

P2 Receptors

Key References

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Overview

The surface receptors for extracellular nucleotides are called P2 receptors. Previously they were called P2 purinoceptors, but it is now realized that some P2 receptors are activated by both pyrimidine and purine nucleotides. Thus, the nomenclature has changed to reflect the varied nature of ligands for P2 receptors. The current nomenclature system for P2 receptors was first proposed by Abbracchio and Burnstock and is based on molecular structure and signal transduction mechanisms, so defining families of ionotropic P2X receptors (ligand-gated ion channels) and metabotropic P2Y receptors (G protein-coupled receptors).

Members of the existing family of ionotropic P2X₁₋₇ receptors show a subunit topology of i) intracellular N- and C-termini possessing consensus binding motifs for protein kinases, ii) two transmembrane spanning regions (TM1 and TM2), the first involved with channel gating and second lining the ion pore, iii) large extracellular loop, with 10 conserved cysteine residues forming a series of disulphide bridges, iv) hydrophobic H5 region close to the pore vestibule, for possible receptor/channel modulation by cations (magnesium, calcium, zinc, copper and protons ions) and v) an ATP-binding site, which may involve regions of the extracellular loop adjacent to TM1 and TM2. The P2X₁₋₇ receptors show 30-50% sequence identity at the peptide level. The stoichiometry of P2X₁₋₇ receptors is now thought to involve three subunits which form a stretched trimer.

P2X_{1-5,7} receptors can form homomultimeric assemblies by using the same subunits, although in some tissues, P2X

receptors exist as heteromultimeric assemblies (e.g. P2X_{2/3} in nodose ganglia, P2X_{2/6} and P2X_{4/6} in CNS neurons, P2X_{1/5} in some blood vessels). The P2X receptor family shows many pharmacological and operational differences. Agonist potency orders can vary significantly between P2X subtypes, and some (P2X₄ and P2X_{4/6}) are relatively insensitive to known P2 receptor antagonists. The kinetics of activation, inactivation and deactivation also vary considerably amongst P2X receptors. Calcium permeability is high for some P2X subtypes, a property that may be functionally important. The P2X₇ subtype converts to a pore and, in some cases, brings about cell death. Several other P2X receptors (P2X₂, P2X_{2/3}, P2X₄ and P2X_{2/6}) also show time-dependent changes in their ion permeability properties of their intrinsic ion channel.

Metabotropic P2Y₁₋₁₂ receptors are characterized by a subunit topology of i) extracellular N-terminus and intracellular C-terminus, the latter possessing consensus binding motifs for protein kinases, ii) seven transmembrane spanning regions which help to form the ligand docking pocket, iii) high level of sequence homology between some transmembrane spanning regions, particularly TM3, 6 and 7 and iv) structural diversity of intracellular loops and C-terminus amongst P2Y subtypes, so influencing the degree of coupling with G_{q/11}, G_s and G_i proteins. Each P2Y receptor binds to a single heterotrimeric G protein (typically G_{q/11}), although P2Y₁₁ can couple to both G_{q/11} and G_s whereas P2Y₁₂ couples to G_i. P2Y receptors may form homo- and heteromultimeric assemblies under some conditions, and many tissues express multiple P2Y subtypes.

P2Y receptors show a low level of sequence homology at the peptide level (19-55% identical) and, consequently, show significant differences in their pharmacological and operational profiles. Some P2Y receptors are activated principally by nucleoside diphosphates (P2Y_{1,6,12}), while others are activated mainly by nucleoside triphosphates (P2Y_{2,4}). Some P2Y receptors are activated by both purine and pyrimidine nucleotides (P2Y_{2,4,6}), and others by purine nucleotides alone (P2Y_{1,11,12}). In response to nucleotide activation, recombinant P2Y receptors either activate phospholipase C and release intracellular calcium or affect adenylyl cyclase and alter cAMP levels. There is scant evidence to indicate P2Y_{5,9,10} sequences are nucleotide receptors or affect intracellular signaling cascades. Endogenous P2Y receptors show a greater diversity in intracellular signaling and can activate phospholipases A₂, C and D, MEP/MAP kinase, Rho-dependent kinase and tyrosine kinase, as well as coupling both positively and negatively to adenylyl cyclase.

