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Topical Review

Cloned Ligand-gated Channels Activated by Extracellular ATP (P2X Receptors)

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Introduction

Adenosine 5′ triphosphate (ATP) is copackaged in exocytotic granules and secreted with a number of neurotransmitters and local mediators from many cell types (Whittaker, 1982). Indeed, the exocytosis of ATP evokes fast synaptic potentials in the central and peripheral nervous system (Edwards, Gibb & Colquhoun, 1992; Evans, Derkach & Surprenant, 1992; Silinsky, Gerzanich & Vauner, 1992). ATP can also be released from the cell cytosol via nonsynaptic mechanisms, for example by diffusion after sudden rupture of intact cells by tissue injury. Moreover, cytosolic ATP can be translocated to the extracellular medium by active transporters under hypoxic conditions (Clemens & Forrester, 1981; Forrester & Williams, 1977). Extracellular ATP exerts its diverse effects by binding to membrane proteins termed P2 receptors (Dubyak & El-Moatassim, 1993). P2 receptors have been classified in two families according to amino acid sequence homology and transduction mechanisms: (i) a P2X family consisting of ligand-gated channels, of which seven subunits have been cloned ($P2X_{1-7}$) (Buell, Collo & Rassendren, 1996*b*) and, (ii) a P2Y family consisting of G-protein coupled receptors with eight reported members ($P2Y_{1-8}$) (Burnstock, 1996; Burnstock & King, 1996). Studies on native P2 receptors defined an additional class represented by the P2Z receptor. Its activation leads to the opening of large-conductance nonselective pores which results in cell lysis (Tatham & Landau, 1990). However, heterologously expressed $P2X_7$ receptor presents functional properties that strongly resembles the behavior of native P2Z receptor (Surprenant et al., 1996*a*).

The characterization of cloned P2X receptors constitutes a basic framework for a precise description of their physiological function. The aim of this review is to summarize recent advances in the cloning, functional characterization and tissue distribution of the P2X receptor subunits.

Structural Features of P2X Receptors

The first two P2X receptor subunits were isolated from rat vas deferens smooth muscle ($rP2X_1$) (Valera et al., 1994) and PC12 cells ($rP2X_2$) (Brake, Wagenbach & Julius, 1994) by expression cloning. Five additional members of the P2X family were subsequently cloned from rat tissues ($rP2X_3$ to $rP2X_7$) (Table 1) either by screening of diverse libraries, by polymerase chain reaction (PCR)-techniques or by using a combination of both (*see* Buell et al., 1996*b* and references therein). The global amino acid identity among the different subunits (35–50%) and the lack of homology with other ligand-gated ion channels (North, 1996) indicate that they constitute a new family of membrane receptors.

The molecular characterization of P2X receptors has been further expanded with the cloning of various homologous human cDNAs: $hP2X_1$ (Valera et al., 1995),

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$hP2X_3$ (Garcia-Guzman et al., 1997*b*), $hP2X_4$ (Garcia-Guzman et al., 1997*a*; Talabot et al., 1997), and $hP2X_7$ (Rassendren et al., 1997). The human sequences exhibit 89%, 93%, 87% and 80% identity to the putative rat homologue, respectively. Additionally, a human cDNA encoding a protein with 66% identity to the $rP2X_5$ subunit (named $hP2X_5$) has been isolated (Talabot et al., 1997). However, the low amino acid identity with the rat counterpart suggests that it may represent a new member of the P2X family. The mouse homologue of $P2X_1$ has also been isolated and its amino acid sequence shows high identity to the $rP2X_1$ (98%) (Valera et al., 1995). Figure 1 shows the alignment of the amino acid sequences of all known P2X receptors including those cloned from human tissues.

