

# International Union of Pharmacology. XXIV. Current Status of the Nomenclature and Properties of P2X Receptors and Their Subunits

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**Abstract**—ATP acts as a humoral mediator to control cell function extracellularly. The receptors that mediate the actions of ATP belong to two classes, the metabotropic P2Y receptors and the transmitter-gated, ion channel P2X receptors. This review describes the structure, distribution, function, and ligand recognition characteristics of P2X receptors, which comprise seven distinct subunits that can function as both homo- and

hetero- polymers. The pharmacology of P2X receptors is complicated by marked differences between species orthologues. The current nomenclature is based largely on recombinant receptor studies and detailed knowledge of endogenous P2X receptors in native tissues is limited because of lack of good selective agonists and antagonists for each receptor type.

## I. Introduction

ATP is probably found in every living cell, representing the major energy source among organic phosphate com-

pounds utilized in metabolism (Hinkle and McCarty, 1978; Gibson, 1982). Perhaps because of this, organisms have evolved specialized cell surface receptors to detect ATP

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when it is released extracellularly. These receptors have been collectively called P2 receptors because their endogenous agonist, ATP, is a purine and they are distinct from P1 receptors, which are activated by the other equally ubiquitous, endogenous purine, adenosine (Burnstock and Kennedy, 1985; Fredholm et al., 1994). In turn, P2 receptors can be subdivided into those that are of the ligand-gated ion channel-type, namely P2X receptors, and those that are G protein-coupled, called P2Y receptors (Burnstock and Kennedy, 1985; Fredholm et al., 1994). This review covers the pharmacological properties of recombinant and native P2X receptor types and their nomenclature. Mammalian P2X receptors belong to a family of at least seven proteins (P2X<sub>1</sub>–P2X<sub>7</sub>) that are found throughout the body, are abundantly expressed in the nervous system, underlie fast purinergic synaptic transmission, and are involved in diseases of the nervous system and periphery. This perspective reveals the pharmacological basis by which distinct P2X receptors can be discriminated.

## II. Overall P2X Subunit Topology

Seven P2X subunits (P2X<sub>1</sub>–P2X<sub>7</sub>) define the simplest transmitter-gated ion channel family. Their identities range between 26 to 47%, and each subunit is between

379 and 595 amino acids in length, P2X<sub>6</sub> being the smallest and P2X<sub>7</sub> the longest (see Fig. 1). In terms of numbers, the P2X family is comparable in size to other transmitter-gated cation channels, and 11 different P2X receptor subunit combinations have been studied, but many more heteromeric receptors are possible than have been electrophysiologically characterized. P2X subunits have two transmembrane domains of sufficient length to cross the plasma membrane, placing most of the protein extracellularly (see Fig. 1; Brake et al., 1994; Valera et al., 1994; Newbolt et al., 1998; Torres et al., 1998a; Rettinger et al., 2000). The extracellular loop contains the ATP binding site (Ennion et al., 2000; Jiang et al., 2000) and sites for antagonists and modulators (Buell et al., 1996a; Garcia-Guzman et al., 1997; Clarke et al., 2000). Substituted cysteine accessibility mutagenesis has been used extensively to identify residues that line the channel walls (Rassendren et al., 1997a; Egan et al., 1998). In brief, transmembrane domain 2 (TM2) lines the pore and the narrowest part of the channel pore is probably near a conserved glycine (at position 342 of P2X<sub>2</sub>), about half way through TM2 (Egan et al., 1998).

Site-directed mutagenesis experiments suggest an important role for residues in the C terminal tail of rat

