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REVIEW

P2X receptor-mediated ATP purinergic signaling in health and disease

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Abstract: Purinergic P2X receptors are plasma membrane proteins present in a wide range of mammalian cells where they act as a cellular sensor, enabling cells to detect and respond to extracellular adenosine triphosphate (ATP), an important signaling molecule. P2X receptors function as ligand-gated Ca^{2+}-permeable cationic channels that open upon ATP binding to elevate intracellular Ca^{2+} concentrations and cause membrane depolarization. In response to sustained activation, P2X receptors induce formation of a pore permeable to large molecules. P2X receptors also interact with distinct functional proteins and membrane lipids to form specialized signaling complexes. Studies have provided compelling evidence to show that such P2X receptor-mediated ATP-signaling mechanisms determine and regulate a growing number and diversity of important physiological processes, including neurotransmission, muscle contraction, and cytokine release. There is accumulating evidence to support strong causative relationships of altered receptor expression and function with chronic pain, inflammatory diseases, cancers, and other pathologies or diseases. Numerous high throughput screening drug discovery programs and preclinical studies have thus far demonstrated the proof of concepts that the P2X receptors are druggable targets and selective receptor antagonism is a promising therapeutics approach. This review will discuss the recent progress in understanding the mammalian P2X receptors with respect to the ATP-signaling mechanisms, physiological and pathophysiological roles, and development and preclinical studies of receptor antagonists.

Keywords: extracellular ATP, ion channel, large pore, signaling complex, chronic pain, inflammatory diseases

Introduction to the P2X receptors

It has become well established that adenosine triphosphate (ATP) released from healthy cells as well as from damaged or dying cells acts as an important extracellular signaling molecule.[1–5] The fast actions of extracellular ATP are largely mediated by the purinergic P2X receptors that are ligand-gated ion channels on the cell surface.

There are seven different mammalian genes encoding a family of P2X proteins or receptor subunits, namely, P2X1-P2X7, with the human P2X subunits being 377 (hP2X6) to 595 amino acid residues (hP2X7) in length.[6] They all have two transmembrane domains (TM1 and TM2) and a large extracellular cysteine-rich domain with short N-terminus and C-terminus of considerably variable length residing intracellularly. A various number of alternative splicing variants have been identified.[6–12] Studies of heterologously expressed P2X receptors suggest that all P2X subunits except the P2X6 can form homomeric receptors[6,13,14] and that two different subunits can assemble heteromeric receptors with P2X1/2, P2X1/4, P2X1/5, P2X2/3, P2X2/5,

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P2X2/6, and P2X4/6 being reported so far.[6,15] Such studies have also gathered considerable evidence to consistently support that the P2X receptor is a trimer and ATP binds to a novel site located at the subunit interface.[13,16–20] Such receptor stoichiometry and inter-subunit ATP-binding sites have been confirmed in the recently published crystal structures of the zebrafish P2X4 receptors with the intracellular N- and C-termini being truncated,[21,22] an exciting breakthrough in P2X receptor research in recent years.

The significant sequence homology to the zebrafish P2X4 receptor has allowed for the first time to generate by computational modeling the structures of the mammalian P2X receptors.[23–26] Figure 1 shows a structural model of the human P2X7 receptor. In an analogy, it adopts a chalice-like architecture, and each subunit bears a dolphin-like shape with the large extracellular domain and the TM helices akin to the body and the fluke, respectively. The P2X receptor ion channels are permeable to Ca^{2+}, Na^{+}, and K^{+}. As has been recently reviewed,[23,24] such structural information has greatly helped to build up a more insightful view on the ATP-binding site, ion-conducting pathway, and other structure–function relationships of the mammalian P2X receptors based on the results from the functional studies of mutant receptors over the past decade.

The P2X receptors, as a family of ligand-gated ion channels, in terms of their subunit membrane topology and receptor stoichiometry, differ from the other two classical ligand-gated ion channels, ie, the cysteine-loop receptors such as the nicotinic acetylcholine (nACh) and γ-aminobutyric acid (GABA) receptor ion channels, which are pentamers with four transmembrane domains in each subunit, and the glutamate receptor ion channels, which are tetramers with three transmembrane and one re-entrant loop domains in each subunit.[5] It was striking however to discover by structural determination that the P2X receptor ion channels and the acid-sensing ion channels, despite no sequence relatedness, have a virtually identical ion-conducting pathway as well as the same subunit membrane topology and receptor stoichiometry.[27]

This review article will discuss the recent progress in understanding the mammalian P2X receptors on the ATP signaling mechanisms, physiological and pathophysiological roles, and development and preclinical studies of receptor antagonists.

P2X receptor-mediated ATP signaling mechanisms

P2X receptor expression at the mRNA, protein, and/or functional levels has been documented in a vast variety of cells, including neurons and glial cells in the central (CNS) and peripheral (PNS) nervous systems, muscle cells, epithelial cells, endothelial cells, endocrine cells, bone cells, and immune cells, and such widespread distribution suggests a role for the P2X receptors in ATP signaling in a diversity of physiological processes.[1,3,6] The P2X receptors mediate ATP signaling mainly through three mechanisms: they function

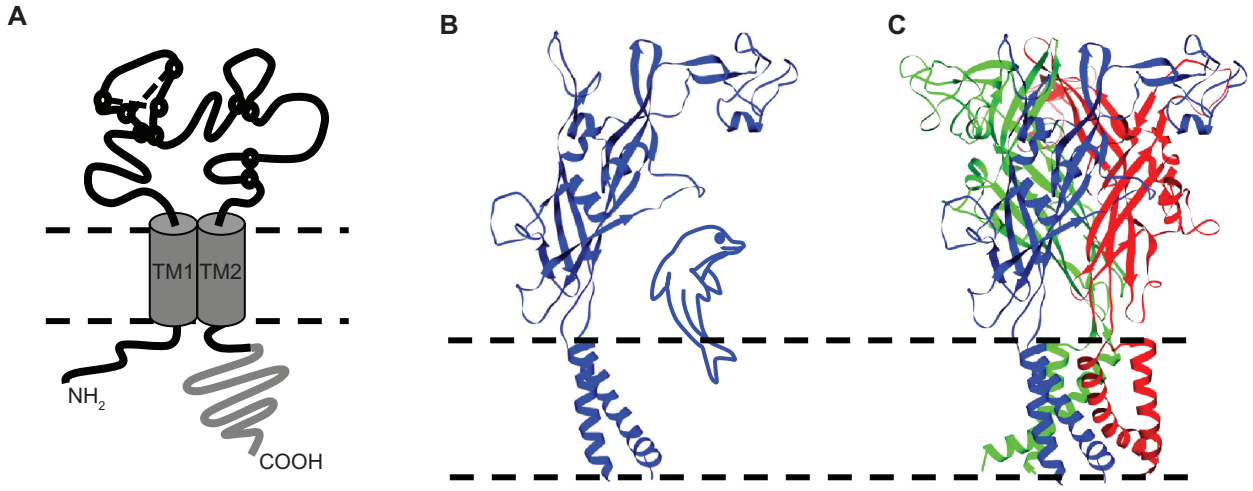

Figure 1 Structural features of P2X receptors. (**A**) A cartoon presentation showing that a mammalian P2X receptor subunit consists of two transmembrane domains (TM1 and TM2) linked by a large extracellular domain and intracellular N- and C-termini. There are 10 conserved cysteine residues denoted by open circles that form five pairs of intra-subunit disulfide bonds denoted by dotted lines. The P2X7 receptor subunit has an exceptionally long C-terminus compared with other P2X receptor subunits. (**B** and **C**) The human P2X7 receptor subunit (**B**) and receptor (**C**) generated by computational modeling using the crystal structure of the zebrafish P2X4 receptor in the closed state.