In vertebrates, P2X receptors constitute a new structural class of ligand-gated ion channels (Barnard, 1996), whose putative topology is depicted in Fig. 2. Several lines of evidence support this assumption: (i) the hydrophobicity plot of primary P2X sequences predict two hydrophobic regions long enough to cross the membrane (M1 and M2 in Fig. 2) (Valera et al., 1994; Brake et al., 1994; Buell et al., 1996*b*), (ii) the absence of a leader peptide consensus sequence favors a cytoplasmic localization of the amino- and carboxy-terminal ends, and (iii) the large loop connecting the two transmembrane domains is likely to be located extracellularly, as suggested by mutational studies on the P2X subunits. Those studies have demonstrated that: (i) the sensitivity to the ATP analog, α,β-methylen-ATP (α,βmeATP) can be exchanged between $rP2X_1$ and $rP2X_2$ by swapping the extracellular loop (Werner et al., 1997), (ii) the effect of the antagonists suramin and pyridoxalphosphate-6-azophenyl-2′, 4′-disulfonic acid (PPADS) can be modified by single point mutations of amino acid residues located in the putative extracellular loop (Buell et al., 1996*a*; Collo et al., 1996*a*; Garcia-Guzman et al., 1997*a*) and by chimeric constructions in which domains of the extracellular loop are exchanged between P2X receptors with different antagonist sensitivity (Garcia-Guzman et al., 1997*a*), and (iii) $rP2X_1$ protein is glycosylated in in vitro translation experiments. The asparagine amino acid responsible for this glycosylation has been localized by single point mutation (N184) and lies in the putative extracellular domain (Buell et al., 1996*b*). Interestingly, this glycosylated site is the only one conserved among all of the P2X receptors with the exception of $P2X_5$ (Fig. 1).

Despite the low global amino acid identity between members of the P2X family, some motifs are conserved in all subunits (Fig. 1). There is high sequence conservation of certain areas of the loop connecting the two transmembrane domains. This loop contains a number of conserved amino acids, largely glycines and lysines. The absence of a consensus sequence for the binding of ATP initiated the speculation that these small residues may be implicated in the formation of the ATP binding site (Buell et al., 1996*b*). Additionally, the extracellular loop contains 10 cystein residues that are conserved in all P2X subunits, suggesting a role in maintaining the tertiary structure of the protein, possibly by forming disulfide bridges.

In contrast to other ligand-gated channels, the putative transmembrane domains show a significant variability between the different P2X receptor subunits: one and two amino acids conserved in M1 and M2, respectively. The lack of primary sequence conservation of the P2X subunits putative transmembrane domains will argue against any of them constituting part of the channel pore. However, some evidence indicates that M2, with a sequence in which every third residue is either polar or possessing a small side-chain enabling the formation of an amphipatic α-helix (Brake et al., 1994), could be facing the ion conduction pathway (Rassendren et al., 1996). The C-terminal domain presents no significant sequence conservation indicating that it might provide specific properties. Specifically, the carboxy-terminal of the protein is implicated in the permeability characteristics of homomeric receptors for $rP2X_7$ and $hP2X_7$ (Surprenant et al., 1996; Rassendren et al., 1997). The predicted membrane topology of P2X places the C-terminal domain in the cytosol, allowing interaction with intracellular proteins. For instance, the $rP2X_2$ C-terminal domain presents a proline-rich motif that resembles an SH3 binding domain (Yu et al., 1994).

The precise architecture of any given P2X gene is not known. Nevertheless, the isolation of cDNA sequences corresponding to splicing variants of some genes indicate that the genomic open reading frame is interrupted by intronic sequences. This fact further increases the molecular diversity generated by the cloned P2X subunits by differential splicing of the corresponding pre-mRNAs. In a clone isolated from pituitary and cochlea, an alternated exon inserted downstream of M2 introduces a stop codon and creates a shorter version of $rP2X_2$ (Housley et al., 1995). One additional splicing variant of $hP2X_1$ lacking a portion of the putative extracellular domain (amino acid 176 to 193) but otherwise preserving the consensus open reading frame has been

Fig. 1. Amino acid sequence alignment of the cloned rat and human P2X receptor subunits. The alignment was made using the LaserGene-Megalin software (DNAstar, Madison, WI). Identical amino acids are indicated by shaded boxes and conserved amino acids are depicted inside boxes. The two putative transmembrane segments (M1 and M2) are overlined. Filled circles above the alignment indicate the ten conserved cystein residues and the square marks the position of the conserved glycosylation site.