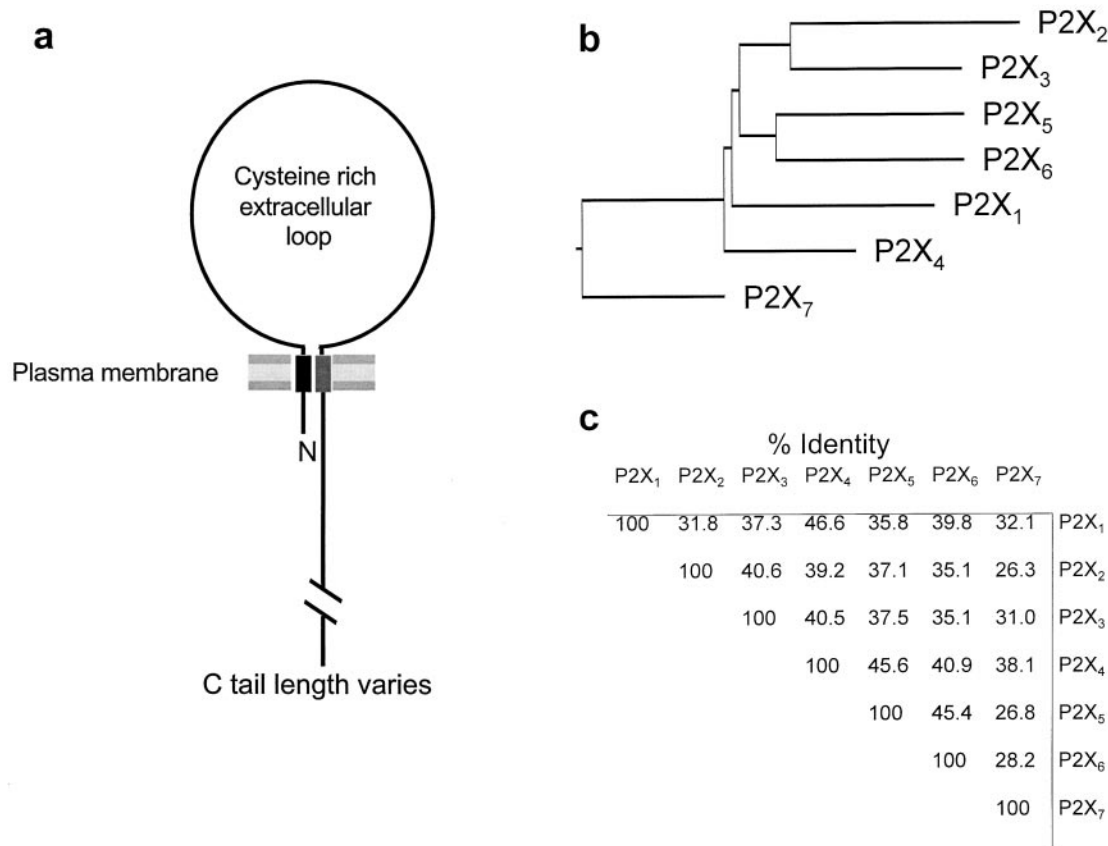


FIG. 1. Topology of, and similarity between, P2X receptor subunits. a, diagrammatic representation of presently understood P2X subunit topology (see text for details); b, relations among the seven rat P2X subunits. The alignment was made using default parameters in DNASTAR (<http://www.dnastar.com/>). The protein sequences were retrieved from the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/Entrez/>) and had the following accession numbers: P2X<sub>1</sub> NP\_037129, P2X<sub>2</sub> s50859, P2X<sub>3</sub> P49654, P2X<sub>4</sub> P51577, P2X<sub>5</sub> P51578, P2X<sub>6</sub> P51579, P2X<sub>7</sub> NP\_062129. c, percent identity between the seven rat P2X subunits determined from alignments performed in DNASTAR.

P2X<sub>2</sub> channels in determining the rate of desensitization (King et al., 1996; Brandle et al., 1997; Simon et al., 1997; Koshimizu et al., 1998, 1999; Smith et al., 1999). The C terminal tails are not the sole determinants of desensitization, and other domains of P2X channels may also contribute (Werner et al., 1996; Boué-Grabot et al., 2000), as one may expect for allosteric proteins.

P2X receptors, like other ion channels, are oligomeric proteins composed of more than one subunit per functional receptor. The number of subunits per receptor—the receptor stoichiometry—is at present unclear; as few as three or four subunits or as many as six may contribute to the receptor, but decisive experiments are required (Kim et al., 1997; Nicke et al., 1998; Stoop et al., 1999). The evidence in favor of a trimer as a minimal unit for P2X receptors is supported by electrophysiological studies (Stoop et al., 1999).

### III. Electrophysiological Properties of P2X Receptors

All P2X receptors are cation-selective channels with almost equal permeability to Na<sup>+</sup>, K<sup>+</sup>, and significant permeability to Ca<sup>2+</sup> (Evans et al., 1996). Quantitative experiments on Ca<sup>2+</sup> permeability are usually performed in two ways: 1) the fraction of the total agonist-evoked current carried by Ca<sup>2+</sup> is determined in physiological solutions, or 2) the permeability of Ca<sup>2+</sup> relative to Na<sup>+</sup> is measured using reversal potentials. Both are valid approaches and have been used for P2X channels (see Table 1). For example, native superior cervical ganglion (SCG<sup>2</sup>) neuron P2X receptors are most like homomeric P2X<sub>2</sub> receptors and carry a fractional Ca<sup>2+</sup> current of ~6.5% in physiological solutions (Rogers and Dani, 1995; Rogers et al., 1997). This is higher than the fractional Ca<sup>2+</sup> current carried by nicotinic,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and kainate receptors (fractional currents of ~4.5, 4, and 2%, respectively) but lower than that of *N*-methyl-D-aspartate receptors, which carry a fractional calcium current of ~12%. Heterologously expressed P2X<sub>1</sub>, P2X<sub>2</sub>, P2X<sub>3</sub>, P2X<sub>4</sub>, P2X<sub>2/3</sub>, and P2X<sub>1/5</sub> receptors are all permeable to Ca<sup>2+</sup> (see Table 1). Ca<sup>2+</sup> permeation through P2X receptors is probably an important component of the physiological responses mediated by P2X receptors in vivo, and perhaps aberrant Ca<sup>2+</sup> entry through P2X receptors can also contribute to P2X receptor-associated pathology. However, it is notable that P2X<sub>2</sub> single channels do not carry a significant Ca<sup>2+</sup> current (Ding and Sachs, 1999a), whereas the macroscopic P2X<sub>2</sub> channel current does (see Table 1).

Calcium ions do have other actions at P2X receptors. Ca<sup>2+</sup> blocks and modulates ATP-evoked currents at endogenously and heterologously expressed P2X receptors

<sup>2</sup> Abbreviations: SCG, superior cervical ganglion; CNS, central nervous system; PPADS, pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid.

TABLE 1  
Known Ca<sup>2+</sup> permeabilities of P2X receptors in relation to some other transmitter-gated cation channels

Receptor	pCa <sup>2+</sup> /pNa <sup>+</sup>	Fractional Ca <sup>2+</sup> Current	Reference
		%	
P2X <sub>1</sub>	~5		Valera et al., 1994; Evans et al., 1996
P2X <sub>2</sub>	2.7	6.5	Rogers and Dani, 1995; Evans et al., 1996
P2X <sub>3</sub>	1.2–4		Lewis et al., 1995; Virginio et al., 1998a
P2X <sub>4</sub>	4.2	8.2	Buell et al., 1961; Soto et al., 1996b; Garcia-Guzman et al., 1997
P2X <sub>2/3</sub>	1.3		Virginio et al., 1998a
P2X <sub>1/5</sub>	1.1		Surprenant et al., 2000
NMDA	10.4	8–12	Burnashev, 1998
AMPA	0.09	1–4	
$\alpha_7$ -Nicotinic	6.6–20		Seguela et al., 1993; Fucile et al., 2000
5HT <sub>3A</sub> <sup>a</sup>	1.08		Davies et al., 1999
5HT <sub>3A/B</sub> <sup>a</sup>	0.62		

AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate.