Notes: The architecture of the single subunit and the trimeric receptor is an analogy to the rising dolphin and the chalice, respectively. The three subunits are represented in different colors.

as ligand-gated Ca^{2+}-permeable cationic channels, induce formation of a large pore, and form signaling complexes with interacting proteins and membrane lipids. For simplicity, these mechanisms are discussed separately next, but the reader should bear in mind that such mechanisms are not mutually exclusive and, as a matter of fact, commonly operate in an interrelated manner.

Ligand-gated Ca^{2+}-permeable cationic channels

All mammalian P2X receptor ion channels are Ca^{2+}-permeable. As a whole, the P2X receptor ion channels mediate more Ca^{2+} influx than the glutamate receptor ion channels and the nACh receptor ion channels.[28] Conceivably, activation of the P2X receptors opens an important route for extracellular Ca^{2+} influx to elevate cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). The P2X receptor ion channels equally well permeate Na^+ and K^+. Na^+ influx and/or K^+ efflux can depolarize the plasma membrane potential, which in turn activates the voltage-gated Ca^{2+} channels (Ca_V) to enhance the Ca^{2+} influx. By contrast, P2X receptor-mediated membrane depolarization can reduce Ca^{2+} influx through the store-operated Ca^{2+} (SOC) channels that open following activation of ATP-sensitive G-protein-coupled P2Y receptors and subsequent depletion of the intracellular Ca^{2+} store.[29]

The above P2X receptor-mediated mechanisms and particularly the increases in the $[Ca^{2+}]_c$ represent the key and widespread ATP signaling mechanism in a diversity of physiological processes; for example, release of neurotransmitters such as glutamate, GABA, and ATP, and synaptic plasticity and neuron–glia communications,[30–34] smooth-muscle contraction,[35–37] flow-induced Ca^{2+} signaling in endothelial cells and flow-dependent control of vascular tone and remodeling,[38] cell proliferation,[7,39] and generation of vascular endothelial growth factors from monocytes[40] and ATP release from satellite glial cells.[41] K^+ efflux is also implicated in interleukin (IL)-1β release from immune cells.[42]

Large pore formation

While the P2X receptor small ion-permeable channels open upon brief stimulation with ATP (<1 second), sustained receptor activation can induce membrane permeabilization or formation of a large pore.[6] The P2X7 receptor, corresponding to the formerly named P2Z receptor in the immune cells,[43] is the prototypic ionotropic receptor with such an unusual functional property. The pore formation can be experimentally demonstrated by intracellular accumulation of fluorescent cationic dyes, such as YO-PRO1 (molecular weight [MW]: 375 Da) and ethidium (MW: 314 Da), or alternatively by progressive increase in the permeability to positively charged organic cations, such as N-methyl-D-glucamine (MW: 195 Da). To date, the pore-forming property has been also reported for the P2X2, P2X4, P2X2/3, and P2X2/5 receptors, although less frequently or consistently in some cases.[15,44–46]

Evidence exists to suggest that the P2X7 receptor-dependent pore is mediated by separate P2X7 receptor-interacting protein(s). In this aspect, the pannexin-1 channel has attracted substantial attention. Several studies have shown that the pannexin-1 channel inhibitor, carbenoxolone, and the mimetic peptide, ^{10}Panx, can strongly suppress the pore formation without affecting the small ion channel in HEK293 cells heterologously expressing the P2X7 receptors and in macrophages endogenously expressing the P2X7 receptors.[47,48] The P2X7 receptor-dependent dye uptake was virtually abolished in astrocytes from the pannexin-1 knockout (KO) mice.[49] Intriguingly, however, there was no significant change in the P2X7 receptor-dependent dye uptake in macrophages from the pannexin-1 KO mice.[50] The pannexin-1 channel, when expressed alone in HEK293 cells and activated in hypotonic solutions, permeated only the small ions but not the fluorescent dye and, in addition, it had no or very mild modulation of P2X7 receptor-dependent dye uptake when co-expressed with the mouse P2X7 receptors in HEK293 cells.[51] The pannex-1 channel has been shown to make no detectable contribution to the pore formation following activation of the P2X2 receptor heterologously expressed in HEK293 cells[52] and the P2X4 receptor endogenously expressed in microglia.[53] These results thus disfavor the idea that the pannexin-1 forms the pore. Mutation of the completely conserved glycine residue in the P2X2 (Gly^{342}), P2X4 (Gly^{347}), and P2X7 (Gly^{345}) receptors to residues with different side-chains and thus physiochemical properties increased, decreased, or completely abolished pore formation.[44,45,54] These remarkable and contrasting mutational effects on pore dynamics, together with location of such a residue within the TM2 helix and being extracellular to the gating hinge during channel opening,[22,55] support the notion that the pore is intrinsic to the P2X receptors.

Regardless of the underlying mechanisms, studies have shown that the P2X7 receptor-dependent pore formation is important in mediating ATP signaling in several physiological and pathophysiological functions, such as lymphocyte death,[56] microglial proliferation,[54,57] and chronic neuropathic and inflammatory pain.[48] The functional role of the pore associated with the other P2X receptors is still unclear.

Signaling complexes

There is also growing evidence to indicate that the P2X receptors interact with structurally and functionally diverse proteins and lipids to form ATP signaling complexes. For example, in addition to the pore formation, sustained activation of the P2X7 receptors induces dramatic changes in cell morphology such as microvesicle shedding and membrane blebbing.[58–61] While the microvesicle shedding was previously shown to mediate ATP-induced IL-1β release from immune cells,[59] formation of multivesicular bodies and exosome release of IL-1β is suggested as the underlying mechanism in a more recent study.[61] The irreversible membrane blebbing disrupts the membrane integrity, leading to cell death.[58,60] Furthermore, activation of the P2X7 receptors in microglia has been proposed to induce formation of a Ca^{2+}-independent signaling complex and changes of the microglial cytoskeleton to attenuate microglial phagocytosis.[62] A recent study has shown that a neuronal signaling complex containing the P2X7 receptors, pannexin-1 channels, and caspases is responsible for inflammation-induced enteric neuron death.[63] A number of P2X7 receptor-interacting proteins have been identified, and several such interacting proteins have been shown to impose significant functional modulation of the P2X7 receptors.[47,64–66] For example, upon prolonged activation, the P2X7 receptor became dephosphorylated at Tyr^{343} by receptor protein tyrosine phosphatase, resulting in reduction in P2X7 receptor currents and delay in membrane blebbing.[64] The Ca^{2+} sensor, calmodulin (CaM), interacts with the rat P2X7 receptor via a CaM-binding motif in the C-terminus to form a signaling complex in a receptor activation- and Ca^{2+}-dependent manner and thereby confer Ca^{2+}-dependent facilitation of the receptor activity and membrane blebbing.[66] The P2X7 receptor functional properties such as the agonist sensitivity and functional roles in inducing such as the pore formation and IL-1β release are strongly modulated by lipids.[67]

Other P2X receptors also form ATP signaling complexes. For example, the P2X1 receptors endogenously expressed in smooth muscles and heterologously expressed in HEK293 cells have been shown to associate with cholesterol- or flotillin-rich lipid rafts and cytoskeleton, and such associations are crucial in regulating the P2X1 receptor ion channel functions and its role in mediating arterial vasoconstriction.[68–70] The neuronal Ca^{2+} sensor, VILIP1, interacts with the P2X2 receptor to form a signaling complex in a receptor activation- and Ca^{2+}-dependent manner and thereby modulates the ATP sensitivity, surface expression, and mobility of the P2X2 receptor.[71] The P2X2, P2X2/3, and P2X4 receptors interact with nACh and $GABA_{A/C}$ receptors, resulting in a mutual functional inhibition,[72–77] which may be involved in modulating synaptic strengthening.[78] The P2X4 receptor function is subject to modulation by phosphoinositides through direct interactions.[79]

Physiological roles of P2X receptors

The pioneering studies in early 1990 provided the first evidence to show an important role for the P2X receptors in mediating excitatory synaptic transmission in the CNS and PNS.[80,81] Our understanding of the role of the P2X receptors in mediating ATP signaling was greatly enriched during the following decade as was elegantly reviewed previously.[6] However, the progress in dissecting contribution of the individual P2X receptors had been hampered by the lack of selective receptor antagonists, which remained largely unchanged until 2000 when the transgenic P2X gene KO mice became available.

