Signaling at Purinergic P2X Receptors

Annmarie Surprenant and R. Alan North

Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, United Kingdom; email: a.surprenant@manchester.ac.uk, alan.north@manchester.ac.uk

Annu. Rev. Physiol. 2009. 71:333–59

First published online as a Review in Advance on October 13, 2008

The *Annual Review of Physiology* is online at physiol.annualreviews.org

This article's doi: 10.1146/annurev.physiol.70.113006.100630

0066-4278/09/0315-0333$20.00

Key Words

ATP, ligand-gated ion channels, transgenic mice, structure-function, mutagenesis, cell signaling

Abstract

P2X receptors are membrane cation channels gated by extracellular ATP. Seven P2X receptor subunits ($P2X_{1-7}$) are widely distributed in excitable and nonexcitable cells of vertebrates. They play key roles in inter alia afferent signaling (including pain), regulation of renal blood flow, vascular endothelium, and inflammatory responses. We summarize the evidence for these and other roles, emphasizing experimental work with selective receptor antagonists or with knockout mice. The receptors are trimeric membrane proteins: Studies of the biophysical properties of mutated subunits expressed in heterologous cells have indicated parts of the subunits involved in ATP binding, ion permeation (including calcium permeability), and membrane trafficking. We review our current understanding of the molecular properties of P2X receptors, including how this understanding is informed by the identification of distantly related P2X receptors in simple eukaryotes.

INTRODUCTION

AMPA: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid

EPSP: excitatory postsynaptic potential

P2X receptors came to our attention as the receptor involved in transmitting the actions of ATP released from sympathetic nerves onto the smooth muscles of the vas deferens and small intestinal arterioles (1). These receptors were distinguished from G protein–coupled metabotropic P2Y receptors (2). The historical development of thinking as to P2X receptors, and the initial associated skepticism, has been recently reviewed (3).

The cloning of P2X receptor subunit cDNAs in 1994 (4, 5) led directly to two major avenues of progress. The first is the demonstration that the proteins have a very widespread tissue distribution in vertebrates and indeed in eukaryotes. This finding in turn has led to the development of receptor antagonists and of mice in which receptor subunits have been genetically deleted; the use of such tools has indicated wide-ranging and hitherto unexpected functional roles for P2X receptors. Second, P2X receptors do not resemble other ion channels at the molecular level. This has spurred many studies to determine the molecular details of the operation of these receptors in the cell membrane. This review focuses on these two areas of research.

PHYSIOLOGICAL ROLES FOR P2X RECEPTORS

Central Nervous System

P2X receptors are widely distributed on central nervous system neurons and glia at the mRNA (6–8) and protein (8, 9) levels. At the protein level, the inadequacy of several of the existing antibodies has handicapped progress, particularly in the case of the $P2X_7$ receptor (10, 11). Two approaches dominate the literature: the use of either slices of brain tissue or cells dissociated and maintained in culture. The first includes studies of the effects of exogenous ATP and analogs to nerve cells and glia, measuring either the direct effects on membrane currents or intracellular calcium (postsynaptic effects) or the alteration of transmitter release (presynaptic effects). The second complementary approach is to deduce roles for endogenous ATP in intercellular signaling by the use of pharmacological antagonists or knockout mice.

Postsynaptic effects. A careful immunohistochemical study using the postembedding technique for electron microscopy showed that $P2X_4$ and $P2X_6$ subunits are found in perisynaptic locations on the hippocampal CA1 pyramidal and cerebellar Purkinje cells (9). There are several reports of residual excitatory postsynaptic potentials (EPSPs) in the presence of high concentrations of blockers of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid), kainate, and NMDA subtypes of glutamate receptor. These EPSPs are rather small in amplitude, and their attribution to ATP is based on blockade by antagonists of limited specificity [habenula (12), hippocampus (13, 14), somatosensory cortex (15–17)]. Pankratov et al. (17) concluded that in the mouse cortex, some glutamate-containing vesicles also contained ATP, whereas others did not. In the lateral hypothalamus of the mouse (and neonatal chick), GABA and ATP are coreleased, and each evokes its own postsynaptic current that can be distinguished pharmacologically (18). There is a clear need for a systemic comparison of the properties of such EPSPs between wild-type mice and mice lacking P2X receptor subunits. In the case of the $P2X_4$ knockout mouse, long-term potentiation in the hippocampus is of reduced amplitude as compared with the wild-type animals, but the underlying cellular mechanism for this remains unclear (19).

Direct postsynaptic effects on neurons of exogenously applied ATP have been described in many parts of the central nervous system and are not reviewed here (see References 8, 20, and 21). More recently, there have been reports of ATP actions on glial cells that are attributable to P2X receptors. Fellin et al. (22) reported ATP-evoked currents in hippocampal astrocytes, although Jabs et al. (23) did not. Astrocytes in the mouse cortex respond to ATP with inward currents exhibiting many of the properties of

the $P2X_{1/5}$ heteromer, and single-cell RT-PCR shows the predominant expression of the two subunits (24).

Presynaptic effects. Presynaptic effects of ATP have been reported at several sites in the central nervous system. The most prevalent effect is an increase in the spontaneous release of glutamate, as detected by increased frequency of spontaneously occurring excitatory postsynaptic currents (EPSCs) in the presence of tetrodotoxin (21). For example, Khakh & Henderson (25) found that ATP enhances glutamate release onto neurons in the motor nucleus of the fifth cranial nerve in the rat but that the receptor involved has properties different from those of the cell bodies of the sensory input neurons that give rise to these presynaptic terminals (located in the mesencephalic nucleus of the fifth nerve) (26). The presynaptic facilitatory effect is rather specific in the hippocampus: ATP facilitates glutamate release at synapses onto inhibitory interneurons, but not at synapses onto CA1 pyramidal cells. This effect was not seen in mice lacking the $P2X_2$ subunit (27).

In the nucleus tractus solitarius, calcium entry through presynaptic P2X receptors elicits glutamate release sufficient to drive postsynaptic cell firing (28). This effect does not involve voltage-gated calcium channels or tetrodotoxin-sensitive sodium channels. The spontaneous EPSCs elicited by ATP are larger in average amplitude than those occurring spontaneously, suggesting that P2X receptor–induced calcium entry evokes the release of a distinct subpopulation of glutamate-containing vesicles. αβmeATP (α,β-methylene adenosine 5′-triphosphate) (≥30 μM) mimics the effect of ATP, and TNP-ATP [2′,3′-*O*-(2,4,6-trinitrophenyl) adenosine 5′-triphosphate] ($IC_{50} \approx 1$ μM) blocks these effects. These results are consistent with the involvement of $P2X_1$- or $P2X_3$-containing receptors but do not completely exclude other subtypes. It will be important to determine the molecular basis through which P2X receptors appear to access a pool of releasable glutamate distinct from that which occurs spontaneously. Release of GABA by presynaptic P2X receptors has also been described in the cerebellum (29), and spontaneous release of glycine release has been observed in the dorsal horn of the spinal cord (30).

ATP depolarizes pituitary gonadotrophs, and the properties of the corresponding inward current conform to those expected of $P2X_2$ and/or $P2X_4$ subtypes (31). This results in a release of luteinizing hormone. In the neurohypophysis, ATP elicits inward currents on vasopressin terminals, but not on oxytocin-releasing terminals (32, 33). The response has features most similar to those expected for $P2X_2$ receptors. In both the anterior pituitary and posterior pituitary, the overall physiological context of these ATP effects remains obscure.

EPSC: excitatory postsynaptic current

αβmeATP: α,β-methylene adenosine 5′-triphosphate

TNP-ATP: 2′,3′-*O*-(2,4,6-trinitrophenyl) adenosine 5′-triphosphate

Afferent Signaling

Action potentials in afferent nerve fibers are often initiated by the release of a specific chemical from specialized sensory cells that then acts on receptors near the peripheral terminals of the nerve. ATP has been implicated as one such chemical.

Taste. Recent work has indicated that homomeric and/or heteromeric $P2X_2$ and $P2X_3$ receptors are essential players in taste transduction. A single mammalian taste bud consists of approximately 100 cells of four distinct types. Type I supporting cells wrap around type II and type III cells to form the characteristic taste bud clusters. Type II cells are the taste receptors expressing distinct G protein–coupled receptors specific for the five differentiated tastes (sweet, sour, salty, bitter, and umami), but type II cells do not synapse with afferent nerves within the taste buds. Presynaptic type III cells synapse, via $5\text{-}HT_3$ neurotransmission, onto afferent taste nerves. The physiology of type IV cells is not clear, although they are known to function in the regeneration of taste cells (34, 35).

Single-cell RT-PCR of isolated mouse taste bud cells found $P2X_2$, $P2X_4$, and $P2X_7$ subunits (36). Immunohistochemical studies using well-characterized antibodies localized $P2X_2$ to

SNARE: a multiprotein scaffold between exocytotic vesicle and plasma membrane that controls vesicle/plasma membrane fusion and transmitter release

afferent nerve fibers and type III presynaptic cells, localized $P2X_3$ to afferent fibers only, and showed no $P2X_4$ protein in taste bud cells or nerve fibers (36–38). ATP release in response to tastants has been measured by luciferase assays from isolated segments of lingual epithelia (39) and also from single isolated type II taste cells via a biosensor assay (40, 41). Release of ATP from these cells is not via classic SNARE-based exocytosis, and both Huang et al. (40) and Romanov et al. (41) have suggested that release is via a hemichannel-like pore made up of proteins of the pannexin or connexin family. Finger et al. (39) recorded afferent firing from both chorda tympani and glossopharyngeal gustatory nerve trunks in mice lacking both $P2X_2$ and $P2X_3$ receptors. These researchers found an almost complete absence of nerve response to tastants applied to the oral cavity; responses to mechanical and other chemical stimuli were not altered. Moreover, behavioral responses to sweeteners, glutamate, and bitter substances were diminished or absent in these double-knockout mice. Finger et al. (39) also found moderately diminished neural and behavioral responses to tastants in single-knockout ($P2X_3$ or $P2X_2$) mice. Taken together, these studies provide convincing evidence for the role of ATP as the primary neurotransmitter in taste perception, acting on homomeric $P2X_2$ receptors, homomeric $P2X_3$ receptors, and heteromeric $P2X_{2/3}$ receptors.

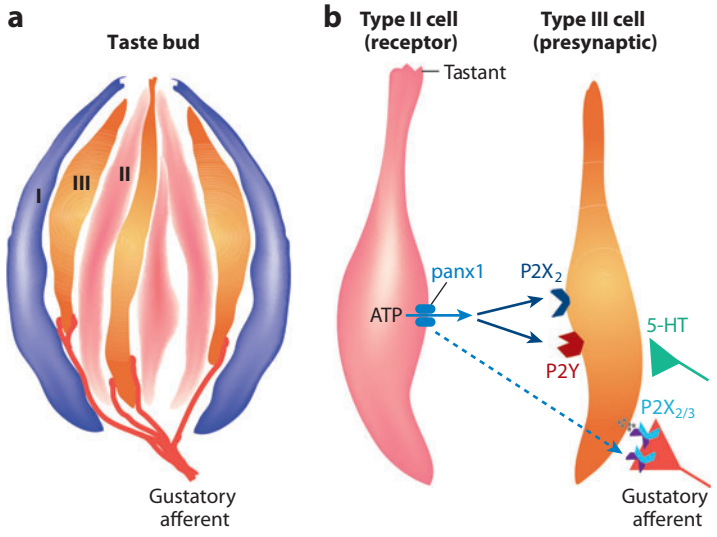

Figure 1

$P2X_2$ and $P2X_3$ receptors are involved in taste transduction. (*a*) Diagram of a single taste bud indicating three of the four types of taste cells: type I supporting cells, type II receptor cells, and type III presynaptic cells; type IV basal cells are not shown (35). (*b*) In response to tastants, specific type II cells release ATP via pannexin-1 (panx1) hemichannels (40, 41). The released ATP acts in a paracrine fashion to activate $P2X_2$ and/or G protein–coupled P2Y receptors in type III cells; this leads to increased intracellular calcium, which evokes classical synaptic release of ATP onto presynaptic $P2X_{2/3}$ receptors on afferent fibers (39). ATP released from type II cells may also act in a paracrine manner to activate presynaptic afferent $P2X_{2/3}$ receptors (*dashed arrow*). Depolarization of type III cells by ATP also leads to the release of 5-HT onto gustatory afferents (35).

However, ATP neurotransmission in mouse taste buds (**Figure 1**) may be atypical. Rong et al. (42) used an isolated, intra-arterially perfused rat tongue with separated gustatory (chorda tympani) and general sensory (lingual) nerves. They observed $P2X_2$ receptor and $P2X_3$ receptor responses only from lingual nerves that did not respond to tastants applied to the tongue, whereas chorda tympani nerves responded robustly to salty or acidic solutions but not to ATP or ATP analogs. There may be technical difficulties in the use of an isolated tongue preparation that can account for the contrasting results, or this study may serve as a caution against the overwhelming domination of mouse tissue and mouse models in physiology research today.

Hayato et al. (36) have demonstrated the presence of functional $P2X_7$ receptors on subpopulations of taste bud cells, although the specific cell type has not been ascertained. Taste bud cells are among the shortest-lived of all mammalian cells, having turnover rates of only several days, whereby old taste cells are removed via apoptosis. Because prolonged $P2X_7$ receptor activation leads to apoptotic cell death, this demonstration of functional $P2X_7$ receptors in taste buds indicates a possible role for $P2X_7$ receptors in taste cell regeneration.

Hearing. Immunohistochemical and electrophysiological studies have been carried out in mouse, rat, and guinea pig cochlea (reviewed in References 43 and 44). These studies have provided evidence for ATP as a neurotransmitter

or neuromodulator in afferent (but not efferent) auditory transmission. Patch-clamp recordings from inner and outer hair cells show ATP-gated currents that are likely mediated by $P2X_2$ receptors, whereas neuronal spiral ganglion cells show $P2X_3$ receptor–mediated inward currents (43–48). The electrophysiological data correspond to the immunolocalization of these receptor subunits. Housley and coworkers have amassed a large body of data that implicates P2X receptors, primarily $P2X_2$, in modulation of the endocochlear potential (43–45). However, there are currently no publications regarding the hearing phenotype of mice lacking $P2X_2$ and/or $P2X_3$ receptors or studies using selective $P2X_3$ antagonists to hearing behavior in animals or humans.