<sup>a</sup> The value given is pCa<sup>2+</sup>/pCs<sup>+</sup>, and pNa<sup>+</sup>/pCs<sup>+</sup> is 0.8 for 5HT<sub>3A</sub>, and 0.83 for 5HT<sub>3A/B</sub>.

(Nakazawa and Hess, 1993; Evans et al., 1996; Surprenant et al., 1996; Virginio et al., 1997, 1998a, 1999a,b; Cook et al., 1998; Ding and Sachs, 1999b, 2000; Khakh et al., 1999a). The amino acid residue(s) involved in Ca<sup>2+</sup> permeation are probably part of the pore lining segments (Rassendren et al., 1997a; Egan et al., 1998), but the molecular players in Ca<sup>2+</sup> block and modulation of P2X receptors are unknown, and may include residues in the extracellular loop.

Some P2X receptors are also permeable to large organic cations. It has been known for some time that P2Z receptors (P2X<sub>7</sub>) display interesting permeation properties (Cockcroft and Gomperts, 1979; Nuttle and Dubyak, 1994; Surprenant et al., 1996). Ionic selectivity has generally been viewed as fixed, with good evidence for many ion channels (Fox, 1987), but one interesting difference between P2X receptors and other transmitter-gated cation channels is the time-dependent decrease in ionic selectivity for P2X receptors (Surprenant et al., 1996). In fact the change in ionic selectivity occurs with P2X<sub>2</sub>, P2X<sub>4</sub>, and P2X<sub>7</sub> receptors, as measured electrophysiologically and with dye uptake studies (Surprenant et al., 1996, 2000; Khakh and Lester, 1999; Khakh et al., 1999b, 2000b; Virginio et al., 1999a,b), as well as with native P2X receptors studied in the same way. The possible mechanisms for ion-selectivity changes for different ion channels have recently been discussed (Khakh and Lester, 1999).

### IV. Functional Properties of Homomeric Receptors

The pharmacological properties of recombinant homomeric P2X receptors have recently been reviewed (Khakh et al., 2000a; North and Surprenant, 2000; see

Table 2). All seven P2X subunits form functional receptors when expressed alone in various expression systems, but homomeric P2X<sub>6</sub> receptors have been particularly hard to study because of poor and erratic expression (Collo et al., 1996; Le et al., 1998a; Khakh et al., 1999b; Torres et al., 1999; King et al., 2000). Early studies of homomeric P2X receptors identified clear pharmacological distinctions between them, which endure and are now supported by further data. However, distinctions between homomeric P2X receptors based on differences in desensitization are less clear, especially in multicellular preparations and in vivo. We argue against the use of desensitization criteria to define P2X receptor because 1) the underlying mechanisms are poorly understood, 2) channel sub-states may have different kinetics, 3) sub-states may be subject to differential post-translational modification, and 4) desensitization is difficult to measure and quantify over a time-scale of hundreds of milliseconds in multicellular preparations. One initial observation, the finding that P2X<sub>1</sub> and P2X<sub>3</sub> receptors desensitize much more rapidly (within ~1 s in all expression systems) than other homomeric P2X receptors, has been exploited with success to identify heteromeric receptors in single cells (Lewis et al., 1995; Torres et al., 1998b; Haines et al., 1999; Le et al., 1999). Sensitivity to the agonist  $\alpha\beta$ -meATP continues to be an important tool for studying P2X receptors in single cells. Unlike ATP,  $\alpha\beta$ -meATP is resistant to enzymatic degradation (Humphrey et al., 1995; Kennedy and Leff, 1995) and thus can be used to identify P2X receptors in multicellular preparations including whole

animals (Humphrey et al., 1995; Khakh et al., 1995b; Bland-Ward and Humphrey, 1997; McQueen et al., 1998; Kirkup et al., 1999; Tsuda et al., 1999). Weak sensitivity to the ATP receptor antagonists suramin and PPADS2 are diagnostic features of homomeric rat P2X<sub>4</sub> receptors, although important species differences exist (Bo et al., 1995; Buell et al., 1996a; Séguéla et al., 1996; Soto et al., 1996a; Le et al., 1998a). Rat P2X<sub>4</sub> receptors are potentiated by ivermectin (Khakh et al., 1999b), but ivermectin also modulates  $\gamma$ -aminobutyric acid and nicotinic receptors (Krusek and Zemkova, 1994; Krause et al., 1998).

In the absence of potent radioligands, the few binding studies reported have utilized radiolabeled ATP or its structural analogs. However complications due to metabolism have necessitated that such studies be carried out at room temperature or in the absence of divalent cations so comparison with functional data is confounded. Nevertheless it has been possible to label recombinant P2X<sub>1</sub>-P2X<sub>4</sub> receptors using [<sup>35</sup>S]ATP $\gamma$ S and [<sup>3</sup>H] $\alpha\beta$ -meATP (Bo et al., 1992; Michel et al., 1996b, 1997). Although the affinity estimates for agonists are much higher than expected from functional studies (see Humphrey et al., 1998; Chessell et al., 2001), studies with antagonists have provided affinity estimates that are similar in both binding and functional studies (Khakh et al. 1994; Michel et al., 1997). The selective affinity of  $\beta,\gamma$ -methylene-L-ATP was first detected in binding studies, and its selectivity as an agonist for P2X<sub>1</sub> relative to P2X<sub>3</sub> receptors confirmed in functional studies (Trezise et al., 1995; Rae et al., 1998). Radioli-

