British Journal of Pharmacology (2007) 151, 571–579
 0007–1188/07 $30.00
www.brjpharmacol.org

REVIEW

Discovery of $P2X_7$ receptor-selective antagonists offers new insights into $P2X_7$ receptor function and indicates a role in chronic pain states

DL Donnelly-Roberts and MF Jarvis

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL, USA

ATP-sensitive $P2X_7$ receptors are localized on cells of immunological origin including peripheral macrophages and glial cells in the CNS. Activation of $P2X_7$ receptors leads to rapid changes in intracellular calcium concentrations, release of the proinflammatory cytokine interleukin-1β and following prolonged agonist exposure, the formation of cytolytic pores in plasma membranes. Both the localization and functional consequences of $P2X_7$ receptor activation indicate a role in inflammatory processes. The phenotype of $P2X_7$ receptor gene-disrupted mice also indicates that $P2X_7$ receptor activation contributes to ongoing inflammation. More recently, $P2X_7$ receptor knockout data has also suggested a specific role in inflammatory and neuropathic pain states. The recent discovery of potent and highly selective antagonists for $P2X_7$ receptors has helped to further clarify P2X receptor pharmacology, expanded understanding of $P2X_7$ receptor signaling, and offers new evidence that $P2X_7$ receptors play a specific role in nociceptive signaling in chronic pain states. In this review, we incorporate the recent discoveries of novel $P2X_7$ receptor-selective antagonists with a brief update on $P2X_7$ receptor pharmacology and its therapeutic potential.
British Journal of Pharmacology (2007) **151,** 571–579; doi:10.1038/sj.bjp.0707265; published online 30 April 2007

Keywords: purines; $P2X_7$ receptor; calcium influx; Yo-Pro uptake; IL-1β; AZ11645373; A-438079; A-740003; allodynia; neuropathic pain; inflammatory pain

Abbreviations: AMP, adenosine 5′-monophosphate sodium salt; ATP, adenosine triphosphate; ATP-γs, adenosine 5′-[γ-thio]triphosphate tetralithium salt; 2-MeS-ATP, 2-methlthioadenosine 5′-triphosphate; αβ-me-ATP, α,β-methylene-adenosine 5′-triphosphate; A-438079, 3-(5-(2,3-dichlorophenyl)-1*H*-tetrazol-1-yl)methyl pyridine; A-740003, *N*-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide; AZ11645373, 3-(1-(3′-nitrobiphenyl-4-yloxy)-4-(pyridine-4-yl)butan-2-yl)thiazolidine-2,4-dione; ADP, adenosine 5′-diphosphate monopotassium salt dihydrate; BBG, Brilliant Blue G; BzATP, 2,3-*O*-(4-benzoylbenzoyl)-ATP; CFA, complete Freund's adjuvant; FLIPR, fluorometric imaging plate reader; IL-1β, interleukin 1β; KN62, (1-[*N*,*O*-bis(5-isoquinoline-sulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine); LL37, human cathelicidin-derived peptide; MAPK, mitogen-activated protein kinase; MTX, maitotoxin; NF-kappa B, nuclear factor-κB; oATP, ATP 2′,3′-dialdehyde; pp38 MAPK, phosphorylated p38 MAPK; PLA_2, phospholipase A_2; PPADS, pyridoxal phosphate-6-azophenyl-2-4-disulfonic acid; TNF-α, tumor necrosis factor-α; UTP, uridine 5′-triphosphate

Introduction

$P2X_7$ receptors belong to the family of ATP-sensitive ionotropic P2X receptors, which include seven homomeric receptor subtypes ($P2X_1$–$P2X_7$; North, 2002). Some of these receptor subunits also natively exist as functional heteromeric receptor combinations, such as $P2X_{2/3}$ (North, 2002). However, $P2X_7$ receptors are unique among the P2X receptor family as they are functional in only homomeric forms and are activated by high concentrations of ATP ($>100\,\mu M$) (Jacobson *et al.*, 2002). In addition, prolonged agonist exposure leads to formation of large cytolytic pores in the cell membrane (Surprenant *et al.*, 1996). The $P2X_7$ subunit, previously termed as the '$P2_Z$ receptor' (Di Virgilio *et al.*, 2001), was initially cloned from rat (Surprenant *et al.*, 1996), and then from human brain (Collo *et al.*, 1997) and human macrophages (Rassendren *et al.*, 1997a). $P2X_7$ receptors are selectively expressed on cells of hematopoietic lineage including mast cells, lymphocytes, erythrocytes, fibroblasts,

Correspondence: Dr MF Jarvis, Neuroscience Research, Global Pharmaceutical Research and Development, R4PM, Abbott Laboratories, AP9A/2, 100 Abbott Park Road, Abbott Park, IL 60064-6123, USA.
E-mail: michael.jarvis@abbott.com
Received 2 February 2007; revised 2 March 2007; accepted 27 March 2007; published online 30 April 2007

peripheral macrophages and epidermal Langerhans cells (Surprenant *et al*., 1996). Within the central nervous system, functional $P2X_7$ receptors are localized on microglia and Schwann cells as well as on astrocytes (Ferrari *et al*., 1996; Collo *et al*., 1997; Sim *et al*., 2004). The existence of functional $P2X_7$ receptors on peripheral or central neurons remains controversial owing to the poor specificity of both antibodies and ligands targeting the rat $P2X_7$ receptor (Anderson and Nedergaard, 2006). In rat peripheral sensory ganglia (dorsal root), $P2X_7$ receptors appear to be selectively localized on glia cells, but not neurons (Zhang *et al*., 2005).

Both the distribution of $P2X_7$ receptors and the fact that high concentrations of ATP are required to activate the receptor have led investigators to consider the possibility that this P2X receptor functions as a 'danger' sensor (Ferrari *et al*., 2006). However, efforts to establish a generalized function for $P2X_7$ receptors are complicated by another distinguishing feature of this receptor subtype; at least 250 polymorphic forms of the $P2X_7$ receptor have been identified (Ferrari *et al*., 2006). Among these variations, both gain-of-function (Cabrini *et al*., 2005) and loss-of-function (Wiley *et al*., 2003) single nucleotide polymorphisms (SNPs) have been reported. Although some of these SNPs may have a predictive utility as biomarkers for some forms of leukemia (Wiley *et al*., 2002) or extrapulmonary tuberculosis (Fernando *et al*., 2007), to date, no clear disease association has been linked to a specific $P2X_7$ receptor polymorphism.

The multiplicity of polymorphic forms of the $P2X_7$ receptor is associated with the extended length of the C-terminal region of the receptor relative to other P2X receptors (Surprenant *et al*., 1996). This region is essential for receptor-mediated cytolytic pore-forming activity (Surprenant *et al*., 1996) and also contains putative interaction sites for lipopolysaccharide (Denlinger *et al*., 2001), SH_2 domains (Kim *et al*., 2001) and α-actin (Kim *et al*., 2001). The mechanism(s) by which $P2X_7$ receptor activation leads to the formation of large cytolytic pores in cell membranes has not been definitively established. The available evidence indicates that the minimum stoichiometric conformation of P2X receptors is a trimer (Egan *et al*., 2006). However, this work has been based on an analysis of channel conductance properties of P2X receptors rather than on potential downstream signaling mechanisms. It should also be noted that investigations of P2X receptor stoichiometry have primarily studied P2X receptor subtypes other than $P2X_7$ (Egan *et al*., 2006). While originally it was hypothesized that pore formation resulted from intrinsic dilation of the channel (North, 2002), the inability of some cell types that express the cation permeable receptor to show agonist-evoked pore formation *in vitro* has led to the generation of data showing that pore formation results from $P2X_7$ receptor-mediated downstream signaling (Donnelly-Roberts *et al*., 2004; Faria *et al*., 2005) that ultimately may depend on the opening of nonselective hemichannels at the cell surface (Pelegrin and Surprenant, 2006).