P2X Subtypes (Ion Channel Family)

CURRENT NAME	P2X ₁	P2X ₂	P2X ₃	P2X ₄	P2X ₅	P2X ₆	P2X ₇
ALTERNATE NAME	P2X (SM) ^a	P2X (N) ^b	—	—	—	—	P2Z ^c
STRUCTURAL INFORMATION ^d	399 aa (human)	471 aa (human)	397 aa (human)	388 aa (human)	455 aa (rat)	379 aa (rat) ^e	595 aa (human)
SELECTIVE AGONISTS ^f	α,β -MeATP (M 6517) BzATP (B 6396) HT-AMP PAPET-ATP Ap ₅ A (D 8013)	2-MeSATP (A-023) ATP γ S (A 1388) Ap ₄ A (D 1262)	α,β -MeATP (M 6517) D- β,γ -MeATP (M 7510) HT-AMP PAPET-ATP Ap ₅ A (D 8013)	2-MeSATP (A-023) CTP (C 1506)	2-MeSATP (A-023) dATP (weak) (D 6500)	—	BzATP (B 6396) ATP (A 2383)
SELECTIVE ANTAGONISTS ^g	isoPPADS Ip ₅ I (D 6938) MRS 2159 (M 7684) NF023 (N 8652) NF279 PPNDS (P 2738) Suramin (S 2671) TNP-ATP (T 4193)	RB-2 (R-115) Suramin (S 2671)	isoPPADS Ip ₅ I (D 6938) NF023 (N 8652) Suramin (S 2671) TNP-ATP (T 4193)	BBG (B 5133)	Suramin (S 2671)	—	KN-62 (I 2142) ^h HMA (A 9561) ^h Oxidized-ATP (A 6779) BBG (B 5133) ^h
SIGNAL TRANSDUCTION MECHANISMS ⁱ	Cation channel (pCa ²⁺ /pNa ⁺ ~4)	Cation channel (pCa ²⁺ /pNa ⁺ ~2)	Cation channel (pCa ²⁺ /pNa ⁺ ~4)	Cation channel (pCa ²⁺ /pNa ⁺ ~4)	Cation channel	—	Cation channel/ pore formation
DESENSITIZATION RATE	Rapid	Very slow	Rapid	Slow	Very slow/none	—	None
RADIOLIGANDS OF CHOICE ^j	[³ H]- α,β -MeATP	[³⁵ S]-ATP γ S	[³⁵ S]-ATP γ S	[³⁵ S]-ATP γ S	—	—	—

ABBREVIATIONS

Ap₄A: Diadenosine tetrakisphosphate
Ap₅A: Diadenosine pentakisphosphate
ATP: Adenosine 5'-triphosphate
ATP γ S: Adenosine 5'-O-(3-thiotriphosphate)
BBG: Brilliant blue G
BzATP: 3'-Benzoylbenzoyl adenosine 5'-triphosphate
dATP: 2'-Deoxyadenosine 5'-triphosphate
HMA: Hexamethylene amiloride
HT-AMP: 2-Hexylthioadenosine 5'-monophosphate
Ip₅I: Diinosine pentakisphosphate
isoPPADS: Pyridoxal-5'-phosphate-6-azophenyl-2',5'-disulphonic acid
KN-62: 1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine

2-MeSATP: 2-Methylthioadenosine 5'-triphosphate
 α,β -meATP: α,β -Methylene-adenosine-5'-triphosphate
D- β,γ -meATP: D- β,γ -Methylene-adenosine-5'-triphosphate
MRS 2159: Pyridoxal-5'-phosphate-6-azophenyl-4'-carboxylate
NF023: 8,8'-(carbonylbis(imino-3,1-phenylene carbonylimino)bis(1,3,5-naphthalenetrisulfonic acid))
NF279: 8,8'-(Carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino)bis(1,3,5-naphthalenetrisulfonic acid))
PAPET-ATP: 2-[2-(4-Aminophenyl)ethylthio]adenosine 5'-triphosphate
PPNDS: Pyridoxal-5'-phosphate-6-(2'-naphthylazo-6-nitro-4',8'-disulphonate)
RB-2: Reactive blue 2
TNP-ATP: 2',3'-O-(2,4,6-Trinitrophenyl) adenosine triphosphate

a P2X₁ resembles the P2X receptor in smooth muscle (SM) cells.

b P2X₂ resembles the P2X receptor in neurons (N).

c P2X₇ is the cytolytic P2Z receptor.

d Length of individual subunits in amino acid residues (aa). Functional channels require three subunits, and most subunits can form functional homomultimeric assemblies in expression systems (*Xenopus* oocytes, HEK293 cells, CHO cells). Endogenous P2X receptors also can exist as heteromultimeric assemblies comprising two or three different subunits.

e P2X₆ subunits cannot form a homomultimeric receptor, but can form heteromultimeric ion channels with other subunits (e.g. P2X_{2/6} and P2X_{4/6}).

f There are no truly selective agonists for P2X receptors, although each subtype can be distinguished by potency ratios for ATP and two or more nucleotide agonists. Agonist potency is enhanced under acidic conditions at P2X₂ receptors, but reduced at P2X_{1,3,4,7} receptors.

g There are no truly selective antagonists for P2X subtypes, except for P2X₇ which is blocked by KN-62 and hexamethylene amiloride (HMA). Human P2X₄ receptors are relatively insensitive to known P2 receptor antagonists, and rat P2X₄ even more so, although hP2X₄ can be blocked by BBG. Suramin is particularly effective at P2X₂ receptors under acidic conditions (pH 5.5).

h KN-62 and HMA are more potent at human than rat P2X₇ receptors, and vice versa for BBG.

i P2X_{1,5,7} receptors form intrinsic cation channels that are permeable to Na⁺, K⁺ and Ca²⁺ ions. The permeability properties of some P2X receptors change during prolonged activation. The P2X₇ converts from an ion channel to a pore that is permeable to large molecules (400-900 Da). P2X receptor subtypes can be distinguished by their desensitization rates.

j [³⁵S]ATP γ S should be used in the absence of divalent cations.

FOOTNOTES

P2Y Subtypes (G Protein Family)

CURRENT NAME ^a	P2Y ₁	P2Y ₂	P2Y ₄	P2Y ₆
ALTERNATE NAME	P2Y	P2U	Pyrimidinoceptor	Pyrimidinoceptor
STRUCTURAL INFORMATION	372 aa (human)	376 aa (human)	365 aa (human)	328 aa (human)
SELECTIVE AGONISTS ^e	ADPβS (A 8016) 2-MeSADP (M-152) 2-MeSATP (A-023) PAPET-ATP	UTPγS ATPγS (A 1388)	UTP (U 1006)	UDP (U 4125) UDPβS
SELECTIVE ANTAGONISTS	A3P5PS (A 1651) PPADS (P-178) RB-2 (R-115) MRS 2179 (M 3808) MRS 2279	Suramin (S 2671)	PPADS (P-178) (weak)	RB-2 (R-115) PPADS (P-178) Suramin (S 2671)
SIGNAL TRANSDUCTION MECHANISMS	G _{q/11} (increase IP ₃ /DAG)	G _{q/11} (increase IP ₃ /DAG) possibly G _i	G _{q/11} (increase IP ₃ /DAG) possibly G _i	G _{q/11} (increase IP ₃ /DAG)
RADIOLIGANDS OF CHOICE	[³⁵ S]-dATPαS [³⁵ S]-ADPβS	—	—	—

ABBREVIATIONS

A3P5PS: Adenosine 3'-phosphate 5'-phosphosulphate

ADPβS: Adenosine 5'-O-(2-thiodiphosphate)