Fig. 1

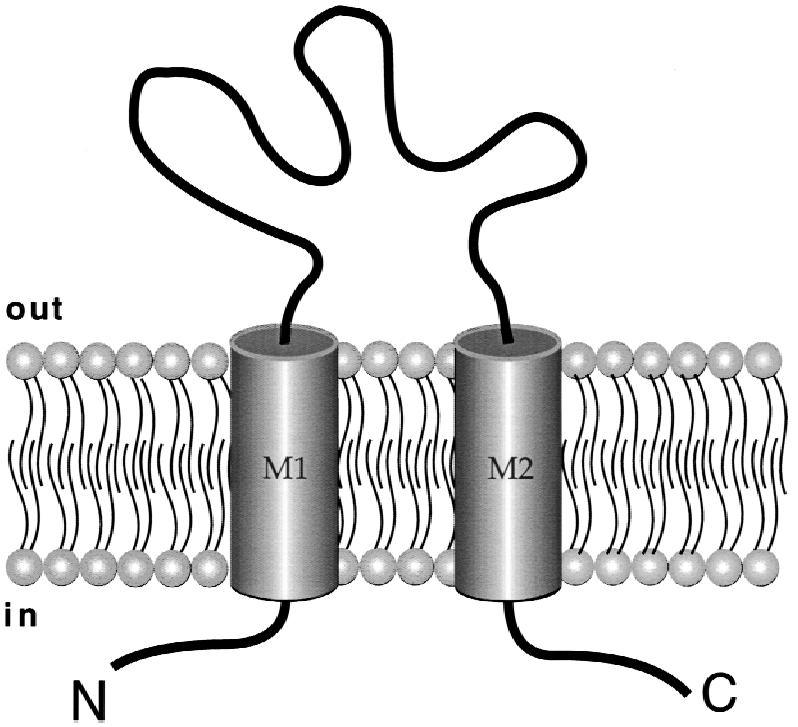

Fig. 2. Schematic representation of the putative two-transmembrane domain topology of P2X receptors.

isolated from human heart (F. Soto and M. Garcia-Guzman, *unpublished observations*).

Chromosomal Localization of Human P2X Receptor Genes

The genes for the cloned human P2X receptors have been mapped to three different chromosomes. The $hP2X_3$ gene is localized in chromosome 11 region q12(Garcia-Guzman et al., 1997*b*). Some of the P2X genes occur in clusters in a similar fashion to several nAChR and GABAR subunit genes (Boulter et al., 1990; McLean et al., 1995). Indeed, the $hP2X_5$ gene has been located in chromosome 17p13.3 (F. Talabot et al., *in preparation*) where the locus for $hP2X_1$ was previously described (Valera et al., 1995). The $hP2X_4$ and $hP2X_7$ genes colocalize in chromosome 12p24 (Garcia-Guzman et al., 1997*b*; Talabot et al., 1997). Attempts to correlate possible mutations in P2X receptors with human diseases have been made. Radiation hybridization mapping has linked the localization of $hP2X_1$, $hP2X_4$, $hP2X_5$ and $hP2X_7$ to several polymorphic markers (Talabot et al., 1997) and thus to some inherited human disorders. However, a detailed analysis of family pedigrees must be performed before any P2X gene mutation may be assigned to a specific human pathology.

Functional Characterization of Heterologously Expressed P2X Receptors

Individual P2X subunits form functional ligand-gated cationic channels of unknown stoichiometry when expressed in heterologous systems. However, the efficiency in forming a functional P2X receptor differs from one subunit to another. For instance, transient transfection of $rP2X_6$ cDNA renders a low percentage of cells expressing functional receptors (1–5%) (Collo et al., 1996*a*) and the expression of an ATP-gated receptor was not detected after injection of the corresponding RNA in *Xenopus* oocytes (Soto et al., 1996*b*). Transiently transfected cell lines expressing $rP2X_5$ produced currents that were 5–10% of the peak currents detected with any other P2X subunit (Collo et al., 1996*a*; Garcia-Guzman et al., 1996). The low amount of current elicited after stimulation of $rP2X_5$ can be also explained if the homomeric receptor has a low open probability or single-channel conductance. Indeed, native P2X receptors with a single-channel conductance of less than 1pS have been reported (Bean, 1992). Simultaneous expression of two different P2X subunits has been attempted for all combinations of the $rP2X_1$-$rP2X_4$ subunits. Only the coexpression of $rP2X_2$ and $rP2X_3$ cDNAs results in ligand-gated channels with functional and pharmacological properties that can solely be explained by the heteropolymerization of both subunits (Lewis et al., 1995). The resulting phenotype has electrophysiological properties that strongly resemble the behavior described for fast activated P2 receptors in native sensory neurons from adult rat (Lewis et al., 1995).

P2X receptors activate with a latency of a few milliseconds and the current rise time varies between 2 and 25 msec depending on the specific subunit tested and the concentration of agonist applied (Surprenant et

al., 1996*b*; Collo et al., 1996*a*). In the continuous presence of the agonist, the currents elicited by heterologously expressed $P2X_1$ and $P2X_3$ receptors decline rapidly. $P2X_1$ desensitization can be fitted to a monoexponential decay (τ = 100–300 msec) whereas $P2X_3$ desensitization kinetics are biexponential with decay constants of 50 msec and 1 sec (Collo et al., 1996*a*). For $P2X_3$, the degree of desensitization increases with the concentration of ATP while for $P2X_1$ the amount of desensitization is almost independent of the amount of agonist applied (Werner et al., 1997).