Taken together, there is a growing body of data to indicate a pathophysiological role for $P2X_7$ receptors in inflammatory responses (Ferrari *et al*., 2006). Interestingly, recent data from the $P2X_7$ receptor knockout mouse (Chessell *et al*., 2005) and the development of new potent and selective $P2X_7$ receptor antagonists (Lappin *et al*., 2005; Nelson *et al*., 2006; Honore *et al*., 2006b) also indicate a role for $P2X_7$ receptors in the onset and persistence of certain types of chronic pain. This article provides an update of the current pharmacology for the $P2X_7$ receptor, recent advances in understanding $P2X_7$ receptor signaling pathways as well as new insights into the therapeutic potential of $P2X_7$ receptor antagonist ligands for pain relief. Other recent reviews by Fields and Burnstock (2006), Ferrari *et al.* (2006), Gever *et al*. (2006) and Khakh and North (2006) provide extensive overviews of P2X receptor physiology and pharmacology.

$P2X_7$ receptor pharmacology

Agonists. Brief agonist activation (<10 s) of the $P2X_7$ receptor results in rapid and reversible channel opening that is permeable to Na^+, K^+ and Ca^{2+} (Surprenant *et al*., 1996). Acute $P2X_7$ receptor activation also triggers a series of cellular responses, such as activation of caspases, cytokine release, cell proliferation, and apoptosis (Perregaux *et al*., 2000; Panenka *et al*., 2001; Chakfe *et al*., 2002; North, 2002; Verhoef *et al*., 2003; Kahlenberg and Dubyak, 2004). Unlike other members of the P2 receptor superfamily, homomeric $P2X_7$ receptors are activated by high concentrations of ATP ($>100\,\mu$M) and 2′,3′-*O*-(4-benzoylbenzoyl)-ATP (BzATP), which has significantly greater potency ($EC_{50}=20\,\mu$M) than ATP ($EC_{50}>100\,\mu$M) (Jacobson *et al*., 2002). The rank order agonist potency for activation of the $P2X_7$ receptor is BzATP$\gg$ATP, with 2MeSATP, ATPγS and ADP being essentially inactive (Jacobson *et al*., 2002). The chemical structures of these agonists are shown in Figure 1.

However, $P2X_7$ receptors can also be primed and undergo agonist plasticity such that brief agonist exposure resulted in an apparent increase in potency to subsequent agonist exposure (Chakfe *et al*., 2002). Furthermore, agonist priming also expands the agonist pharmacology such that both ADP and AMP can now activate the receptor (Chakfe *et al*., 2002). Although BzATP is the most potent agonist for $P2X_7$ receptors, it is not a selective agonist for this receptor. BzATP is 100–1000 times more potent as an agonist at both $P2X_1$ and $P2X_3$ receptors than at $P2X_7$ receptors (Bianchi *et al*., 1999). Consequently, a rank order of potency of nonselective purine agonists (BzATP$\gg$ATP$\gg$UTP$\gg\alpha\beta$MeATP) has been utilized to identify $P2X_7$ receptors in native cell lines and tissue (Bianchi *et al*., 1999; Anderson and Nedergaard, 2006).

Antagonists. The prototypic nonselective P2X receptor antagonists, suramin and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonate (PPADS; Figure 2), both block $P2X_7$ receptors with low affinity ($K_i>10\,\mu$M; Table 1) and typically show noncompetitive antagonism (Jacobson *et al*., 2002). Brilliant Blue G (BBG; $pIC_{50}=5.1$; Figure 2 and Table 1) is a more potent and selective antagonist with a 30- to 50-fold greater selectivity for rat versus human $P2X_7$ receptors (Jiang *et al*., 2000). Large cationic inhibitors of calcium/calmodulin-dependent protein kinase II such as the isoquinoline, (1-[*N*,*O*-bis(5-isoquinoline-sulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine) (KN62) (Figure 2 and Table 1), potently block $P2X_7$ receptor function in a noncompetitive fashion (Gargett and Wiley, 1997). KN62, like other putative $P2X_7$ receptor antagonists, also shows significant species differences in

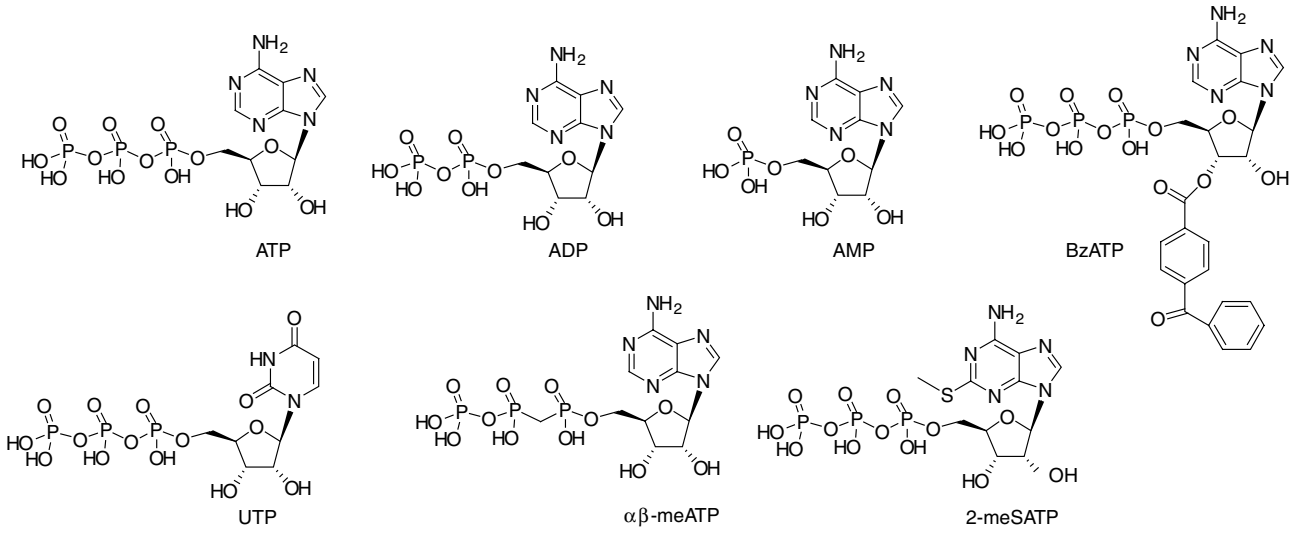

Figure 1 Structures of prototypical adenine nucleotides modified on the phosphate moiety that have been investigated as P2 receptor agonists.

in vitro assays (Humphreys *et al*., 1998). Analysis of the magnitude of apparent species differences for $P2X_7$ antagonists is also complicated by the end point that is measured, cation flux and pore formation (by large dye, that is YO-Pro uptake), which requires different agonist incubation times (Gever *et al*., 2006) with the latter end point being host-cell dependent (North, 2002).

ATP 2′,3′-dialdehyde (oxidized-ATP, oATP; Figure 2) is an irreversible inhibitor of $P2X_7$ receptors that requires a 1- to 2-h incubation to inhibit functional activation of $P2X_7$ receptors (Di Virgilio, 2003). In light of its original use as a ligand for nucleotide binding proteins (Lowe and Beechey, 1982), oATP was initially used to block $P2X_7$ receptor-mediated responses in mouse macrophages (Murgia *et al*., 1993). However, it must be noted that oATP has many other pharmacological actions including blockade of $P2X_1$ and $P2X_2$ receptors (Evans *et al*., 1995) as well as inhibition of nuclear factor-κB (NF-κB) and cytokine release (Murgia *et al*., 1993; Beigi *et al*., 2003; Ferrari *et al*., 2006). Consequently, these newer findings raise further doubt on the utility of oATP to properly interrogate $P2X_7$-related function in other than selective recombinant receptor expression systems (Beigi *et al*., 2003; Di Virgilio, 2003).

