *Physiological Reviews* 82: 1013–1067, with permission from the American Physiological Society.

to TM1 and TM2 (see Figure 1(b)). The $P2X_{1-7}$ receptors show 30–50% sequence identity at the peptide level. The stoichiometry of $P2X_{1-7}$ receptors is thought to involve three subunits, which form a stretched trimer or a hexamer of conjoined trimers. All of the P2X receptor subunits have consensus sequences for N-linked glycosylation.

The pharmacology of the recombinant P2X receptor subtypes expressed in oocytes or other cell types is often different from the pharmacology of P2X-mediated responses in naturally occurring sites. Several contributing factors may account for these differences. First, heteromultimers as well as homomultimers are involved in forming the trimer ion pore. $P2X_{2/3}$, $P2X_{1/2}$, $P2X_{1/5}$, $P2X_{2/6}$, $P2X_{4/6}$, and $P2X_{1/4}$ receptor heteromultimers have been identified (Table 2). $P2X_7$ does not form heteromultimers, and $P2X_6$ will not form a functional homomultimer. Second, spliced variants of P2X receptor subtypes might play a part.

There are seven genes for P2X receptor subunits. $P2X_4$ and $P2X_7$ subunit genes are located close to the tip of the long arm of chromosome 12. $P2X_4$ and $P2X_7$ subunits are among the most closely related pairs in amino acid sequences. $P2X_1$ and $P2X_5$ genes are also very close together on the short arm of chromosome 13. The remaining genes are on different chromosomes (Table 3). The genes vary considerably in size (e.g., $mP2X_3 = 40$ kb; $hP2X_6 = 12$ kb). The full-length forms have 11–13 exons, and all share a common structure, with well-conserved intron/exon boundaries. Many spliced forms of the receptor subunits (or fragments) have been described. Several full-length nonmammalian vertebrate sequences have been identified. There are no reports of homologous sequences from invertebrate species, although there is considerable functional evidence that extracellular ATP and other nucleotides can directly gate ion channels in invertebrates.

Recent advances have been made by the preparation of knockout mice for $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, and $P2X_7$ receptors, and transgenic mice that overexpress the $P2X_1$ receptor.

P2X Receptor Subtypes

$P2X_1$ receptors A cDNA encoding the $P2X_1$ receptor was isolated by direct expression in *Xenopus* oocytes, beginning with a cDNA library made from rat vas deferens. Human and mouse cDNAs have also been cloned and expressed. The homomeric $P2X_1$ receptor is a cation-selective channel that shows little selectivity for sodium over potassium. It has a relatively high permeability to calcium.

A major property of the $P2X_1$ receptor is the mimicry of the agonist actions of ATP by α,β-methylene ATP (α,β-meATP), which distinguishes $P2X_1$ and $P2X_3$ receptors from the other homomeric forms. 2′,3′-*O*-(benzoyl-4-benzoyl)-ATP (BzATP) is also an effective agonist. $P2X_1$ receptors are blocked by suramin and pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), but there are newer antagonists that are more $P2X_1$-selective (see Table 1). A valuable antagonist at $P2X_1$ receptors is 2′,3′-*O*-(2,4,6-trinitrophenyl)-ATP (TNP-ATP), which has an IC_{50} of about 1 nM.

Desensitization means the decline in the current elicited by ATP during the continued presence of ATP. In some P2X receptors this decline occurs in milliseconds (fast desensitization: $P2X_1$, $P2X_3$), and in others it occurs 100–1000 times more slowly (slow desensitization: $P2X_2$, $P2X_4$). Recovery from desensitization is extremely slow. Treatment with

apyrase allows $P2X_1$ receptors to recover from desensitization. Adenoviral expression of a $P2X_1$ receptor–green fluorescent protein construct in vas deferens shows the receptor to be localized in clusters, with larger ones apposing nerve varicosities.