On the other hand, ATP evokes sustained currents at $P2X_2$ receptors (Brake et al., 1994; Evans et al., 1996) and $P2X_5$ receptors (Collo et al., 1996*a*; Garcia-Guzman et al., 1996) even during agonist applications lasting tens of seconds (Brake et al., 1994). $P2X_4$ and $P2X_6$ receptors show an intermediate phenotype, with approximately 60% of the peak current remaining after a 2–sec application of ATP to transfected mammalian cells (Collo et al., 1996*a*).

The structural domains involved in the desensitization of $P2X_1$ and $P2X_3$ receptors have been localized by engineering chimeric constructs of $rP2X_1$, $rP2X_3$ and $rP2X_2$ subunits. Desensitization can be introduced in $rP2X_2$ by replacing the putative transmembrane domains (M1 and M2) in combination with 11–15 amino acids towards the N- and C-terminal end with the equivalent $rP2X_1$ or $rP2X_3$ sequence (Werner et al., 1997). The mechanisms governing the desensitization of P2X receptors are not completely understood although recent data indicates that its kinetics might be a regulated process. Thus, removal of extracellular Ca^{2+} completely abolishes $rP2X_3$ desensitization (King et al., 1997). Injection of calcineurin inhibitory peptide decreases desensitization on consecutive applications of ATP. It has been proposed that Ca^{2+}, entering through $rP2X_3$ will produce desensitization through calcineurin-mediated dephosphorylation of N-terminal residues that are phosphorylated on $rP2X_3$ receptors under resting conditions (King et al., 1997).

Heterologously expressed P2X receptors do not strongly distinguish between monovalent cations, in a similar fashion to that already described for native systems (Edwards & Gibb, 1993). The Ca^{2+} permeability of several P2X subunits has been determined by reversal potential displacement when the extracellular concentration of the divalent ion was changed. Thus, $rP2X_1$ and $hP2X_1$ (Valera et al., 1994; Evans et al., 1995), $rP2X_3$ (Lewis et al., 1995) and $rP2X_4$ and $hP2X_4$ (Soto et al., 1996*a*; Garcia-Guzman et al., 1997*a*) present a permeability ratio (P_{Ca}/P_{Na}) of approximately 4. The Ca^{2+} permeability of $rP2X_2$ is lower (P_{Ca}/P_{Na} = 2.2) (Evans et al., 1996). In addition, Ca^{2+} strongly inhibits the currents evoked by ATP at $rP2X_2$ receptor at relatively low concentrations (2 mM), possibly by allosteric modulation of the ATP binding affinity (Evans et al., 1996). However, flickery block of native P2X receptors by Ca^{2+} has been reported (Nakazawa et al., 1996). Single-channel analysis on $rP2X_2$ transfected mammalian cells will be required to solve the nature of the Ca^{2+} effect.

A quantitative estimation of the amount of current carried by Ca^{2+} through $hP2X_4$ stable transfected HEK cells was obtained by applying a combination of patch-clamp techniques and Ca^{2+} influx measurements using the Ca^{2+} sensitive dye Fura-2 (Garcia-Guzman et al., 1997*a*). Under physiological ionic conditions, the percentage of the current carried by Ca^{2+} is 8%. Using similar conditions, a value of 6.5% was reported for native ATP currents in sympathetic neurons from rat superior cervical ganglia (Rogers & Dani, 1995). Among the ligand-gated channels, a higher Ca^{2+} fractional current has been only reported for some subunit combinations of NMDA receptors (8–12%) (Burnashev et al., 1995; Rogers & Dani, 1995). Influx of Ca^{2+} is of particular interest because it activates second messenger systems. Moreover, by way of its Ca^{2+}-permeable receptor channel, ATP may play a role in neuronal toxicity similar to glutamate through NMDA receptors with the additional characteristic that membrane depolarization is not necessary to produce the influx of Ca^{2+} (Edwards, 1996).