More recently, decavanadate, a polymeric form of vanadate ($H_2V_{10}O_{28}^{-4}$), was reported also to be a reversible and competitive $P2X_7$ receptor antagonist (Michel *et al*., 2006a). However, decavanadate also displays nonselective activity since it blocks $P2X_2$ and $P2X_4$ receptors, and at nanomolar concentrations, interacts with a number of other targets including activation of 5′-nucleotidase, inhibition of inositol 1,4,5-trisphospate (IP_3) binding and inhibition of ribonuclease A (Michel *et al*., 2006a). Thus, like oATP, the usefulness of decavanadate as a pharmacological tool for $P2X_7$ receptors is limited.

In 2003, two novel series of $P2X_7$ antagonists, composed of cyclic imides (Alcaraz *et al*., 2003) and adamantane amides (Baxter *et al*., 2003; Figure 2), were shown to be effective $P2X_7$ receptor antagonists and useful starting points for directed structure–function studies. However, these compounds have not yet been evaluated for activity at P2 or other cell surface receptors. Stokes *et al*. (2006) recently published a more complete characterization of the cyclic imide $P2X_7$ antagonist, AZ11645373 (3-(1-(3′-nitrobiphenyl-4-yloxy)-4-(pyridine-4-yl)butan-2-yl)thiazolidine-2,4-dione; Figure 2), which was shown to potently ($IC_{50} = 10$–90 nM; Table 1) block human $P2X_7$ receptor-mediated cation influx and interleukin-1β (IL-1β) release. However, like KN62, AZ11645373 shows preferential affinity for the human versus the rat $P2X_7$ receptor thus limiting its usefulness as an *in vivo* tool to examine $P2X_7$ receptor function in rat preclinical models.

In 2006, our group at Abbott Laboratories disclosed two novel series of $P2X_7$ antagonists: disubstituted tetrazoles and cyanoguanidines (Nelson *et al*., 2006; Honore *et al*., 2006b). Both series demonstrate enhanced potency and selectivity as antagonists at rat and human $P2X_7$ receptors compared to previous antagonists (Nelson *et al*., 2006; Honore *et al*., 2006b). Unlike other $P2X_7$ antagonists, these compounds are also reversible and competitive blockers. A-438079, 3-((5-(2,3-dichlorophenyl)-1*H*-tetrazol-1-yl)methyl pyridine; Figure 2), from a disubstituted tetrazole series, potently blocked BzATP-stimulated changes in intracellular calcium concentrations ($IC_{50} = 100$ and 300 nM at rat and human $P2X_7$ receptors, respectively; Table 1) and was essentially devoid of activity ($IC_{50} \gg 10\,\mu$M) at other P2 receptors (Nelson *et al*., 2006). Additionally, this compound showed little or no activity at a wide array of other cell-surface receptors and ion channels (Nelson *et al*., 2006). A-438079 and several analogs were also shown to inhibit BzATP-stimulated IL-1β release and pore formation in human THP-1 cells differentiated with LPS and IFN-γ into a macrophage-like phenotype (Nelson *et al*., 2006).

Another novel $P2X_7$ antagonist pharmacophore from a cyanoguanidine series is represented by A-740003 ((*N*-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide) (Figure 2; Honore *et al*., 2006b). A-740003 is a highly specific and potent antagonist for both rat and human $P2X_7$ receptors

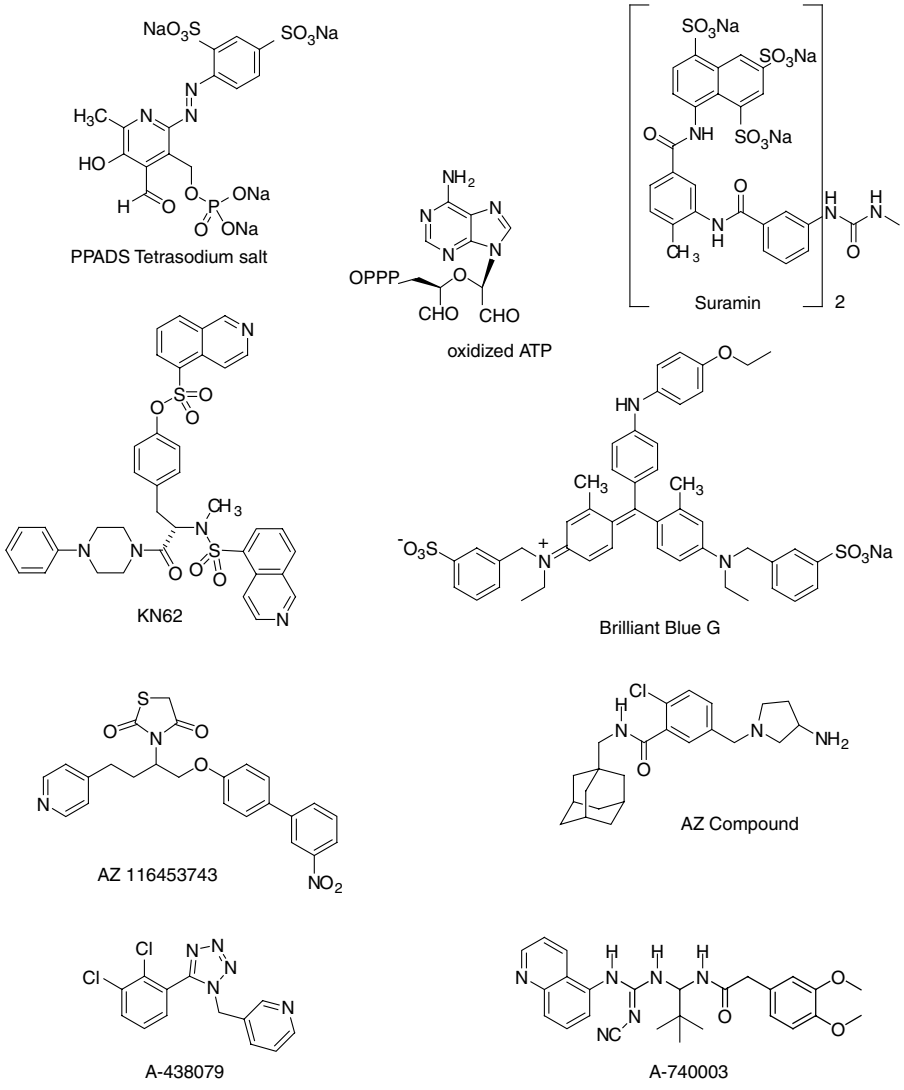

Figure 2 Structures of prototypical and novel antagonists of $P2X_7$ receptors. PPADS and suramin are nonselective antagonists, BBG has higher potency for rat versus human $P2X_7$ receptors whereas KN62 and the AZ compounds exhibit the reverse potency of human > rat $P2X_7$ receptors. A-740003 and A-438079 exhibited equivalent potency to both the rat and human $P2X_7$ receptors (see Table 1). BBG, Brilliant Blue G; PPADS, pyridoxal phosphate-6-azophenyl-2-4-disulfonic acid.

with potencies of 18–40 nM, respectively (Honore *et al.*, 2006b; Table 1). Similar to A-438079, A-740003 blocked $P2X_7$ receptor-mediated changes in intracellular calcium concentrations in a competitive fashion and was shown to be highly selective for $P2X_7$ compared to other P2 receptors as well as other cell-surface receptors and ion channels. In addition, A-740003 was more potent in blocking BzATP-evoked IL-1β release and pore formation in differentiated human THP-1 cells than A-438079 (Honore *et al.*, 2006b). An additional feature of these compounds is that they have sufficient bioavailability following intraperitoneal administration to allow for their use in *in vivo* investigations on the role of $P2X_7$ receptors in various disease models (Nelson *et al.*, 2006; Honore *et al.*, 2006b).