Physiological roles of P2X receptors inferred from KO mice studies

P2X1, P2X2, P2X3, P2X4, and P2X7 single KO mice and P2X2 and P2X3 double KO mice have been generated. Studies using these transgenic KO mice have substantiated the long-suspected roles in ATP signaling for specific P2X receptors; for example, P2X1 receptor in mediating vas deferens contraction evoked by ATP and sympathetic nerve stimulation,[35] P2X3 and P2X2/3 receptors in sensory neurons in pain,[82,83] and P2X7 receptor in IL-1β release.[84] Moreover, studies using these KO mice have revealed more previously unrecognized roles, such as P2X3 and P2X2/3 receptors in mediating taste transduction,[85] P2X4 receptor in flow-induced changes in vascular tone and remodeling,[38] and P2X7 receptor in cathepsin release from macrophages.[86] Many of these studies have been reviewed recently.[3] Table 1 provides a summary of the physiological functions of the P2X receptors inferred from the KO mice studies.

Physiological roles of P2X7 receptors revealed by studies of splicing variants

A number of splicing variants have been recently identified for the human and mouse P2X7 receptors, and characterizations of them have shed light on additional physiological roles of the P2X7 receptors.[7–11] The human P2X7B variant is of particular interest because of its wide tissue distribution and several-fold higher mRNA expression than the P2X7A variant encoding the original and full-length receptor subunit. The P2X7B variant retains an intron between exons 10 and 11

Table 1 Physiological functions of P2X receptors inferred from knockout mice studies

Receptor	Physiological functions
P2X1	Vas deference contraction and male fertility;[35] renal microvascular auto-regulation;[169] platelet aggregation and thrombosis;[170] neutrophil chemotaxis[171]
P2X2	Enteric neurotransmission and peristalsis in small intestine;[172] modulation of excitatory synapses onto interneuron in hippocampus;[31] skeletal neuromuscular junction formation;[173] ATP secretion in taste buds;[174] vasopressin release at hypothalamic neurohypophysial terminals;[175] sperm function and male fertility[176]
P2X3, P2X2/3	Inflammatory and neuropathic pain;[82,83,96] mechanotransduction in urinary bladder;[82,83,177] enteric neurotransmission and peristalsis in small intestine;[178] chemoreception in carotid body;[179] temperature sensation;[180] taste transduction;[85] long-term depression[181]
P2X4	Long-term potentiation in hippocampus;[30,97] flow-induced endothelial Ca^{2+} signaling and control of vascular tone and remodeling;[38] BDNF release in microglia and neuropathic pain;[99] prostaglandin E2 release from tissue-residing macrophages and inflammatory pain[101]
P2X7	Interleukin-1β release from immune cells;[42,84,103,104] changes in volume and shape of monocytes and lymphocytes and L-selectin shedding;[42] macrophage and microglia death;[103,104] mechanic loading-induced prostaglandin E2 release and membrane blebbing in osteoblasts and osteogenesis, and nuclear factor-κB signaling in osetoclast formation;[182–185] nicotinamide adenine dinucleotide-induced T lymphocyte cell death;[186] inflammatory and neuropathic pain;[89,163] ATP release and astrocytic intercellular Ca^{2+} signaling;[187] regulation of natural killer T cell function in autoimmune liver injury;[188] regulation of exocrine gland secretion;[189,190] release of cathepsins from macrophages[86]

Abbreviations: ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor.

that introduces a premature stop codon and results in replacement of the last 249 residues in the C-terminus of the P2X7A subunit with different 18 residues. When heterologously expressed in HEK293 cells, the P2X7B receptor operated as a Ca^{2+}-permeable ion channel but lacked the pore-forming ability.[7] Expression of the P2X7B receptor in HEK293 cells increased the endoplasmic reticulum Ca^{2+} level, activation of transcription factor NFATc1 and intracellular ATP content, and potentiated HEK293 cell proliferation.[7] Furthermore, P2X7B and P2X7A subunit co-assembly enhanced agonist-induced Ca^{2+} influx, pore formation, membrane blebbing, and ATP release. These results suggest a role for the P2X7B variant in regulating cell proliferation and P2X7A-mediated functions.[7] However, the P2X7B-mediated cell proliferation appears to differ from the above-mentioned microglial proliferation, which is absolutely dependent of the pore-forming ability of the P2X7A receptor.[54]

The P2X7k is a mouse P2X7 splicing variant that results from alternative exon 1 and encodes a P2X7 subunit with the N-terminus and intracellular half of the TM1 helix different from the original and full-length P2X7a subunit.[8] When heterologously expressed in HEK293 cells, the P2X7k receptor exhibited a greater sensitivity to BzATP, an ATP analogue, than the P2X7a receptor and a significant sensitivity to nicotinamide adenine dinucleotide (NAD), and receptor activation induced much more rapid pore formation.[8,51] The P2X7k variant is mainly expressed in lymphocytes. These results have led to the proposal that the P2X7k receptor mediates NAD-induced phosphatidylserine flip and cell death that were observed in lymphocytes, but not in macrophages.[8,51,87,88] The P2X7k has escaped disruption in the Glaxo KO mice generated by inserting a LacZ gene and neomycin cassette (Neo) into exon 1,[89] which may also explain the paradoxical observation[90] that BzATP-induced phosphatidylserine exposure and pore formation were greater and faster in lymphocytes from the Glaxo KO mice, but not from the Pfizer KO mice generated by inserting a Neo cassette in exon 13.[42,84]

In summary, studies of the recently identified splicing variants reveal additional roles for the P2X7 receptors and also explain some of the unanticipated results from studies using the existing KO mice. Generation of the truly P2X7 KO mice is desirable to better understand the physiological significance of different variants and clarify the discrepancies; for example, regarding their role in IL-6 generation from macrophages[84,89] and the ongoing controversy surrounding the P2X7 receptor expression in CNS neurons.[91,92]

Pathophysiological roles of P2X receptors

Steady progress in understanding how alterations in the P2X receptor expression and function contribute to diseases has been made, largely from studies of rodent disease models in conjunction with using the KO mice or selective receptor antagonists, and also from genetic linkage analysis of single nucleotide polymorphisms (SNP) to human diseases. A brief discussion is presented below, focusing on the evidence that supports the roles for the P2X receptors in pain and for the P2X7 receptor in inflammatory diseases and cancers. Table 2 provides a summary of more pathophysiological or diseased conditions associated with the P2X receptors.

P2X receptors in pain

ATP was known to induce pain in humans long before molecular cloning of the receptors.[93] When tissues are injured and

Table 2 Pathological or diseased conditions associated with the P2X receptors

Receptor	Pathological or disease conditions
P2X1	Thromboembolism;[170] hypertension[191]
P2X3, P2X2/3	Inflammatory and neuropathic pain;[82,96,138] cystitis;[192] bone cancer pain[141]
P2X4	Neuropathic and inflammatory pain[98–101]
P2X7	Arthritis;[42] inflammatory and neuropathic pain;[89,146,150,163] tuberculosis;[104,193–195] hepatitis;[188] obstructive nephropathy;[196] pulmonary fibrosis;[105] Huntington's disease;[197] Alzheimer's disease;[198–202] glomerulonephritis;[203] spinal cord injury;[164] graft-versus-host disease;[204] morphine tolerance;[205] amyotrophic lateral sclerosis;[206] seizure;[207] irritable bowel syndrome;[208] inflammatory bowel diseases;[63] cystitis;[209] chronic lymphocytic leukemia;[106,108,115] mood disorders;[210–213] ischemic heart disease and stroke[214]

inflamed, ATP released from damaged or dying cells depolarizes the sensory neurons to elicit pain. In this context, since molecular identification, the P2X3 has attracted substantial attention because of its highly restricted expression in the sensory neurons.[94,95] Its role in pain has been subsequently substantiated in studies using P2X3 KO mice; P2X3 deficiency reduces pain behaviors, such as lifting, licking, and biting, evoked by injection of ATP, and inflammatory pain induced by injection of formalin.[82,96] P2X2 KO mice and P2X2 and P2X3 double KO mice also exhibit significant reduction in formalin-induced inflammatory pain.[83]