$P2X_2$ receptors The rat $P2X_2$ receptor cDNA was isolated from a library constructed from nerve growth factor (NGF)-differentiated PC12 cells by testing pools for functional expression in *Xenopus* oocytes. The human receptor cDNA was amplified from pituitary gland. There are no agonists currently known that are selective for $P2X_2$ receptors. However, $P2X_2$ receptors are potentiated by protons and by low concentrations of zinc and copper. There are no selective antagonists for $P2X_2$ receptors. The $P2X_2$ receptor is generally described as nondesensitizing, compared with the $P2X_1$ and $P2X_3$ receptors.

When oocytes are injected with RNAs encoding $P2X_2$ receptors, and also the $\alpha 3$ and $\beta 4$ subunits of nicotinic receptors, they show responses to both ATP and acetylcholine; these can be selectively antagonized with appropriate receptor blockers. However, with concomitant application of both agonists, the resultant current is less than the expected sum of the two independent currents, indicating an interaction between the two receptors.

Heteromeric $P2X_{1/2}$ receptors $P2X_1$ and $P2X_2$ receptor subunits have been co-expressed in defolliculated *Xenopus* oocytes and the resultant receptors were studied under voltage clamp conditions. Co-expression yielded a mixed population of homomeric and heteromeric receptors, with a subpopulation of novel pH-sensitive P2X receptors showing identifiably unique properties that indicate the formation of heteromeric $P2X_{1/2}$ ion channels. It has been claimed that trimeric $P2X_{1/2}$ receptors incorporate one $P2X_1$ and two $P2X_2$ subunits.

$P2X_3$ receptors $P2X_3$ receptor subunit cDNAs were isolated from rat dorsal root ganglion cDNA libraries, from a human heart cDNA library, and from a zebra fish library. The mimicry of ATP by α,β-meATP makes these receptors similar to $P2X_1$ and distinct from the other homomeric forms. 2-MethylthioATP is as potent as or more potent than ATP at $P2X_3$ receptors. The antagonists suramin, PPADS, and TNP-ATP do not readily distinguish between $P2X_1$ and $P2X_3$ receptors, but NF023 is about 20 times less effective at $P2X_3$ than at $P2X_1$ receptors. Similar to $P2X_1$ receptors, desensitization is fast and recovery is very slow. $P2X_3$ receptors are prominently expressed on nociceptive sensory neurons.

Heteromeric $P2X_{2/3}$ receptors Direct association between $P2X_2$ and $P2X_3$ receptor subunits has been shown by co-immunoprecipitation. $P2X_{2/3}$ heteromeric channels can be defined on the basis of a sustained current elicited by α,β-meATP. $P2X_{2/3}$ receptor channels and, like homomeric $P2X_2$ receptors, are potentiated by low pH, and do not desensitize rapidly. The $P2X_{2/3}$ heteromer, like the homomeric $P2X_3$ receptor, is blocked by TNP-ATP, as well as PPADS and suramin. IP_5I is much more potent for blocking $P2X_1$ and $P2X_3$ homomers than for blocking the $P2X_{2/3}$ heteromers and is therefore useful to distinguish between $P2X_3$ and $P2X_{2/3}$ receptors. $P2X_{2/3}$ receptors have been identified in subpopulations of sensory neurons, sympathetic ganglion cells, and brain neurons.

$P2X_4$ receptors cDNAs for the rat $P2X_4$ receptor were isolated independently from superior cervical ganglion, brain, hippocampus, and pancreatic islet cells. Human, mouse, chick, and *Xenopus* cDNAs have also been isolated. Homomeric $P2X_4$ receptors are activated by ATP, but not by α,β-meATP. The most useful distinguishing feature of ATP-evoked currents at $P2X_4$ receptors is their potentiation by ivermectin.

When the application of ATP is of short duration, $P2X_4$ receptors operate as cation-selective channels; the calcium permeability is relatively high. When the application of ATP is continued for several seconds, the $P2X_4$ receptor channel becomes increasingly permeable to larger organic cations such as *N*-methyl-D-glucamine (NMDG). Desensitization at $P2X_4$ receptors is intermediate between that observed at $P2X_1$ and $P2X_2$.