$P2X_7$ receptor signaling

As noted above, activation of $P2X_7$ receptors results in a rapid but reversible channel opening that is permeable to Ca^{2+}, Na^+ and K^+ ions. $P2X_7$ receptor-mediated changes in intracellular potassium concentrations lead to the activation of caspase-1 and the rapid maturation and release of the proinflammatory cytokine, IL-1β (Sanz and DiVirgilio, 2000; Kahlenberg and Dubyak, 2004; Perregaux *et al.*, 2000; Solle *et al.*, 2001; Ferrari *et al.*, 2006). ATP-stimulated IL-1β release is independent of cytolysis, does not require $P2X_7$-mediated pore formation, and is blocked by $P2X_7$ receptor antagonists (Grahames *et al.*, 1999; Chessell *et al.*, 2001). Increased IL-1β concentrations, in turn, trigger the induction of nitric oxide synthase, cycloxygenase-2 and tumor necrosis factor-α (TNF-α) (Woolf *et al.*, 1997; Samad *et al.*, 2001; Parvathenani *et al.*, 2003; Burnstock, 2006). Activation of caspase 3 has also been linked to $P2X_7$ receptor activation and may underlie receptor-associated cytolytic mechanisms including pore formation (Perregaux *et al.*, 2001). Inhibitors of caspase 1 and 3 effectively block $P2X_7$ receptor-mediated IL-1β release and Yo-Pro uptake, respectively (Donnelly-Roberts *et al.*, 2004).

Table 1 Functional pharmacological evaluation

Antagonist	*Rat P2X$_7$*	*Human P2X$_7$*
[Ca^{2+}]$_i$ pIC$_{50}$±s.e.m.		
A-740003	7.75±0.03	7.36±0.04
A-438079	6.50±0.20	6.90±0.20
AZ11645373	>4	8.15[a]
AZ compound[b]	ND	ND
PPADS	5.10±0.02	5.45±0.03
KN62	<4	4.88±0.18
BBG	5.08±0.07	<4
Suramin	<4	<4

Antagonist	*Rat P2X$_7$*	*Human P2X$_7$*
Yo-Pro activity pIC$_{50}$±s.e.m.		
A-740003	7.00±0.02	7.03±0.04
A-438079	6.25±0.20	6.70±0.10
AZ11645373	>4	7.70[a]
AZ compound[b]	6.54±0.32	8.38±0.12
PPADS	5.92±0.09	5.88±0.03
KN62	<4	6.67±0.02
BBG	6.22±0.03	5.71±0.09
Suramin	<4	<4

Antagonist	*THP-1 cells/Human P2X7*
IL-1β activity pIC$_{50}$±s.e.m.	
A-740003	7.00±0.02
A-438079	6.40±0.30
AZ11645373	7.68[a]
AZ compound[b]	6.82±0.08
PPADS	5.92±0.20
KN62	7.12±0.16
BBG	6.22±0.03

Abbreviations: BBG, Brilliant Blue G; PPADS, pyridoxal phosphate-6-azophenyl-2-4-disulfonic acid; ND, not determined; s.e.m., standard error of the mean.
[a]pIC50 values derived from published results (Stokes *et al.*, 2006).
[b]Data from Fonfria *et al.* (2005).

Activation of P2X$_7$ receptors has also been associated with other downstream signaling pathways including phospholipase D (Humphreys and Dubyak, 1996), phospholipase A$_2$ (PLA$_2$), NF-κB (Ferrari *et al.*, 1997; Aga *et al.*, 2002) and mitogen-activated protein kinases (MAPKs) (Aga *et al.*, 2002; Armstrong *et al.*, 2002; Bradford and Soltoff, 2002). Studies have shown that different MAPK kinases are affected by P2X$_7$ activation that vary depending on host cell systems. For example, in human astrocytoma 1321 cells, recombinant P2X$_7$ receptor activation mediates ERK1/2 upregulation (Gendron *et al.*, 2003) but not in differentiated human THP-1 cells (Donnelly-Roberts *et al.*, 2004). Recent work by Monterio da Cruz *et al.* (2006) supported the latter finding by demonstrating that ERK1/2 is activated by a number of extracellular nucleotides and that this activation is not dependent on ATP-induced pore formation.

Recent studies provide data showing that activation of P2X$_7$ receptors leads to downstream activation of p38 MAPK (Ono and Han, 2000; Armstrong *et al.*, 2002; Donnelly-Roberts *et al.*, 2004). For example, BzATP-evoked increases in phosphorylated p38 expression in THP-1 cells that had been differentiated into a macrophage phenotype (Donnelly-Roberts *et al.*, 2004). This effect was fully blocked by p38 MAPK inhibitors and by KN62 as well as a novel cyanoguanidine P2X$_7$ receptor-selective antagonist (Donnelly-Roberts *et al.*, 2004, unpublished observations). Like its pore-forming activity (North, 2002) and other P2X$_7$ receptor downstream signaling mechanisms (Donnelly-Roberts *et al.*, 2004; Faria *et al.*, 2005), P2X$_7$ receptor-mediated activation of p38 MAPK is likely to be cell type-dependent. While some recent reports have demonstrated this possibility in THP-1 cells (Michel *et al.*, 2006b), a clear interpretation of the experimental data is complicated by the use of suboptimal cell differentiation conditions for p38 MAPK expression (Carter *et al.*, 2001) and the use of oATP as a P2X$_7$ antagonist, which has many other non-P2X$_7$ receptor-mediated activities.

Other reports (MacKenzie *et al.*, 2001; Verhoef *et al.*, 2003) have also implicated that many of the functional sequelae to P2X$_7$ receptor activation including IL-1β release, membrane blebbing and pore formation may be mediated by parallel rather than convergent intracellular signal transduction pathways (North, 2002). These data are consistent with the specific involvement of p38 MAPK in P2X$_7$ receptor-mediated pore formation as this mechanism does not contribute to pore formation induced by maitotoxin (MTX), a marine toxin isolated from dinoflagellates (Schilling *et al.*, 1999), or by human cathelicidin-derived peptide (LL37) (Elssner *et al.*, 2004). Further, pore formation is not a unique property of P2X$_7$ receptors as prolonged agonist activation of other nondesensitizing P2X receptors such as P2X$_{2a}$, P2X$_{2/3}$ and P2X$_4$, also leads to the formation of cell-surface pores (Khakh *et al.*, 1999; Virginio *et al.*, 1999; North, 2002; Donnelly-Roberts *et al.*, 2004), although with different time courses and pore sizes (Virginio *et al.*, 1999).

P2X$_7$ receptor-mediated Yo-Pro uptake does not occur in all cell types and may be dependent upon receptor density (North 2002). Some cell types such as the B-lymphocytes (Gu *et al.*, 2000), retinal muller cells (Pannicke *et al.*, 2000) and non-neuronal cells of the dorsal root ganglion (Zhang *et al.*, 2005) express functional P2X$_7$ receptors but yet do not form cytolytic pores. Thus, this disconnection has indicated the possibility that these two functional properties are not intrinsic to the P2X$_7$ receptor itself but require the assistance of secondary molecules that are host cell-dependent (North, 2002).

The ability of P2X$_7$ receptor activation to generate cytolytic pores has been clearly linked to interactions within the C-terminal domain of the receptor (Rassendren *et al.*, 1997b). Removal of this portion of the channel results in pore dilating properties similar to P2X$_2$ and P2X$_4$ receptors based on measuring time-dependent changes in NMDG$^+$ permeability (Khakh *et al.*, 1999). Recent studies have shed light on differences in P2X$_7$ receptor-mediated pore formation between Yo-Pro and NMDG$^+$ entry. North and co-workers (Jiang *et al.*, 2005) have shown that NMDG$^+$ and Yo-Pro do not enter P2X$_7$ receptor-expressing cells by the same route further supporting the hypothesis that although pore formation is triggered by P2X$_7$ receptor activation, it is dependent on the involvement of downstream signaling interactions not involved in channel opening.