TABLE 2  
Functional properties of recombinant homomeric P2X receptors

	Homomeric Receptor Type						
	P2X <sub>1</sub>	P2X <sub>2</sub>	P2X <sub>3</sub>	P2X <sub>4</sub>	P2X <sub>5</sub>	P2X <sub>6</sub>	P2X <sub>7</sub>
Agonist EC <sub>50</sub> ( $\mu$ M)							
ATP	1	1–30	1	10	10	12	100
2MeSATP	1	3	0.3	10–100	10	9	10
$\alpha\beta$ -meATP	1–3	>300	1	>300	>300	>100	>300
BZ-ATP	3	30		>500	>500		3
Antagonist IC <sub>50</sub> ( $\mu$ M)							
Suramin	1	10	3	>500	4	>100	500
PPADS	1	1	1	>500	3	>100	50
TNP-ATP	0.006	1	0.001	15			>30
NF023	0.2	>10	8.5	>100			
Calmidazolium							0.065
IP <sub>5</sub> I	0.003	No block	2.8	Potentialiation			
Brilliant Blue G	>5	1.4	>10	>10			0.01
Modulator EC <sub>50</sub> ( $\mu$ M)							
Ivermectin		>30	>30	0.25			>30
Cibacron blue	Block IC <sub>50</sub> ~0.7 $\mu$ M		Potentialiation	Potentialiation and block			
Ion effects							
Zn <sup>2+</sup>		Increase EC <sub>50</sub> 6.9 $\mu$ M		Increase 2 $\mu$ M			Decrease 10 $\mu$ M
H <sup>+</sup>	Decrease pKa 6.3	Increase pKa 7.3	Decrease pKa 6.0	Decrease pKa 6.8			Decrease pKa 6.1
Ca <sup>2+</sup>	No effect >100 mM	Block IC <sub>50</sub> 87 mM	Block IC <sub>50</sub> 15 mM		Decrease 7 mM		Decrease 3 mM

Data are derived from the following references: agonists and antagonists (Valera et al., 1994; Bo et al., 1995; Chen et al., 1995; Lewis et al., 1995; Buell et al., 1996b; Collo et al., 1996, 1997; Séguéla et al., 1996; Soto et al., 1996a,b; Surprenant et al., 1996; Radford et al., 1997; Rassendren et al., 1997b; Virginio et al., 1997; Chessell et al., 1998a; Egan et al., 1998; Le et al., 1998a, 1999; Torres et al., 1998b; Alexander et al., 1999; Haines et al., 1999; King et al., 1999; Townsend-Nicholson et al., 1999; Boue-Grabot et al., 2000; Dunn et al., 2000; Jones et al., 2000); modulators (Miller et al., 1998; Khakh et al., 1999b); ionic effects (Stoop et al., 1997; Virginio et al., 1997, 1998a; Wildman et al., 1997, 1998, 1999; Ding and Sachs, 1999b, 2000; Xiong et al., 1999); and species differences—note marked species differences between various orthologues for both agonist and antagonist potencies (see text and Chessell et al., 1998a,b; Hibell et al., 2000; Jones et al., 2000).



gand binding studies have also provided evidence for allosteric regulation of the P2X<sub>4</sub> receptor by cibacron blue, which correlated with potentiation of responses in functional studies on the rat recombinant receptor (Michel et al., 1997; Miller et al., 1998).

A long-awaited development has been the discovery of high-affinity (nanomolar) antagonists. TNP-ATP, TNP-ADP, and TNP-AMP block P2X<sub>1</sub>, P2X<sub>3</sub>, and P2X<sub>2/3</sub> receptors with 1000-fold higher affinity than at other homomeric P2X receptors (Thomas et al., 1998; Virginio et al., 1998b). Together with IP<sub>5</sub>I, which blocks P2X<sub>1</sub> and P2X<sub>3</sub> receptors, but not P2X<sub>2</sub> and P2X<sub>2/3</sub> receptors (King et al., 1999; Dunn et al., 2000), they are useful *in vitro* tools because their use allows direct demonstration of P2X receptor heterogeneity in sensory neurons (Burgard et al., 1999; Grubb and Evans, 1999; Dunn et al., 2000; Patel et al., 2001). However, the use of TNP-ATP in multicellular preparations is probably quite limited by its susceptibility to enzymatic breakdown (Lewis et al., 1998). Ecto-ATPases have an impressive ability to rapidly metabolize nucleotides [within 200 ms in a hippocampal brain slice (Dunwiddie et al., 1997)], but we expect that ecto-ATPases are not a complicating factor under conditions where agonists have been applied rapidly (Evans and Kennedy, 1994; Zimmermann, 1994; Humphrey et al., 1995; Kennedy and Leff, 1995; Khakh et al., 1995b; Zimmermann and Braun, 1996). Considerable effort has been devoted to defining the ligand recognition characteristics of each of the seven homomeric P2X channels using rapid application of agonists and antagonists (Table 2). The properties of some of these presumed homomeric channels are very similar to P2X channels that have been identified in native cell types, suggesting that native cell types may express homomeric P2X channels as well (compare Table 2 and Table 4).