The rat $P2X_4$ receptor is unusual among the P2X receptors in its relative insensitivity to blockade by the conventional antagonists suramin and PPADS. Currents evoked by ATP at the mouse $P2X_4$ receptor are actually increased by PPADS and suramin, probably because of their ectonucleotidase inhibitory activity.

Heteromeric $P2X_{1/4}$ receptors Co-injection of $P2X_1$ and $P2X_4$ subunits into *Xenopus* oocytes showed that both subunits were present in trimeric complexes of the same size. Voltage clamp experiments revealed functional P2X receptors with kinetic properties resembling those of homomeric $P2X_4$ receptors and a pharmacological profile similar to that of homomeric $P2X_1$ receptors. Preliminary results show that the $P2X_1$ receptor from the vas deferens and the $P2X_4$ receptor from salivary gland form complexes of the same size as the recombinant trimeric complexes expressed in oocytes.

$P2X_5$ receptors The $P2X_5$ receptor cDNA was first isolated from cDNA libraries constructed from rat celiac ganglion and heart. A P2X receptor was also cloned from embryonic chick skeletal muscle. The only human cDNAs reported are missing exon 10 ($hP2X_{5a}$) or exons 3 and 10 ($hP2X_{5b}$).

A feature of the currents elicited by ATP in cells expressing the rat $P2X_5$ receptor is their small amplitude, compared with the currents observed with $P2X_1$, $P2X_2$, $P2X_3$, or $P2X_4$ receptors expressed under similar conditions. The currents otherwise resemble those seen at $P2X_2$ receptors: they show little desensitization, are not activated by α,β-meATP, and are blocked by suramin and PPADS. $P2X_5$ mRNA is highly expressed in developing skeletal muscle.

Heteromeric $P2X_{1/5}$ receptors $P2X_1$ and $P2X_5$ subunits can be co-immunoprecipitated and the defining phenotype of this heteromer is a sustained current evoked by α,β-meATP, which is not seen for either of the homomers when expressed separately. Cells expressing the heteromeric receptor are very sensitive to ATP, concentrations as low as 3 or 10 nM evoking measurable currents. The sensitivity to the antagonist TNP-ATP is intermediate between the sensitive homomeric $P2X_1$ receptor and the insensitive homomeric $P2X_5$ receptor.

$P2X_6$ receptors The rat $P2X_6$ receptor was cloned from superior cervical ganglion cDNA and from rat brain. The human equivalent was isolated from peripheral lymphocytes as a p53-inducible gene. This was originally designated P2XM to reflect its abundance in human and mouse skeletal muscle. The $P2X_6$ receptor appears to be a 'silent' subunit, in the sense that no currents are evoked by ATP when it is expressed as a homomultimer in oocytes or HEK293 cells. It appears that the $P2X_6$ subunit is only functionally expressed as a heteromultimer.

Heteromeric $P2X_{2/6}$ receptors $P2X_2$ and $P2X_6$ receptors have been found to co-immunoprecipitate after expression in HEK293 cells. Oocytes expressing this combination have subtly different responses to ATP as compared to oocytes expressing only $P2X_2$ receptors. The most convincing of these differences is the fact that at pH 6.5 the inhibition of the current by suramin is clearly biphasic; one component has the high sensitivity of homomeric $P2X_2$ receptors, whereas the other component is less sensitive. $P2X_{2/6}$ receptors are prominently expressed by respiratory neurons in the brain stem.

Heteromeric $P2X_{4/6}$ receptors $P2X_4$ and $P2X_6$ receptors form a heteromeric channel when co-expressed in oocytes. The subunits can be co-immunoprecipitated from oocytes and HEK293 cells. The principal functional evidence for co-expression is that currents elicited by ATP are larger in oocytes 5 days after injection of mRNAs for $P2X_4$ and $P2X_6$ than after injection of $P2X_4$ alone. However, the phenotype of the heteromer differs only in minor respects from that of $P2X_4$ homomers. $P2X_{4/6}$ receptors are prominent in adult trigeminal mesencephalic nucleus and in hippocampal CA1 neurons.