Both intrinsic P2X$_7$ receptor channel dilation and P2X$_7$ receptor-dependent recruitment of accessory proteins have

been proposed as two potential mechanisms necessary for pore formation (North, 2002). Data demonstrating that $P2X_7$ receptor pore formation is progressive, occurs in multiple cell types and has an identical pharmacology for the opening of the cation channel support the intrinsic receptor hypothesis. But recent data strongly suggest that pore formation involves accessory proteins such as heat shock proteins (for example, HSP90), chaperone-like proteins or hemichannels such as pannexins. Studies of the ubiquitously expressed HSP90 protein have shown that HSP90 might act as a negative regulator of $P2X_7$ receptor function because, geldanamycin, a HSP90 inhibitor, produced a twofold increase on $P2X_7$ receptor activation by agonists (Adinolfi *et al*., 2003). A more recent report has demonstrated that the recruitment of pannexin-type hemichannels that are involved in gap junction formation (Barbe *et al*., 2006) may contribute to pore formation following $P2X_7$ receptor activation (Pelegrin and Surprenant, 2006; Locovei *et al*., 2007). Interestingly, pannexin-1 signaling is also required for the processing of caspase-1 and the subsequent $P2X_7$ receptor-mediated release of mature IL-1β (Pelegrin and Surprenant, 2006). It was also demonstrated in these studies that pannexin siRNA selectively blocks $P2X_7$ receptor-induced pore formation, but not ionic currents further supporting separate pathways for these two distinct $P2X_7$ receptor-mediated functional events (Pelegrin and Surprenant, 2006). These recent data strongly suggest that the pannexin hemichannel may serve as a portal for $P2X_7$ receptor pore formation to occur and unlock the release of IL-1β as well as to permit other downstream signaling events to proceed such as the upregulation of p38 MAPK.

Therapeutic significance

It is now well established that ATP acting at $P2X_7$ receptors serves as an efficient secondary stimulus for the maturation and release of IL-1β from proinflammatory cells (Perregaux and Gabel, 1994; Mackenzie *et al*., 2001; Ferrari *et al*., 2006). As a consequence, $P2X_7$ receptor activation may function as a danger signal in the context of tissue trauma (Ferrari *et al*., 2006). However, validation of this concept in disease models has been slow due to the previous lack of appropriate pharmacological tools. Additionally, initial phenotypic data from a $P2X_7$ receptor null mouse revealed unremarkable changes (Sikora *et al*., 1999).

A detailed analysis of an independently generated $P2X_7$ receptor knockout mouse revealed that $P2X_7$ (−/−) mice show a disruption in cytokine signaling cascades with perturbation of ATP-induced processing of pro-IL-1β in macrophages (Solle *et al*., 2001). Using a monoclonal antibody-induced model of collagen arthritis, these investigators also demonstrated that $P2X_7$ (−/−) mice show a decreased incidence and severity of arthritis in this model as compared with wild-type control mice (Labasi *et al*., 2002). A more recent study has demonstrated that $P2X_7$ knockout mice also show reduced pain sensitivity following both complete Freund's adjuvant-induced inflammation and partial injury of the sciatic nerve (Chessell *et al*., 2005).

The finding that disruption of $P2X_7$ receptors not only altered inflammatory pain but also reduces pain associated with frank nerve injury (Chessell *et al*., 2005), provided new insights into the potential physiological role of $P2X_7$ receptors in sensory functions. Additionally, these data are also consistent with the mechanistic role of $P2X_7$ receptors in modulating IL-1β release and the ability of IL-1β to alter pain sensitivity in experimental models. Previous data have shown that endogenous IL-1 levels are increased in the nervous system in response to trauma associated with mechanical damage, ischemia, seizures and hyperexcitability (Touzani *et al*., 2002). Increased IL-1 levels are also associated with enhanced nociceptive signaling in a concentration-related fashion (Bianchi *et al*., 1998; Horai *et al*., 2000). At the level of the spinal cord, blockade of IL-1 receptors with the IL-1 receptor antagonist (IL-1ra), results in reduced nociception in animal models of inflammation and nerve injury-induced pain (Maier *et al*., 1993; Safieh-Garabedian *et al*., 1995; Sommer *et al*., 1999). Other genetic manipulations of IL-1 signaling including targeted gene disruption of the IL-1 type I receptor or the IL-1 accessory protein (IL-1acp) as well as transgenic overexpression of the IL-1ra (Wolf *et al*., 2004) or IL-1$\alpha\beta$ double knockout (Honore *et al*., 2006a) have generated mice that show reduced nociceptive responses relative to wild-type animals.

Complementing these genetic data are recent studies using receptor-selective antagonists. Collectively, these studies indicate a specific role for $P2X_7$ receptor activation in pain signaling. Early work by Dell'Antonio *et al*. (2002a, b) showed that local administration of oATP reduced inflammation-induced mechanical hyperalgesia in rats. These results were attributed to pharmacological blockade of $P2X_7$ receptors. However, as noted above, oATP has weak affinity for $P2X_7$ receptors, slow kinetics and many other pharmacological actions that limit interpretations about $P2X_7$ receptor specificity. More direct support for a role of $P2X_7$ receptors in pain modulation are provided by the recent $P2X_7$ knockout study (Chessell *et al*., 2005) and studies using selective antagonists (Nelson *et al*., 2006; Honore *et al*., 2006b). Similar to the nociceptive phenotype of mice lacking $P2X_7$ receptors (Chessell *et al*., 2005) or lacking both isoforms of IL-1 (Honore *et al*., 2006a), systemic administration of $P2X_7$ receptor-selective antagonists (for example, A-438079 and A-740003) produced dose-dependent antinociceptive effects in models of neuropathic (Nelson *et al*., 2006; Honore *et al*., 2006b) and inflammatory pain (Honore *et al*., 2006a). Consistent with their *in vitro* potencies, A-740003 was more potent than A-438079 at reducing mechanical allodynia observed 2 weeks after spinal L5/L6 nerve ligation. These data are also consistent with an independent study of one of the adamantane $P2X_7$ antagonists that showed dose-dependent antinociception in an inflammatory pain model (Lappin *et al*., 2005). These data illustrate the potential role of $P2X_7$ receptor modulation of IL-1β in reducing nociception in neuropathic pain models.

The robust antinociceptive effects of $P2X_7$ antagonists in inflammatory pain models does not appear to be secondary to an anti-inflammatory effect as A-740003 was more efficacious in reducing nociception compared with paw edema in inflammation models (Honore *et al*., 2006b). It should be noted, however, that the anti-inflammatory activity of $P2X_7$ antagonists may be more pronounced in

arthritis models as compared with acute (carrageenan) and subacute complete Freund's adjuvant (CFA) inflammatory models, where the contribution of IL-1β to ongoing inflammatory processes may be more prominent than in chronic arthritis (Labasi *et al*., 2002).

Summary and perspective

The discovery of $P2X_7$ receptor-selective antagonists has provided data demonstrating that the acute *in vivo* blockade of $P2X_7$ receptors significantly reduced nociception in animal models of persistent neuropathic and inflammatory pain. While there is growing appreciation for the role of $P2X_7$ receptor modulation of proinflammatory IL-1 processing (Ferrari *et al*., 2006), the analgesic activity of $P2X_7$ receptor antagonists (Lappin *et al*., 2005; Honore *et al*., 2006b; Nelson *et al*., 2006), indicates a specific role for $P2X_7$ receptors in neural–glial cell interactions associated with ongoing pain (Zhang *et al*., 2005).

In addition to the analgesic effects of $P2X_7$ receptor antagonists, there exist multiple P2 receptor-based mechanisms by which ATP can alter nociceptive sensitivity following tissue injury (Fields and Burnstock, 2006). Evidence from a variety of experimental strategies including genetic disruption and the development of other selective antagonists has indicated that the activation of several P2X receptors, including $P2X_3$, $P2X_{2/3}$, $P2X_4$ and $P2X_7$, and P2Y ($P2Y_2$) receptors can modulate pain. For example, A-317491, a selective $P2X_3$ antagonist (Jarvis *et al*., 2002) effectively blocks both CFA-induced inflammatory thermal hyperalgesia as well as mechanical and thermal hyperalgesia resulting from chronic constriction of the sciatic nerve. Intrathecally delivered antisense oligonucleotides specifically targeting $P2X_4$ receptors have been shown to decrease tactile allodynia following nerve injury (Tsuda *et al*., 2003). In addition, activation of $P2Y_2$ receptors leads to sensitization of transient receptor potential vanilloid-1 (TRPV1) receptors (Tominaga *et al*., 2001; Lakshmi and Joshi, 2005). Thus, ATP acting at multiple purinergic receptors either directly on neurons (for example, $P2X_3$, $P2X_{2/3}$ and P2Y receptors) or through indirect neural–gial cell interactions ($P2X_4$ and $P2X_7$) appear to alter nociceptive sensitivity (Tsuda *et al*., 2003; Inoue *et al*., 2004; Zhang *et al*., 2005).