CTP: Cytidine 5'-triphosphate

2-MeSADP: 2-Methylthioadenosine-5'-diphosphate

MRS 2179: 2'-Deoxy-N⁶-methyladenosine-3',5'-bisphosphate

MRS 2279: (N)-Methanocarba-N⁶-methyl-2-chloro-2'-deoxyadenosine-3',5'-bisphosphate

PPADS: Pyridoxal-5'-phosphate-6-azophenyl-2',4'-disulphonic acid

UDP: Uridine 5'-diphosphate

UDPβS: Uridine 5'-O-(2-thiodiphosphate)

UTP: Uridine 5'-triphosphate

UTPγS: Uridine 5'-O-(2-thiotriphosphate)

FOOTNOTES

a The P2Y_{1-n} series comprises 14 putative G protein-coupled receptors: P2Y₃ may be an ortholog of P2Y₆; P2Y_{5,9,10} are not yet proven to be functional P2 receptors; P2Y₇ is a LTB₄ leukotriene receptor and was misnamed; another receptor (turkey p2y) may be related to P2Y₄; P2Y₈ (532 aa) was cloned from *Xenopus laevis*, where it occurs mainly in early development during neurogenesis and is activated by CTP (and other nucleoside triphosphates) and weakly antagonized by suramin.

b P2Y₁₂ is the previously named P2Y_{AC} receptor that couples negatively to adenylyl cyclase.

c P2Y_{Ap4A} is a temporary name until the P2D receptor is cloned.

d The P2Y_T receptor is best fitted by a three-receptor model comprising P2Y₁₂ coupled negatively to adenylyl cyclase, P2Y₁ activating phospholipase C and P2X₁ coupled to an ion channel permeable to Na⁺ and Ca²⁺ ions.

e ATP is an agonist at P2Y_{2,8,11}, but a partial agonist and/or antagonist at human P2Y₁. Several subtypes (P2Y_{1,6} and P2Y_{ADP}) are preferentially activated by nucleoside diphosphates.

P2Y Subtypes (G Protein Family) (continued)

CURRENT NAME ^a	P2Y ₁₁	P2Y ₁₂ ^b	P2Y _{Ap4A} ^c
ALTERNATE NAME	P2Y	P2T ^d P2Y _T	P2D
STRUCTURAL INFORMATION	371 aa (human)	342 aa (human) + P2Y ₁ + P2X ₁	—
SELECTIVE AGONISTS ^e	AR-C67085MX dATP (D 6500) BzATP (B 6396)	2-MeSADP (M-152) ADP (A 5285)	Ap ₄ A (D 1262)
SELECTIVE ANTAGONISTS	Suramin (S 2671) RB-2 (R-115)	AR-C67085MX AR-C69931MX C1330-7 2-MeSAMP	Ip ₅ I (D 6938)
SIGNAL TRANSDUCTION MECHANISMS	G _{q/11} (increase IP ₃ /DAG) G _s (increase cAMP)	G _i (cAMP modulation)	G _{q/11} (increase IP ₃ /DAG)
RADIOLIGANDS OF CHOICE	—	β-[³² P]-2-MeSADP	[³ H]-Ap ₄ A

ABBREVIATIONS

AR-C67085MX: 2-Propylthio-D-β,γ-dichloromethylene-ATP

AR-C69931MX: N⁶-[2-(methylthio)-ethyl]-2-(3,3,3-trifluoropropyl)thio-5'-adenylic acid

C1330-7: N¹-(6-Ethoxy-1,3-benzothiazol-2-yl)-2-(7-ethoxy-4-hydroxy-2,2-dioxo-2H-2-⁶benzo[4,5][1,3]thiazolo[2,3-c][1,2,4]thiadiazin-3-yl)-2-oxo-1-ethanesulfonamide

CTP: Cytidine 5'-triphosphate

2-MeSADP: 2-Methylthioadenosine-5'-diphosphate

2-MeSAMP: 2-Methylthioadenosine-5'-monophosphate

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