From the original papers, we report the antagonist IC<sub>50</sub> values reported for various homomeric P2X receptors (Table 2). These data present a general view of the differences between receptor types, but IC<sub>50</sub> values are without any quantitative definition, and cannot be equated with the desired measurement of affinity. IC<sub>50</sub> values are often used in radioligand binding experiments, but in functional experiments, their use is more limited. For example, even if one assumes competition between agonist and antagonist for one binding site, we expect the antagonist IC<sub>50</sub> to change with agonist concentration. This makes it problematic to compare antagonist IC<sub>50</sub> values between studies, because many investigators have used different agonist concentrations. Moreover, even at any one agonist concentration, assuming competition, the antagonist IC<sub>50</sub> is without quantitative or mechanistic definition, because unlike with radioligand binding experiments, in functional studies there are multiple steps, subsequent to binding, that lead to the final parameter—the open state (Colquhoun, 1998; Grosman et al., 2000). These prob-

lems are even more of a concern because many ATP receptor antagonists are clearly not competitive, e.g., PPADS (Li, 2000). It should also be emphasized that suramin is a poor antagonist of low potency and questionable selectivity, even blocking glutamate channels (Nakazawa et al., 1995). Suramin also inhibits ecto-ATPases, like other antagonists such as PPADS, which complicates assessment of antagonist potency against hydrolyzable agonists in isolated tissue studies (see Humphrey et al., 1995). Better antagonists are eagerly awaited; in the meantime IC<sub>50</sub> values should be treated with caution for all the various reasons discussed, although the general trends are informative (compare Tables 2 and 4). However, the data is further confounded by interspecies differences in agonist and antagonist potencies, notably at P2X<sub>4</sub> and P2X<sub>7</sub> receptors (Chessell et al., 1998a,b; Hibell et al., 2000; Jones et al., 2000).

#### A. P2X<sub>1</sub>

The rP2X<sub>1</sub> subunit cDNA was isolated by expression cloning from *vas deferens* smooth muscle (Valera et al., 1994), and P2X<sub>1</sub> mRNA and protein are abundantly expressed in smooth muscle preparations (Valera et al., 1994; Bo et al., 1998; Chan et al., 1998; Nori et al., 1998). These observations extend electrophysiological studies demonstrating ATP-activated currents in smooth muscle myocytes (Suzuki, 1985; Benham et al., 1987; Benham and Tsien, 1987; Nakazawa and Matsuki, 1987; Friel, 1988; Friel and Bean, 1988; Benham, 1989; Honore et al., 1989; Inoue and Brading, 1990). The properties of P2X receptors expressed in smooth muscle preparations are most like those of recombinant P2X<sub>1</sub> receptors. This suggests that homomeric P2X<sub>1</sub> receptors constitute native P2X receptors in smooth muscle preparations (Evans and Kennedy, 1994; Khakh et al., 1995b; Lewis et al., 1998; Lewis and Evans, 2000). Evidence to confirm that *vas deferens* smooth muscle myocytes express P2X<sub>1</sub> receptors was obtained by ablation of the P2X<sub>1</sub> gene in mice; predictably there were no ATP-evoked responses in *vasa deferentia* of these mice (Mulryan et al., 2000). P2X<sub>1</sub> receptors are present on smooth muscle cells of blood vessels where they can be activated by neuronally released ATP, and mediate blood vessel constriction (Evans and Surprenant, 1992). In a surprise, but preliminary result, blood pressure was normal in mice lacking P2X<sub>1</sub> receptors; one possibility is that P2X<sub>1</sub> receptors are not the sole vascular P2X receptors (Nori et al., 1998; Lewis and Evans, 2000). Other explanations including developmental compensation are possible and need to be systematically addressed. P2X<sub>1</sub> receptors also exist in the immature rat brain (Kidd et al., 1995), cerebellum (Loesch and Burnstock, 1998), dorsal horn spinal neurons (Vulchanova et al., 1996), and platelets (Clifford et al., 1998; Scase et al., 1998).

#### B. P2X<sub>2</sub>

Electrophysiological studies reveal that P2X receptors that have properties most similar to homomeric P2X<sub>2</sub>

receptors, are widely expressed in the nervous system. For example, myenteric neurons (Zhou and Galligan, 1996), rat and guinea pig SCG neurons (Boehm, 1999; Khakh et al., 1995a; Surprenant et al., 1995; Zhong et al., 2000a), mouse pelvic neurons (Zhong et al., 1998), mouse and rat celiac neurons (Zhong et al., 2000b), guinea pig chromaffin cells (Liu et al., 1999), guinea pig cochlea (Housley et al., 1999; Chen et al., 2000), dorsal horn neurons (Hugel and Schlichter, 2000), auditory neurons (Salih et al., 1999), cerebellar Purkinje neurons (Garcia-Lecea et al., 1999), possibly retinal ganglion neurons (Taschenberger et al., 1999), rat SCG nerve terminals (Boehm, 1999), and neurohypophysial terminals (Troade et al., 1998) all express functional P2X<sub>2</sub>-like receptors. Although these studies suggest a major role for homomeric P2X<sub>2</sub> receptors in these cell types, they do not prove it. Heteromeric assemblies in which P2X<sub>2</sub> receptors dominate or coexpression of more than one type of P2X receptor cannot be fully dismissed in many cases. P2X<sub>2</sub> subunit mRNA is widely expressed in the CNS (Collo et al., 1996) raising the possibility that more native homomeric P2X<sub>2</sub> receptors exist. Recent work on the P2X<sub>5</sub> mRNA rich trigeminal mesencephalic nucleus neurons (Collo et al., 1996; Cook et al., 1997; Khakh et al., 1997, 1999b; Patel et al., 2001) shows that they express functional P2X receptors that may comprise P2X<sub>2</sub> and P2X<sub>5</sub> receptor subunits.

### C. P2X<sub>3</sub>

Early observations of ATP-evoked currents in sensory neuronal soma (Jahr and Jessell, 1983; Krishtal et al., 1983, 1988a,b), and axons (Trezise et al., 1994a,b) were important for the general appreciation that ATP could directly activate receptors with integral ion pores in sensory neurons. Homomeric P2X<sub>3</sub> receptors, or heteromeric P2X<sub>2/3</sub> receptors, mediate a major component of these sensory neuron ATP responses. The evidence is based on gene cloning, electrophysiology, in situ hybridization, and immunocytochemistry (Chen et al., 1995; Lewis et al., 1995; Collo et al., 1996; Robertson et al., 1996; Vulchanova et al., 1996, 1997; Virginio et al., 1998a,b; Grubb and Evans, 1999). There is increasing support for the hypothesis that ATP, acting via P2X<sub>3</sub> receptors, is involved in certain types of pain; we do not discuss this topic further because it has been extensively reviewed (Burnstock, 1996, 2000; McCleskey and Gold, 1999).