As this article illustrates, the discovery of receptor-selective antagonists for individual P2X receptor subtypes has led to important insights into the roles of individual P2X receptors in chronic pain states. From a pharmacological perspective, there is presently a wider structural diversity of non-nucleotide $P2X_7$ receptor antagonist pharmacophores than have been described for $P2X_3$ receptors (Jarvis, 2003; Gever *et al*., 2006) or for $P2X_4$ receptors, for which, no potent antagonists have yet been identified (Gever *et al*., 2006). While both $P2X_4$ and $P2X_7$ receptors have been implicated in glial-based modulatory mechanisms involved in sensory neurotransmission (Tsuda *et al*., 2003; Zhang *et al*., 2005), the discovery of selective $P2X_4$ antagonists are needed to differentiate the respective roles of these receptors in pain signaling. The identification of novel $P2X_7$ receptor-selective antagonists has provided new research tools for both *in vitro* and *in vivo* studies of $P2X_7$ receptor pharmacology and has led to the generation of new data that indicates an expanded role for this receptor in pain signaling associated with nerve injury and inflammation.

Acknowledgements

We are grateful to Derek Nelson for his assistance with the figures and comments on earlier versions of this manuscript.

Conflict of interest

These authors are employees of Abbott Laboratories.

References

Adinolfi E, Kim M, Young MT, Di Virgillio F, Surprenant A (2003). Tyrosine phosphorylation of HSP90 within the $P2X_7$ receptor complex negatively regulates $P2X_7$ receptors. *J Biol Chem* **278**: 37344–37351.

Aga M, Johnson CJ, Hart AP, Guadarrama AG, Suresh M, Svaren J *et al.* (2002). Modulation of monocyte signaling and pore formation in response to agonists of the nucleotide receptor $P2X_7$. *J Leukocyte Biol* **72**: 222–232.

Alcaraz L, Baxtrer A, Bent J, Bowers K, Braddock M, Cladingboel D *et al.* (2003). Novel $P2X_7$ receptor antagonists. *Bioorg Med Chem Lett* **13**: 4043–4046.

Anderson CM, Nedergaard M (2006). Emerging challenges of assigning P2X(7) receptor function and immunoreactivity in neurons. *Trends Neurosci* **29**: 257–262.

Armstrong JN, Brust TB, Lewis RG, MacViar BA (2002). Activation of presynaptic $P2X_7$-like receptors depresses mossy fiber-CA3 synaptic transmission through p38 mitogen-activated protein kinase. *J Neurosci* **22**: 5938–5945.

Barbe MT, Monyer H, Bruzzone R (2006). Cell–cell communication beyond connexins: the pannexin channels. *Physiol* **21**: 103–114.

Baxter A, Bent J, Bowers K, Braddock M, Brough S, Fagura M *et al.* (2003). Hit-to-lead studies: the discovery of potent adamantane amide $P2X_7$ receptor antagonists. *Bioorg Med Chem Lett* **13**: 4047–4050.

Beigi RD, Kertesy SB, Aquilina G, Dubyak GR (2003). Oxidized ATP (oATP) attenuates proinflammatory signaling via P2 receptor-independent mechanisms. *Br J Pharmacol* **140**: 507–519.

Bianchi BR, Lynch KJ, Touma E, Niforatos W, Burgard EC, Alexander KM *et al.* (1999). Pharmacological characterization of recombinant human and rat P2X receptor subtypes. *Eur J Pharmacol* **376**: 127–138.

Bianchi M, Dib B, Panerai AE (1998). Interleukin-1 and nociception in the rat. *J Neurosci Res* **53**: 645–650.

Bradford MD, Soltoff SP (2002). $P2X_7$ receptors activate protein kinase D and p42/p44 mitogen-activated protein kinase (MAPK) downstream of protein kinase C. *Biochem J* **366**: 745–755.

Burnstock G (2006). Pathophysiology and therapeutic potential of purinergic signaling. *Pharmacol Rev* **58**: 58–78.

Cabrini G, Flazonni S, Forchap SL, Pellegatti P, Balboni A, Agostini P *et al.* (2005). A His-155 to tyr polymorphism confers gain-of-function to the human $P2X_7$ receptor of human leukemic lymphocytes. *J. Immunol* **175**: 82–89.

Carter AB, Tephly LA, Hunnunghake GW (2001). The absence of activator protein1-dependent gene expression in THP-1 macrophages stimulated with phorbol esters is due to lack of p38 mitogen-activate protein kinase activation. *J Biol Chem* **276**: 33826–33832.

Chakfe Y, Seguin R, Antel JP, Morissette C, Malo D, Henderson D *et al.* (2002). ADP and AMP induce interleukin-1 beta release from microglia cells through activation of ATP-primed $P2X_7$ receptor channels. *J Neurosci* **22**: 3061–3069.

Chessell IP, Grahames CBA, Michel AD, Humphrey PPA (2001). Dynamics of P2X7 receptor pore dilation: pharmacological and functional consequences. *Drug Dev Res* **53**: 60–65.

Chessell IP, Hatcher J, Bountra C, Michel AD, Hughes JP, Green P *et al*. (2005). Disruption of the $P2X_7$ purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* **114**: 386–396.

Collo G, Neidhart S, Kawashima E, Kosco-Vibois M, North RA, Buell G (1997). Tissue distribution of the $P2X_7$ receptor. *Neuropharmacology* **36**: 1277–1283.

Dell'Antonio A, Quattrini A, Dal Cin E, Fulgenzi A, Ferrero ME (2002a). Antinociceptive effect of a new P2Z/$P2X_7$ antagonist, oxidized ATP, in arthritic rats. *Neurosci Lett* **327**: 87–90.

Dell'Antonio A, Quattrini A, Dal Cin E, Fulgenzi A, Ferrero ME (2002b). Relief of inflammatory pain in rats by local use of the selective $P2X_7$ ATP receptor inhibitor, oxidized ATP. *Arthritis Rheum* **46**: 3378–3385.

Denlinger LC, Fisette PL, Sommer JA, Watters JJ, Prabhu U, Dubyak GR *et al*. (2001). The nucleotide receptor $P2X_7$ contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. *J Immunol* **167**: 1871–1876.

Di Virgilio FD, Vishawanath V, Ferrari D (2001). On the role of the $P2X_7$ receptor in the immune system. In: Abbracchio MP and Williams M (eds). *Purinergic and Pyrimidinergic Signalling*. Springer Verlag: Heidelberg, GR, pp 355–373.

Di Virgilio F (2003). Novel data point to a broader mechanism of action of oxidized ATP: the $P2X_7$ receptor is not the only target. *Br J Pharmacol* **140**: 441–443.

Donnelly-Roberts DL, Namovic M, Faltynek CR, Jarvis MF (2004). Mitogen-activated protein kinase and caspase signaling pathways are required for $P2X_7$ receptor ($P2X_7R$)-induced pore formation in human THP-1 cells. *J Pharmacol Exp Ther* **308**: 1053–1061.

Egan TM, Samways DSK, Li Z (2006). Biophysics of P2X Receptors. *Eur J Physiol* **452**: 501–512.