Chemoreceptors. The carotid body contains glomus cells that release a primary afferent transmitter onto primary afferent nerves in response to hypoxia. The identity of this transmitter (as also the primary hypoxia sensor) has been the subject of long-standing debate (49). Rong et al. (50) reported that ATP is released from type 1 glomus cells in response to hypoxia, that ATP activates primary afferent nerves (probably through a $P2X_{2/3}$ heteromeric receptor), and that the excitation of primary afferent nerves by hypoxia is much reduced in mice lacking the $P2X_2$ subunit (see Reference 51).

Bladder. Accumulating evidence indicates a role for P2X receptors in the initiation of primary afferent signaling in hollow viscera and particularly the urinary bladder. Distension of the bladder results in ATP release from urothelial cells (52). ATP and αβmeATP increase the excitability of bladder afferent nerves (53). Bladder afferent nerves express $P2X_3$ receptor subunits, and in the anesthetized $P2X_3$ knockout mouse the bladder must be distended to a greater volume than in wild-type mouse to initiate the voiding reflex (54, 55).

Pain. ATP acting at P2X receptors has been implicated in pain sensation at both peripheral and central sites within the nervous system. ATP applied within the skin evokes pain (56), and $P2X_3$ subunits are expressed on a subset of primary afferent neurons implicated in pain sensation (57, 58). Three lines of evidence implicate $P2X_3$ receptor subunits in some aspects of neuropathic and inflammatory pain perception: Some, although by no means all, pain-related behavior is blocked by antagonists selective for $P2X_3$ subunit–containing receptors (59), by reduction in $P2X_3$ subunit expression by RNA knockdown (60), and by genetic elimination of the $P2X_3$ subunits (reviewed in detail in Reference 61).

BDNF: brain-derived nerve growth factor

A role for $P2X_4$ receptors in neuropathic (but not inflammatory) pain has more recently been proposed. $P2X_4$ subunit expression is increased in microglia of the dorsal horn of the spinal cord following spinal nerve ligation, a commonly used model for neuropathic pain. The mechanical allodynia resulting from the ligation was reduced by intrathecal TNP-ATP and by intrathecal antisense oligonucleotides that somewhat reduced $P2X_4$ receptor expression (62). Conversely, intrathecal administration of cultured microglia that had been stimulated with ATP could induce allodynia in naive rats. It was proposed that spinal nerve ligation activates dorsal horn microglia by a mechanism involving $P2X_4$ receptors and that they release brain-derived nerve growth factor (BDNF). BDNF then acts on spinal cord interneurons to stimulate the expression of the potassium-chloride cotransporter KCC2, and the resultant reduction of intracellular chloride converts the usually inhibitory action of GABA into excitation (63). One difficulty with the attribution of these effects to the activation of $P2X_4$ receptors is that TNP-ATP has several nonspecific actions and is in any event a weak blocker of $P2X_4$ receptors (64). It will be important to repeat these studies in mice in which the $P2X_4$ and $P2X_7$ receptors have been knocked out, or with currently available $P2X_7$ receptor antagonists.

More recently, $P2X_7$ receptors have also been implicated in pain perception. Disruption of the $P2X_7$ receptor gene markedly reduces both neuropathic and chronic inflammatory

UVEC: umbilical vein endothelial cell

IL-1β: interleukin-1 beta

IL-1Ra: a naturally occurring protein that binds to IL-1 receptor without activating it and prevents binding of IL-1β

pain in mice (65), and antagonists with high selectivity for $P2X_7$ receptors strongly inhibit neuropathic pain in rats (66). The mechanism of these actions is as yet unclear but presumably involves the rearrangement of sensory processing pathways and decreased release of proinflammatory cytokines from microglia in the spinal cord.

Cardiovascular System

P2X receptors have widespread expression throughout tissues of the cardiovascular system (6). Our understanding of their function in two tissues—(*a*) the endothelium and its role in the control of blood pressure and (*b*) the platelet with respect to blood coagulation—has advanced rapidly in recent years.

Vascular endothelium. Vascular endothelium cells coexpress $P2X_4$ and $P2X_7$ receptors: mRNA and protein levels for the former are typically 5–20-fold higher than for the latter (67). The heterogeneity of vascular endothelia should be kept in mind when functional roles of any endothelial protein are discussed. One of the clearest examples of this heterogeneity is a microarray study performed by Chi et al. (68) on 53 different human endothelial cell lines obtained from various large and small arteries and veins; distinct patterns of gene expression were detected on the basis of not only tissue site, developmental stage, and artery versus vein type but also specific functions such as left/right asymmetry. Nevertheless, in endothelial cells increased intracellular calcium directly leads to nitric oxide (NO) production and release with consequent smooth muscle vasodilation; therefore, calcium assays (using Fura2 or Indo1) are confidently used in endothelial cells as indirect readouts of NO activity (69). Human and bovine umbilical vein endothelial cells (UVECs) have been the most commonly used preparations for in vitro studies of purinoceptor function in endothelia.

Ando and colleagues (70) have presented evidence for autocrine activation of $P2X_4$ receptors in human and bovine UVECs via shear stress–induced release of ATP from endothelial cells. The key evidence for $P2X_4$ receptor involvement is that antisense oligonucleotides that decreased protein levels abrogated shear stress–induced calcium transients and that heterologous expression of $P2X_4$ receptors in human embryonic kidney 293 cells resulted in a shear stress–induced calcium transient. Surprisingly, the ATP-induced responses blocked by $P2X_4$ receptor antisense oligonucleotide treatment were observed only with low ATP concentrations (<1 μM), concentrations that do not activate homomeric human or rodent $P2X_4$ receptors or heteromeric rat $P2X_{4/6}$ receptors (71).

In contrast, Wilson et al. (67) found no evidence for $P2X_4$ receptor–mediated membrane currents or calcium transients in human UVECs even under conditions (interferon-γ and TNFα stimulation) that upregulated $P2X_4$ receptor mRNA and protein by 10–75-fold; these investigators found that >95% of total $P2X_4$ protein is intracellular. The predominantly intracellular localization of the $P2X_4$ receptor has been documented in several other cell types and appears to be the default expression pattern of this receptor (72, 73). After activation by interferon-γ and TNFα, these human UVECs released small amounts of proinflammatory interleukin-1 beta (IL-1β) in response to $P2X_7$ receptor stimulation but simultaneously released anti-inflammatory IL-1Ra; the net effect was anti-inflammatory.

Although these in vitro studies of P2X receptor function in UVECs may seem contradictory, Ando and colleagues have provided conclusive evidence for $P2X_4$ receptor involvement in endothelial function in studies, using wild-type and $P2X_4$ receptor knockout mice (74). First, they found that flow-induced increases in calcium and NO production in cultured endothelia obtained from lung microvessels were virtually absent in preparations from $P2X_4$ receptor knockout mice and that these responses were regained with the reintroduction of $P2X_4$ receptor by adenoviral constructs. Second, these researchers found that in vivo vasodilation of preconstricted cremaster

arterioles induced by intra-arterial ATP (but not acetylcholine), and the associated increased blood flow, was decreased in knockout mice; the decrease (30–100%) depended on ATP concentration and flow rate. A similar inhibition of ATP-induced vasodilation occurred in isolated mesenteric arteries from knockout mice. Third, $P2X_4$ receptor knockout mice had significantly higher blood pressure and lower basal plasma concentrations of nitrites and nitrates (an indicator of NO levels) than did wild-type controls. Fourth, Ando and coworkers found that the resting carotid artery diameter of $P2X_4$ receptor knockout mice was significantly smaller and that their smooth muscle layers were thicker compared with wild type. Moreover, these investigators used the carotid artery ligation to elicit neointimal depositions and long-term decreased vessel diameter, which is commonly employed as an animal model of atherosclerotic disease; $P2X_4$ receptor knockout mice showed no further decrease in vessel diameter. Chamberlain et al. (75) performed similar carotid artery ligation experiments on $P2X_7$ receptor knockout mice and found no differences in responses, thus ruling out $P2X_7$ receptor involvement in this animal model of vascular disease. Taken overall, the vascular phenotype of $P2X_4$ receptor knockout mice resembles that seen in endothelial nitric oxide synthase (eNOS) knockout mice (74), supporting the idea that the critical functional role of $P2X_4$ receptors in mouse vascular endothelia resides in the modulation of NO production and release. This finding has implications for the possible use of $P2X_4$ receptor antagonists in atherosclerosis.

Thrombosis. The study of ADP acting on P2Y receptors on platelets has a long history that has brought considerable clinical utility. The main players are the $P2Y_1$ and $P2Y_{12}$ receptors; the latter is blocked by the antithrombotic agents such as clopidogrel (76). Platelets also express $P2X_1$ receptors, and their activation by $\alpha\beta$meATP induces a rapid calcium influx associated with a transient shape change (77, 78). The bleeding time of $P2X_1$ receptor knockout mice is normal, suggesting little impairment of normal hemostasis; however, thrombosis associated with injury of the walls of small arterioles is reduced in the knockout mice (79). Thus, the $P2X_1$ receptor may play a role in thrombus formation under conditions of high shear stress, suggesting that a $P2X_1$-selective antagonist may be particularly effective in inhibiting thrombosis in conditions of stenosis, in which shear stress is high, while having little effect on normal hemostasis, in which shear stress is less (76).

Respiratory System

An important recent development has been the discovery that P2X receptors expressed by the ciliated epithelial cells of the bronchi can control ciliary beat frequency and thus the clearance of mucus from the airways. The subject has obvious importance for a range of lung diseases, including cystic fibrosis. A second area of substantive progress has been the central control of respiration. Sophisticated assays of ATP release combined with electrophysiological recordings have strongly supported a role for P2X receptors in the ventral surface of the medulla.

Ciliated epithelia. Ciliated epithelia play a critical role throughout the airways in clearing mucous secretions. Agents that increase ciliary beating, such as β_2-adrenergic and $P2Y_2$ receptor agonists, provide key therapies in several acute and chronic respiratory diseases (80). ATP increases ciliary beating in ciliated epithelia from nose, trachea, and other airway epithelia in several species via activation of both P2Y and P2X receptors (80, 81). Silberberg and coworkers have determined the properties of P2X responses in rabbit airway ciliary epithelia (82, 83). They have concluded that a novel P2X receptor, which they term $P2X_{cilia}$, underlies the ionotropic purinergic response. The functional responses reported for rabbit $P2X_{cilia}$ are the same as those reported for rodent $P2X_7$ receptors in most respects. These responses include agonist dose responses, divalent cation sensitivity, facilitation of response

N-methyl-D-glucamine (NMDG): the largest soluble cation (molecular weight 195.2) used in biophysical studies to estimate channel pore size

during prolonged agonist application, and inhibition by extracellular sodium ions. The key differences are that the rabbit cilia do not show uptake of dyes (such as propidium), do not show increases in *N*-methyl-D-glucamine (NMDG) permeability with prolonged agonist application, and have currents that are potentiated by zinc (1 μM) (83). Because dye-permeable pore formation and zinc inhibition are typical properties of $P2X_7$ receptors, these workers ruled out (homomeric) $P2X_7$ receptor involvement. However, the dye/NMDG-permeable properties of $P2X_7$ receptor function depend on the density of $P2X_7$ receptor protein expression (84) and/or the presence of other associated interacting proteins (85), and zinc modulation of many ligand-gated ion channels, including P2X receptors, commonly shows biphasic potentiation and inhibition of agonist-evoked responses depending on zinc concentration (21). The complete concentration dependence of the action of zinc in airway epithelial cells remains to be determined.

The Silberberg group (82, 83) also found that ivermectin potentiates the amplitude of the ATP-induced cationic current. Because ivermectin potentiation is a selective characteristic of $P2X_4$ receptors (21), this research group's general conclusion was that $P2X_{cilia}$ most likely resulted from a heteromeric $P2X_{4/7}$ receptor. However, ivermectin potentiated only the amplitude, and not the duration, of the ATP response in rabbit ciliary epithelia, whereas studies on ectopic or endogenous $P2X_4$ receptors have also shown a marked prolongation of the duration of $P2X_4$ receptor responses. We have recently observed that ivermectin significantly potentiates the amplitude of mouse (but not rat) $P2X_7$ receptor–induced current (J. Sim, unpublished observations) in a manner similar to that observed by Silberberg and colleagues in rabbit cilia.

Central control of respiration. Dale and colleagues (86) have cleverly developed an enzymatic/amperometric electrode to measure local ATP concentration. In one application of this device (87), they measured ATP concentrations in the ventral medulla during hypoxia. In vivo, they detected ATP release only in those discrete regions of the medullary surface known to correspond to classical CO_2 chemosensitivity. Slices of medulla maintained in vitro also released ATP in response to increased CO_2. Higher concentrations of CO_2 were required to stimulate respiratory activity in the presence of P2X receptor antagonists. Moreover, exogenous ATP itself mimicked the effect of increased CO_2 levels. These findings led the authors to suggest that ATP released in response to increased CO_2 acts on P2X receptors expressed on the dendrites of ventral respiratory column neurons and thus determines the adaptive responses in breathing (51, 87). This implies a key role for ATP released from peripheral chemosensors to initiate action potentials in primary afferent nerves (see above section, Afferent Signaling) and a very similar role for ATP in the ventral medulla to drive the central respiratory response. In both cases, an understanding of the mechanism of ATP release is urgently needed.

Genitourinary System

ATP acting at P2X receptors was first recognized as the efferent transmitter process from sympathetic nerves to the vas deferens (3), and more recently it has become clear that ATP released from urothelial cells can activate afferent nerves in a bladder distention reflex. More recent work implicates P2X receptors in the initial stage of urine formation, namely in the feedback mechanism for maintaining urinary filtration at the glomerulus in a wide range of physiological conditions.