$P2X_7$ receptors A chimeric cDNA encoding the rat $P2X_7$ receptor was first constructed from overlapping fragments isolated from superior cervical ganglion and medial habenula; full-length cDNAs were subsequently isolated from a rat brain cDNA library. Human and mouse cDNAs were cloned from monocyte and microglial cells, respectively. The main feature of the $P2X_7$ receptor is that in addition to the usual rapid opening of the cation-selective ion channel, with prolonged exposure to high concentrations of ATP it becomes permeable to larger cations (e.g., NMDG) and later undergoes a channel-to-pore conversion to allow the passage of large dye molecules such as ethidium and YO-PRO-1, and this usually leads to cell death. Evidence for $P2X_7$ receptor activation includes inward currents and increase in $[Ca^{2+}]_i$; other end points involve uptake of YO-PRO-1 or similar fluorescent dyes which bind to nucleic acid, and structural changes in the cell, such as membrane blebbing.

BzATP is a potent agonist at the $P2X_7$ receptor. There are five main types of blockers (see **Table 1**): ions (calcium, magnesium, zinc, copper, and protons), the suramin analog NF279, Coomassie brilliant blue G (which is most effective at rat $P2X_7$ receptors), oxidized ATP, and KN62, which is selective for the human $P2X_7$ receptor.

ATP or BzATP induces remarkable changes in the appearance of HEK293 cells transfected with the rat $P2X_7$ receptor. After continuous application of BzATP (30 μM) for about 30 s, the plasma membrane begins to develop large blebs, and after 1 or 2 min, these become multiple and sometimes coalesce. Blebs are usually preceded by the appearance of smaller vesicles (<1 μm in diameter), which are shed from the cell and appear to release inflammatory cytokines.

P2Y Receptors

Molecular Structure

The first P2Y receptors were cloned in 1993. Since then several other subtypes have been isolated by homology cloning and are assigned a subscript on the basis of cloning chronology ($P2Y_4$, $P2Y_6$, $P2Y_{11}$). The long-awaited G_i-coupled ADP receptor ($P2Y_{12}$) of platelets was finally isolated by expression

cloning in 2001, while $P2Y_{13}$ and $P2Y_{14}$ receptors were characterized later during a systematic study of orphan receptors. At present, there are eight accepted human P2Y receptors: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ (see Table 1). The missing numbers represent either nonmammalian orthologs, or receptors having some sequence homology to P2Y receptors, but for which there is no functional evidence of responsiveness to nucleotides. p2y3 may be a chicken ortholog of $P2Y_6$, while p2y8 and tp2y could be the *Xenopus* and turkey orthologs of $P2Y_4$, respectively. p2y7 is a leukotriene B4 receptor. p2y5 and p2y10 are considered as orphan receptors. A p2y8 receptor cloned from the frog embryo appears to be involved in the development of the neural plate. p2y9 was reported to be a novel receptor for lysophosphatidic acid, distant from the Edg family. $P2Y_{15}$ was recently introduced to designate the orphan receptor GPR80/GPR99 on the basis that it would be a receptor for adenosine 5′-monophospahte (AMP), but it is now firmly established that it is not a P2Y receptor, but rather a receptor for α-ketoglutarate, as recently underlined in a report by the International Union of Basic and Clinical Pharmacology (IUPHAR) P2Y Subcommittee.

In contrast to P2X receptors, P2Y receptor genes do not contain introns in the coding sequence, except for the $P2Y_{11}$ receptor. Site-directed mutagenesis of the $P2Y_1$ and $P2Y_2$ receptors has shown that some positively charged residues in TM3, TM6, and TM7 (see Figure 1(c)) are crucial for receptor activation by nucleotides. From a phylogenetic and structural (i.e., protein sequence) point of view, two distinct P2Y receptor subgroups characterized by a relatively high level of sequence divergence have been identified. The first subgroup includes $P2Y_{1,2,4,6,11}$ subtypes and the second subgroup encompasses the $P2Y_{12,13,14}$ subtypes (see dendrogram in Figure 2).

