

P2 Receptors: P2X Ion Channel Family

Key References

- Abbracchio, M.P. and Burnstock, G., Purinoceptors: Are there families of P2X and P2Y purinoceptors., *Pharmacol. Ther.*, **64**, 445-475 (1994).
- Barrera, N.P., et al., Atomic force microscopy imaging demonstrates that P2X₂ receptors are trimers but that P2X₆ receptor subunits do not oligomerize., *J. Biol. Chem.*, **280**, 10759-10765 (2005).
- Buell, G., et al., P2X receptors: an emerging channel family., *Eur. J. Neurosci.*, **8**, 2221-2228 (1996).
- Chessell, I.P., et al., Disruption of the P2X₇ purinoceptor gene abolishes chronic inflammatory and neuropathic pain., *Pain*, **114**, 386-396 (2005).
- Khakh, B.S., Molecular physiology of P2X receptors and ATP signalling at synapses., *Nature Reviews*, **2**, 165-174 (2001).
- Kucenas, S., et al., Molecular characterization of the zebrafish P2X receptor subunit gene family., *Neurosci.*, **121**, 935-945 (2003).
- Mulryan, K., et al., Reduced *vas deferens* contraction and male infertility in mice lacking P2X₁ receptors., *Nature*, **403**, 86-89 (2000).
- North, R.A., Molecular physiology of P2X receptors., *Physiol. Rev.*, **82**, 1013-1067 (2002).
- Roberts, J.A. and Evans, R.J., ATP binding at human P2X₁ receptors. Contribution of aromatic and basic amino acids revealed using mutagenesis and partial agonists., *J. Biol. Chem.*, **279**, 9043-9055 (2004).
- Silberberg, S.D., et al., Secondary structure and gating rearrangements of transmembrane segments in rat P2X₄ receptor channels., *J. Gen. Physiol.*, **125**, 347-359 (2005).
- Torres, G.E., et al., Hetero-oligomeric assembly of P2X receptor subunits. Specificities exist with regard to possible partners., *J. Biol. Chem.*, **274**, 6653-6659 (1999).
- Yan, Z., et al., Molecular determinants of the agonist binding domain of a P2X receptor channel., *Mol. Pharmacol.*, **67**, 1078-1088 (2005).

Overview

Once known as P2 purinoceptors, surface receptors for extracellular nucleotides are now called P2 receptors. This subtle change in nomenclature reflects the more varied nature of nucleotidic ligands, other than those containing a purine moiety, that are capable of activating these surface receptors. The current nomenclature system for P2 receptors is also based on their molecular structure and signal transduction mechanisms, so defining a family of ionotropic P2 receptors (P2X Ligand-Gated Ion Channels, LGICs) and another family of metabotropic P2 receptors (P2Y G Protein-Coupled Receptors, GPCRs).

The seven subunits forming P2X receptors show a common topology of i) intracellular N- and C-termini which possess consensus binding motifs for protein kinases, ii) two transmembrane spanning regions (TM1 and TM2) which line the ion pore, iii) a large extracellular loop, possibly folded into a β -sheet, with 10 conserved cysteine residues forming a series of disulphide bridges, iv) a hydrophobic H5 region close to the pore vestibule, for receptor/channel modulation by extracellular cations (e.g. magnesium, calcium, zinc, copper and hydrogen ions) and v) an ATP-binding site, which involves amino acid residues in the extracellular loop immediately adjacent to TM1 and TM2. The P2X₁₋₇ receptor subunits show 30-50% sequence identity at the peptide level. The stoichiometry of assembled P2X₁₋₇ receptors is thought to involve three subunits, arranged as a stretched trimer.