The P2X4 receptor is widely regarded as a neuronal P2X receptor involved in eg, synaptic plasticity.[97] However, an early study using the rat model of neuropathic pain induced by spinal nerve ligation (SNL) provided evidence to indicate that the P2X4 receptor is engaged in chronic neuropathic pain resulting from peripheral nerve injury. More surprisingly, it is the de novo or upregulated expression of the P2X4 receptor in activated spinal microglia but not neuron that mediates the tactile allodynia.[98] P2X4 KO mice are insensitive to SNL-induced neuropathic pain experienced by the wild-type littermates. In addition, brain-derived neurotrophic factor (BDNF) release in microglia is completely abolished and BDNF signaling in the spinal cord is impaired in P2X4 KO mice, suggesting that the microglial P2X4 receptors mediate neuropathic pain through a central inflammatory pathway dependent of BDNF signaling.[99] Studies have also provided evidence to support a role for the P2X4 receptor in chronic inflammatory pain. P2X4 KO mice show attenuation or complete loss of tactile allodynia in inflammatory pain induced by injection of inflammatory stimuli such as formalin, carrageenan, and complete Freund's adjuvant (CFA).[100,101] P2X4 deficiency prevents inflammatory stimuli-induced production of prostaglandin E2 (PGE2) from macrophages residing in the peripheral tissues.[101] PGE2 is the major prostaglandin produced during inflammatory responses that induces pain hypersensitivity by sensitizing and overexciting the nociceptive neurons.[102] In addition, neuropathic and inflammatory pain can be elicited in naive animals by injecting ATP-primed microglia and macrophages, respectively.[98,101] Thus, the P2X4 receptor contributes to chronic neuropathic and inflammatory pain by mediating different molecular and cellular mechanisms.

The P2X7 receptor is required for ATP-induced release of proinflammatory IL-1β from microglia and glial–neuron interaction in the brain as well as ATP-induced IL-1β release from macrophages and other immune cells in the peripheral tissues,[84,103,104] implying a crucial role for the P2X7 receptor in chronic neuropathic and inflammatory pain. Indeed, the P2X7 KO mice exhibit complete loss of tactile allodynia and thermal hyperalgesia induced by nerve injury and injection of CFA.[89] Interestingly, a recent genome-wide linkage study has shown that the mice expressing the P2X7 receptor deficient of inducing the pore formation were less sensitive to chronic neuropathic pain induced by nerve injury than the mice expressing the P2X7 receptor able to induce the pore formation.[48] Furthermore, this study has found that within a cohort of patients after mastectomy and a separate cohort of patients suffering from inflammatory disease osteoarthritis, individuals expressing the P2X7 receptors deficient of pore formation reported a lower amount of pain than those expressing the P2X7 receptors with the pore-forming ability.[48] These results suggest that the pore formation rather than the small ion channel opening is key to the role of the P2X7 receptor in determining the sensitivity to chronic neuropathic and inflammatory pain. Such a finding bears importance to rationalizing development of pain therapeutics targeting the P2X7 receptor and also raises questions such as whether the pore-forming ability is critical in chronic neuropathic and inflammatory pain mediated by the P2X2/3 and P2X4 receptors.

In summary, the P2X3, P2X2/3, P2X4 and P2X7 receptors play an important role in mediating ATP signaling in chronic neuropathic and inflammatory pain, pointing to these P2X receptors as promising targets for pain therapeutics.

P2X7 receptor in inflammatory diseases

Altered P2X7 receptor expression and function are causatively associated with numerous inflammatory diseases (Table 2). For example, an early study using the mouse arthritis model induced by monoclonal anti-collagen antibody showed that

the P2X7 KO mice manifested significant reduction in both incidence and severity of lipopolysaccharide (LPS)-induced swelling in the paws, lesions in joint cartilage, loss of proteoglycan content, and collagen degradation,[42] supporting a critical role for the P2X7 receptor in arthritis.

Pulmonary fibrosis is another devastating inflammatory disease currently without effective treatments. Bronchoalveolar lavage fluid from pulmonary fibrosis patients has been found in a recent study to contain a substantially higher level of ATP than that from control.[105] This study further using the bleomycin-induced lung fibrosis mouse model has shown that inhibition of the P2X7 receptor using antagonist A740003 or inhibition of the pannexin-1 channel using carbenoxolone and ^{10}Panx reduced bleomycin-induced ATP release from pulmonary epithelial cells.[105] The bleomycin-administrated P2X7 KO mice exhibited substantially reduced lung inflammation and lung fibrosis. These results suggest that the P2X7 receptor and pannexin-1 channel play an important role in mediating ATP signaling in the pathogenesis of lung fibrosis. Another recent study using the mouse experimental colitis model has revealed that the P2X7 receptor and pannexin-1 are critical components of an ATP-signaling complex that contribute to inflammation-induced loss of enteric neurons and progression of inflammatory bowel diseases.[63]

P2X7 receptors in cancers

Alterations in the expression and function of the P2X receptors and particularly the P2X7 receptor have also been documented by numerous studies of a variety of cancerous tissues and cells.[10,106–114] An early study investigating the human P2X7 receptor in lymphocytes from chronic lymphocytic leukemia (CLL) patients reveals a strong correlation of the P2X7 expression and function with the CLL severity.[107] In a separate group of CLL patients, there was a threefold greater prevalence of the non-synonymous SNP (ns-SNP) 1513A-C which introduces loss-of-functional E496A mutation in the C-terminus, and higher frequency of the 1513C allele.[108,115] Moreover, the P2X7 receptor-dependent dye uptake, detected in lymphocytes from the CLL patients expressing the 1513A/A alleles or the Glu^{496}-carrying P2X7 receptor, was reduced by approximately half in lymphocytes expressing the 1513A/C alleles or both the Glu^{496}- and Ala^{494}-carrying P2X7 receptor subunits, and completely lost in lymphocytes expressing the 1513C/C alleles or the Ala^{496}-carrying P2X7 receptor.[108] Survival for the CLL patients with the 1513A/A alleles was found to be significantly lower than those with the 1513A/C alleles.[115] Another interesting ns-SNP, 559A-G, was identified in human J6-1 leukemia cells, resulting in N187D mutation in the extracellular domain.[106] When heterologously expressed in K562 cells void of endogenous P2X7 expression, the P2X7 mutant receptor is three times less sensitive to ATP than the wild-type receptor. Expression of the mutant receptor accelerated K562 cell proliferation and decreased BzATP-induced apoptosis. Furthermore, the nude mice subcutaneously implanted with K562 cells expressing the N187D mutant receptor produced tumors significantly faster, and the size and weight of the tumors were larger and heavier. Angiogenesis and macrophage infiltration were also elevated in the tumor tissues formed by the cells expressing the mutant receptor. These results provide strong evidence to support a role for the P2X7 receptor with altered functional properties in determining the pathogenesis and progression of human hematopoietic malignancies such as CLL.

A recent study has shown that the P2X7 expression is highly expressed in the aggressive human breast cancer cells MDA-MB-435s, and ATP stimulates cell migration and particularly cell invasion.[116] Such ATP-induced effects were prevented by reducing the P2X7 expression using small interference RNA or inhibiting the P2X7 receptor using receptor selective antagonists KN62 and A740003. Furthermore, using a zebrafish metastases model, this study has elegantly demonstrated that the P2X7 receptor activity is crucial in determining the invasiveness of such breast cancer cells.