Most of the P2Y receptor subtypes are still lacking potent and selective synthetic agonists and antagonists. However, ADPβS is a potent agonist of $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors. 2-Methylthio-ADP (2-MeSADP) is a potent agonist (EC_{50}, in nM) at $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors. Selective antagonists have been identified for some P2Y receptor subtypes (see Table 1). P2Y receptor-mediated responses occur in nonneuronal and nonmuscular cell types, as well as in the nervous system, involved in both short- and long-term signaling.

Second Messenger Systems and Ion Channels

$P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors couple to G-proteins to increase inositol trisphosphate (IP_3) and cytosolic calcium. Activation of the $P2Y_{11}$ receptor by ATP leads to a rise in both cAMP and in IP_3, whereas activation by uridine 5′-triphosphate (UTP) produces calcium mobilization without IP_3 or cAMP increase. The $P2Y_{13}$ receptor can simultaneously couple to G_{16}, G_i, and, at high concentrations of ADP, G_s. The activation of several P2Y receptors is commonly associated with the stimulation of several mitogen-activated protein (MAP) kinases, in particular extracellular signal-regulated protein kinase-1/2.

In recent years, GPCRs in neurons and other excitable cells have been found to modulate the activity of voltage-gated ion channels in the cell membrane through certain actions of activated G-proteins. Such actions are now well established in closing (or, in certain cases, in opening or potentiating) various classes of K^+ channels and voltage-gated Ca^{2+} channels. ATP (or UTP, or their products ADP or UDP) present at synapses, plus ATP diffusing from astrocytes, activates P2Y receptors on distinct subsets of brain neurons, regulating their activities by the coupling of those receptors to specific ion channels. While ion channel couplings of P2Y receptors are primarily of importance in neurons, they have in a few cases been detected also in various other tissues (e.g., in cardiac muscle cells). Among the channels with which the superior cervical ganglion cell membrane is well endowed are two types of voltage-gated channels, which are important in receptor-based regulation of neuronal activity, the Ca^{2+} channel of the N-type and the M-current K^+ channel.

P2Y Receptor Subtypes

$P2Y_1$ receptors Human, rat, mouse, cow, chick, turkey, and *Xenopus* $P2Y_1$ receptors have been cloned and characterized. In most species, ADP is a more potent agonist than ATP is and their 2-methylthio derivatives are even more potent. UTP, UDP, CTP, and GTP are inactive. At present, the most potent and selective agonist known is the *N*-methanocarba analog of 2-MeSADP, MRS2365 (EC_{50} of 0.4 nM). The most effective antagonists to display selectivity for the $P2Y_1$ receptor are MRS2179, MRS2279, and MRS2500 (see Table 1).

Site-directed mutagenesis studies on the human $P2Y_1$ receptor have shown that amino acid residues in TM3, TM6, and TM7 are critical determinants in the binding of ATP. Four cysteine residues in the extracellular loops, which are conserved in P2Y receptors, are essential for proper trafficking of the human $P2Y_1$ receptor to the cell surface. $P2Y_1$ mRNA expression is highest in various regions of the brain, prostate gland, and placenta, and has also been detected at varying levels in other organs.