Tubuloglomerular feedback. Strong evidence now exists for a critical role of $P2X_1$ receptors in renal autoregulation. Autoregulation is the process whereby the kidney is able to maintain constant renal blood flow and glomerular filtration rate over changes in arterial pressure from approximately 80 to 180 mm Hg in the absence of changes in sympathetic input and/or vasoactive substances (88).

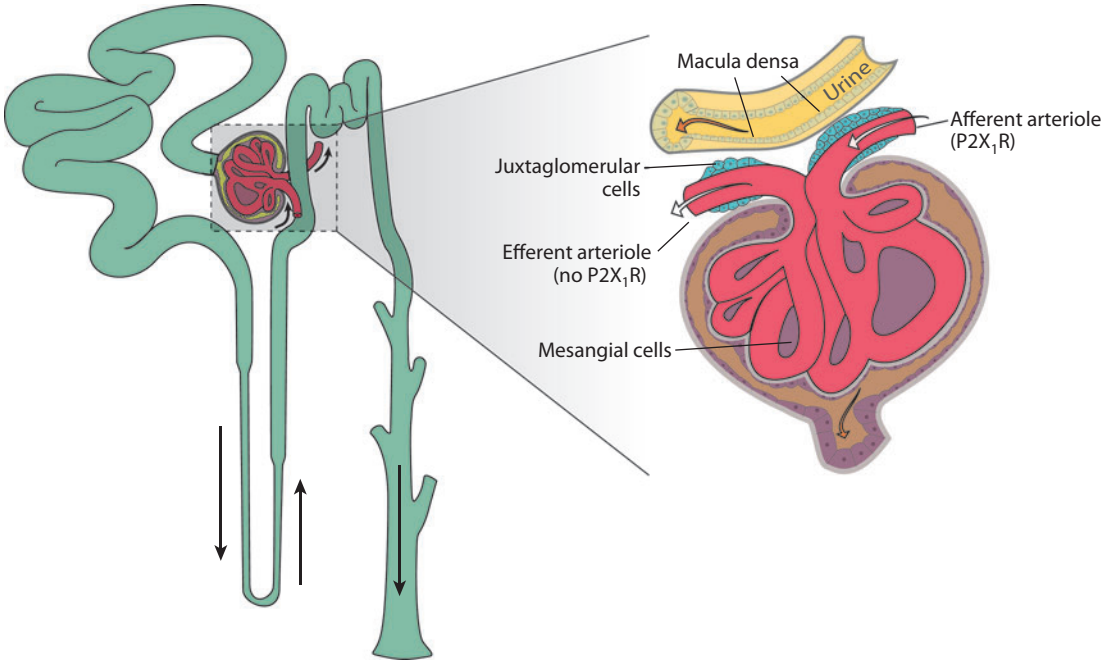

Figure 2

$P2X_1$ receptors are involved in renal autoregulation via tubuloglomerular feedback. To the left is a single nephron, with the glomerular apparatus enlarged on the right. Changes in tubular fluid flow rate occur in response to changes in glomerular filtration rate or the rate of reabsorption in the proximal tubule. Changes in glomerular filtration rate are sensed by macula densa cells that release ATP onto $P2X_1$ receptors on afferent arteriolar smooth muscle, triggering an increase in afferent arteriolar resistance. This reduces pressure in the glomerular capillaries and decreases glomerular filtration (i.e., tubuloglomerular feedback).

This autoregulation is due to both intrinsic myogenic response of smooth muscle and tubuloglomerular feedback, and $P2X_1$ receptors appear to be involved in the latter process. Tubuloglomerular feedback involves the juxtaglomerular apparatus, consisting of the macula densa, the glomerulus, and both efferent and afferent arterioles of the same nephron (**Figure 2**) (89). Apical macula densa cells are exquisitely sensitive to increases in NaCl concentration in the distal tubular fluid; as little as 5–10 mEq l^{-1} leads to afferent arteriolar vasoconstriction via tubuloglomerular feedback (90). How does this occur? Bell and colleagues (91, 92) used $P2X_2$ receptor–expressing PC12 cells as ATP biosensors. When they placed whole-cell patch-clamped (or Fura2-loaded) PC12 cells into contact with rabbit macula densa cells, they recorded ($P2X_2$-activated) currents (or calcium transients) when luminal NaCl was increased by 5- to 10-fold; the responses corresponded to the release of ~10 μM ATP. Smaller changes in NaCl concentration were not examined in this assay. However, the Navar group made in vivo microdialysis measurements of canine renal interstitial ATP (and adenosine) in response to changes in renal arterial pressure within the autoregulatory range (90, 93). They found a tight correlation between interstitial concentrations of ATP (but not adenosine) and tubuloglomerular feedback in response to small stepwise changes in renal arterial pressure. Taken together, these results show conclusively that ATP is released from the macula densa in response to luminal changes in NaCl. Although the molecular mechanism of the ATP release remains unknown, it is currently hypothesized that this ATP release triggers tubuloglomerular feedback.

However, there is considerable debate as to how such tubuloglomerular feedback is achieved. On one side of the debate is the adenosine A_1 receptor hypothesis: Specific ectonucleotidases hydrolyze released ATP to adenosine with subsequent activation of vasoconstrictor A_1 receptors on afferent arterioles (94). On the other side of the debate is the $P2X_1$ receptor hypothesis: Released ATP directly activates vasoconstrictor $P2X_1$ receptors on afferent arteriolar smooth muscle (89). The main support for the former hypothesis consists of the following. (*a*) The specific ectonucleotidases required to break down ATP to adenosine are highly expressed in the juxtaglomerular apparatus, (*b*) specific A_1 receptor antagonists inhibit or abolish tubuloglomerular feedback, and (*c*) A_1 receptor knockout mice do not exhibit tubuloglomerular feedback (94–97). Equally strong support for the latter model comes from findings that (*a*) $P2X_1$ receptors are expressed only on afferent arterioles but not on efferent arterioles or other kidney cells, (*b*) a selective $P2X_1$ receptor antagonist (NF279) abolishes tubuloglomerular feedback, and (*c*) $P2X_1$ receptor knockout mice do not exhibit tubuloglomerular feedback (89, 98, 99). Interpretations of results from blockade of A_1 receptor with antagonists or knockout mice may be more complicated than for results from similar blockade of $P2X_1$ receptors because of the widespread expression of A_1 receptors in the kidney and renal vasculature and because of the concomitant vasodilatory actions of the similarly widespread expression of A_2 receptor throughout the renal vasculature (100). It is also likely that either or both ATP and adenosine underlie tubuloglomerular feedback, depending on subpopulations of nephrons and their individual metabolic state; there are more than 300,000 individual nephrons in a rodent kidney. Moreover, subtle differences in in vivo microdialysis and other in vitro techniques and protocols among laboratories may also contribute to experimental differences. This A_1 receptor/$P2X_1$ receptor puzzle may be resolved by exchange of the knockout mice among the relevant groups and parallel experiments in the same laboratory using both sets of mice, along with a more quantitative comparison of the kinetics and pharmacology of inhibition of tubuloglomerular feedback by A_1 and $P2X_1$ receptor antagonists.

Renal fibrosis. The abnormal accumulation of extracellular matrix (i.e., fibrosis) is associated with all chronic renal diseases. Renal mesangial cells are primarily responsible for extracellular matrix deposition via the secretion of transforming growth factor β (TGFβ), which leads to increased accumulation of fibronectin and other extracellular matrix proteins (101). Thus, mesangial cell dysfunction is a key contributor to interstitial fibrosis and, as such, has attracted recent interest in regard to the potential roles purinoceptors may play in normal and diseased mesangial cells.

Schulze-Lohoff et al. (102) first showed that $P2X_7$ receptor activation induces mesangial cell apoptosis. Harada et al. (103) showed that ATP exerts dual actions on isolated rat mesangial cells; $P2Y_2$ receptor and/or $P2Y_4$ receptor activation stimulated mesangial cell proliferation, and $P2X_7$ receptor stimulation with BzATP [2′,3′-*O*-(4-benzoyl-benzoyl) adenosine 5′-triphosphate] led to increased apoptosis. Soloni et al. (104) confirmed these findings and found that $P2X_7$ receptor stimulation increased secretion of TGFβ and subsequent fibronectin levels, effects that were significantly enhanced when cells were grown in a high-glucose medium (mimicking hyperglycemic conditions). Goncalves et al. (105) used an animal model of interstitial fibrosis, unilateral ureteral obstruction, in both wild-type and $P2X_7$ receptor knockout mice. This group found that the classical features of unilateral ureteral obstruction (high macrophage invasion into the interstitium, high TGFβ secretion, increased mesangial cell apoptosis, and interstitial fibrosis) were all significantly abrogated in the $P2X_7$ receptor knockout mice. Taken together, these results suggest a physiologically significant role for $P2X_7$ receptors in renal fibrosis and may point to the therapeutic

potential of $P2X_7$ receptor antagonists in the treatment of chronic renal diseases.

However, Soloni et al. (106) used human mesangial cells from normal segments of kidneys obtained at nephrectomy and repeated experiments they had carried out on rat mesangial cells, with surprising results. They found that ATP clearly induced significant apoptosis in human mesangial cells, but could find no evidence for the presence or involvement of $P2X_7$ receptor; moreover, their pharmacological data were not readily compatible with the involvement of known human P2Y or P2X receptors. Only high concentrations of ATP (>1 mM), but not of BzATP, induced the apoptotic response, suggesting that ATP breakdown to adenosine may have been responsible; it has been shown that apoptosis of a hepatic epithelial cell line by ATP and ADP is due to the activation of adenosine A_3 receptors in response to hydrolysis of these nucleotides to adenosine (107). In any event, results from this study serve as caution against overreliance on, and overinterpretation of, results obtained from rodent tissues and cells.

Gastrointestinal System

The nonadrenergic, noncholinergic inhibitory transmission to the smooth muscle of the intestine was one of the very first demonstrations of a functional role for extracellular ATP (reviewed in References 1 and 3). Further, quite distinct roles in the gastrointestinal tract are now emerging. These include accessory structures such as salivary glands, liver, and exocrine pancreas. They also include signaling between nerve cells of the enteric nervous system within the gut wall.

Exocrine glands. Luminal ATP has long been known to activate purine receptors on several secretory epithelia with the consequent secretion of chloride, potassium, bicarbonate, or hormonal peptides (80, 81). The majority of studies on nucleotide-induced epithelial secretion show clearly that the P2Y family of purinoceptors, particularly the $P2Y_2$ receptor, is primarily responsible (80, 81). Additional involvement of $P2X_7$ receptors and/or $P2X_4$ receptors has also been suggested, but the interpretation is confounded by marked species differences in the physiological and pharmacological properties of these two receptors. Submandibular gland acinar and ductal cells from rat and mouse have been the most studied. Patch-clamp recordings show both $P2X_4$ receptor– and $P2X_7$ receptor–mediated currents, whereas Fura2 intracellular calcium measurements show predominantly $P2X_7$ receptor–mediated responses, with a very small contribution via $P2X_4$ receptors (<5% of total calcium response). Stimulation of $P2X_7$ receptors, but not of $P2X_4$ receptors, induces kallikrein secretion from ductal cells (108). Dehaye and colleagues (108–110) have built up a strong case for $P2X_7$ receptor signaling to salivary secretion through the modulation of phospholipid signaling processes, particularly phospholipase A2 and phospholipase D. The strongest evidence comes from a study comparing responses from $P2X_7$ receptor wild-type and knockout mice, in which (*a*) ATP no longer induced any phospholipid signaling, (*b*) the ATP-induced calcium signal was decreased by ~90–95%, and (*c*) the saliva from the knockout mice showed a significantly decreased potassium content (110). This study thus directly demonstrated the involvement of $P2X_7$ receptor in salivary secretions, but the evidence for phospholipid signaling as the underlying mechanism remains correlative.

The difficulty of matching P2X phenotype in epithelia with P2X protein expression is highlighted in a recent study on human gallbladder and rat liver bile duct cells, in which ATP and BzATP potently stimulated chloride secretion when applied to the apical surfaces of these epithelia (111). In these bile ducts, $P2X_4$ but not $P2X_7$ receptors were expressed by RT-PCR, immunoblot, and immunohistochemistry. However, the properties of the secretory response and whole-cell patch clamp recordings were more typical of activating $P2X_7$ than $P2X_4$ receptors. The recent availability of

BzATP: 2′,3′-*O*-(4-benzoyl-benzoyl) adenosine 5′-triphosphate

highly selective and potent rodent and human $P2X_7$ receptor antagonists may resolve some of these discrepancies.

Intestinal motility. Afferents traveling by the vagus nerve or the dorsal root ganglia signal intestinal activity to the central nervous system. The role of P2X receptors on such nerves is described above (see Afferent Signaling section). In contrast, considerable evidence exists for a key role for P2X receptors in signaling within the enteric nervous system that underlies coordinated movements of the intestine. The principal intrinsic afferent neuron of the enteric nervous system is the AH cell (112). AH cells (which can be identified by immunoreactivity for calbindin D28K) express $P2X_2$ and $P2X_3$ subunits; there is evidence that the afferent terminals of these neurons, in the mucosal layer and in the muscle layers, are activated by ATP through P2X receptors, but the subtype has not been conclusively identified (113). Another main class of neuron, the descending interneurons on the pathway to the circular muscle motoneurons, also expresses $P2X_2$ subunits; in these cells ATP substantially contributes to the fast EPSP when presynaptic fibers are stimulated electrically, as shown by comparing wild-type and knockout mice (114). Peristalsis of the small intestine recorded in vitro is clearly impaired in the ileum taken from $P2X_3$ receptor knockout mice, suggesting most likely that the afferent neurons are less sensitive to the initiating distension (115).