The amount of ATP necessary to elicit a half maximal response (EC_{50}) varies between submicromolar concentrations ($P2X_1$ and $P2X_3$) and low micromolar concentrations ($P2X_2$, $P2X_4$-$P2X_6$) (Table 2). The calculated Hill coefficient ranges between 0.9 and 1.4. However, a Hill coefficient of 2 was reported for homomeric $rP2X_2$ receptors expressed in *Xenopus* oocytes (Brake et al., 1994) as well as for native P2X receptors from superior cervical ganglia (Khakh, Humphrey & Surprenant, 1995). ATP is not a full agonist at $P2X_7$, the EC_{50} concentrations being 100 μM and 1 mM for the $rP2X_7$ and $hP2X_7$, respectively. The most widely used P2X agonist in in vivo studies, α,βmeATP mimics the action of ATP on $P2X_1$ and $P2X_3$ subunits. However, it is very ineffective as an agonist for the remaining subunits tested (Table 2).

The existence of P2X receptors insensitive to antagonists was not described before the $rP2X_4$ and $rP2X_6$ receptors were cloned and led the search of antagonist-insensitive ATP-activated ligand-gated channels in native rat tissues. Electrophysiological techniques have been utilized to demonstrate that epithelial cells from the rat submandibular gland contain P2X receptors that are not blocked by suramin or PPADS (Buell et al., 1996*a*). Two different behaviors can be observed between P2X receptors which are sensitive to PPADS (IC_{50} = 1–5 μM) (Table 2). $P2X_1$ and $P2X_2$ receptors are blocked by PPADS in a pseudoirreversible fashion, several minutes (15–30 min) of perfusion with control solution being necessary before the blocking action is completely re-

Table 1. Structural properties of the cloned P2X subunits

Gene	Species	Tissue of cloning	Length (aa)	Potential N-glycosylation sites	Genbank/EMBL accession number	Reference
$P2X_1$	Rat	Vas deferens	399	5	X80477	Valera et al., 1994
	Human	Urinary bladder	399	5	X83688	Valera et al., 1995
	Human	Heart*	348	5		
	Mouse	Urinary bladder	399	5	X84896	Valera et al., 1995
$P2X_2$	Rat	PC12 cells	472	3	U14414	Brake et al., 1994
	Rat	Pituitary*	361	3		Housley et al., 1995
$P2X_3$	Rat	Dorsal root ganglia	397	4	X90651	Chen et al., 1995
	Rat	Dorsal root ganglia	397	4	X91167	Lewis et al., 1995
	Human	Heart	397	4	Y07683	Garcia-Guzman et al., 1997*b*
$P2X_4$	Rat	Hippocampus	388	5	X91200	Bo et al., 1995
	Rat	Superior cervical ganglia	388	5	X87763	Buell et al., 1996
	Rat	Brain	388	5	U32497	Seguela et al., 1996
	Rat	Brain	388	5	X93565	Soto et al., 1996*a*
	Rat	Pancreatic islets	388	5	U47031	Wang et al., 1996
	Human	Brain	388	5	Y07684	Garcia-Guzman et al., 1997*a*
	Human	Placenta	388	5		Talabot et al., 1997
$P2X_5$	Rat	Celiac ganglia	455	3	X92070	Collo et al., 1996
	Rat	Heart	455	3	X97376	Garciz-Guzman et al., 1996
	Human	Fetal brain	398	2		Talabot et al., 1997
$P2X_6$	Rat	Superior cervical ganglia	379	3	X92070	Collo et al., 1996
	Rat	Brain	379	3	X97376	Soto et al., 1996*b*
$P2X_7$	Rat	Autonomic ganglia	595	6	X95882	Surprenant et al., 1996
	Human	Monocytes	595	6	Y09561	Rassendren et al., 1997

* splicing variants

moved (Evans et al., 1995). In contrast, block by PPADS of $rP2X_3$ and $rP2X_5$ is rapidly washed out (<3 min). Mutation of a single amino acid residue (E249 of $rP2X_4$, L251 of $rP2X_6$) to lysine renders the $rP2X_4$ and $rP2X_6$ subunits sensitive to PPADS with the slow recovery from the block typical of $rP2X_2$ and $rP2X_1$ (Buell et al., 1996*a*, Collo et al., 1996*a*). However, when the reciprocal mutation was performed in $rP2X_2$ (K246E) the block was rapidly reversed but the homomeric receptor was still sensitive to PPADS (Buell et al., 1996*a*). In contrast, the block by suramin was rapidly reversible for all the P2X subunits and the single point mutations discussed above did not change the IC_{50} (Collo et al., 1996*a*; Buell et al., 1996*b*), suggesting that other structural domains of the protein are implicated in the suramin block.