Elssner A, Duncan M, Gavrillin M, Wewers MD (2004). A novel P2X7 receptor activator, the human cathelicidin-derived peptide LL37, induces IL-1β processing and release. *J Immunol* **172**: 4987–4994.

Evans RJ, Lewis C, Buell G, Vlaera S, North RA, Surprenant A (1995). Pharmacological characterization of heterologously expressed ATP-gated cation channels (P2X purinoceptors). *Mol Pharmacol* **48**: 178–183.

Faria RX, DeFarias FP, Alves LA (2005). Are second messengers crucial for opening the pore associated with $P2X_7$ receptor? *Am J Physiol Cell Physiol* **288**: C260–C271.

Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Golbberg H, Marks GB *et al*. (2007). A polymorphism in the P2X7 gene increases susceptibility to extrapulmonary tuberculosis. *Am J Respir Crit Care Med* **175**: 360–366.

Ferrari D, Villalba M, Chiozzi P, Falzoni S, Ricciardi-Castagnoli P, DiVirgilio F (1996). Mouse microglial cells express a plasma membrane pore gated by extracellular ATP. *J. Immunol* **156**: 1531–1539.

Ferrari D, Wesselborg S, Bauer M, Schulze-Osthoff K (1997). Extracellular ATP activates transcription factor NF-[kappa]B through the P2Z purinoreceptor by selectively targeting NF-[kappa]B. *J Cell Biol.* **139**: 1635–1643.

Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, Idzko M *et al*. (2006). The P2X7 receptor: a key player in IL-1 processing and release. *J Immunol* **176**: 3877–3883.

Fields RD, Burnstock G (2006). Purinergic signaling in neuron–glia interactions. *Nature Review* **7**: 423–436.

Fonfria E, Chambers LJ, Demont EH, Roman SA, Skaper SD, Michel AD (2005). Species and temperature dependent effects of a novel P2X7 receptor antagonist on recombinant and native P2X7 receptors. *Soc Neurosci* Abstr no. 958.1.

Gargett CE, Wiley JS (1997). The isoquinoline derivative KN-62 is a potent antagonist of the $P2X_Z$-receptor of human lymphocytes. *Br J Pharmacol* **120**: 1483–1490.

Gendron F-P, Neary JT, Theiss PM, Sun GY, Gonzalez FA, Weismann GA (2003). Mechanisms of $P2X_7$ receptor-mediated ERK1/2 phosphorylation in human astrocytoma cells. *Am J Physiol Cell Physiol* **284**: C571–C581.

Gever JR, Cockayne DA, Dillon MP, Burnstock G, Ford APDW (2006). Pharmacology of P2X channels. *Eur J Physiol* **452**: 513–537.

Grahames GBA, Michel AD, Chessell IP, Humpreys PPA (1999). Pharmacological characerization of ATP-and LPS-induced IL-1β release in human monoctyes. *Br J Pharmacol* **127**: 1915–1921.

Gu BJ, Zhang WY, Bendall LJ, Chessell IP, Buell GN, Wiley JS. (2000). Expression of P2X7 purinoceptors on human lymphocytes and monoctyes: evidence for nonfunctional P2X7 receptors. *Am J Physiol* **279**: C1189–C1197.

Honore P, Wade CL, Zhong C, Harris RR, Wu C, Ghayur T *et al*. (2006a). Interleukin-1$\alpha\beta$ gene-deficient mice show reduced nociceptive sensitivity in models of inflammatory and neuropathic pain but not post-operative pain. *Behav Brain Res* **167**: 355–364.

Honore PM, Donnelly-Roberts D, Namovic M, Hsieh G, Zhu C, Mikusa J *et al*. (2006b). A-740003 (N-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide, a novel and selective P2X7 receptor antagonist dose-dependently reduces neuropathic pain in the rat. *J Pharmacol Exp Ther* **319**: 1376–1385.

Horai R, Saijo S, Tanioka H, Nakae S, Sudo K, Okahara A *et al*. (2000). Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* **191**: 313–320.

Humphreys BD, Dubyak GR (1996). Induction of the P2z/$P2X_7$ nucleotide receptor and associated phospholipase D activity by lipopolysaccharide and IFN-γ in the human THP-1 monocytic cell line. *J Immunol* **157**: 5627–5637.

Humphreys BD, Virginio C, Surprenant A, Rice J, Dubyak GR (1998). Isoquinolines as antagonists of the $P2X_7$ nucelotide receptor: high selectivity for the human versus rat receptor. *Mol Pharmacol* **54**: 22–32.

Inoue K, Tsuda M, Koizumi S (2004). ATP- and adenosine-mediated signaling in the central nervous system: chronic pain and microglia: involvement of the ATP receptor $P2X_4$. *J Pharmacol Sci* **94**: 112–114.

Jacobson KA, Jarvis MF, Williams M (2002). Perspective: purine and pyrimidine. (P2) receptors as drug targets. *J Med Chem* **45**: 4057–4093.

Jarvis MF, Burgard EC, McGaraughty S, Honore P, Lynch K, Brennan TJ *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 rats. *Proc Natl Acad Sci USA* **99**: 17179–17184.

Jarvis MF (2003). Contributions of $P2X_3$ homomeric and heteromeric channels to acute and chronic pain. *Expert Opin Ther Targets* **7**: 513–522.

Jiang LH, MacKenzie AB, North RA, Surprenant A (2000). Brilliant blue G selectively blocks ATP-gated rat $P2X_7$ receptors. *Mol Pharmacol* **58**: 82–88.

Jiang LH, Rassendren F, MacKenzie AB, Zhang YH, Surprenant A, North RA (2005). *N*-methyl-D-glucamine and propidium dyes utilize different permeation pathways at rat P2X7 receptors. *Am J Physiol Cell Physiol* **289**: C1295–C1302.

Kahlenberg J, Dubyak GW (2004). Mechanisms of caspase-1 activation by $P2X_7$ receptor-mediated K^+ release. *Am J Cell Physiol* **286**: C1100–C1108.

Khakh BS, Bao XR, Labarca C, Lester HA (1999). Neuronal P2X transmitter-gated cation channels change their ion selectivity in seconds. *Nature Neurosci* **2**: 322–330.

Khakh BS, North RA (2006). P2X receptors as cell-surface ATP sensors in health and disease. *Nature* **442**: 527–532.

Kim M, Jiang LH, Wilson HL, North RA, Suprenant A (2001). Proteomic and functional evidence for a $P2X_7$ receptor signaling complex. *EMBO J* **20**: 6347–6358.

Labasi JM, Petrushova N, Donovan C, McCurdy S, Lira P, Payette MM *et al*. (2002). Absence of the $P2X_7$ receptor alters leukocyte function and attenuates an inflammatory response. *J Immunol* **168**: 6436–6445.

Lakshmi S, Joshi PG (2005). Co-activation of P2Y2 receptor and TRPV channel by ATP: implications for ATP induced pain. *Cell Mol Neurobiol* **25**: 819–832.

Lappin SC, Winyard LA, Clayton N, Chambers LJ, Demont EH, Chessell IP *et al*. (2005). Reversal of mechanical hyperalgesia in a rat model of inflammatory pain by a potent and selective $P2X_7$ antagonist. *Soc Neurosci* Abstr no. 958.2.

Locovei S, Scemes E, Qiu F, Spray DC, Dahl G (2007). Pannexin1 is part of the pore forming unit of the P2X(7) receptor death complex. *FEBS Lett* **581**: 483–488.

Lowe PN, Beechey RB (1982). Interactions between the mitochondrial adenosinetriphosphatase and periodate-oxidized adenosine 5′-triphosphate, an affinity label for adenosine 5′-triphosphate binding sites. *Biochemistry* **21**: 4073–4082.

MacKenzie A, Wilson HL, Kiss-Tosh E, Dower SK, North RA, Surprenant A (2001). Rapid secretion of interleukin-1β by microvesicle shedding. *Immunity* **8**: 825–835.

Maier SF, Wietelak EP, Martin D, Watkins LR (1993). Interleukin-1 mediates the behavioral hyperalgesia produced by lithium chloride and endotoxin. *Brain Res* **623**: 321–324.