Immune System

$P2X_1$, $P2X_4$, and $P2X_7$ receptor proteins coexist in most immune cells, including mast cells, B and T lymphocytes, monocytes, macrophages, microglia, and osteoclasts (8). Cation currents and calcium influx with the features expected for each of these P2X receptors have been demonstrated in all these immune cells. However, only $P2X_7$ receptors are thus far established as having physiological roles in the immune system. There is a long history of $P2X_7$ receptor involvement in inflammation via the unique ability of the $P2X_7$ receptor to induce the rapid activation of caspase-1 with subsequent release of the proinflammatory cytokine IL-1β from activated macrophage and microglia (116, 117). The underlying mechanism(s) by which $P2X_7$ receptor activation leads to caspase-1 activation and IL-1β release remains a matter of considerable debate and study, but abrogation of inflammatory responses by inhibition of $P2X_7$ receptors is directly linked to concomitant decreased levels of IL-1β in plasma or in the area of injury (116, 117).

Two lines of mice have been bred with disrupted $P2X_7$ receptors. In one [Glaxo mice (65)] 5′ exons were disrupted by a neomycin-resistant vector. In the other [Pfizer mice (118)] the gene was disrupted in the portion encoding the cytoplasmic tail, leaving open the possibility of expression of a truncated receptor. In both lines, ATP did not induce the release of IL-1β or other proinflammatory members of the IL-1 family (IL-18 and IL-1α) from endotoxin-activated macrophages. Moreover, mechanical hypersensitivity (allodynia) in response to adjuvant injection into the paw, as well as in response to partial nerve ligation, was absent, and levels of IL-1β from the area of paw inflammation systemically were reduced. Recently, similar results have been obtained from wild-type mice treated with the selective $P2X_7$ antagonist A740003 (119). Researchers have described other potent and selective $P2X_7$ receptor antagonists that show either human or rodent specificity (119, 120). One such molecule (AZD9056) produces clinically relevant improvements in patient assessments of symptoms, and reduced swollen and tender joints, in a Phase II clinical trial of 75 patients with active rheumatoid arthritis treated for one month (121). These early results hold promise that $P2X_7$ receptor antagonists in inflammation may be the first therapeutic benefits to emerge from P2X receptor research.

Killing intracellular mycobacteria. Stimulation of macrophage $P2X_7$ receptors can lead to the death of several types of intracellular bacteria, and the killing of mycobacterium

tuberculosis is of much clinical relevance (122). The killing of intracellular bacilli directly parallels $P2X_7$ receptor–mediated macrophage apoptosis (122). Because IL-1β leads to apoptosis of surrounding cells, the direct cause of macrophage apoptosis and death of intracellular pathogens may be the $P2X_7$ receptor–induced release of IL-1β. Studies with RAW 264.7 murine macrophages have ruled out this possibility. These macrophages do not show caspase-1 activation/IL-1β release because they lack a key adaptor protein (ASC) required to form the multiprotein complex (the inflammasome; see **Figure 3**) (123). Yet these macrophages express high levels of $P2X_7$ receptor, and prolonged activation leads to macrophage apoptosis and concomitant killing of intracellular pathogens (123, 124). It is not known whether macrophage apoptosis is necessary for killing of intracellular mycobacteria. However, the $P2X_7$ receptor–mediated apoptotic process is associated with the formation of

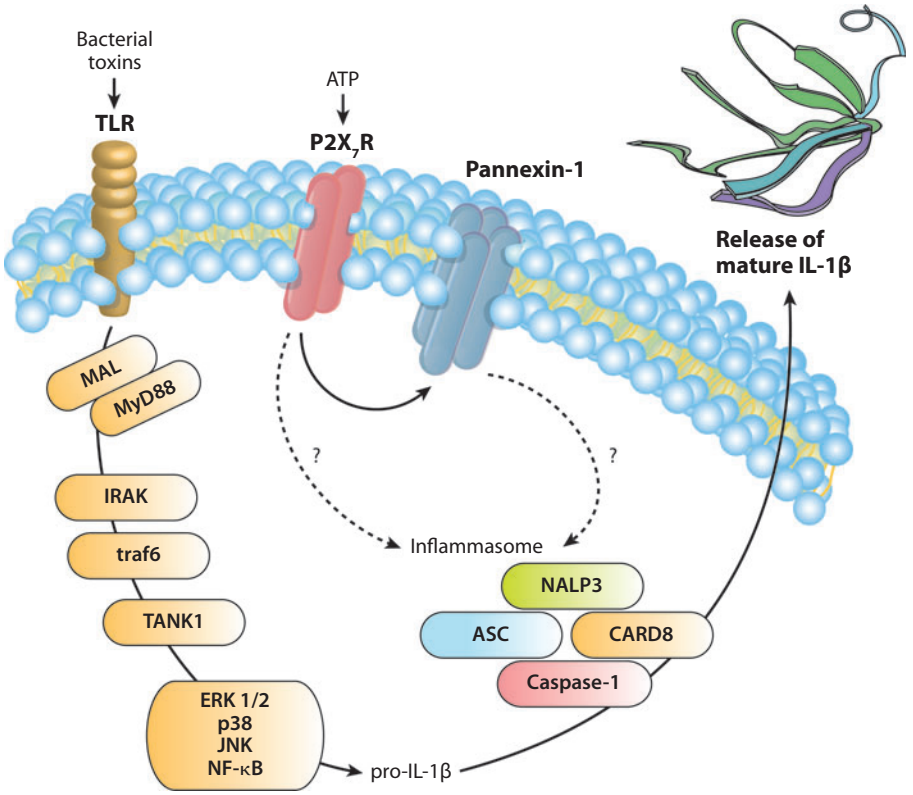

Figure 3

$P2X_7$ receptor ($P2X_7R$) and the NALP3 inflammasome. An initial inflammatory insult (e.g., bacterial endotoxins) activates Toll-like receptors (TLRs) on monocytes, macrophages, and microglia to initiate the classical nuclear factor-κB (NF-κB) cascade and the synthesis of pro-interleukin-1β (pro-IL-1β). However, in the absence of a secondary stimulus, little or no processing or release of IL-1β occur (116, 117). High concentrations of extracellular ATP, present at sites of inflammation, activate a $P2X_7R$/pannexin-1 protein complex (172). This leads to the activation of caspase-1, with the subsequent formation of a multiprotein intracellular complex, the NALP3-type inflammasome (173). Activated caspase-1 within the inflammasome scaffold now cleaves pro-IL-1β, and the release of mature, bioactive IL-1β follows. Blockade of either $P2X_7R$ or pannexin-1 independent of $P2X_7R$ prevents rapid IL-1β processing and release (117, 172). Mechanisms by which $P2X_7R$/pannexin-1 activation leads to caspase-1 activation and the release of mature IL-1β remain uncertain and are likely to be tissue/cell and stimulus specific.

large intracellular vacuoles and with the fusion of lysosomes with the bacilli-containing phagosomes, thus providing a means for killing the mycobacteria via lysosomal enzymes (122, 124, 125).

Osteoclasts. Several studies have implicated $P2X_7$ receptors in the formation of multinucleated giant cells in granulomatous diseases and in normal and pathological formation of multinucleated osteoclasts in bone (126). In one study, the Pfizer $P2X_7$ receptor knockout mouse showed abnormal bone formation and resorption in the form of a reduced periosteal circumference and decreased cortical bone content (127). In another study using Glaxo $P2X_7R$ knockout mice, there was no difference between wild-type and knockout mice in the ability of mononuclear phagocytes to generate multinucleated osteoclasts (128). The $P2X_7$ receptor antagonist AZ11657312 significantly reduced bone resorption, synovial inflammation, and the number of osteoclastic giant cells (129). Thus, although it has not yet been determined whether $P2X_7$ receptor alters bone metabolism via alterations in the formation of multinucleated giant cells, recent studies provide strong evidence for its role in modulating the function of osteoclasts in bone.

$P2X_7$ polymorphisms and immune function. The human $P2X_7$ receptor is associated with more than 500 single-nucleotide polymorphisms, some of which render the $P2X_7$ receptor nonfunctional. Wiley and colleagues (124, 130) have examined five specific $P2X_7$ receptor polymorphisms known to result in nonfunctional $P2X_7$ receptors when examined in heterologous expression systems. In a cohort of more than 700 mainly Caucasian adults, these investigators found that 51% showed the wild-type gene at the five alleles they examined, 40% were heterozygous at one of these alleles, and 25% of the heterozygous adults showed reduced functional readouts compared with wild type. Approximately 3% of the population was homozygous for T357S/E496A without any functional $P2X_7$ receptor response in their blood-derived macrophages (130). Macrophages obtained from individuals consistently show "high, inducible, or no" response to $P2X_7$ receptor stimulation (125, 130), and specific polymorphisms in the $P2X_7$ receptor gene may explain these observations. The molecular identity of individual $P2X_7$ receptors may of course alter their susceptibility to newly developed therapeutic antagonists.

MOLECULAR PROPERTIES OF P2X RECEPTORS

Direct structural approaches with single-particle electron microscopy and atomic force microscopy strongly support the view that the P2X receptor channel forms as a trimer (131, 132). This stoichiometry was originally based on polyacrylamide gel electrophoresis under nondenaturing conditions (133) and is supported by measurements of single-channel kinetics (134), or currents evoked by ATP through channels formed from concatenated subunits with reporter mutations (21, 135).

In the past few years, deduced P2X receptor sequences have become available from the genomes of several simple eukaryotes. Where these have been shown to function as ATP-gated ion channels, the limited sequence relatedness helps to identify those residues or parts of the molecule that are most critical for function. **Figure 4** illustrates this for five P2X receptors. These are green algae (*Ostreococcus tauri*), choanoflagellate (*Monosiga brevicollis*), slime mold (*Dictyostelium discoideum*), trematode worm (*Schistosoma mansoni*), and the vertebrate *Rattus norvegicus* (21, 136–138). The first two are single-celled organisms that are phylogenetically close to the origins of the divergence of plant and animal cells, the third has a life cycle with both single-celled and multicellular components, the fourth is a segmented worm, and the fifth is man.

Several points can be taken from this comparison of sequences of functional receptors. First, only relatively few residues, notably tyrosines, lysines, and glycines, are completely conserved. In almost all cases, these conserved

N terminus, TM1, post-TM1

```
rP2X2   ----MVRRLARGCWSAFWDYETPKVIVVRNRRLGFVHRMVQLLILLYFVWYVFIVQKSYQDSETGPESSIITKVKG 072
schis   ---------MVKGIAVLFEYETPKLVQISNIKIGVTQRLLQLVILIYVVCWVMIYEKGYQENDI-AKSAVTTKVKG 066
choan   MAASGFWGSIQQGIYSMLEYDTLKTVHIRSKKVGLIFRLLQITILAYVVGYGIIYQKGYQEVDS-AVSTVLTKVKG 075
algae   --------------MGLSYTTQKSVVIRSWRLGALYYGLVAVVLAYVGFFLVYVERGYQRTSR-AVGNIGLKVKG 059
dicty   -------MGFSFDWDDIFQYSTVKIVRIRDRRLGILHLSFLVGIVAYIVVYSAIIKKGYLFTEV-PIGSVRTSLKG 078
```

Ectodomain

```
rP2X2   ---------------ITMSEDKVWDVEEYVKPPEGGSVVSIITRIEVTPSQTLG----------TCPESMRVHSST 123
schis   -------VGFTNFSHIPGIGMRSWDVADYIVPPLGNNALFVITNLVKTERQSLS----------KCQESSWVPEAA 125
choan   IAITCDNTDISSMNDCVPGDLRVWDTPDYIKPAQENDAFFVVSNSIQTSKQTQRAEGWDEDPAA--PVTGSASAFN 149
algae   ---------QATLRDATTGATLVYDANDLVMYEPSG---FFIATALATTLQARG------------RCPGMDEDET 112
dicty   --------PNTFASNLTYCSNQQHNGSTYPFTPLECNY-WDEQLALFPVGQDSTF-------TCTTRVRLSKQEAN 128

rP2X2   CHSDD-DCIAGQLDMQGNGIRTGHCVPYYHG-----DSKTCEVSAWCPVEDG---TSDNH------FLGKMAPNFT 184
schis   CYKDS-DCKPYFISHLGNGAHTGKCIIKPGN-----DIGSCEIYSWCPLENDTLPLGRKS------FLFPMVYNYT 189
choan   CTSDA-DCPR--FATSRNGALTGECNT---------TTERCRIYGWGPVESKDEDDRATTDGLFYARHMPAVKNFT 213
algae   CTDAS-ACVVGTFSPS--GRMTGQCVATALKDEDGKVVKRCEVEGWCPGEPEKDEVTVLEN----------VGNFT 175
dicty   CNFTDPTCKFVDEPGSAKN-------------------------------------------------IYIADIESFT 132

rP2X2   ILIKNSIHYPKFKFSKGNIASQKSDY-LKHCTFDQDS--DPYCPIFRLGFIVEKAGEN-------------FTEL 243
schis   LLIKNDINFEKFGIHRRNIQNWASKKFLRTCLYNKTDPENRFCPIFQFGTIFEEANVD-------------QSIF 251
choan   VYIKNTVFFQRFGAKFG-STDESDKVDVYTCTWSPTG-LERHCPIFKIDTILNEAGIT------------DFENQA 275
algae   VFTRISVEFPGIPDEDGEGNMLWTNLNGTKP-TLGWNLWTINDLLESGGMSVKEVARK--VTPYTLKEVARKGWDG 236
dicty   ILIDHTMYASSSGSQFNAVDLHGYILNQDGDEVQIDANGTSIGVSGKPDIMTIGQLLSFGGVSLDQASPV--LDQA 224

rP2X2   AHKGGVIGVIINWNCDLDLSESECNPKYSFRRLDPKYVPAS-SGYNFRFAKYYKINGTTTT--------------- 303
schis   IS-GGVIGIDIDWKCDLDWDVQYCNPTYSFRRLDDAHAKIA-SGFNFRYAHFYSENGTNY---------------- 309
choan   MRNGALITIQVNYDCNLDSSAHTCSPTYKFTRLD-TKSDLS-AGYNFRFANYQIDPPA----------------- 331
algae   RVDVEF-------DCNLDRGIDACAPKTPYTLKQVMHPNTLSEGFNIRWISGQNVGEPSAQAGVVYSNETANGPGKDV 307
dicty   SPVDSNVSIR--YDGVVLFVFITYSNTYTYSTSDFKYVYSVQQIANTIYDVPETIILESIHS-------------- 284
```