The blocking efficiency of both antagonists for $hP2X_1$ and $hP2X_3$ is in the same range of the published IC_{50} for the rat homologues (Table 2). This is not the case for all the cloned human P2X receptors. Thus, there are marked differences between $hP2X_4$ and $rP2X_4$ sensitivity to antagonists, the human homologue being more efficiently blocked by either compound. The sensitivity to PPADS can be reversed when a portion of the putative extracellular domain (amino acid 81 to 183) is exchanged between both proteins. The only lysine residue of $hP2X_4$ in this domain (K127), which is not conserved in $rP2X_4$, is not responsible for the differing effect of PPADS (Garcia-Guzman et al., 1997*a*).

P2X Receptors are Regulated by Different Agents

Zn^{2+} is contained in presynaptic vesicles from neurons of diverse regions of the brain and is released to the extracellular space upon nerve stimulation (Frederikson, 1989). As has been shown for native responses (Li et al., 1993; Cloues, Jones & Brown, 1993), modulation of heterologously expressed P2X receptors by Zn^{2+} has been described for $rP2X_2$ (Brake et al., 1994), $rP2X_4$ (Soto et al., 1996*a*; Séguéla et al., 1996) and $hP2X_4$ (Garcia-Guzman et al., 1997*a*). At low micromolar concentrations (between 5 and 10 μM), Zn^{2+} increases the affinity for ATP but does not alter the maximal response. At higher concentrations of Zn^{2+} (mM), a voltage-independent block of the currents through $hP2X_4$ receptors has been described (Garcia-Guzman et al., 1997*a*). Interestingly, $hP2X_3$ is blocked by Zn^{2+} but the affinity for ATP is not increased by the divalent cation (M. Garcia-Guzman, *unpublished observations*). In dissociated neurons from rat nodose ganglia, two types of responses of the ATP-activated currents to Zn^{2+} have been found. In the majority of neurons, Zn^{2+} potentiates the response to ATP whereas in a subset of neurons, the ATP-

Table 2. Functional characteristics of heterologously expressed P2X subunits

P2X receptor	ATP EC_{50} (μM)	Agonist rank order of potency	Antagonist IC_{50} (μM)		Reference
			Suramin	PPADS	
$rP2X_1$	1*	2MeSATP ⩾ ATP > αβmeATP > ADP	<10	<30	Valera et al., 1994
$hP2X_1$	0.8*	ATP = 2MeSATP = 2ClATP > αβmeATP	1–5	1–5	Evans et al., 1995
$rP2X_2$	60*	ATP ⩾ 2MeSATP >> CTP (ββmeATP ins.)			Brake et al., 1994
	10**	2ClATP = 2MeSATP ⩾ ATP >> ADP (αβmeATP ins.)	1–5	1–5	Evans et al., 1995
$rP2X_3$	0.5**	ATP ⩾ 2MeSATP > ATPγS >> ADP	3	1.5	Lewis et al., 1995
	1.2*	2MeSATP >> ATP > αβmeATP > ATPγS > CTP			Chen et al., 1995
$hP2X_3$	0.8*	2MeSATP ⩾ ATP > αβmeATP > CTP > ADP	15	2	Garcia-Guzman et al., 1997*b*
$rP2X_4$	10*	ATP > ATPγS > 2MeSATP > ADP = αβmeATP	>50	>50	Bo et al., 1995
	10**	ATP = 2MeSATP >> ADP (αβmeATP ins.)	>100	>100	Buell et al., 1996*a*
	20*	ATP > 2MeSATP ⩾ CTP >> αβmeATP > dATP	<100	<100	Seguela et al., 1996
	7*	ATP > 2MeSATP ⩾ CTP >> αβmeATP > dATP	>500	>100	Soto et al., 1996*a*
	63*	ATP = 2MeSATP >> αβmeATP	<500		Wang et al., 1996
$hP2X_4$	7*	ATP > 2MeSATP ⩾ CTP >> αβmeATP > dATP	180	28	Garcia-Guzman et al., 1997*a*
$rP2X_5$	15*	ATP ⩾ 2MeSATP > ATPγS >> ADP	4	3	Collo et al., 1996
	8*	ATP ⩾ 2MeSATP > AMP ≈ ADP > dATP ≈ βγmeATP	13	7	Garcia-Guzman et al., 1996
$rP2X_6$	12**	ATP ⩾ 2ClATP > 2MeSATP > ATPγS (αβmeATP ins.)	>100	>100	Collo et al., 1996
$rP2X_7$	115**	BzATP >> ATP > 2MeSATP > ATPγS >> ADP	>300	45	Suprenant et al., 1996
$hP2X_7$	1000**	BzATP >> ATP	78	51	Rassendren et al., 1997
$rP2X_2$+$rP2X_3$	1**	ATP >> αβmeATP	2	2	Lewis et al., 1995