Michel AD, Xing M, Thompson KM, Jones CA, Humphrey PPA (2006a). Decavanadate, a P2X receptor antagonist, and its use to study ligand interactions with $P2X_7$ receptors. *Br J Pharmacol* **534**: 19–26.

Michel AD, Thompson KM, Simon J, Boyfield I, Fonfria E, Humphrey PPA (2006b). Species and response dependent differences in the effects of MAPK inhibitors on $P2X_7$ receptor function. *Br J Pharmacol* **149**: 948–957.

Monterio da Cruz C, Ventura ALM, Schachter J, Costa-Junior HM, da Silva Souza HA, Gomes FR *et al*. (2006). Activation of ERK1/2 by extracellular nucleotides in macrophages is mediated by multiple P2 receptors independently of $P2X_7$-associated pore or channel formation. *Br J Pharmacol* **147**: 324–334.

Murgia M, Hanau S, Pizzo P, Rippa M, Di Virgilio F (1993). Oxidized ATP. An irreversible inhibitor of the macrophage purinergic P2Z receptor. *J Biol Chem* **268**: 8199–81203.

Nelson DW, Gregg RJ, Kort ME, Perez-Medrano A, Voight EA, Wang Y *et al*. (2006). Structure–activity relationship studies on a series of novel, substituted 1-benzyl-5-phenyltetrazole $P2X_7$ antagonists. *J Med Chem* **49**: 3659–3666.

North AR (2002). Molecular physiology of P2X receptors. *Physiol Rev* **82**: 1013–1067.

Ono K, Han J (2000). The p38 signal transduction pathway: activation and function. *Cell Signal* **12**: 1–13.

Panenka W, Jijon H, Herx LM, Armstrong N, Feighan D, Wei T *et al*. (2001). $P2X_7$-like receptor activation in astrocytes increases chemokine monocyte chemoattractant protein-1 expression via mitogen-activated protein kinase. *J Neurosci* **21**: 7135–7142.

Pannicke T, Fisher W, Biefermann B, Schadlich H, Grosche J, Faude F *et al*. (2000). $P2X_7$-receptors activation in Mueller glial cells from the human retina. *J Neurosci* **20**: 5965–5972.

Parvathenani LK, Svetlana T, Greco CR, Roberts SB, Robertson B, Posmantur R (2003). $P2X_7$ mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. *J Biol Chem* **278**: 13309–13317.

Pelegrin P, Surprenant A (2006). Pannexin-1 mediates large pore formation and interleukin-1b release by the ATP-gated $P2X_7$ receptor. *EMBO J* **25**: 5071–5082.

Perregaux DG, Gabel CA (1994). Interleukin-1β maturation and release in response to ATP and nigericin. *J Biol Chem* **269**: 15195–15203.

Perregaux DG, McNiff P, Laliberte R, Conklyn M, Gabel CA (2000). ATP acts as an agonist to promote stimulus-induced secretion of IL-1β and IL-18 in Human Blood. *J Immunol* **165**: 4615–4623.

Perregaux DG, Labasi J, Laliberte R, Stam E, Solle M, Koller B *et al*. (2001). Interleukin-1β posttranslational processing-exploration of $P2X_7$ receptor involvement. *Drug Dev Res* **53**: 83–90.

Rassendren F, Buell GN, Virginio C, Collo G, North RA, Surprenant A (1997a). The permeabilizing ATP receptor, $P2X_7$: cloning and expression of a human cDNA. *J Biol Chem* **272**: 5482–5486.

Rassendren F, Buell GN, Newbolt A, North RA, Surprenant A (1997b). Identification of amino acid residues contributing to the pore of a P2X receptor. *EMBO J* **16**: 3446–3454.

Safieh-Garabedian B, Poole S, Allchorne A, Winter J, Woolf CJ (1995). Contribution of interleukin-1 to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. *Br J Pharmacol* **115**: 1265–1275.

Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S *et al*. (2001). Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. *Nature* **410**: 471–475.

Sanz JM, DiVirgilio F (2000). Kinetics and mechanism of ATP-dependent IL-1β release from microglia cells. *J. Immunol* **164**: 4893–4898.

Schilling WP, Sinkins WG, Estacion M (1999). Maitotoxin activates a nonselective cation channel and a P2Z/$P2X_7$-like cytolytic pore in human skin fibroblasts. *Am J Physiol Cell Physiol* **277**: C755–C765.

Sikora A, Li J, Brosnan C, Buell G, Chessel I, Bloom BR (1999). Cutting edge: purinergic signaling regulates radical-mediated bacterial killing mechanisms in macrophages through a $P2X_7$-independent mechanism. *J Immunol* **163**: 558–561.

Sim JA, Young MT, Sung HY, North RA, Surprenant A (2004). Reanalysis of $P2X_7$ receptor expression in rodent brain. *J Neurosci* **24**: 6307–6314.

Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, Koller BH *et al*. (2001). Altered cytokine production in mice lacking P2X7 receptors. *J Biol Chem* **276**: 125–132.

Sommer C, Petrausch S, Lindenlaub T, Toyka K (1999). Neutralizing antibodies to interleukin1-receptor reduce pain-associated behavior in mice with experimental neuropathy. *Neurosci Lett* **270**: 25–28.

Stokes L, Jiang L-H, Alcaraz L, Bent J, Bowers K, Fagura M *et al*. (2006). Characterization of a selective and potent antagonist of human $P2X_7$ receptors, AZ11645373. *Br J Pharmacol* **149**: 880–887.

Surprenant A, Rassendren F, Kawashima E, North RA, Buell G (1996). The Cytolytic P2z receptor for extracellular ATP identified as a P2X receptor (P2X7). *Science* **272**: 735–738.

Tominaga M, Wada M, Masu M (2001). Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. *Proc Natl Acad Sci USA* **98**: 6951–6956.

Touzani O, Boutin H, LeFeurve R, Parker L, Miller A, Luheshi G *et al*. (2002). Interleukin-1 influences ischemic brain damage in the mouse independently of the interleukin-1 type 1 receptor. *J Neurosci* **22**: 38–43.

Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter M *et al*. (2003). $P2X_4$ receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* **424**: 778–783.

Verhoef PA, Estacion M, Schilling W, Dubyak GR (2003). $P2X_7$ receptor-dependent blebbing and the activation of Rho-effector kinases, caspases, and IL-1 release. *J Immunol* **170**: 5728–5738.

Virginio C, MacKenzie A, Rassendren FA, North RA, Suprenant A (1999). Kinetics of cell lysis, dye uptake and permeability changes in cells expressing the rat $P2X_7$ receptor. *Nature Neurosci* **2**: 315–321.

Wiley JS, Dao-Ung LP, Gu BJ, Sluyter R, Shemon AN, Li C *et al*. (2002). A loss of function polymorphic mutation in the cytolytic $P2X_7$ receptor gene and chronic lymphocytic leukemia: a molecular study. *Lancet* **359**: 1114–1119.

Wiley JS, Dao-Ung LP, Li C, Shemon AN, Gu BJ, Smart ML *et al*. (2003). An Ile-568 to Asn polymorphism prevents normal trafficking and function of the human P2X7 receptor. *J Biol Chem* **278**: 17108–17113.

Wolf G, Yirmiya R, Goshen I, Iverfeldt K, Holmlund L, Takeda K *et al*. (2004). Impairment of interleukin-1 (IL-1) signaling reduces basal pain sensitivity in mice: genetic, pharmacological and developmental aspects. *Pain* **104**: 471–480.

Woolf CJ, Allchorne A, Safieh-Garabedian B, Poole S (1997). Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor alpha. *Br J Pharmacol* **121**: 417–424.

Zhang X-F, Han P, Faltynek CR, Jarvis MF, Shieh C-C (2005). Functional expression of $P2X_7$ receptors in non-neuronal cells of rat dorsal root ganglion. *Brain Res* **1052**: 63–70.