Pre-TM2, TM2, post-TM2/C terminus

```
rP2X2   RTLIKAYGIRIDVIVHGQAGKFSLIPTIINLATALTSIGVGSFLCDWILLTFMN-KNKLYSHKKFDKVRTPKHPS 377
schis   RDLIKAYGIRFVIHVSGEAGKFHLLPLTMNIGSGLALLGLAPTVCDIIALNLLR-SRDIYQRAKFETIAEEQAHL 383
choan   RDLYKVYGLRFVFVVSGTAGRFSMVPLLVALGSGLGLLGLATVIADLLVTKCIR-NANVYYGLKYQVVDEEDIDR 405
algae   RLLVKGYGPRIRFEVTGVGRKFDWLTLSTTVGAGVAFLGIASLVVNAVMMYCSGPKSKQYESWLFAEFHDTPYGS 382
dicty   RLLYKRHGIRVIFIQTGTIGSFHFQTLLLTLVSGLGLLAVATTVVDQLAIRLLP-QRKSYSSLKFQVTESMSNPM 358
```

Figure 4

Conservation of P2X receptors among disparate organisms. Sequence alignment of five P2X receptors that form functional ATP-gated channels when expressed in HEK cells. Overlines denote proposed transmembrane domains. Red denotes residues conserved among five sequences; blue denotes residues similar among five sequences (similar are IVLM, FYWH, KRH, EQDN). Sequences (with Genbank accessions) are rP2X2, *Rattus norvegicus* $P2X_2$ (P49653); schis, *Schistosoma mansoni* (CAH04147); choan, *Monosiga brevicollis* (EDQ92249); algae, *Ostreococcus tauri* (CAL54489); dicty, *Dictyostelium discoideum* (XP_645378). Further C termini (5–95 residues) are divergent and not shown.

residues have previously been shown to be essential for function in mammalian receptors (21, 135). Second, the highly conserved residues are in or close to the two transmembrane domains. This applies to the highly conserved intracellular Y-X-X-X-K motif that is found both ~10 residues before the start of transmembrane domain 1 (TM1) and ~10 residues after the end of transmembrane domain 2 (TM2). Such a positioning pattern also applies to the highly conserved extracellular positively charged amino acids that are located ~15 residues after the end of TM1 (Lys^{69} and Lys^{71} in $P2X_2$) as well as ~20 residues before the start of TM2 (Arg^{304} and Lys^{308}). Third, the great bulk of the ectodomain is not conserved. This includes the ten cysteine residues. The lack of conservation extends also to several residues that, on the basis of mutagenesis in vertebrate receptors, had been considered to contribute to an ATP-binding site

(see 135). The key residues that had been proposed to represent homology with the ATP-binding site of class II tRNA synthases (139) are also mostly missing in the simpler P2X receptors.

N Terminus

The most striking feature of the N terminus of the P2X receptor family is the Y-X-T-X[KR] motif that ends some 10 residues prior to the start of TM1. The T-X-K conforms to the protein kinase C consensus, and phosphorylation of Thr^{18} in the $P2X_2$ receptor has been demonstrated for $P2X_2$ (140) but not for $P2X_1$ receptors (141); mutations at this site alter peak currents and desensitization at the $P2X_1$ receptor (141). In the $P2X_2$ receptor, replacement of Tyr^{16} by cysteine prevents channel function, although such a substitution is tolerated at Thr^{18} or Lys^{20} (142).

Transmembrane Domain 1

Each residue of TM1 has been systematically substituted by cysteine [$P2X_2$ (142–144)], alanine [$P2X_2$ (145, 146)], or tryptophan [$P2X_4$ (147)]. The results indicate in general that (*a*) TM1 is very likely α-helical, (*b*) residues Gly^{30} and Tyr^{43} ($P2X_2$ numbering) are completely conserved and critical for function, (*c*) TM1 likely moves outward with respect to TM2 during channel gating (148), and (*d*) residues in TM1 seem not to contribute directly to the permeation pathway (146).

Post–Transmembrane Domain 1

The 20 amino acids immediately following TM1 do not show strong conservation (**Figure 4**) except for Tyr^{55} and Gln^{56}, which are essential for receptor function (142, 148), and the KΦKG motif Lys^{69} and Lys^{71} in the $P2X_2$ receptor, where Φ is any hydrophobic amino acid (142). Abundant evidence now supports the view that one or both of these Lys residues play a critical role in ATP binding. The three main lines of evidence are as follows. (*a*) Changing the charge on the nearby Ile^{67} can affect ATP potency in a manner that is consistent with a direct electrostatic effect on the binding of a negatively charged agonist (142), (*b*) comparing the effects of ATP with those of the partial agonist BzATP suggest a direct binding rather than a gating role (149), and (*c*) Lys^{69} is also required for channel activation by TNP-ATP at the constitutively active $P2X_2$ receptor carrying a T339S mutation (150). Experiments with coexpressed $P2X_2$ subunits, in which one contains Lys^{69} and the other does not (i.e., $P2X_2$[K69A]), strongly suggest that two rather than three such lysines are required for agonist activation of the channel and, moreover, that the ATP-binding site likely forms between lysines provided by two different subunits (151).

Ectodomain

Figure 4 illustrates that most parts of the ectodomain that are conserved among vertebrates are not found in the *Dictyostelium* sequence; this includes the ten cysteine residues. This indicates that much of the ectodomain is not critical for function as an ATP-gated channel and presumably subserves other roles such as interactions with proteins provided by the matrix or by neighboring cells. The pattern of disulfide bond formation has been deduced. However, although the cysteines play a role in transport to the cell membrane, none of the cysteines are critical for channel function (152, 153). Zinc potentiates currents evoked by ATP at $P2X_2$ receptors. By using concatenated cDNAs to express subunits joined in tandem, Hume and colleagues (154) showed that His^{120} and His^{213} form the zinc-binding site and that different subunits contribute to the compositions of these two histidines. When these histidines are replaced by cysteine, an intersubunit disulfide bond can form. Similar intersubunit ectopic disulfide bonds between pairs of cysteines introduced into the $P2X_1$ receptor (Lys^{68} and Phe^{291}) have been demonstrated (155), as was first shown for cysteines at the outer ends of the transmembrane domains of the $P2X_2$ receptor [Val^{48} to Ile^{328} (148)]. One conserved motif

is present in all known sequences, including the *Dictyostelium* sequence: [EQP]-[NDS]-F-T-Φ-Φ-Φ-[KD]-NH]-[STN] (where Φ = any hydrophobic residue). The FT (Phe^{183}-Thr^{184} in $P2X_2$) residues may play a role in stacking against the adenine moiety of ATP (149, 156). A similar role has also been ascribed to the NFR motif (Asn^{288}-Phe^{289}-Arg^{290} in $P2X_2$) (156), but this seems less likely given that these residues are missing from the *Dictyostelium* sequence (137).

Pre–Transmembrane Domain 2

This region extends from the highly conserved Arg^{304} ($P2X_2$) to the beginning of TM2 (around Ile^{328}). The first half of this region shows particularly high sequence conservation (**Figure 4**). The lysine residue at position 308 (in $P2X_2$; position 309 in $P2X_1$ and position 313 in $P2X_4$) is required for channel function and has been implicated in ATP binding (21, 135, 142, 149, 157). For the $P2X_{2/3}$ heteromer, Wilkinson et al. (158) suggested that the ATP-binding site may have contributions from Lys^{69} of one subunit and Lys^{308} of another. Other work suggests a more fundamental role for Lys^{308} in channel gating. Yan et al. (157) favor a role for this residue (Lys^{313} in $P2X_4$ receptor) in contributing to intersubunit interactions that accompany gating, rather than contributing directly to binding. Cao et al. (150) have reported that this residue is critical for channel activation in a slightly modified channel ($P2X_2$[T339S]) that opens spontaneously in the complete absence of any agonist. $P2X_2$[T339S] is also activated by suramin, but $P2X_2$[T339S,K308A] is not; this finding is also best interpreted to indicate a key role for Lys^{308} in channel gating. With the exception of Arg^{304} and Lys^{308}, substitution of any individual residue in pre-TM2 by cysteine (in $P2X_1$) does not markedly affect the ability of ATP to evoke inward currents (159). In most cases, these cysteines were accessible to labeling with MTSEA-biotin, suggesting their exposure on the protein surface. Yan et al. (157) studied alanine substitutions (in $P2X_4$) and have speculated that the pre-TM2 segment functions as a "signal transduction module" from ATP-binding site to channel gate. Taken together, it appears that this highly conserved region may subserve roles other than ATP binding and channel gating.

Transmembrane Domain 2

The consensus view from cysteine scanning (21, 135), alanine scanning (145), and tryptophan scanning (147) is that TM2 does not cross the membrane as an uninterrupted α-helix. Cysteine is tolerated at almost all positions through TM2 when substituted individually (160, 161). Tryptophan is tolerated in the outer half of TM2, and the positions causing the greatest perturbation of function (actually an increase in sensitivity to ATP) align along one surface of a helix ($P2X_4$) (147). Near the center of TM2, tryptophan is not tolerated at Ala^{349}, Leu^{352}, Cys^{353}, Asp^{354}, Val^{357}, and Leu^{358} ($P2X_4$; positions correspond to $P2X_2$ Gly^{344}, Leu^{347}, Cys^{348}, Asp^{349}, Leu^{352}, and Leu^{353}, respectively), suggesting to these authors (147) "a tightly packed and relatively delicate structure that poorly tolerates substitution" at this position in the protein.

Early studies with cysteine scanning and the effects of methanethiosulfonates had indicated that the gate might form in the middle of TM2 [between Thr^{339} and Asp^{349} on the $P2X_2$ receptor (160)], and studies on the permeability of the P2X receptor now support this interpretation. Migita et al. (162) identified Thr^{339} and Ser^{340} of the $P2X_2$ receptor as having the greatest effect on cation permeability. More recently, the fraction of the inward current carried by calcium ions (*Pf*%) has been determined by combination of measurement of charge with measurement of change in calcium concentration (163). Measurements of *Pf*% also implicate residues Thr^{339} and Ser^{340} as the main deep determinants of calcium flux. Calcium *Pf*% varies among homomeric P2X receptors; $P2X_1$ and $P2X_4$ receptors show the highest values [11–15%, similar to calcium *Pf*% for NMDA receptors; see Egan & Khakh (163)]. The difference between these two receptors and

others in the family may result in part from the calcium-concentrating effect of fixed negative charges provided by glutamate residues near the extracellular ends of both TM1 and TM2 (164).

The Asp residue at position 349 ($P2X_2$) is completely conserved among P2X receptors other than in the algae *O. tauri* (138). Replacement of Asn by Asp in the algae receptor causes a 50% increase in relative calcium permeability. However, the reverse mutation in $P2X_2$ receptors does not change relative calcium permeability (138, 162). Thus, this aspartate residue does not appear to play a major role in the determination of calcium permeability. In contrast, it does have a key role in the intramembrane interhelix interactions required for channel assembly ($P2X_5$) (165).

C Terminus

The cytoplasmic tails of six of the P2X receptors retain considerable sequence relatedness for the first 25 amino acids (**Figure 4**), but relatedness thereafter disappears completely. A YXXXK motif, beginning ~10 residues from the cytoplasmic end on TM2 (or 28 in $P2X_7$; see below), is very highly conserved among all P2X receptors, including those from single-celled organisms that have poorly conserved ectodomains. This lends a symmetry to P2X receptor sequences, the conserved region of which both begins and ends with YXXXK motifs in the cytoplasmic regions (**Figure 4**). Rassendren and colleagues (166) have shown that the Tyr and the Lys of this motif are required to stabilize the P2X receptor subunit in the plasma membrane; in heteromeric $P2X_{2/3}$ receptors the motif needs to be present on only one of the partners. The $P2X_7$ receptor differs from the other six in this region by the presence of an 18-amino-acid cysteine-rich insertion. Removal of this insertion prevents the $P2X_7$ receptor from undergoing the increase in NMDG permeability that normally occurs when the ATP application is continued for several seconds. Deletion of this cysteine-rich segment has no effect on the properties of the channel in normal extracellular sodium concentrations, and it does not affect the ability of the $P2X_7$ receptor to drive uptake of fluorescent dyes (167).

To the C-terminal side of the YXXXK motif, the sequences of P2X receptors are subunit specific. For the $P2X_2$ subunit, the C terminus binds to Fe65 (168), a multidomain protein first identified by its ability to bind to β-amyloid precursor protein. Both proteins colocalize to the postsynaptic membranes of excitatory synapses in the hippocampus. Coexpression in oocytes showed that the presence of Fe65 slows the rate of pore dilation (increase in NMDG permeability) that occurs when the ATP application is applied for several minutes (see Reference 21). $P2X_4$ subunits have a motif (YXXGΦ) that directly binds the μ2 subunit of the AP2 adaptor protein (73). This interaction is responsible for $P2X_4$ receptor endocytosis, despite the presence of a nearby YXXΦ motif that is commonly involved in the binding of AP2 to other proteins.

$P2X_7$ receptors interact with several partners, including cytoskeletal proteins (85). One of the interactors is receptor protein tyrosine phosphatase β, which has a clear effect on the currents through the $P2X_7$ receptor when it is activated repeatedly (85). Other partners include epithelial membrane proteins (169). Motifs have been identified in conserved regions of the $P2X_7$ receptor C terminus that resemble sequences in the SH3 domain–binding proteins *Mycoplasma genitalium* cytadherence accessory protein HMW3 and *Caenorhabditis elegans* protein C18H2.1 (170). However, the most intriguing binding partner may be the bacterial endotoxin lipopolysaccharide (LPS) (170). A motif very close to the tail of the $P2X_7$ sequence (residues 573 to 590) is well conserved among all known $P2X_7$ subunits and closely resembles a sequence in soluble LPS-binding protein. Bertics and his colleagues showed that a peptide corresponding to this motif (rat $P2X_7$ 573 to 590: CRWRIRKEFPKTQGQYSG) directly binds FITC-labeled LPS, as does the LPS-binding protein itself. The same two basic amino acids in the $P2X_7$

peptide and in LPS-binding protein are essential for LPS binding (170), and mutation of these two residues impairs both receptor trafficking and channel function in HEK293 cells (171). It will be important to test the possibility that this region of the $P2X_7$ receptor is directly responsible for those actions of bacterial LPS that are independent of Toll-like receptor 4 (TLR4).