Triplets of identical P2X subunits form homomeric assemblies and produce the P2X₁₋₇ receptors. Additionally, some P2X receptors exist as heterotrimeric assemblies (e.g. P2X_{1/2} in SCG neurons, P2X_{2/3} in sensory ganglia, P2X_{2/6} and P2X_{4/6} in CNS

neurons, P2X_{1/5} in some blood vessels). Members of this extended P2X family of homomeric and heteromeric assemblies show many pharmacological and operational differences. For example, the potency orders for nucleotidic agonists vary significantly between P2X receptor subtypes. With blocking agents, some P2X subtypes (P2X₄ and P2X_{4/6}) are relatively insensitive to potent antagonists of other P2X receptor subtypes. Agonism and antagonism at some P2X receptors, notably P2X₂ and P2X_{2/3}, are affected by extracellular H⁺ ions. The kinetics of activation, inactivation and deactivation vary considerably among P2X receptor subtypes. Calcium permeability is high for some P2X receptor subtypes, amounting to some 6-10% of the ionic current carried. The P2X₇ receptor converts from an ion channel to a pore and, in some cases, this conversion brings about cell death. Other P2X receptors (P2X₂, P2X_{2/3}, P2X₄ and P2X_{2/6}) show reversible and time-dependent changes in the ion permeability properties of their intrinsic ion channel but here, this phenomenon is not as profound as seen with P2X₇.

The loci of the P2X₁₋₇ genes have been defined in the genome of human, rat and, importantly, mice. Some P2X receptors also have been identified from cDNA libraries of chimp, dog, guinea-pig, frog and zebrafish, as well from invertebrates such as *Schistosoma mansoni*. Notably, the zebrafish genome contains nine P2X genes of which two are paralogues for P2X₃ and P2X₄ receptors. All P2X genes show a complex organization, with the encoding sequences interrupted by up to 14 introns. Occasionally, errors occur in P2X gene transcription, which yield splice variants that are either dominant negative, modulatory or functionless. Using mice, gene deletion

has been achieved for P2X₁, P2X₂, P2X₃ and P2X₇ receptors. None are lethal, but P2X₁-null homozygote males are infertile. Disruption of either P2X₂ or P2X₃ genes causes hyporeflexia, whereas knock-out of the P2X₇ gene has a profound effect on neuropathic pain.

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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)	444 aa (human)	379 aa (rat), ^e	595 aa (human)
SELECTIVE AGONISTS ^f	2-MeSATP (A023), α,β-MeATP (M6517), L-β,γ-MeATP, HT-AMP, PAPET-ATP, Ap ₅ A (D8013), CTP (C1506)	2-MeSATP (A023), ATPγS (A1388), Ap4A (D1262)	2-MeSATP (A023), α,β-MeATP (M6517), D-β,γ-MeATP (M7510), HT-AMP, PAPET-ATP, Ap ₅ A (D8013), UTP (U1006)	2-MeSATP (A023), ATPγS (A1388), CTP (C1506)	2-MeSATP (A023), α,β-MeATP (M6517), BzATP (B6396), GTP (G5884)	α,β-MeATP (M6517)	2-MeSATP (A023), BzATP (B6396)
SELECTIVE ANTAGONISTS ^g	isoPPADS, IpsI (D6938), MRS 2159 (M7684), NF023 (N8652), NF279, NF449 (N4784), Phenol red (P4758), PPNDS (P2738), Suramin (S2671), TNP-ATP (T4193)	NF279, PPADS (P1599), RB-2 (R115), Suramin (S2671), TNP-ATP (T4193)	A317491 (A2979), isoPPADS, IpsI (D6938), NF023 (N8652), Phenol red (P4758), Suramin (S2671), TNP-ATP (T4193)	BBG (B5133), Phenolphthalein (P9750), TNP-ATP (T4193)	BBG (B5133), PPADS (P1599), Suramin (S2671), TNP-ATP (T4193)	isoPPADS, TNP-ATP (T4193)	KN-62 (I2142), ^h KN-04, ^h HMA (A9561), ^h BBG (B5133), ^h Chelerythrine (C2932), Oxidized-ATP (A6779)
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 (pCa ²⁺ /pNa ⁺ 1.5)	Cation channel	Cation channel/ pore formation
DESENSITIZATION RATE	Rapid	Very slow	Rapid	Slow	Very slow	Very slow	None
RADIOLIGANDS OF CHOICE ^j	[³ H]-α,β-MeATP	[³⁵ S]-ATPγS	[³ H]-A317491	[³⁵ S]-ATPγS	Not known	[³ H]-α,β-MeATP	Not known
TISSUE EXPRESSION	Blood vessels, Urogenital Smooth muscle, platelets, heart, cerebellar neurons	DRG neurons, myenteric neurons, symp. ganglia, CNS neurons, retina, cochlea	DRG neurons, myenteric neurons, symp. ganglia, heart, cochlea	CNS neurons, myenteric neurons, symp. ganglia, smooth muscle, epithelia, microglia	Sensory neurons, spinal motoneurons, symp. ganglia, heart, thymus	CNS neurons, sensory neurons, symp. ganglia, epithelia	Immune cells, mast cells, osteoclasts
PHYSIOLOGICAL FUNCTION	Depolarization, calcium channel	Depolarization, pH sensor, ROS sensor, transmitter release, sensory transduction	Depolarization, calcium channel, transmitter release, sensory transduction,	Depolarization, calcium channel, transmitter release, secretion	Modulatory subunit	Modulatory subunit	Inflammation, cell death, MC degranulation
DISEASE RELEVANCE	Infertility, bleeding disorders	Deafness, hyporeflexia	Pain, hyporeflexia	Allodynia, cholestasis/gallstones	Not known	Not known	Osteoporosis, inflammation/pain