Therefore, accumulating evidence indicates the expression and function of the P2X7 receptors have strong causative relationships to cancer cell proliferation, migration, and invasion. The P2X7 receptor may represent a novel diagnostics biomarker and a promising therapeutic target for cancers.

Experimental P2X receptor antagonists and therapeutic potential

As mentioned above, the P2X receptor research was hindered by the lack of selective antagonists for a long time. Early efforts to search for selective receptor antagonists, largely focusing on structural modification of the existing antagonists, eg, suramin, to improve the specificity, and also detailed characterization of the antagonists implied by previous studies, such as KN-62 and Brilliant blue G (BBG), led to identification of several potent and selective P2X receptor antagonists, which have been proved as useful experimental tools aiding in-vitro and even in-vivo studies, as highlighted later. However, many of them have poor physiochemical properties that thus predict poor pharmacokinetics and limited therapeutic potential. The growing evidence

that supports the importance of the P2X receptors in health and disease has triggered interest in developing therapeutic P2X receptor antagonists. There have been a number of high-throughput screening (HTS) drug discovery programs driven by pharmaceutical companies, mainly focusing on the P2X3, P2X2/3, and P2X7 receptors. Such efforts have led to discovery of numerous novel potent and selective P2X receptor antagonists with drug-like properties. Many of these compounds have been shown to exhibit weak or no activity at a large number of G-protein-coupled receptors, enzymes, transporters, and other ion channels. Discussed below are the pharmacological properties of the well characterized P2X receptor antagonists and some recently developed drug-like P2X receptor antagonists, and example in-vitro and in-vivo studies and clinical trials of P2X receptor antagonists. Table 3 provides a summary of the pharmacological properties of the main P2X receptor antagonists that are commercially available. Figure 2 shows the chemical structures of representative drug-like P2X receptor antagonists that have been recently developed.

P2X receptor selective antagonists

NF023, NF279, and NF449 (Table 3) were developed by structural modification of suramin, a generic P2 receptor antagonist (Table 3), with significantly improved specificity towards the P2X receptors and particularly the P2X1 receptor. The antagonism is competitive. NF023 inhibits the P2X1 receptor with a half-maximal inhibitory concentration (IC_{50}) of 210–240 nM, and is less potent at the other P2X receptors with IC_{50} of 9–29 μM at the P2X3 receptor, 1.4 μM at the P2X2/3 receptor, >50 μM at the P2X2 receptor and >>100 μM at the P2X4 receptor.[117] NF279 antagonizes the P2X1 receptor, with an IC_{50} of 19–50 nM,[118,119] and far less potent at the P2X2, P2X3, P2X4, and P2X7 receptors, with IC_{50} of 0.8 μM, 1.6 μM, >300 μM, and 2.8 μM, respectively. NF449 is more potent than NF023 and N279 in inhibiting the P2X1 receptor, with an IC_{50} of 0.3 nM. It is equally potent at the P2X1/5 receptor, with an IC_{50} of 0.7 nM, whereas three or more orders of magnitude higher concentrations are required for inhibiting the other P2X receptors, with IC_{50} of 47 μM, 1.8 μM, 0.3 μM, and >300 μM at the P2X2, P2X3, P2X2/3, and P2X4 receptors, respectively.[120–122]

Diinosine pentaphosphate (IP5I) (Table 3) is a potent P2X1 receptor antagonist, with an IC_{50} of 3 nM, exhibiting surmountable antagonism at ≤100 nM and a more complex action at higher concentrations.[123] IP5I also blocks the P2X3 receptor but at much higher concentrations with an IC_{50} of 2.8 μM. It does not however inhibit the P2X2/3 receptor even at 30 μM;[124] such selectivity between the P2X3 and P2X2/3 receptors results from preferential binding to the desensitized state of the P2X3 receptor.[125]

TNP (2′,3′-O-(2,4,6-trinitrophenyl)-substituted)-ATP (Table 3) represents a highly potent antagonist for inhibiting the P2X1, P2X1/5, P2X3, and P2X2/3 receptors with IC_{50} of 0.4–7.0 nM.[126] It is a competitive antagonist when examined at the P2X2/3 receptor[127] or a slowly desensitizing P2X2-3 chimera receptor in which the N-terminus and TM1 helix of the P2X3 subunit is replaced with the corresponding parts of the P2X2 subunit.[128] TNP-ATP inhibits the P2X2, P2X4, and P2X7 receptors, but it is much less effective with IC_{50} of ≥2 μM.[126,129]

There are several P2X7 receptor antagonists, some of which display striking species differences. For example, the CaMK-II inhibitor KN-62 (Table 3) and its inactive analogue KN-04 are the first potent human P2X7 receptor antagonists.[130,131] KN-62 antagonizes agonist-induced Ca^{2+} influx and dye uptake with IC_{50} of ~40 nM and 100 nM in HEK293 cells expressing the human P2X7 receptor, but even at 1 μM it has no effect on the responses mediated by the rat P2X7 receptor. The inhibition is competitive. To prove the pharmacokinetic properties, several series of KN-62 derivatives have been tested,[132–134] leading to identification of several phenylpiperazine derivatives with approximately ten-fold greater potency. However, these studies also indicate the isoquinolinesulfonyl group to be essential for the potent antagonism, suggesting that identification of KN-62-related P2X7 receptor antagonists with desirable pharmacokinetics remains a challenging if not impossible mission.

BBG (Table 3) is a potent and selective P2X7 receptor antagonist.[12,135] It is particularly potent at the rat P2X7 receptor, with an IC_{50} of ~10 nM, >100-fold less effective at the rat P2X2 receptor, with an IC_{50} of 1.4 μM, and inactive at the rat P2X1, P2X1/5, P2X3, P2X2/3, and P2X4 receptors, with IC_{50} of >5 μM. BBG is also potent in inhibiting the human P2X7 receptor, with an IC_{50} of 270 nM, but it is less discriminative among the human P2X receptors as it blocks the human P2X5 receptor, with an IC_{50} of 500 nM. It is much less potent at the human P2X4 receptor, with an IC_{50} of 3.2 μM, and ineffective at the human P2X1 and P2X2/3 receptors, with IC_{50} of >5 μM. The antagonism of BBG at the P2X7 receptors is insurmountable.

Recently developed P2X receptor antagonists

RO-1 (or RO-0437626) is a selective P2X1 receptor antagonist (Table 3 and Figure 2).[136,137] It inhibits the human