CONCLUDING THOUGHTS AND FUTURE ISSUES

The field continues to be limited by the availability of agonists, antagonists, and modulators with high selectivity for one or another P2X receptor, but there have been notable advances. Particularly in the cases of $P2X_1$, $P2X_3$, and $P2X_7$ receptors, there are now antagonists available with nanomolar affinity. There remain two important caveats to their application. The first is the issue of species specificity, which seems particularly important to take into account for $P2X_7$ antagonists. The second is the problem associated with actions at receptors other than P2X receptors. For example, suramin analogs and ivermectin have several such effects. Physiologists studying the functional roles of P2X receptors would welcome further progress in the development of such compounds.

The availability of mice lacking $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, and $P2X_7$ receptors has impacted the discovery of new functional roles. The difficulties here are those familiar to all such approaches—the species specificity and the possibilities of compensatory changes. Tissue-specific and inducible knock-in and knockout constructs would be most valuable.

LPS: bacterial lipopolysaccharide

At the molecular level, progress continues to be made with respect to our understanding of the permeation pathway and the ATP-binding site. The former will benefit from single-channel recordings combined with mutagenesis, and the latter from the sequencing of receptor fragments cross-linked to appropriate agonists or antagonists. Similar mass spectroscopic approaches need to be further extended to identify the range of proteins that interact directly with P2X receptors. These will lead us to an improved understanding of the subcellular trafficking and localization of P2X receptors, in which context it will be important to seek further evidence for intracellular functional roles such as those reported for *Dictyostelium*. And finally, high-resolution structure of a crystallized receptor would of course provide the field with its greatest impetus.

SUMMARY POINTS

1. $P2X_1$ receptors are neurotransmitter receptors at sympathetically innervated smooth muscle and play functional roles on platelets and in the juxtaglomerular apparatus of the kidney.
2. $P2X_2$ and $P2X_3$ subunits (and $P2X_{2/3}$ heteromeric receptors) are necessary for the initiation of sensory signaling in pathways subserving taste, chemoreception, visceral distension, and neuropathic pain.
3. $P2X_4$ receptors are involved in the function of vascular endothelium. Mice lacking the $P2X_4$ receptor gene are hypertensive and have smaller-diameter arteries. Microglial $P2X_4$ receptors in the spinal cord have also been implicated in neuropathic pain.
4. Activation of $P2X_7$ receptors drives the release of proinflammatory cytokines from macrophages primed with lipopolysaccharides. $P2X_7$ antagonists appear beneficial in the inflammation of rheumatoid arthritis.
5. In the central nervous system there are widespread presynaptic and postsynaptic actions of ATP, but the importance of P2X signaling remains unclear at the cellular level.

6. P2X receptors in a simple eukaryote are intracellular and involved in osmoregulation. Thus, functions on intracellular membranes in mammalian cells may be worth seeking. The distant sequence relatedness of these primitive P2X receptors also indicates that substantial parts of the receptor ectodomain are not needed for receptor function.
7. Single-channel recordings and mutagenesis are identifying the regions of the P2X receptor involved in ion permeation, ATP binding, and trafficking to the cell surface, but higher-resolution structural information would greatly accelerate this work.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by The Wellcome Trust and the BBSRC.

LITERATURE CITED

1. Burnstock G. 2007. Physiology and pathophysiology of purinergic neurotransmission. *Physiol. Rev.* 87:659–797
2. Burnstock G, Kennedy C. 1985. Is there a basis for distinguishing two types of P2-purinoceptor? *Gen. Pharmacol.* 16:433–40
3. **Burnstock G. 2006. Historical review: ATP as a neurotransmitter. *Trends Pharmacol. Sci.* 27:166–76.**
4. Valera S, Hussy N, Evans RJ, North RA, Surprenant A, et al. 1994. A new class of ligand-gated ion channel defined by P2X receptor for extracellular ATP. *Nature* 371:516–19
5. Brake AJ, Wagenbach MJ, Julius D. 1994. New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature* 371:519–23
6. Collo G, North RA, Kawashima E, Merlo-Pich E, Neidhart S, et al. 1996. Cloning of $P2X_5$ and $P2X_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J. Neurosci.* 16:2495–507
7. Collo G, Neidhart S, Kawashima E, Kosco-Vilbois M, North RA, Buell G. 1997. Tissue distribution of the $P2X_7$ receptor. *Neuropharmacology* 36:1277–83
8. Burnstock G, Knight GE. 2004. Cellular distribution and functions of P2 receptor subtypes in different systems. *Int. Rev. Cytol.* 240:31–304
9. Rubio ME, Soto F. 2001. Distinct localization of P2X receptors at excitatory postsynaptic specializations. *J. Neurosci.* 21:641–53
10. Sim JA, Young MT, Sung HY, North RA, Surprenant A. 2004. Reanalysis of $P2X_7$ receptor expression in rodent brain. *J. Neurosci.* 24:6307–14
11. Anderson CM, Nedergaard M. 2006. Emerging challenges of assigning $P2X_7$ receptor function and immunoreactivity in neurons. *Trends Neurosci.* 29:257–62
12. Edwards FA, Gibb AJ, Colquhoun D. 1992. ATP receptor-mediated synaptic currents in the central nervous system. *Nature* 359:144–46
13. Pankratov Y, Castro E, Miras-Portugal MT, Krishtal O. 1998. A purinergic component of the excitatory postsynaptic current mediated by P2X receptors in the CA1 neurons of the rat hippocampus. *Eur. J. Neurosci.* 10:3898–902
14. Mori M, Heuss C, Gähwiler BH, Gerber U. 2001. Fast synaptic transmission mediated by P2X receptors in CA3 pyramidal cells of rat hippocampal slice cultures. *J. Physiol.* 535:115–23

3. Engaging and authoritative personal perspective on the initiation and progress of the field of purine receptor biology.

15. Pankratov Y, Lalo U, Krishtal O, Verkhratsky A. 2002. Ionotropic P2X purinoreceptors mediate synaptic transmission in rat pyramidal neurones of layer II/III of somato-sensory cortex. *J. Physiol.* 542:529–36
16. Pankratov Y, Lalo U, Krishtal O, Verkhratsky A. 2003. P2X receptor-mediated excitatory synaptic currents in somatosensory cortex. *Mol. Cell. Neurosci.* 24:842–49
17. Pankratov Y, Lalo U, Verkhratsky A, North RA. 2007. Quantal release of ATP in mouse cortex. *J. Gen. Physiol.* 129:257–65
18. Jo YH, Role LW. 2002. Coordinate release of ATP and GABA at in vitro synapses of lateral hypothalamic neurons. *J. Neurosci.* 22:4794–804
19. Sim JA, Chaumont S, Jo J, Ulmann L, Young MT, et al. 2006. Altered hippocampal synaptic potentiation in $P2X_4$ knock-out mice. *J. Neurosci.* 26:9006–9
20. Sperlágh B, Vizi ES, Wirkner K, Illes P. 2006. P2X receptors in the nervous system. *Prog. Neurobiol.* 78:327–46
21. North RA. 2002. Molecular physiology of P2X receptors. *Physiol. Rev.* 82:1013–67
22. Fellin T, Pozzan T, Carmignoto G. 2006. Purinergic receptors mediate two distinct glutamate release pathways in hippocampal astrocytes. *J. Biol. Chem.* 281:4274–84
23. Jabs R, Matthias K, Grote A, Grauer M, Seifert G, Steinhäuser C. 2007. Lack of P2X receptor mediated currents in astrocytes and GluR type glial cells of the hippocampal CA1 region. *Glia* 55:1648–55
24. Lalo U, Pankratov Y, Wichert SP, Rossner MJ, North RA, et al. 2008. $P2X_1$ and $P2X_5$ subunits form the functional P2X receptor in mouse cortical astrocytes. *J. Neurosci.* 28:5473–80
25. Khakh BS, Henderson G. 1998. ATP receptor-mediated enhancement of fast excitatory neurotransmitter release in the brain. *Mol. Pharmacol.* 54:372–78
26. Patel MK, Khakh BS, Henderson G. 2001. Properties of native P2X receptors in rat trigeminal mesencephalic nucleus neurones: lack of correlation with known, heterologously expressed P2X receptors. *Neuropharmacology* 40:96–105
27. Khakh BS, Gittermann D, Cockayne DA, Jones A. 2003. ATP modulation of excitatory synapses onto interneurons. *J. Neurosci.* 23:7426–37
28. Shigetomi E, Kato F. 2004. Action potential-independent release of glutamate by Ca^{2+} entry through presynaptic P2X receptors elicits postsynaptic firing in the brainstem autonomic network. *J. Neurosci.* 24:3125–35
29. Donato R, Rodrigues RJ, Takahashi M, Tsai MC, Soto D, et al. 2008. GABA release by basket cells onto Purkinje cells, in rat cerebellar slices, is directly controlled by presynaptic purinergic receptors, modulating Ca^{2+} influx. *Cell Calcium* 44:521–32
30. Rhee JS, Wang ZM, Nabekura J, Inoue K, Akaike N. 2000. ATP facilitates spontaneous glycinergic IPSC frequency at dissociated rat dorsal horn interneuron synapses. *J. Physiol.* 524:471–83
31. Zemkova H, Yan Z, Liang Z, Jelinkova I, Tomic M, Stojilkovic SS. 2007. Role of aromatic and charged ectodomain residues in the $P2X_4$ receptor functions. *J. Neurochem.* 102:1139–50
32. Troadec JD, Thirion S, Nicaise G, Lemos JR, Dayanithi G. 1998. ATP-evoked increases in $[Ca^{2+}]_i$ and peptide release from rat isolated neurohypophysial terminals via a $P2X_2$ purinoceptor. *J. Physiol.* 511:89–103
33. Knott TK, Velázquez-Marrero C, Lemos JR. 2005. ATP elicits inward currents in isolated vasopressinergic neurohypophysial terminals via $P2X_2$ and $P2X_3$ receptors. *Pflüg. Arch.* 450:381–89
34. Yoshida R, Yasumatsu K, Shigemura N, Ninomiya Y. 2006. Coding channels for taste perception: information transmission from taste cells to gustatory nerve fibers. *Arch. Histol. Cytol.* 69:233–42
35. Roper SD. 2007. Signal transduction and information processing in mammalian taste buds. *Pflüg. Arch. Eur. J. Physiol.* 454:759–76
36. Hayato R, Ohtubo Y, Yoshii K. 2007. Functional expression of inotropic purinergic receptors on mouse taste bud cells. *J. Physiol.* 584:473–88
37. Bo X, Alavi A, Xiang Z, Oglesby I, Ford A, Burnstock G. 1999. Localization of ATP-gated $P2X_2$ and $P2X_3$ receptor immunoreactive nerves in rat taste buds. *Neuroreport* 10:1107–11
38. Kataoka S, Toyono T, Seta Y, Toyoshima K. 2006. Expression of ATP-gated $P2X_3$ receptors in rat gustatory papillae and taste buds. *Arch. Histol. Cytol.* 69:281–88

39. Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, et al. 2005. ATP signaling is crucial for communication from taste buds to gustatory nerves. *Science* 310:1495–99

40. Huang YJ, Maruyama Y, Dvoryanchikov G, Pereira E, Chaudhari N, Roper SD. 2007. The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. *Proc. Natl. Acad. Sci. USA* 104:6436–41
41. Romanov RA, Rogachevskaja OA, Bystrova MF, Jiang P, Margolskee RF, Kolesnikov SS. 2007. Afferent neurotransmission mediated by hemichannels in mammalian taste cells. *EMBO J.* 26:657–67
42. Rong W, Burnstock G, Spyer KM. 2000. P2X purinoceptor-mediated excitation of trigeminal lingual nerve terminals in an in vitro intra-arterially perfused rat tongue preparation. *J. Physiol.* 524:891–902
43. Housley GD, Jagger DJ, Greenwood D, Raybould NP, Salih SG, et al. 2002. Purinergic regulation of sound transduction and auditory neurotransmission. *Audiol. Neurootol.* 7:55–61
44. Housley GD, Marcotti W, Navaratnam D, Yamoah EN. 2006. Hair cells—beyond the transducer. *J. Membr. Biol.* 209:89–118
45. Munoz DJB, Thorne PR, Housley GD. 1999. P2X receptor-mediated changes in cochlear potentials arising from exogenous adenosine 5′-triphosphate in endolymph. *Hear. Res.* 138:56–64
46. Salih SG, Jagger DJ, Housley GD. 2002. ATP-gated currents in rat primary auditory neurones in situ arise from a heteromultimeric P2X receptor subunit assembly. *Neuropharmacology* 42:386–95
47. Lee JH, Chiba T, Marcus DC. 2001. $P2X_2$ receptor mediates stimulation of parasensory cation absorption by cochlear outer sulcus ells and vestibular transitional cells. *J. Neurosci.* 21:9168–74
48. Dulon D, Jagger DJ, Lin X, Davis RL. 2006. Neuromodulation in the spiral ganglion: shaping signals from the organ of Corti to the CNS. *J. Memb. Biol.* 209:167–75
49. Prabhakar NR. 2000. Oxygen sensing by the carotid body chemoreceptors. *J. Appl. Physiol.* 88:2287–95
50. Rong W, Gourine AV, Cockayne DA, Xiang Z, Ford AP, et al. 2003. Pivotal role of nucleotide $P2X_2$ receptor subunit of the ATP-gated ion channel mediating ventilatory responses to hypoxia. *J. Neurosci.* 23:11315–21
51. Spyer KM, Dale N, Gourine AV. 2004. ATP is a key mediator of central and peripheral chemosensory transduction. *Exp. Physiol.* 89:53–59
52. Ferguson DR, Kennedy I, Burton TJ. 1997. ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes—a possible sensory mechanism? *J. Physiol.* 505:503–11
53. Yu Y, de Groat WC. 2008. Sensitization of pelvic afferent nerves in the in vitro rat urinary bladder-pelvic nerve preparation by purinergic agonists and cyclophosphamide pretreatment. *Am. J. Physiol. Ren. Physiol.* 294:F1146–56

54. Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, et al. 2000. Urinary bladder hyporeflexia and reduced pain-related behaviour in $P2X_3$-deficient mice. *Nature* 407:1011–15

55. Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, et al. 2001. $P2X_3$ knock-out mice reveal a major sensory role for urothelially released ATP. *J. Neurosci.* 21:567–77
56. Hamilton SG, Warburton J, Bhattacharjee A, Ward J, McMahon SB. 2000. ATP in human skin elicits a dose-related pain response which is potentiated under conditions of hyperalgesia. *Brain* 123:1238–46
57. Lewis C, Neidhart S, Holy C, North RA, Buell G, Surprenant A. 1995. Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. *Nature* 377:432–35
58. Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN. 1995. A P2X purinoceptor expressed by a subset of sensory neurons. *Nature* 377:428–31
59. Jarvis MF, Burgard EC, McGaraughty S, Honore P, Lynch K, et al. 2002. A-317491, a novel potent and selective non-nucleotide antagonist of $P2X_3$ and $P2X_{2/3}$ receptors, reduces chronic inflammatory and neuropathic pain in the rat. *Proc. Natl. Acad. Sci. USA* 99:17179–84
60. Honore P, Kage K, Mikusa J, Watt AT, Johnston JF, et al. 2002. Analgesic profile of intrathecal $P2X_3$ antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats. *Pain* 99:11–19
61. Wirkner K, Sperlagh B, Illes P. 2007. $P2X_3$ receptor involvement in pain states. *Mol. Neurobiol.* 36:165–83
62. Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, et al. 2003. $P2X_4$ receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 424:778–83
63. Trang T, Beggs S, Salter MW. 2006. Purinoceptors in microglia and neuropathic pain. *Pflüg. Arch.* 452:645–52

39. $P2X_{2/3}R$ double-knockout mice reveal the critical importance of $P2X_{2/3}$ receptors in afferent signaling from taste buds.

54. The first description of pain phenotype from $P2X_3R$ knockout mice and the discovery of its importance in afferent signaling in bladder reflexes.

64. Virginio C, Robertson G, Surprenant A, North RA. 1998. Trinitrophenyl-substituted nucleotides are potent antagonists selective for $P2X_1$, $P2X_3$, and heteromeric $P2X_{2/3}$ receptors. *Mol. Pharmacol.* 53:969–73
65. Chessell IP, Hatcher JP, Bountra C, Michel AD, Hughes JP, et al. 2005. Disruption of the $P2X_7$ purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* 114:386–96
66. Honore P, Donnelly-Roberts D, Namovic MT, Hsieh G, Zhu CZ, et al. 2006. A-740003 [*N*-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective $P2X_7$ receptor antagonist, dose-dependently reduces neuropathic pain in the rat. *J. Pharmacol. Exp. Ther.* 319:1376–85
67. Wilson HL, Varcoe RW, Stokes L, Holland KL, Francis SE, et al. 2007. P2X receptor characterization and IL-1/IL-rA release from human endothelial cells. *Br. J. Pharmacol.* 151:96–108
68. Chi JT, Chang HY, Haraldsen G, Jahnsen FL, Troyanskaya OG, et al. 2003. Endothelial cell diversity revealed by global expression profiling. *Proc. Natl. Acad. Sci. USA* 100:10623–28
69. Tran QK, Watanabe H. 2006. Calcium signaling in the endothelium. *Handb. Exp. Pharmacol.* 176:145–87
70. Yamamoto K, Korenaga R, Kamiya A, Qi Z, Sokabe M, Ando J. 2000. $P2X_4$ receptors mediate ATP-induced calcium influx in human vascular endothelial cells. *Am. J. Physiol.* 27:H285–92
71. Garcia-Guzman M, Soto F, Gomez-Hernandez JM, Lund P, Stuhmer W. 1997. Characterization of recombinant human $P2X_4$ receptor reveals pharmacological differences to the rat homologue. *Mol. Pharmacol.* 51:109–18
72. Bobanovic LK, Royle SJ, Murrell-Lagnado RD. 2002. P2X receptor trafficking in neurons is subunit specific. *J. Neurosci.* 22:4814–24
73. Royle SJ, Qureshi OS, Bobanović LK, Evans PR, Owen DJ, Murrell-Lagnado RD. 2005. Non-canonical YXXGΦ endocytic motifs: recognition by AP2 and preferential utilization in $P2X_4$ receptors. *J. Cell Sci.* 118:3073–80
74. Yamamoto K, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, et al. 2006. Impaired flow-dependent control of vascular tone and remodeling in $P2X_4$-deficient mice. *Nat. Med.* 12:133–37
75. Chamberlain J, Evans D, King A, Dewberry R, Dower S, et al. 2006. Interleukin-1β and signaling of interleukin-1 in vascular wall and circulating cells modulates the extent of neointimal formation in mice. *Am. J. Pathol.* 168:1396–403
76. Gachet C. 2008. P2 receptors, platelet function and pharmacological implications. *Thromb. Haemost.* 99:466–72
77. Rolf MG, Brearley CA, Mahaut-Smith MP. 2001. Platelet shape change evoked by selective activation of $P2X_1$ purinoceptors with α,β-methylene ATP. *Thromb. Haemost.* 85:303–8
78. Rolf MG, Mahaut-Smith MP. 2002. Effects of enhanced $P2X_1$ receptor Ca^{2+} influx on functional responses in human platelets. *Thromb. Haemost.* 88:495–502
79. Hechler B, Lenain N, Marchese P, Vial C, Heim V, et al. 2003. A role of the fast ATP-gated $P2X_1$ cation channel in thrombosis of small arteries in vivo. *J. Exp. Med.* 198:661–67
80. Leipziger J. 2003. Control of epithelial transport via luminal P2 receptors. *Am. J. Physiol.* 284:F419–32
81. Schwiebert EM, Zsembery A. 2003. Extracellular ATP as a signalling molecule for epithelial cells. *Biochim. Biophys. Acta* 1615:7–32
82. Ma W, Korngreen A, Uzlaner N, Priel Z, Silberberg SD. 1999. Extracellular sodium regulates airway ciliary motility by inhibiting a P2X receptor. *Nature* 400:894–97
83. Ma W, Korngreen A, Weil S, Cohen EB, Priel A, et al. 2006. Pore properties and pharmacological features of the P2X receptor channel in airway ciliated cells. *J. Physiol.* 571:503–17
84. Fujiwara Y, Kubo Y. 2004. Density-dependent changes of the pore properties of the $P2X_2$ receptor channel. *J. Physiol.* 558:31–43
85. Kim M, Jiang LH, Wilson HL, North RA, Surprenant A. 2001. Proteomic and functional evidence for a $P2X_7$ receptor signaling complex. *EMBO J.* 20:6347–58
86. Llaudet E, Hatz S, Droniou M, Dale N. 2005. Microelectrode biosensor for real-time measurement of ATP in biological tissue. *Anal. Chem.* 77:3267–73
87. Gourine AV, Llaudet E, Dale N, Spyer KM. 2005. Release of ATP in the ventral medulla during hypoxia in rats: role in hypoxic ventilatory response. *J. Neurosci.* 25:1211–18

74. $P2X_4$R knockout mice provide striking evidence for the role of $P2X_4$R in endothelial cell function.

88. Schnermann J, Levine DZ. 2003. Paracrine factors in tubuloglomerular feedback: adenosine, ATP and nitric oxide. *Annu. Rev. Physiol.* 65:501–29
89. Guan Z, Osmond DA, Inscho EW. 2007. P2X receptors as regulators of the renal microvasculature. *Trends Pharmacol. Sci.* 28:646–52
90. Nishiyama A, Majid DSA, Taher KA, Miyatake A, Navar LG. 2000. Relation between renal interstitial ATP concentrations and autoregulation-mediated changes in renal vascular resistance. *Circ. Res.* 86:656–62
91. Komlosi P, Fintha A, Bell PD. 2005. Renal cell-to-cell communication via extracellular ATP. *Physiology* 20:86–90
92. Bell PD, Lapointe J, Sabirov R, Hayashi S, Peti-Peterdi J, et al. 2003. Macula densa cell signaling involves ATP release through a maxi anion channel. *Proc. Natl. Acad. Sci. USA* 100:4322–27
93. Nishiyama A, Majid DSA, Walker M, Miyatake A, Navar LG. 2001. Renal interstitial ATP responses to changes in arterial pressure during alterations in tubuloglomerular feedback activity. *Hypertension* 37(Pt. 2):753–59
94. Castrop H. 2007. Mediators of tubuloglomerular feedback regulation of glomerular filtration: ATP and adenosine. *Acta Physiol.* 189:3–14
95. Sun D, Samuelson LC, Yang T, Huang Y, Paliege A, et al. 2001. Mediation of tubuloglomerular feedback by adenosine: evidence from mice lacking adenosine 1 receptors. *Proc. Natl. Acad. Sci. USA* 98:9983–88
96. Brown R, Ollerstam A, Johansson B, Skott O, Gebre-Mehin S, et al. 2001. Abolished tubuloglomerular feedback and increased plasma rennin in adenosine A1 receptor-deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281:1362–67
97. Ren Y, Garvin JL, Liu R, Carretero OA. 2004. Role of macula densa adenosine triphosphate (ATP) in tubuloglomerular feedback. *Kidney Int.* 66:1479–85
98. Zhao X, Cook AK, Field M, Edwards B, Zhang S, et al. 2005. Impaired Ca^{2+} signaling attenuates P2X receptor-mediated vasoconstriction of afferent arterioles in angiotensin II hypertension. *Hypertension* 46:562–68

99. Inscho EW, Cook AK, Imig JD, Vial C, Evans RJ. 2003. Physiological role for $P2X_1$ receptors in renal microvascular autoregulatory behavior. *J. Clin. Investig.* 112:895–905

99. $P2X_1R$ knockout mice provide convincing evidence for their essential role in renal autoregulation via tubuloglomerular feedback.

100. Feng M, Navar LG. 2007. Adenosine A2 receptor activation attenuates afferent arteriolar autoregulation during adenosine receptor saturation in rats. *Hypertension* 50:744–49
101. Hillman KA, Burnstock G, Unwin RJ. 2005. The $P2X_7$ ATP receptor in the kidney: a matter of life or death? *Nephron Exp. Nephrol.* 101:24–30
102. Schulze-Lohoff E, Hugo C, Rost S, Arnold S, Gruber A, et al. 1998. Extracellular ATP causes apoptosis and necrosis of cultured mesangial cells via $P2Z/P2X_7$ receptors. *Am. J. Physiol.* 275:F962–71
103. Harada H, Chan CM, Loesch A, Unwin R, Burnstock G. 2000. Induction of proliferation and apoptotic cell death via P2Y and P2X receptors, respectively, in rat glomerular mesangial cells. *Kidney Int.* 57:949–58
104. Solini A, Iacobini C, Ricci C, Chiozzi P, Amadio L, et al. 2005. Purinergic modulation of mesangial extracellular matrix production: role in diabetic and other glomerular diseases. *Kidney Int.* 67:875–85

105. Goncalves RG, Gabrich L, Rosario A, Takiya CM, Ferreira ML, et al. 2006. The role of purinergic $P2X_7$ receptors in the inflammation and fibrosis of unilateral ureteral obstruction in mice. *Kidney Int.* 70:1599–606

105. Using $P2X_7R$ knockout mice, this study provides evidence for the role of $P2X_7R$ in collagen deposition and renal fibrosis in response to ureteral obstruction.