### D. P2X<sub>4</sub>

The P2X<sub>4</sub> subunit is found extensively in the CNS and perhaps is a major target for ATP released in CNS synapses. The ATP receptor antagonists suramin and PPADS (see Tables 1 and 2) have conflictingly been shown to be ineffective or weak antagonists at the rP2X<sub>4</sub> receptor (Buell et al., 1996a; Soto et al., 1996a), to completely block ATP-evoked currents (Séguéla et al., 1996), or to potentiate them (Bo et al., 1995). PPADS is notably

more effective as an antagonist at the human P2X<sub>4</sub> and mouse P2X<sub>4</sub> channels than at the rat isoform (Garcia-Guzman et al., 1997; Jones et al., 2000). Recent studies demonstrate that ivermectin is a selective allosteric modulator of rat P2X<sub>4</sub> channels expressed in *Xenopus* oocytes (EC<sub>50</sub> = 250 nM), although all possible homomeric or heteromeric P2X subunit assemblies were not tested. A lack of block by suramin and potentiation by ivermectin may be a useful diagnostic feature of rat P2X<sub>4</sub> channels (Khakh et al., 1999b). Rat P2X<sub>4</sub> channel currents are potentiated by cibacron blue between 3 and 30 μM (Miller et al., 1998), but another study shows that cibacron blue blocks them with an IC<sub>50</sub> of 128 μM (Garcia-Guzman et al., 1997), with no obvious potentiation being reported. Mouse P2X<sub>4</sub> channel currents are potentiated by suramin, reactive blue2, and PPADS; concentrations of PPADS >3 μM may also block mouse P2X<sub>4</sub> receptor currents (Townsend-Nicholson et al., 1999).

Rat P2X<sub>4</sub> subunits form a heteromeric receptor with P2X<sub>6</sub> subunits when coexpressed in *Xenopus* oocytes and HEK293 cells (Le et al., 1998a), but to date there is no evidence for an equivalent native receptor. The splice variant mouse P2X<sub>4(a)</sub> expresses poorly on its own but may contribute in a heteromeric assembly with full-length mouse P2X<sub>4</sub> receptor (Townsend-Nicholson et al., 1999). Native P2X receptors in the brain may be like P2X<sub>4</sub> receptors, largely because P2X<sub>4</sub> receptors are abundantly expressed in the brain (Bo et al., 1995; Collo et al., 1996; Soto et al., 1996a; Le et al., 1998b). However, in a preliminary investigation the properties of P2X receptors in the hippocampus CA1 region (Proctor and Dunwiddie, 1998; Khakh et al., 1999b; Proctor et al., 1999) are unlike P2X<sub>4</sub> receptors, despite the fact that the most abundant mRNA species is P2X<sub>4</sub>.

### E. P2X<sub>5</sub>

The P2X<sub>5</sub> subunit mRNA is present in some parts of the brain, heart, spinal cord, and adrenal medulla, as well as thymus and lymphocytes (Collo et al., 1996; Garcia-Guzman et al., 1996; Le et al., 1997). When heterologously expressed as a homomer, minimal desensitization is observed with the rat receptor, but the channel currents are of small amplitude (Collo et al., 1996). The full sequence of the human P2X<sub>5</sub> receptor has yet to be confirmed (Le et al., 1997). The recently identified P2X subunit from chick skeletal muscle was tentatively called the chick P2X<sub>5</sub> subunit (Bo et al., 2000), because 1) it has 59% sequence homology to rat and human P2X<sub>5</sub> subunits, and 2) it has unique functional properties. But, further sequence analysis indicates it is a species homolog of P2X<sub>5</sub>. There is clear evidence for a P2X<sub>1/5</sub> heteromeric receptor with properties distinct from P2X<sub>1</sub> and P2X<sub>5</sub> receptors (Torres et al., 1998b; Haines et al., 1999; Le et al., 1999). The trigeminal mesencephalic nucleus is rich in P2X<sub>5</sub> mRNA and electrophysiological recordings show that neurons from this nucleus express two populations of functional P2X channels, one of

which is most like homomeric P2X<sub>5</sub> receptors (Cook et al., 1997; Khakh et al., 1997; Patel et al., 2001). A second population of P2X receptors contain the P2X<sub>2</sub> subunit (Patel et al., 2001).

#### F. P2X<sub>6</sub>

In initial studies, when the P2X<sub>6</sub> subunit was heterologously expressed as a homomer, ATP-evoked currents were observed in only a very small proportion of HEK293 cells (Collo et al., 1996). Currently the emerging picture is that homomeric P2X<sub>6</sub> channels are not readily expressed in *Xenopus* oocytes or mammalian transfected cells (Collo et al., 1996; Le et al., 1998a; Khakh et al., 1999b; Torres et al., 1999; King et al., 2000; North and Surprenant, 2000).