Table 3 Pharmacological properties of commercially available P2X antagonists

Antagonist	Target receptor: IC_{50}	Further information or comments	References
Suramin	P2X1: 1 μM; P2X1/5: 1.6 μM; P2X2: 10 μM; P2X3: 3 μM; P2X5: 2.4–4.0 μM	P2X7: ~80 μM; P2X4: >500 μM; active at $P2Y_2$: 29 μM[b] and particularly $P2Y_{11}$	215–217
PPADS	P2X1: ≤1 μM; P2X1/5: 0.6 μM; P2X2: 2–5 μM; P2X2/3: ~5 μM; P2X3: 1.5 μM	P2X7: ~60 μM, 1.3 μM (r); P2X4: 10–27, ≥500 μM (r); active $P2Y_2$-like: 900 μM	215,217,218
NF023	P2X1: 210 (h), 240 nM (r)[b]	P2X2: >50 μM[b]; P2X2/3: 1.4 μM[b]; P2X3: 1.6–8.5 (r), 29 μM (h)[b]; P2X4: >>100 μM[b]	117
NF157	P2X1: 63 nM (h), 80 nM (r)[b]	P2X2: 138 nM[b]; P2X3: 800 nM (h)[b]; P2X4: >1 μM[b]; P2X7: >30 μM[b]; active at $P2Y_{11}$: 460 nM[b]	216
NF279	P2X1: 19–50 nM[b]	P2X2: 800 nM[b]; P2X3: 1.6 μM[b]; P2X4: >300 μM[b]; P2X7: 2.8 μM[b]	118,119
NF449	P2X1: 0.3 nM[b]	P2X1/5: 0.7 nM[b]; P2X2: 47 μM[b]; P2X2/3: 300 nM[b]; P2X3: 1.8 μM[b]; P2X4: >300 μM[b]; P2X7: 40 μM[b]	120–122,219
NF110	P2X1: 201 nM[b]; P2X2/3: 55 nM[b]; P2X3: 90 nM[b]	P2X2: 6.2 μM[b]; P2X4: >100 μM[b]; P2X7: >30 μM[b]	220
IP5I	P2X1: 3 nM[b]	P2X2: >>30 μM[b]; P2X2/3: >30 μM[b]; P2X3: 2.8 μM[b]; facilitation of P2X4 receptor responses	123,124
TNP-ATP	P2X1: 6 nM[b]; P2X1/5: 0.4 nM[b]; P2X2/3: 7 nM[b]; P2X3: 0.9 nM[b]	P2X2: 2 μM[b]; P2X4: 15 μM[b]; P2X7: >30 μM[b]	126,129
RO-1	P2X1: 3 μM[a]	P2X2, P2X2/3, P2X3: >100 μM[a]	136
A-317491	P2X3, P2X2/3: 15–170 nM[b] (K_i: 4–20 nM[b], 9–92 nM[a])	P2X1, P2X2, P2X4 and P2X7: K_i ≥ 2 μM[a]	138
RO-3	P2X3: 125 nM[a]	P2X2/3: 2 μM[a]	137
RO-51	P2X2/3: 5 nM[a]; P2X3: 2 nM[a]	P2X1, P2X2, P2X4, P2X5, P2X7: >10 μM[a]	140
Spinorpin	P2X3: 8.3 pM[b]	P2X1: >10 μM[b]; P2X7: >10 μM[d]	221
5-BDBD	P2X4: 500 nM[b]		222
KN-62	P2X7: ~40–130 nM (h)[b], 86 nM (rm)[b], 23–320 nM (h)[d], 130 nM (gp)[d], 10 nM (d)[d]	hP2X7 in lymphocytes: 13 nM[a], 13 nM[d]; rP2X7: >>1 μM[b], >>3 μM[d]; P2X4: >>3 μM[b]	130,131,218,223,224
BBG	P2X7: ~10 nM (r)[b], 270 nM (h)[b], 158 nM (r)[d], 324 nM (h)[d], 49nM (d)[d], 23 nM (gp)[d]	P2X5: 500 nM[b]; P2X1: >5 μM[b]; P2X1/5: >10 μM[b]; P2X2: >1.4 μM[b]; P2X2/3: 10 μM[b]; P2X3: >10 μM[b]; P2X4: >10 μM (r) and 3.2 μM (h)[b]	12,135,218,223
A-438079	P2X7: 126 nM (h)[a], 321 nM (r)[a], 493 nM (h)[b], 200 nM (h)[d], 297 nM (rm)[b]	P2X7 in THP-1 cells: 398 nM[e]; P2X1, P2X2, P2X2/3, P2X4: inactive at 100 μM[a]	146,224
A-839977	P2X7: 20 nM (h)[a], 42 nM (r)[a], 150 nM (m)[a]	P2X7 in THP-1 cells: 3 nM (h)[d]; 37 nM (h)[e]	147
A-740003	P2X7: 18 nM (h)[a], 40 nM (r)[a]	P2X7 in THP-1 cells: 92 nM (h)[d]; 156 nM (h)[e]; P2X1, P2X2, P2X2/3, P2X4: inactive at 100 μM[a]	148–150
A-804598	P2X7: 11 nM (h)[a], 10 nM (r)[a], 9 nM (m)[a]	P2X7 in THP-1 cells: 8.1 nM (h)[d]; 8.5 nM (h)[e]; P2X1, P2X2, P2X2/3, P2X4: inactive at 100 μM[a]	151
AZ11645373	P2X7: 31 nM[b] (or K_i = 5–20 nM[a,b,d]) (h), 23 nM (rm)[b]	P2X7 in THP-1 cells: 90 nM[e]; rP2X7: >10 μM[b]; P2X1, P2X2, P2X3, P2X2/3, P2X4, P2X5: inactive at 10 μM[b]	153,224
AZ10606120	P2X7: <10 nM (h)[d], >10–1000 nM (r)[d], 58 nM (gp)[d]		156,223,225

Notes: [a]IC_{50} or K_i based on measurements of agonist-induced Ca^{2+} ($/Ba^{2+}$) influx (or P2Y receptor-mediated intracellular release); [b]ionic currents; [c]K^+ efflux; [d]dye uptake; [e]interleukin-1β release.

Abbreviations: IC_{50}, half-maximal inhibitory concentration; K_i, equilibrium dissociation constant/binding affinity; h, human; rm, rhesus macaque monkey; d, dog; r, rat; m, mouse; gp, guinea pig.

P2X1-mediated Ca^{2+} influx, with a modest IC_{50} of 3 μM, and is >30-fold less potent at the human P2X2, P2X3, and P2X2/3 receptors, with IC_{50} of >100 μM.

A-317491 represents the first potent and selective drug-like P2X3 and P2X2/3 receptor antagonist (Table 3 and Figure 2).[128,138] It antagonizes agonist-induced Ca^{2+} influx with an equilibrium dissociation constant/binding affinity (K_i) of 9–92 nM, which is >200-fold more potent in comparison with K_i of ≥2 μM for inhibiting Ca^{2+} influx mediated by the P2X1, P2X2, P2X4, and P2X7 receptors. It also inhibits currents mediated by the P2X3 and P2X2/3 receptors, with IC_{50} of 15–170 nM or K_i of 4–20 nM.

RO-3, RO-4, RO-5, and RO51 are all selective P2X3 and P2X2/3 receptor antagonists.[137,139–141] RO-3 (Table 3 and Figure 2) inhibits the Ca^{2+} influx mediated by the human P2X3 and P2X2/3 receptors, with IC_{50} of 125 nM and