106. Solini A, Santini E, Chimenti D, Chiozzi P, Pratesi F, et al. 2007. Multiple P2X receptors are involved in the modulation of apoptosis in human mesangial cells: evidence for a role of $P2X_4$. *Am. J. Physiol.* 292:F1537–47
107. Wen LT, Knowles AF. 2003. Extracellular ATP and adenosine induce cell apoptosis of human hepatoma Li-7A cells via the A3 adenosine receptor. *Br. J. Pharmacol.* 140:1009–18
108. Alola E, Perez-Etxebarria A, Kabre E, Fogarty DJ, Metioui M, et al. 1998. Activation by $P2X_7$ agonists of two phospholipases A_2 (PLA_2) in ductal cells of rat submandibular gland. *J. Biol. Chem.* 273:30208–17
109. Garcia-Marcos M, Pochet S, Marino A, Dehaye JP. 2006. $P2X_7$ and phospholipid signaling: the search of the "missing link" in epithelial cells. *Cell. Signal.* 18:2098–104
110. Pochet S, Garcia-Marcos M, Seil M, Otto A, Marino A, Dehaye JP. 2007. Contribution of two ionotropic purinergic receptors to ATP responses in submandibular gland ductal cells. *Cell. Signal.* 19:2155–64

111. Doctor RB, Matzakos T, McWilliams R, Johnson S, Feranchak AP, et al. 2005. Purinergic regulation of cholangiocyte secretion: identification of a novel role for P2X receptors. *Am. J. Physiol.* 288:G779–86
112. Furness JB, Jones C, Nurgali K, Clerc N. 2004. Intrinsic primary afferent neurons and nerve circuits within the intestine. *Prog. Neurobiol.* 72:143–64
113. Galligan JJ. 2004. Enteric P2X receptors as potential targets for drug treatment of irritable bowel syndrome. *Br. J. Pharmacol.* 141:1294–302
114. Ren J, Bian X, DeVries M, Schnegelsberg B, Cockayne DA, et al. 2003. $P2X_2$ subunits contribute to fast synaptic excitation in myenteric neurons of the mouse small intestine. *J. Physiol.* 552:809–21
115. Bian X, Ren J, DeVries M, Schnegelsberg B, Cockayne DA, et al. 2003. Peristalsis is impaired in the small intestine of mice lacking the $P2X_3$ subunit. *J. Physiol.* 551:309–22
116. Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, et al. 2006. The $P2X_7$ receptor: a key player in IL-1 processing and release. *J. Immunol.* 176:3877–83
117. Di Virglio F. 2007. Liaisons dangereuses: $P2X_7$ and the inflammasome. *Trends Pharmacol. Sci.* 28:465–72
118. Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, et al. 2001. Altered cytokine production in mice lacking $P2X_7$ receptors. *J. Biol. Chem.* 276;125–32
119. King BF. 2007. Novel $P2X_7$ receptor antagonists ease the pain. *Br. J. Pharmacol.* 151:565–67
120. Donnelly-Roberts DL, Jarvis MF. 2007. Discovery of $P2X_7$ receptor-selective antagonists offers new insights into $P2X_7$ receptor function and indicates a role in chronic pain states. *Br. J. Pharmacol.* 151:571–79
121. McInnes IB, Snell NJ, Perrett JH, Parmar H, Wang MM, et al. 2007. Results of a Phase II clinical trial of a novel $P2X_7$ receptor antagonist, AZD9056, in patients with active rheumatoid arthritis (CREATE study). *Am. College Rheumatol.* Abstr. 2085
122. Placido R, Auricchio G, Falzoni S, Battistini L, Colizzi V, et al. 2006. $P2X_7$ purinergic receptors and extracellular ATP mediate apoptosis of human monocytes/macropahges infected with *Mycobacterium tuberculosis* reducing the intracellular bacterial viability. *Cell. Immunol.* 244:10–18
123. Pelegrin P, Barroso-Gutierrez C, Surprenant A. 2008. $P2X_7$ receptor differentially couples to distinct release pathways for IL-1β in mouse macrophage. *J. Immunol.* 180:7147–57
124. Saunders BM, Fernando L, Sluyter R, Britton WJ, Wiley JS. 2003. A loss-of-function polymorphism in the human $P2X_7$ receptor abolishes ATP-mediated killing of mycobacteria. *J. Immunol.* 171:5442–46
125. Fairbairn IP, Stober CB, Kumararatne DS, Lammas DA. 2001. ATP-mediated killing of intracellular mycobacteria by macrophages is a $P2X_7$-dependent process inducing bacterial death by phagosome-lysosome fusion. *J. Immunol.* 167:3300–7
126. Steinberg TH, Hiken JF. 2007. P2 receptors in macrophage fusion and osteoclast formation. *Purinergic Signal.* 3:53–57
127. Ke HZ, Qi H, Weidema AF, Zhang Q, Panupinthu N, et al. 2003. Deletion of the $P2X_7$ nucleotide receptor reveals its regulatory roles in bone formation and resorption. *Mol. Endocrinol.* 17:1356–67
128. Gartland A, Buckley KA, Hipskind RA, Perry MJ, Tobias JH, et al. 2003. Multinucleated osteoclast formation in vivo and in vitro by $P2X_7$ receptor-deficient mice. *Crit. Rev. Eukaryot. Gene Expr.* 13:243–53
129. Cruwys S, Midha A, Rendall E, McCormick M, Nicol A, et al. 2007. Antagonism of $P2X_7$ receptor attenuates joint destruction in a model of arthritis. *Am. Coll. Rheumatol.* Abstr. 1772
130. Sherman AN, Sluyter R, Fernando SL, Clarke A, Dao-Ung LP, et al. 2006. A Thr^{357} to Ser polymorphism in homozygous and compound heterozygous subjects causes absent or reduced $P2X_7$ function and impairs ATP-induced mycobacterial killing by macrophages. *J. Biol. Chem.* 281:2079–86
131. Mio K, Kubo Y, Ogura T, Yamamoto T, Sato C. 2005. Visualization of the trimeric $P2X_2$ receptor with a crown-capped extracellular domain. *Biochem. Biophys. Res. Commun.* 337:998–1005
132. Barrera NP, Ormond SJ, Henderson RM, Murrell-Lagnado RD, Edwardson JM. 2005. Atomic force microscopy imaging demonstrates that $P2X_2$ receptors are trimers but that $P2X_6$ receptor subunits do not oligomerize. *J. Biol. Chem.* 280:10759–65
133. Nicke A, Bäumert HG, Rettinger J, Eichele A, Lambrecht G, et al. 1998. $P2X_1$ and $P2X_3$ receptors form stable trimers: a novel structural motif of ligand-gated ion channels. *EMBO J.* 17:3016–28
134. Ding S, Sachs F. 1999. Single channel properties of $P2X_2$ purinoceptors. *J. Gen. Physiol.* 113:695–720

121. The first drug targeting a P2XR to make it to Phase II clinical trials, with initial positive results for inflammatory disease.

135. Roberts JA, Vial C, Digby HR, Agboh KC, Wen H, et al. 2006. Molecular properties of P2X receptors. *Pflüg. Arch.* 452:486–500
136. Agboh KC, Webb TE, Evans RJ, Ennion SJ. 2004. Functional characterization of a P2X receptor from *Schistosoma mansoni*. *J. Biol. Chem.* 279:41650–57
137. Fountain SJ, Parkinson K, Young MT, Cao L, Thompson CR, North RA. 2007. An intracellular P2X receptor required for osmoregulation in *Dictyostelium discoideum*. *Nature* 448:200–3
138. Fountain SJ, Cao L, Young MT, North RA. 2008. Permeation properties of a P2X receptor in the green algae *Ostreococcus tauri*. *J. Biol. Chem.* 283:15122–26
139. Freist W, Verhey JF, Stuhmer W, Gauss DH. 1998. ATP binding site of P2X channel proteins: structural similarities with class II aminoacyl tRNA-synthetases. *FEBS Lett.* 434:61–65
140. Boué-Grabot E, Archambault V, Séguéla P. 2000. A protein kinase C site highly conserved in P2X subunits controls the desensitization kinetics of $P2X_2$ ATP-gated channels. *J. Biol. Chem.* 275:10190–95
141. Ennion SJ, Evans RJ. 2002. $P2X_1$ receptor subunit contribution to gating revealed by a dominant negative PKC mutant. *Biochem. Biophys. Res. Commun.* 291:611–16
142. Jiang LH, Rassendren F, Surprenant A, North RA. 2000. Identification of amino acid residues contributing to the ATP-binding site of a purinergic P2X receptor. *J. Biol. Chem.* 275:34190–96
143. Haines WR, Migita K, Cox JA, Egan TM, Voigt MM. 2001. The first transmembrane domain of the P2X receptor subunit participates in the agonist-induced gating of the channel. *J. Biol. Chem.* 276:32793–98
144. Haines WR, Voigt MM, Migita K, Torres GE, Egan TM. 2001. On the contribution of the first transmembrane domain to whole-cell current through an ATP-gated ionotropic P2X receptor. *J. Neurosci.* 21:5885–92
145. Li Z, Migita K, Samways DS, Voigt MM, Egan TM. 2004. Gain and loss of channel function by alanine substitutions in the transmembrane segments of the rat ATP-gated $P2X_2$ receptor. *J. Neurosci.* 24:7378–86
146. Samways DS, Migita K, Li Z, Egan TM. 2008. On the role of the first transmembrane domain in cation permeability and flux of the ATP-gated $P2X_2$ receptor. *J. Biol. Chem.* 283:5110–17
147. Silberberg SD, Chang TH, Swartz KJ. 2005. Secondary structure and gating rearrangements of transmembrane segments in rat $P2X_4$ receptor channels. *J. Gen. Physiol.* 125:347–59
148. Jiang LH, Rassendren F, Spelta V, Surprenant A, North RA. 2001. Amino acid residues involved in gating identified in the first membrane-spanning domain of the rat $P2X_2$ receptor. *J. Biol. Chem.* 276:14902–8
149. Roberts JA, Evans RJ. 2004. ATP binding at human $P2X_1$ receptors. Contribution of aromatic and basic amino acids revealed using mutagenesis and partial agonists. *J. Biol. Chem.* 279:9043–55
150. Cao L, Young MT, Broomhead HE, Fountain SJ, North RA. 2007. A Thr^{339}-to-serine substitution in rat $P2X_2$ receptor second transmembrane domain causes constitutive opening and indiates a gating role for Lys^{308}. *J. Neurosci.* 27:12916–23
151. Wilkinson WJ, Jiang LH, Surprenant A, North RA. 2006. Role of ectodomain lysines in the subunits of the heteromeric $P2X_{2/3}$ receptor. *Mol. Pharmacol.* 70:1159–63
152. Clyne JD, Wang LF, Hume RI. 2002. Mutational analysis of the conserved cysteines of the rat $P2X_2$ purinoceptor. *J. Neurosci.* 22:3873–80
153. Ennion SJ, Evans RJ. 2002. Conserved cysteine residues in the extracellular loop of the human $P2X_1$ receptor form disulfide bonds and are involved in receptor trafficking to the cell surface. *Mol. Pharmacol.* 61:303–11
154. Nagaya N, Tittle RK, Saar N, Dellal SS, Hume RI. 2005. An intersubunit zinc binding site in rat $P2X_2$ receptors. *J. Biol. Chem.* 280:25982–93
155. Marquez-Klaka B, Rettinger J, Bhargava Y, Eisele T, Nicke A. 2007. Identification of an intersubunit cross-link between substituted cysteine residues located in the putative ATP binding site of the $P2X_1$ receptor. *J. Neurosci.* 27:1456–66
156. Roberts JA, Digby HR, Kara M, El Ajouz S, Sutcliffe MJ, et al. 2008. Cysteine substitution mutagenesis and the effects of methanethiosulfonate reagents at $P2X_2$ and $P2X_4$ receptors support a core common mode of ATP action at P2X receptors. *J. Biol. Chem.* 283:20126–36
157. Yan Z, Liang Z, Obsil T, Stojilkovic SS. 2006. Participation of the Lys^{313}-Ile^{333} sequence of the purinergic $P2X_4$ receptor in agonist binding and transduction of signals to the channel gate. *J. Biol. Chem.* 281:32649–59

137. A P2X receptor homolog in a simple eukaryote has an important function as intracellular vacuolar protein.

158. Wilkinson WJ, Jiang LH, Surprenant A, North RA. 2006. Role of ectodomain lysines in the subunits of the heteromeric $P2X_{2/3}$ receptor. *Mol. Pharmacol.* 70:1159–63
159. Roberts JA, Evans RJ. 2007. Cysteine substitution mutants give structural insight and identify ATP binding and activation sites at P2X receptors. *J. Neurosci.* 27:4072–82
160. Rassendren F, Buell G, Newbolt A, North RA, Surprenant A. 1997. Identification of amino acid residues contributing to the pore of a P2X receptor. *EMBO J.* 16:3446–54
161. Egan TM, Haines WR, Voigt MM. 1998. A domain contributing to the ion channel of ATP-gated $P2X_2$ receptors identified by the substituted cysteine accessibility method. *J. Neurosci.* 18:2350–59
162. Migita K, Haines WR, Voigt MM, Egan TM. 2001. Polar residues of the second transmembrane domain influence cation permeability of the ATP-gated $P2X_2$ receptor. *J. Biol. Chem.* 276:30934–41
163. Egan TM, Khakh BS. 2004. Contribution of calcium ions to P2X channel responses. *J. Neurosci.* 24:3413–20
164. Samways DS, Egan TM. 2007. Acidic amino acids impart enhanced Ca^{2+} permeability and flux in two members of the ATP-gated P2X receptor family. *J. Gen. Physiol.* 129:245–56
165. Duckwitz W, Hausmann R, Aschrafi A, Schmalzing G. 2006. $P2X_5$ subunit assembly requires scaffolding by the second transmembrane domain and a conserved aspartate. *J. Biol. Chem.* 281:39561–72
166. Chaumont S, Jiang LH, Penna A, North RA, Rassendren F. 2004. Identification of a trafficking motif involved in the stabilization and polarization of P2X receptors. *J. Biol. Chem.* 279:29628–38
167. Jiang LH, Rassendren F, Mackenzie A, Zhang YH, Surprenant A, et al. 2005. *N*-Methyl-D-glucamine and propidium dyes utilize different permeation pathways at rat $P2X_7$ receptors. *Am. J. Physiol. Cell Physiol.* 289:C1295–302
168. Masin M, Kerschensteiner D, Dümke K, Rubio ME, Soto F. 2006. Fe65 interacts with $P2X_2$ subunits at excitatory synapses and modulates receptor function. *J. Biol. Chem.* 281:4100–8
169. Wilson HL, Wilson SA, Surprenant A, North RA. 2002. Epithelial membrane proteins induce membrane blebbing and interact with the $P2X_7$ receptor C terminus. *J. Biol. Chem.* 277:34017–23
170. Denlinger LC, Fisette PL, Sommer JA, Watters JW, Prabhu U, et al. 2001. The nucleotide receptor $P2X_7$ contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysacharide. *J. Immunol.* 167:1871–76
171. Denlinger LC, Sommer JA, Parker K, Gudipaty L, Fisette PL, et al. 2003. Mutation of a dibasic amino acid motif within the C terminus of the $P2X_7$ nucleotide receptor results in trafficking defects and impaired function. *J. Immunol.* 171:1304–11
172. Pelegrin P, Surprenant A. 2006. Pannexin-1 mediates large pore formation and interleukin-1β release by the ATP-gated $P2X_7$ receptor. *EMBO J.* 25:5071–82
173. Petrilli V, Dotert C, Muruve DA, Tschopp J. 2007. The inflammasome: a danger sensing complex triggering innate immunity. *Curr. Opin. Immunol.* 19:615–22

Annual Review of Physiology
Volume 71, 2009

Contents

SPECIAL TOPIC, ASTHMA, *Jeffrey M. Drazen, Special Topic Editor*

Indexes

Errata

An online log of corrections to *Annual Review of Physiology* articles may be found at http://physiol.annualreviews.org/errata.shtml