#### G. P2X<sub>7</sub>

Recombinant P2X<sub>7</sub> receptors are weakly activated by ATP (EC<sub>50</sub> in the 100 μM range), whereas other P2X channels respond to ATP with an EC<sub>50</sub> in the low micromolar range. Various compounds block P2X<sub>7</sub> receptors with high affinity: calmidazolium appears to block only the initial Na<sup>+</sup> current through P2X<sub>7</sub> receptors and not the secondary "pore" that is permeable to large molecules such as YO-PRO1 and ethidium bromide (Virginio et al., 1997). KN62 (1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine) and KN04 (N-[1-[N-methyl-p-(5-isoquinolinesulfonyl)benzyl]-2-(4-phenylpiperazine)ethyl]-5-isoquinolinesulfonamide) potently block human P2X<sub>7</sub> channels in the tens of nanomolar concentration range, but not rat P2X<sub>7</sub> receptors (Chessell et al., 1998a,b; Humphreys et al., 1998). KN62 is a blocker of CAM (Ca<sup>2+</sup>/calmodulin-dependent protein) kinase II, but the block of human P2X<sub>7</sub> receptors does not involve this enzyme, rather KN62 probably binds to unique residues in the extracellular loop of human P2X<sub>7</sub> receptors (Humphreys et al., 1998). P2X<sub>7</sub> receptor function is potently modulated by

and this must be considered when comparing functional data between different studies.

### V. Functional Properties of Heteromeric Receptors

Distinct P2X subunits assemble to form at least 11 different heteromeric receptors. Often native cell types express multiple P2X subunit mRNA transcripts and multiple P2X subunits (Kidd et al., 1995; Collo et al., 1996; Vulchanova et al., 1996, 1997). Recently, we have come to learn of the prevalence and mechanisms that determine P2X subunit heteromerization (Torres et al., 1999), and these studies are instructive for studies of native systems. A few points warrant attention. P2X<sub>6</sub> subunits form heteromeric but not homomeric channels (Torres et al., 1999). P2X<sub>7</sub> subunits form only homomeric channels. Fewer heteromeric assemblies have been detected than are theoretically possible, even with pairwise comparisons, and this implies some specificity in the partnerships that P2X subunits make. From biochemical studies, 11 different P2X receptors can be formed from the seven known subunits in pairwise comparisons (Torres et al., 1999), and it is important to determine their functional properties (see Table 3). We do not yet understand how many subunits are required in each heteromer, which ones dominate, or if a greater number of heteromers can be formed if cells are given the choice to express more than any two P2X subunits, as may be the case in the spinal cord (Hugel and Schlichter, 2000). The heteromer formed between P2X<sub>2</sub> and P2X<sub>3</sub> subunits is perhaps the best understood; support for it has come from studies of native P2X receptors in sensory neurons (Lewis et al., 1995; Cook et al., 1997; Radford et al., 1997), biochemical studies on P2X<sub>2</sub> and P2X<sub>3</sub> subunits (Radford et al., 1997; Vulchanova et al., 1997; Torres et al., 1999), and electrophysiology on cells

TABLE 3  
Functional properties of recombinant heteromeric P2X receptors

	Heteromeric Receptor Type			
	P2X <sub>2/3</sub>	P2X <sub>4/6</sub>	P2X <sub>1/5</sub>	P2X <sub>2/6</sub>
Agonists EC <sub>50</sub> (μM)				
ATP	1	6	1	30
2MeSATP	1	7	1	35
αβmeATP	1–3	12	3	>100
Antagonists IC <sub>50</sub> (μM)				
Suramin	Block 30–100 μM	Block 10 μM	1.6 μM	6
PPADS	Block 3–300 μM	Block 10 μM	0.6 μM	
TNP-ATP	0.007 μM		0.4 μM	
Brilliant Blue G	>10 μM		>10 μM	
Ion effects				
Zn <sup>2+</sup>				Potentiation EC <sub>50</sub> 6 μM
H <sup>+</sup>	Potentiation at pH 6.3, block at pH 8.3		Block at pH greater or less than pH 7.3	Potentiation pKa 7 Block pH 6.5–4
Ca <sup>2+</sup>	Block IC <sub>50</sub> 15 mM		No effect on peak, but plateau is potentiated	
Co-immunoprecipitation	Yes	Yes	Yes	Yes

The P2X<sub>4/6</sub> heteromer is blocked to a slightly greater degree by suramin and PPADS than homomeric P2X<sub>4</sub> channels (Le et al., 1998a), but IC<sub>50</sub> or affinity estimates are unknown. Data in the table are from the following references: Lewis et al., 1995; Radford et al., 1997; Le et al., 1998a, 1999; Thomas et al., 1998; Torres et al., 1998b, 1999; Virginio et al., 1998a,b; Haines et al., 1999; King et al., 2000; Surprenant et al., 2000.



that coexpress P2X<sub>2</sub> and P2X<sub>3</sub> subunits (Lewis et al., 1995; Radford et al., 1997). Similar approaches have been used to show that P2X<sub>4</sub> and P2X<sub>6</sub> subunits form a heteromer (Le et al., 1998a), as do P2X<sub>2</sub> and P2X<sub>6</sub> subunits (King et al., 2000). A P2X<sub>1/5</sub> heteromer has also been identified on the basis of similar experiments (Haines et al., 1999; Le et al., 1999; Torres et al., 1998b, 1999), and such a channel may exist in discrete areas in the spinal cord and in the gut (Surprenant et al., 2000). Although P2X<sub>4</sub> subunit messenger RNA and protein is widely expressed in the CNS and homomeric P2X<sub>4</sub> receptors are readily formed in heterologous expression systems (Bo et al., 1995; Buell et al., 1996a; Collo et al., 1996; Séguéla et al., 1996; Soto et al., 1996a; Le et al., 1998b), to date there is no decisive evidence for native P2X<sub>4</sub>-like channels in the CNS. Instead, in areas that abundantly express P2X<sub>4</sub> mRNA the functional properties of native P2X receptors are most like other homomeric P2X receptors, a mixture of P2X receptors or possibly novel heteromers (Nabekura et al., 1995; Proctor and Dunwiddie, 1998; Khakh et al., 1999b; Proctor et al., 1999; Hugel and Schlichter, 2000; Patel et al., 2001).