$P2Y_1$ receptor knockout mice have been generated. These mice are viable with no apparent abnormalities affecting their development, survival, and reproduction. Platelet counts are normal, but shape change is abolished. Transgenic mice overexpressing the $P2Y_1$

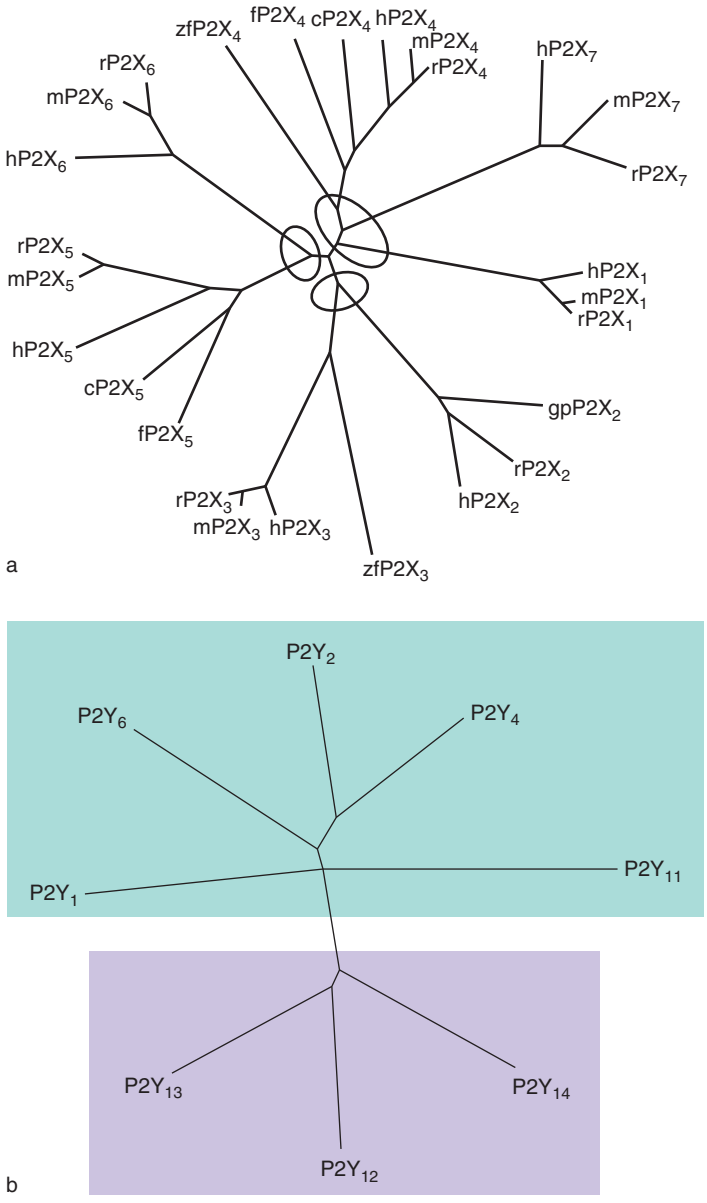

Figure 2 (a) Dendrogram to show relatedness of 29 P2X receptor subunits. Full-length amino acid sequences were aligned with Clustal W software using default parameters. The dendrogram was constructed with Tree View. h, human (*Homo sapiens*); r, rat (*Rattus norvegicus*); m, mouse (*Mus musculus*); gp, guinea pig (*Cavia porcellus*); c, chicken (*Gallus gallus*); zf, zebra fish (*Danio rerio*); f, fugu (*Takifugu rubripes*). The ovals indicate the apparent clustering by relatedness into subfamilies. (b) A phylogenetic tree (dendrogram) showing the relationships among the current members of the P2Y receptor family (human $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ receptors) and the human UDP-glucose receptor (here indicated as the $P2Y_{14}$ receptor). The P2Y receptors can be divided into two subgroups, shown with blue and purple backgrounds. Sequences were aligned using Clustal X and the tree was built using the Tree View software. (a) Reproduced from North RA (2002) Molecular physiology of P2X receptors. *Physiological Reviews* 82: 1013–1067, with permission from the American Physiological Society. (b) Reproduced from Abbracchio MP, Boeynaems J-M, Barnard EA, et al. (2003) Characterization of the UDP-glucose receptor (re-named here the $P2Y_{14}$ receptor) adds diversity to the P2Y receptor family. *Trends in Pharmacological Sciences* 24: 52–55, with permission from Elsevier.

receptor specifically in the megakaryocytic/platelet lineage have also been generated.