* *Xenopus* oocytes

** transiently transfected HEK-293 cells

α,β-methylen-ATP, αβmeATP; β,γ-methylen-ATP, βγmeATP; pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid, PPADS; 2-methylthio-ATP, 2MeSATP; 2′ and 3′-(O)-(4-benzoylbenzoyl)ATP, BzATP, ins., insensitive

activated current is not affected by low concentrations of the divalent cation (Li et al., 1996*a*). This observation together with the existence of P2X subunits insensitive to potentiation by Zn^{2+} strongly argues towards a differential expression of P2X subunits in different neurons of rat nodose ganglia.

Cu^{2+} can be released from cortical and hypothalamic neurons by synaptic stimulation and similar to Zn^{2+} has been shown to potentiate fast responses to ATP in the majority of rat nodose ganglia dissociated neurons (Li, Peoples & Weight, 1996*a*). Both divalent ions seem to allosterically modulate the affinity of some native P2X receptors for ATP by interacting with a common site in the molecule (Li et al., 1996*a*). The effect of Cu^{2+} has not been determined in heterologously expressed homomeric P2X receptors, but it is tempting to speculate that the P2X subunits that are modulated by Zn^{2+} will also be modulated by Cu^{2+}. Furthermore, the extent of potentiation of the ATP response might be controlled by the native P2X receptor subunit composition in different areas of the brain, accordingly modifying the amount of Ca^{2+} entering the neurons upon ATP release.

In rat hippocampus, synaptic transmission also causes a transient acidic pH shift at the synaptic cleft (Chesler & Kaila, 1992). Homomeric receptors composed of $rP2X_2$ subunits have recently been reported to be sensitive to extracellular pH when expressed in *Xenopus* oocytes, with acidosis increasing the affinity of the receptor for ATP (King et al., 1996). Li and coworkers (Li, Peoples & Weight, 1996*b*) described the same effect on native P2X receptors at physiological proton concentrations. The extracellular pH decrease also affects the Zn^{2+} potentiation, decreasing the amount of divalent ions necessary to potentiate the ATP-induced current by tenfold. Therefore, protons can regulate the performance of P2X receptors under physiological and pathological conditions in opposite direction to the pH regulation of NMDA receptors (Traynelis & Cull-Candy, 1990). In addition, a localized extracellular decrease in pH can regulate the effect of Zn^{2+}, and possibly Cu^{2+}, on ATP-gated channels. The possible importance of these mechanisms in synaptic transmission has yet to be determined.

P2X Receptors Localization

Fast responses to ATP have been described in many different tissues and cell lines (Dubyak & El-Moatassim, 1995). Accordingly, at least one of the known members

of the P2X family has been localized in every tissue analyzed making the P2X family the most ubiquitous ligand-gated channels cloned as yet.

The main P2X receptors detected in adult rat CNS by in situ hybridization are $rP2X_4$ (Bo et al., 1995; Séguéla et al., 1996; Buell et al., 1996*a*; Soto et al., 1996*a*) and $rP2X_6$ (Collo et al., 1996*a*; Soto et al., 1996*b*), showing an overlapping distribution in many regions of the brain. Strong hybridization signals have been found in all the regions of the hippocampus, Purkinje and granular cells of the cerebellum, cortex and in brain stem nuclei. The expression of the $rP2X_4$ protein in brain has been confirmed using an antibody against the C-terminus of the protein. Thus, the expression of $rP2X_4$ is confined to postsynaptic membranes with the exception of the olfactory bulb where it is associated to axon terminals (Le et al., 1996). $rP2X_2$ and $rP2X_5$ mRNA have also been found in adult brain although its pattern of expression is limited to restricted areas (Kidd et al., 1995; Vulchanova et al., 1996*a*; Garcia-Guzman et al., 1996; Collo et al., 1996*a*). $rP2X_7$ and $hP2X_7$ receptors transcripts have been found in brain by Northern Blot analysis, but its expression is thought to be confined to microglia cells (Surprenant et al., 1996*b*; Rassendren et al., 1997; Collo et al., 1996*b*).