## VI. Native Receptors in Whole Tissues

### A. Studies in Vitro

Rigorous studies in whole tissues have identified P2X receptors in smooth muscle preparations such as rat bladder and vas deferens and in neuronal preparations such as the isolated vagus nerve (Trezise et al., 1994a,b; Khakh et al., 1995b). These experiments thus extended earlier studies to pharmacologically define P2 receptors. However, all such studies have been confounded by marked ectonucleotidase activity, which interferes with both agonist and antagonist potency estimates (Humphrey et al., 1995). The same is true in brain slice electrophysiological studies, such as in the locus coeruleus, medial vestibular nucleus, and trigeminal mesence-

phalic nucleus, where good evidence for P2X receptors has been provided (Shen and North, 1993; Chessell et al., 1997b; Khakh et al., 1997). Even in single-cell recording systems, where such complications can be reduced, definitive receptor subtype identification remains difficult (Khakh et al., 1995a,b; Robertson et al., 1996). This results largely from the paucity of good selective drug tools. Thus, to better understand native P2X receptors in whole tissues we need more potent and selective agonists and antagonists to profile the functional properties of homomeric (Table 2), as well as heteromeric, P2X receptors (Torres et al., 1999; Table 3), to compare with those of P2X receptors in native systems (Table 4). However, even with the limitations of the drug tools available it is possible to identify cell types that appear to contain particular P2X receptor subtypes, some of which appear to be homomeric and others, such as those in nodose and dorsal root ganglion cells, that appear to be heteromeric (see Table 4).

### B. Studies in Vivo

Studies in vivo have indicated a number of potential physiological and pathophysiological roles for ATP in smooth muscle as well as neurons and inflammatory cells. Thus, in male transgenic mice lacking P2X<sub>1</sub> receptors, contraction of the vas deferens in response to sympathetic nerve stimulation is substantially reduced and their fertility impaired by 90% (Mulryan et al., 2000). A growing body of evidence suggests that P2X receptors on nerve terminals both in the periphery and spinal dorsal horn are involved in sensory processing and nociception at sites throughout the body including the joints, viscera, and cardiovascular system (Burnstock, 1996, 2000; Pelleg and Hurt, 1996; Bland-Ward and Humphrey, 1997, 2000; McQueen et al., 1998; Kirkup et al., 1999; Tsuda et al., 1999). Studies in transgenic mice lacking the P2X<sub>3</sub> have shown that formalin-induced pain behav-

TABLE 4  
Functional properties of some native P2X receptors

	Native Receptor Type						
	P2X <sub>1</sub>	P2X <sub>2</sub>	P2X <sub>3</sub>	P2X <sub>4</sub>	P2X <sub>5</sub>	P2X <sub>7</sub>	P2X <sub>2/3</sub>
Cell type	HL60 cells, mesenteric artery, rat tail artery	PC12 cells <sup>a</sup>	Dorsal root ganglion neurons	Epithelial cells	MNV neurons	NTW-8 cells, J774 cells, human dendritic cells	Nodose and dorsal root ganglion neurons
Agonist EC <sub>50</sub> (μM)							
ATP	3	45	0.7–1.5	10	3	298–500	3
2MeSATP	3.4	81	0.5–3.2			505–1000	0.4
αβmeATP	1.4	>300	1.4–2.0	>300	>30	>300	9
BZ-ATP						30–58	
Antagonist (μM)							
Suramin	IC <sub>50</sub> 30 μM	>100	0.3	>300	>30		>30
PPADS	IC <sub>50</sub> 8 μM	>30	0.4	>300	>30		>10
TNP-ATP	Myocytes IC <sub>50</sub> 2 nM, tissue IC <sub>50</sub> 30 μM		0.3		>10		~2 nM

Predominant subunit considered to be involved on the basis of functional data and receptor localization studies. For antagonists, IC<sub>50</sub> estimates are not available for all receptor types; instead an effective concentration or concentration range is indicated. The table is based on the following references: Khakh et al., 1995a, 1997; Lewis et al., 1995, 1998; Buell et al., 1996a; Michel et al., 1996a; Robertson et al., 1996; Surprenant et al., 1996; Chessell et al., 1997a; Cook et al., 1997; McLaren et al., 1998; Rae et al., 1998; Thomas et al., 1998; Grubb and Evans, 1999; Ueno et al., 1999; Hargreaves and Pollard, 2000; Lewis and Evans, 2000; Patel et al., 2001.

<sup>a</sup> Note that differentiation can change the receptor profile (Arslan et al., 2000).



ior is reduced and that their ability to code the intensity of non-noxious heat stimulation is absent (Cockayne et al., 2000; Souslova et al., 2000). Interestingly these mice also exhibited a decreased urine voiding frequency and an increased bladder capacity associated with a normal intravesical pressure (Cockayne et al., 2000). This suggests a urinary bladder hyporeflexia, whereby the release of ATP on bladder distension no longer excites peripheral primary afferent nerve terminals (Cockayne et al., 2000). Recent studies in a P2X<sub>7</sub>-deficient mouse have confirmed earlier studies, which suggested that receptors may play an important role in initiating the processing and release of IL-1 $\beta$  from inflammatory cells (Ferrari et al., 1997; Grahames et al., 1999; Solle et al., 2001)

## VII. Summary

In the last few years, spectacular advances have been made in our knowledge of P2X receptors and the biology of ATP itself, which appears to act as an important humoral regulator both physiologically and in disease. At the molecular level, we better understand the functional properties and identity of this whole new family of transmitter-gated cation channels. At the cellular level the use of antagonists and receptor knock-out studies are beginning to unravel the functional roles that P2X receptors and ATP play in vivo. Finally, the P2X Receptor Subcommittee of the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) recommends continued use of the present common nomenclature for P2X receptor subunits.

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