$P2Y_2$ receptors $P2Y_2$ receptors have been cloned and pharmacologically characterized from human, rat, mouse, canine, and porcine cells or tissues. $P2Y_2$ receptors are fully activated by ATP and UTP, whereas ADP and UDP are much less effective agonists. The γ-thiophosphate, UTPγS, has been shown to be a potent hydrolysis-resistant agonist of $P2Y_2$

receptors, as is the recently developed $P2Y_2$ receptor agonist, INS37217 (Up_4dC). Suramin acts as a weak competitive antagonist of human and rat $P2Y_2$ receptors. AR-C126313 and the related aminotetrazole derivative AR-C118925, flavanoids, and tangeretin have been claimed recently to be effective antagonists.

$P2Y_2$ receptors can directly couple to PLCβ_1 via $G_{\alpha q/11}$ protein to mediate the production of IP_3 and diacylglycerol, which are second messengers for calcium release from intracellular stores and protein kinase C (PKC) activation, respectively.

Expression of $P2Y_2$ receptor mRNA and protein has been detected in many tissues. $P2Y_2$ receptor activation increases the synthesis and/or release of arachidonic acid, prostaglandins, and nitric oxide. $P2Y_2$ receptor expression in smooth muscle cells is upregulated by agents that mediate inflammation. $P2Y_2$ receptors have been shown to play a role in the wound healing process. $P2Y_2$ receptor activation increases Cl^- secretion and inhibits Na^+ absorption in epithelial cells. A $P2Y_2$ receptor knockout mouse has been produced that is defective in nucleotide-stimulated ion secretion in airway epithelial cells. $P2Y_2$ receptors have been shown to inhibit bone formation by osteoblasts, and N-type calcium currents in neurons.

$P2Y_4$ receptors Human, rat, and mouse $P2Y_4$ receptors have been cloned and characterized. UTP is the most potent activator of the recombinant human $P2Y_4$ receptor. In contrast, the recombinant rat and mouse $P2Y_4$ receptors are activated equipotently by ATP and UTP. Up_4U (INS365) and dCp_4U (INS37217) are agonists of the human $P2Y_4$ receptor. Reactive blue 2 effectively blocks rat $P2Y_4$ receptors, but only partially blocks human $P2Y_4$ receptors. Suramin is a weak antagonist at the $P2Y_4$ receptor. The structural determinants of agonism versus antagonism by ATP are located in the N-terminal domain and the second extracellular loop.

In the human and the mouse, $P2Y_4$ mRNA and protein was most abundant in the intestine, but was also detected in other organs. $P2Y_4$-null mice have apparently normal behavior, growth, and reproduction, but the chloride secretory response of the jejunal epithelium to apical UTP and ATP is abolished.

$P2Y_6$ receptors The mouse, rat, and human $P2Y_6$ receptors are UDP receptors. The uridine β-thiodiphosphate, UDPβS, and Up_3U are selective agonists of the $P2Y_6$ receptor and are more stable to degradation. INS48823 is also a potent $P2Y_6$ agonist. A 1,4-di-(phenylthioureido)butane derivative (MRS2578) has recently been shown to selectively inhibit UDP-induced PLC activity. A unique feature of the $P2Y_6$ receptor is its slow desensitization and internalization. A wide tissue distribution of $P2Y_6$ mRNA and protein has been demonstrated, with the highest expression in spleen, intestine, liver, brain, and pituitary.

$P2Y_{11}$ receptors Among P2Y receptors, the human $P2Y_{11}$ receptor has a unique profile. Its gene is the only P2Y receptor gene that contains an intron in the coding sequence. The potency of its natural agonist ATP is relatively low and it is dually coupled to PLC and adenylyl cyclase upon stimulation. ATPγS is a more potent agonist than ATP is. The $P2Y_{12}$ antagonist AR-C67085MX acts as a potent agonist at the $P2Y_{11}$ receptor. Suramin behaves as a competitive antagonist of the $hP2Y_{11}$ receptor.