The overlapping expression pattern of $rP2X_4$ and $rP2X_6$ in CNS is not reproduced in peripheric tissue. $rP2X_6$ was not detected in RNA isolated from thymus, blood vessels and vas deferens (Soto et al., 1996*b*) where $rP2X_4$ (Soto et al., 1996*a*) and $rP2X_1$ (Valera et al., 1994; Vulchanova et al., 1996*a*) are present. The functional and pharmacological characteristics of homomeric $rP2X_1$ receptor mirror the response mediated by P2X receptors in smooth muscle cells (Surprenant et al., 1995). However, $rP2X_4$ has been identified in organs containing smooth muscle tissue and in blood vessels, implying a contribution of this P2X subunit to the fast responses to ATP in those tissues (Soto et al., 1996*b*). $rP2X_1$ expression is enriched in dexamethasone-induced apoptosis in immature thymocytes (Owens, Hahn & Cohen, 1991), and in differentiated promyelocytes (Buell et al., 1996*c*). The significance for an involvement of the $P2X_4$ subunit in apoptosis is unknown.

$rP2X_2$ and $rP2X_4$ subunits have been found in secretory tissues, such as anterior pituitary (Brake et al., 1994; Soto et al., 1996*a*). In pituitary gonadotrophs, ATP gating of native P2X receptors leads to Ca^{2+} influx and release of luteinizing hormone. The pharmacology of the response to ATP indicates an involvement of $rP2X_5$ and/or $rP2X_2$ in the signal transduction process (Tomic et al., 1996). Since $rP2X_5$ mRNA has not been detected by RT-PCR analysis in RNA isolated from anterior pituitary, $rP2X_2$ alone or in combination with $rP2X_4$ induces gonadotropin secretion in pituitary gonadotrophs.

RNA transcripts for $rP2X_3$ receptors are exclusively found in sensory ganglia including dorsal root, nodose and trigeminal ganglia utilizing Northern blot analysis and in situ hybridization techniques (Collo et al., 1996*a*; Chen et al., 1995; Lewis et al., 1995). In dorsal root ganglia, $rP2X_3$ RNA is confined to a subset of rat dorsal root ganglion neurons. Some of them also express nociceptor-associated markers (Chen et al., 1995). The localization of rat $P2X_3$ in nociceptive sensory ganglia neurons indicates that the activation of $P2X_3$ receptors might play an important role in the transmission of pain. However, immunocytochemical studies using an antibody directed against the C-terminal end of $rP2X_3$ detected protein expression in neurons that have small diameter myelinated fibers (Aβ and Aδ) and are not associated directly to nociception (Vulchanova et al., 1996*b*). Further, messenger RNA for $P2X_{1-6}$ subunits has been detected in sensory ganglia and spinal cord (Collo et al., 1996*a*) suggesting a possible role of yet other P2X subunits in pain transmission. Finally, the situation seems to be different for $hP2X_3$. Indeed, $hP2X_3$ clone was isolated from a heart cDNA library and RNA transcripts have been detected in human heart and spinal cord by RT-PCR analysis (Garcia-Guzman et al., 1997*b*).

Activation by ATP released from damaged cells of P2X receptors located on nociceptive terminals would produce a likely explanation to the ATP-induced pain in the human blister model and to the analgesic action of suramin (Brake et al., 1996; Burnstock et al., 1996*b*). However, a more detailed analysis of the distribution of $hP2X_3$ should be performed before any specific antagonist of this P2X subunit can be used for pain relief.

Outlook

Since the expression cloning of the first two P2X receptor subunits just over two years ago, our understanding of the structure and physiology of these ligand-gated channels has greatly improved. Probably the most important finding is the diversity in responses to agonists and antagonists produced by the functionally expressed receptors and the extended distribution of the various P2X receptor subunits in rat tissues. However, the number of questions to be addressed has been growing since the discovery of new P2X genes:

1. Synaptic transmission mediated by ATP has been described in central and pheripheric nervous system. What is the function of P2X receptors in the brain? The direct contribution of P2X receptors to synaptic transmission is limited to its localization in the postsynaptic membrane. Extrasynaptic or presynaptic localization will indicate a role in the regulation of neuronal performance, e.g., by modifying neurotransmitter release. The

cloning of P2X receptors subunits opens the door to the use of specific antibodies to determine the exact localization of the protein.

2. The protein domains and the mechanisms governing desensitization and block are now better understood. However, the site of agonist binding and the ionic pore have not yet been localized. Mutagenesis studies and the discovery of pore blockers will probably help in solving this task in the future.

3. What is the functional significance of the high Ca^{2+} permeability in the various P2X subtypes?

4. What is the stoichiometry and composition of the native P2X receptors? Are there β-subunits associated with the P2X receptors? Which are the domains responsible for the selective formation of possible heterooligomers?

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