FOOTNOTES

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Abbreviations

A-317491: [3-Phenoxybenzyl-(1,2,3,4-tetrahydro-naphthalene-1-yl)-carbamoyl]-benzene-1,2,4-tricarboxylic acid
Ap₄A: Diadenosine tetraphosphate
Ap₅A: Diadenosine pentaphosphate
ATP: Adenosine 5'-triphosphate
ATP_γS: Adenosine 5'-O-(3-thiotriphosphate)
BBG: Brilliant blue G
BzATP: 3'-Benzoylbenzoyl adenosine 5'-triphosphate
CTP: Cytidine 5'-triphosphate
GTP: Guanosine 5'-triphosphate
HMA: Hexamethylene amiloride
HT-AMP: 2-Hexylthioadenosine 5'-monophosphate
Ip5I: Diinosine pentaphosphate
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
L-β,γ-MeATP: L-β,γ-Methylene-adenosine-5'-triphosphate
MRS 2159: Pyridoxal-5-phosphate-6-azophenyl-4'-carboxylate
MRS 2500: (N)-Methanocarpa-N6-methyl-2-iodo-2'-deoxyadenosine-3',5'-bisphosphate
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)
NF449: 8,8'-(Carbonylbis(imino-5,1,3-benzenetriylbis(carbonylimino))tetrakis-benzene-1,3-disulphonic acid)
PAPET-ATP: 2-[2-(4-Aminophenyl)ethylthio]adenosine 5'-triphosphate
Phenol red: Phenolsulfonphthalein sodium salt
Phenolphthalein: 3,3-bis[4-Hydroxyphenyl]-1(3H)-isofuranone
PPADS: Pyridoxal-5-phosphate-6-azophenyl-2',4'-disulphonic acid
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
UTP: Uridine 5'-triphosphate

FOOTNOTES

- 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 all subunits can form functional homotrimeric assemblies in expression systems (*Xenopus* oocytes, HEK293 cells, CHO cells). Endogenous P2X receptors also can exist as heterotrimeric assemblies comprising two or three different subunits.
e P2X₆ subunits cannot efficiently form a homomultimeric receptor as these subunits are mostly retained in the ER, but they can readily 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 which are all activated by ATP, 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, KN-04 and HMA are more potent at human than rat P2X₇ receptors, and vice versa for BBG.
i P2X₁₋₇ 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_γS should be used in the absence of divalent cations.