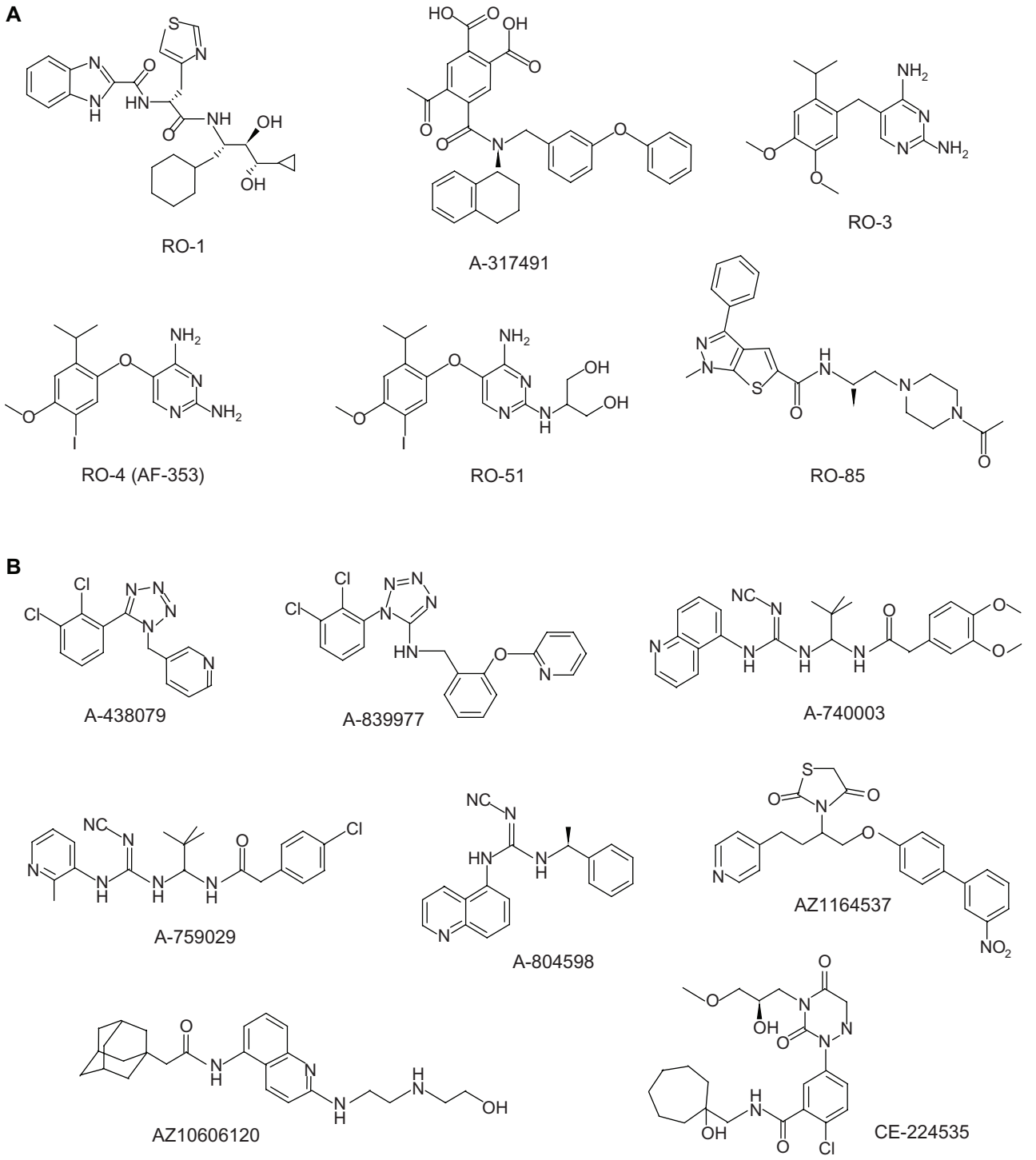

Figure 2 Chemical structures of recently-developed drug-like P2X receptor antagonists. (**A**) P2X2/3 and P2X3 selective antagonists, except for RO-1 and RO-85 that are P2X1 and P2X3 selective antagonists respectively. (**B**) P2X7 selective antagonists.

2 μM, respectively, and its actions on other P2X receptors have not been reported. RO-4 (Figure 2), recently renamed as AF-353, inhibits agonist-induced Ca^{2+} influx, with IC_{50} of 3–10 nM and 18–39 nM at the P2X3 and P2X2/3 receptors, respectively. The inhibition is insurmountable. RO-4 is inactive at the P2X1, P2X2, P2X4, P2X5, and P2X7 receptors, with IC_{50} of >10 μM. RO-5, recently renamed as AF-792, exhibits similar potency in inhibiting the Ca^{2+} influx mediated by the P2X3 and P2X2/3 receptors, with IC_{50} of 6 and 11 nM, respectively. It is inactive in inhibiting

Ca^{2+} influx mediated by the P2X1, P2X2, P2X4, P2X5, and P2X7 receptors, with IC_{50} of >10 μM. RO-51 (Table 3 and Figure 2) inhibits Ca^{2+} influx mediated by the rP2X3 and hP2X2/3 receptors, with IC_{50} of 2 and 5 nM, respectively, and inactive at the P2X1, P2X2, P2X4, P2X5, and P2X7 receptors at 10 μM.

RO-85 (Figure 2) is a selective P2X3 receptor antagonist inhibiting Ca^{2+} influx mediated by the rat and human P2X3 receptors, with IC_{50} of 32 and 400 nM, respectively.[142] It is ineffective at the P2X2/3 and other P2X receptors, with IC_{50} of >10 μM.

A series of tetrazole/triazole-based compounds have been reported as potent, selective, and competitive P2X7 receptor antagonists.[143–146] Two tetrazole compounds, A-438079 and A-839977 (Table 3 and Figure 2), have been extensively characterized.[146,147] A-438079 inhibits BzATP-induced Ca^{2+} influx and dye uptake in 132N1 cells expressing the human P2X7 receptor, with IC_{50} of 126 and 200 nM, respectively, and BzATP-induced IL-1β release in THP-1 cells, with an IC_{50} of 398 nM. It also potently blocks BzATP-induced Ca^{2+} influx in 132N1 cells expressing the rat P2X7 receptors, with an IC_{50} of 321 nM. However, it is inactive in inhibiting agonist-induced Ca^{2+} influx in 132N1 cells expressing the P2X1, P2X2, P2X2/3, and P2X4 receptors at 100 μM. Similarly, A-839977 is very potent in inhibiting BzATP-induced Ca^{2+} influx in 1321N1 cells expressing human, rat, or mouse P2X7 receptors, with IC_{50} of 20, 42, and 150 nM, respectively; the inhibition is competitive with pA2 of 8.1 (or 8 nM). A-839977 blocks BzATP-induced dye uptake and IL-1β release in THP-1 cells, with IC_{50} of 7 and 37 nM, respectively.

A series of cyanoguanidine derivatives have been discovered as potent and selective P2X7 receptor antagonists, with IC_{50} of ~100 nM.[148–151] A-740003, A-759029, and A-804598 represent three of them. A-740003 (Table 3 and Figure 2) inhibits BzATP-induced Ca^{2+} influx, with IC_{50} of 18 and 40 nM in 132N1 cells expressing the human and rat P2X7 receptors, respectively, and BzATP-induced dye uptake, with IC_{50} of 138 and 93 nM, respectively. It also prevents BzATP-induced dye uptake and IL-1β release in THP-1 cells, with IC_{50}s of 92 and 156 nM, respectively. A-740003 at 100 μM has little or no inhibition of agonist-induced Ca^{2+} influx in 132N1 cells expressing the P2X1, P2X2, P2X2/3, and P2X4 receptors. A-759029 (Figure 2) inhibits BzATP-induced Ca^{2+} influx in 132N1 cells expressing the human and rat P2X7 receptors, with IC_{50} of 32 and 40 nM, respectively, and it is inactive at the P2X3 and P2X4 receptors at 10 μM. A-804598 (Table 3 and Figure 2) is most potent among the three compounds. It inhibits BzATP-evoked Ca^{2+} influx in 1321N1 cells expressing the human, rat, and mouse P2X7 receptors, with IC_{50} of 11, 10, and 9 nM, respectively. The antagonism is competitive with pA2 of 7.7 (or 20 nM). It is also effective in suppressing BzATP-evoked dye uptake and IL-1β release in THP-1 cells, with IC_{50} of 8.1 and 8.5 nM, respectively. A-840598 at 100 μM is inactive in preventing agonist-evoked Ca^{2+} influx in 132N1 cells expressing the P2X1, P2X2, P2X2/3, and P2X4 receptors.

A series of cyclic imides have been shown as potent, selective, and competitive human P2X7 antagonists inhibiting BzATP-induced dye uptake, with pA_2 of 6.2–7.7 (or 20–630 nM).[152] One of them, AZ11645373 has been characterized in detail.[153] AZ11645373 inhibits human P2X7 receptor-mediated currents, Ca^{2+} influx, and dye uptake in HEK293 cells, with an IC_{50}/K_i of 5–20 nM depending on the assays, and ATP-induced release of IL-1β from THP-1 cells, with an IC_{50} of 90 nM. The antagonism becomes largely insurmountable at relatively high concentrations. However, it poorly blocks rat P2X7 receptor-mediated currents, with an IC_{50} of >10 μM, and has no significant effect on agonist-induced currents mediated by the P2X1, P2X2, P2X3, P2X2/3, P2X4, and P2X5 receptors expressed in HEK293 cells at 10 μM.