$P2Y_{12}$ receptors The human, rat, and mouse $P2Y_{12}$ receptors have been identified and characterized. ADP is the natural agonist of this receptor. The $P2Y_{12}$ receptor is heavily expressed in platelets, where it is the molecular target of the active metabolite of the antiplatelet drug clopidogrel. Potent direct competitive $P2Y_{12}$ antagonists are also available, including the 5′-triphosphate derivative AR-C69931MX compound, named cangrelor. The $P2Y_{12}$ receptor has also been shown to be expressed in subregions of the brain, glial cells, brain capillary endothelial cells, smooth muscle cells, and chromaffin cells. The $P2Y_{12}$ knockout mice that have been generated display the phenotype of clopidogrel-treated animals.

$P2Y_{13}$ receptors The human, mouse, and rat $P2Y_{13}$ receptors have been identified and characterized. ADP and Ap_3A are naturally occurring agonists of the $P2Y_{13}$ receptor. The $P2Y_{13}$ receptor is primarily coupled to a $G_{i/o}$ protein. However, cangrelor, which is an antagonist of the $hP2Y_{12}$ receptor, is also an antagonist of the human and rat $P2Y_{13}$ receptors. Recently MRS2211, a derivative of PPADS, was shown to selectively antagonize the human $P2Y_{13}$ receptor. The $P2Y_{13}$ receptor is strongly expressed in the spleen, followed by placenta, liver, heart, bone marrow, monocytes, T cells, lung, and various brain regions. $P2Y_{13}$-null mice have been generated recently, but no phenotype has been characterized to date.

$P2Y_{14}$ receptors From a phylogenetic and structural point of view, the $P2Y_{14}$ receptor (previously known as GPR105 or UDP-glucose receptor) is 47% identical to the $P2Y_{12}$ and $P2Y_{13}$ receptors. The gene for this receptor has been found in human chromosome 3q24-3q25, where a cluster of other related GPCRs, consisting of $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors and the orphan receptors GPR87, GPR91, and H963, have been found. The $P2Y_{14}$ receptor couples to the $G_{i/o}$ family of G-proteins and is activated by

UDP-glucose as well as UDP-galactose, UDP-glucuronic acid, and UDP-*N*-acetylglucosamine. At present, no selective antagonists are available. $P2Y_{14}$ mRNA is widely distributed in the human body. Both chemoattractant and neuroimmune functions have been claimed for the $P2Y_{14}$ receptor.

Receptor Dimerization and Cross-Talk

It is now recognized that interactions between GPCRs can take place through the formation of oligomers, or downstream of the receptor through the action of second messengers. The former process is commonly referred to as receptor dimerization. The latter process is known as receptor cross-talk.

There is evidence that the human $P2Y_2$ receptor forms homodimers. An example of dimerization involving P2Y receptors with non-P2Y receptors is rat $P2Y_1$ and adenosine A_1 receptors co-expressed in HEK293 cells. It has also been shown that the $P2Y_1$ and A_1 receptors are co-localized in neurons of the rat cortex, hippocampus, and cerebellum. The formation of oligomers by P2Y receptors is likely to be widespread and to greatly increase the diversity of purinergic signaling. P2X receptors and P2Y receptors are often expressed in the same cells. Thus, there is the possibility of bidirectional cross-talk between these two families of nucleotide-sensitive receptors. For example, the $P2X_1$ receptor may have a priming role in activation of $P2Y_1$ receptors during platelet stimulation.

See also: Adenosine; Adenosine Triphosphate (ATP); Adenosine Triphosphate (ATP) as a Neurotransmitter; Adenosine Receptor Mediated Functions; Calcium Waves: Purinergic Regulation; P2X Receptors; Pharmacology of Sleep: Adenosine; Purinergic Receptors.

Further Reading

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