A series of adamantane amides has been also identified as potent, selective, and competitive human P2X7 antagonists.[154,155] One of the most potent compounds inhibits human P2X7 receptor-dependent dye uptake, with pA_2 of 8.8 (or 1.6 nM), but it is ineffective for blocking rat P2X7 receptor-dependent dye uptake, with pA_2 of <5 (or >10 μM). Further efforts to search for human P2X7 receptor antagonists with better pharmacokinetics and also rodent P2X7 receptor antagonists have led to identification of a compound that inhibits BzATP-induced dye uptake in HEK293 cells expressing the human P2X7 receptor, and IL-1β release from THP-1 cells, with IC_{50} of 10 and 80 nM, respectively. However, it is still less effective in blocking BzATP-induced dye uptake in HEK293 cells expressing the rat P2X7 receptor, with an IC_{50} of 400 nM. This compound is inactive at the P2X1, P2X2, P2X2/3, P2X3, P2X4, and P2X5 at 10 μM. AZ-10606120 (Table 3 and Figure 2), also known as compound-17, is an adamantane amide.[67,156] It is a potent and noncompetitive P2X7 receptor antagonist blocking human P2X7 receptor-dependent dye uptake in HEK293 cells, with an IC_{50} of <10 nM, and rat P2X7 receptor-dependent dye uptake with reduced potency, with an IC_{50} of >10–1000 nM.

A series of 6-azauracil derivatives has been described as potent and selective human P2X7 receptor antagonists based on the inhibition of BzATP-induced IL-1β release in THP-1 cells.[157] One of them, CE-224535 (Figure 2), displays good pharmacokinetic and safety profiles in rat, dog, and monkey, and excellent potency in inhibiting ATP-induced dye uptake and IL-1β release in THP-1 cells, with IC_{50} of 4 and 1 nM, respectively. However, it is inactive in inhibiting ATP-induced IL-1β release in murine macrophages.

In summary, a number of potent and selective drug-like P2X receptor antagonists and particularly those inhibiting the P2X3, P2X2/3, and P2X7 receptors have been developed.

Preclinical studies using rodent disease models

A majority of newly published drug-like P2X receptor antagonists, with the exception of a few, suffer one way or another from unsatisfactory pharmacokinetic properties. Nevertheless, preclinical studies using rodent disease models have demonstrated excellent efficacy of several P2X receptor antagonists. For example, the P2X3 and P2X2/3 receptor antagonist A-317491 is tri-acidic in nature and is predicted to have poor oral bioavailability and high plasma protein binding.[158] Administration of A-317491 induced concentration-dependent suppression of neuropathic and inflammatory pain in rats.[138,159] Other P2X3 and P2X2/3 receptor antagonists AF-353 (RO-4) and AF-792 (RO-5) have significantly better pharmacokinetic properties including CNS penetration.[140–142,160] Systemic administration of AF-353 has been shown to strongly reduce bone cancer pain in rats.[141] A recent study using AF-353 has shown a role for the P2X3 and P2X2/3 receptors in bronchoconstriction-induced activation of vagal nodose C-fibers in the lung that may relate to airway allergic responses.[161] Another study using AF-792 has revealed presynaptic P2X3 and P2X2/3 receptors in the spinal cord as a central purinergic mechanism regulating the visceral activities, suggesting that spinal P2X3 and P2X2/3 receptors are therapeutic targets for treatment of overactive bladder.[162]

Studies have shown that administration of the P2X7 receptor antagonists A-740003[150] and A-438079[146] in rats suppresses chronic neuropathic pain induced by nerve injury and chemotherapy, and inflammatory pain induced by injection of formalin, carrageenan, or CFA. Concomitant injection of A-438079 in the lumbar spinal cord of rats strongly reduced LPS-induced tactile hyperalgesia associated with microglial activation and IL-1β release in the dorsal horn.[163] These studies support a critical role for the P2X7 receptor in mediating glia-neuron interactions in determining the sensitivity to chronic pain, and suggest that CNS-penetratable P2X7 receptor antagonists targeting microglial release of IL-1β in pain-enhanced response states is beneficial in alleviating chronic pain.

BBG as a potent and selective rat P2X7 receptor antagonist has proved useful in revealing previously unrecognized roles in diseases. For example, traumatic spinal cord injury (SCI) is characterized by an acute loss of tissue at the lesion site followed by secondary damage of peri-traumatic tissue due to inflammatory responses induced by persistent release of excessive ATP. Studies using the rat model of thoracic SCI have shown that systemic administration of BBG after SCI significantly prevented inflammation in the tissues surrounding the lesion, prevented injury severity, and accelerated locomotor function recovery,[164] suggesting that inactivation of the secondary injury mechanisms by targeting the P2X7 receptor may offer a therapeutic opportunity to improve the recovery from SCI.

Clinical trials

To date, a small number of P2X receptor antagonists have been tested in clinical trials.[165] Afferent Pharmaceuticals is conducting Phase 2 clinical studies to assess AF-219, a P2X3 receptor antagonist, for treatment of osteoarthritic knee pain (ClinicalTrials.gov identifier: NCT01554579), interstitial cystitis/bladder pain (NCT01569438) and chronic cough (NCT01432730). AstraZeneca has recently completed their study of a Phase 2 clinical trial of AZD9056, a P2X7 receptor antagonist, for treatment of rheumatoid arthritis (NCT00520572); the outcome is rather disappointing as this compound failed to bring about significant therapeutic benefits.[166] AZD9056 has also been in clinical trials for treatment of osteoarthritis, chronic obstructive pulmonary disease, and inflammatory bowel disease. Pfizer terminated their clinical trial of CE-224535, a P2X7 receptor antagonist as mentioned above, against osteoarthritic knee pain because of the lack of efficacy (NCT00418782), and has recently concluded their Phase 2 clinical study of this compound for treatment of rheumatoid arthritis in patients with an inadequate response to methotrexate (NCT00628095), and the data indicate that CE-224535 was not efficacious.[167] Clearly, further more investigations are needed to understand the contribution of P2X7 receptors to the rather complex nature of the disease.

Conclusion and future perspective

Rapid progress has been made in understanding the mammalian P2X receptors in mediating ATP signaling at the

molecular, cellular and systems level. However, there is still more to be learnt in the coming years. The determination of the crystal structures of the zebrafish P2X4 receptors in the closed and open states will revolutionize the approaches to studying the mammalian P2X receptors in the post-structural era. The structure for the mammalian P2X receptors is highly desirable. Research efforts are still required to elucidate the molecular or structural basis that determines, for example, the remarkable plasticity in the membrane permeability following activation of the P2X receptors. The importance of a better understanding of the structure–function relationships and the P2X receptor-mediated ATP signaling mechanisms is nicely highlighted by the interesting finding that the pore formation but not the small ion channel is key to the role of the P2X7 receptors in determining chronic pain sensitivity.[48] The identity of the native P2X receptors largely remains speculative. The P2X transgenic KO mice and also the increasing availability of potent and selective P2X receptor antagonists will surely continue to help to define the native P2X receptors and understand their roles in physiology and pathophysiology. The P2X KO mice studies have provided compelling evidence to support important roles for the P2X3, P2X2/3, P2X4, and P2X7 receptors in chronic pain and for the P2X7 receptors in rheumatoid arthritis, inflammatory bowel disease, and lung fibrosis, which represent the areas full of considerable unmet clinical needs. The failure in a recent extensive attempt to validate the finding that pharmacological blockage of the P2X7 receptors can improve locomotor function and recovery from SCI[168] clearly indicates more preclinical investigations are required. The drug discovery programs and preclinical studies so far have provided strong proof of concept that the P2X receptors are druggable targets but therapeutic drugs have not yet been successfully generated. The increased structural information will rationalize the drug discovery strategies, including virtual HTS of chemical libraries and de nova design of drug-like compounds, which is expected to increase the efficiency and accelerate the pace of identifying potent and selective human P2X receptor antagonists for therapeutics. A better understanding of the P2X receptor downstream signaling may offer further opportunity for identification of specific intervention targets and development of effective therapeutics.

In conclusion, the past decade, particularly the past few years, has witnessed a remarkable progression in gaining a mechanistic understanding of the P2X receptor-mediated ATP signaling in health and disease. Let us hope that the accumulating knowledge from the research bench will bring benefits to the patients on the bedside in the near future.

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