12

P2X Receptors as New Therapeutic Targets

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1. Introduction

P2X receptors are membrane non-selective cation channels that gated in the presence of extracellular adenosine triphosphate (ATP) and related di- and tri-phosphate nucleotides, that more commonly known for providing cells with energy. Binding of ATP to the excellular pocket of P2X triggers the opening of transmembrane pore, which allows sodium, magnesium, potassium, calcium and other organic ions to flow down their electrochemical gradients. Because seven P2X receptor subtypes ($P2X_{1-7}$) are widely distributed in excitable and nonexcitable cells of vertebrates, P2X receptors mediate many physiology processes including synaptic transmission and thrombocyte aggregation. These ion channels are also involved in the pathology of several disease states, playing key roles in inter alia afferent signaling (including neuropathic pain), regulation of renal blood flow, vascular endothelium, and chronic inflammation, and thus are potential targets for drug development. The recent discovery of potent and highly selective antagonists for P2X receptors, through the use of high-throughput screening, have helped to further understand the P2X receptors pharmacology and provided new evidence that P2X receptors play specific roles, such as in chronic pain states. In this review, we place previous work of P2X in the context of three-dimensional (3D) crystal structure of Zebrafish $P2X_{4.1}$ ($\Delta zfP2X_{4.1}$), discuss how the P2X family of ion channels have distinguished themselves as potential new drug targets, and try to differentiate between drugs which are useful research tools, helpful in understanding the physiological roles of these receptors. We also summarize the key questions and challenges, which await researchers' further work as we move forward to the new drug development era of P2X receptors. We are optimistic that safe and effective candidate drugs will be suitable for progression into clinical development.

2. The P2X receptor protein family

2.1 The gene of P2X receptors

Seven P2X receptor subtypes in mammals are different in genes. Their chromosomal locations, amino acid length and mass are summarized (Table 1). $P2X_2$, $P2X_3$ and $P2X_6$ receptor genes are all on different chromosomes. $P2X_1$ and $P2X_5$ subunit genes are located close on the short arm of chromosome 13 (Table 1). $P2X_4$ and $P2X_7$ subunit genes are also both located on the long arm of chromosome 12 (North 2002). From the alignment of amino acid sequences, $P2X_4$ and $P2X_7$ are the most related pairs (North 2002). The full-length of seven P2X receptors have 11-13 exons, and they all share a common structure, with well-

conserved amino acids in the outer loop and transmembrane regions. These seven genes are considerable different in size.

Subunit	Chromosome	Length (AA)	Mass (Da)	Accession Nos.	Reference Nos.
$P2X_1$	17p13.2	399	44,980	P51575	(Longhurst PA 1996, Soledad Valera 1994)
$P2X_2$		471	51,754	Q9UBL9	(Kevin J. Lynch 1999, Lynch et al. 1999)
$P2X_3$	11q12	397	44,289	P56373	(Garcia-Guzman et al. 1997b)
$P2X_4$	12q24.31	388	43,369	Q99571	(Garcia-Guzman et al. 1997a)
$P2X_5$	17p13.3	422	47,205	Q93086	(Le et al. 1997)
$P2X_6$	22q11	441	48,829	O15547	(Urano et al. 1997)
$P2X_7$	12q24.31	595	68,586	Q99572	(Franc¸ois Rassendren 1997)

Table 1. Properties of human P2X receptors

Chromosomal localizations, amino acid length, and Mass are from Protein Knowledge base of UniProtKB (http://www.uniprot.org/). The accession numbers and references are indication for the original submission of cDNA sequences.

2.2 Amino acid sequence and structure

In the mid-1990s, the first cDNA cloning of P2X receptor led to the deduction of P2X receptors' structure and physiology. In 2009, Kawate and colleagues reported the crystal structure of a truncated mutation of the Zebrafish $P2X_{4.1}$ receptor ($\Delta zfP2X_{4.1}$) (Toshimitsu Kawate 2009), which is the first 3D crystal structure of P2X receptors. It represents a step change in our understanding of these membrane ion channels, where previously only low-resolution structure data and inferences from indirect structure-function studies were available. Due to these indirect and direct biochemical and pharmacological data, approximate structure and topology of seven distinct $P2X_1$–$P2X_7$ subtypes can be elucidated. Three individual subunits assemble to form functional homomeric (except $P2X_6$) or heteromeric receptors (except $P2X_7$). These subunit peptides are difference in size ranging from 388 ($hP2X_4$) to 595 ($hP2X_7$) amino acids long. The protein of these seven subtypes are pairwise identical (from 40% to 55%) (North 2002). All functional P2X receptors have the following topology: intracellular N-terminus/first transmembrane segment (TM1)/extracellular segment/second transmembrane segment (TM2)/intracellular C-terminus.

2.3 TM domains and the pore region

Every P2X protein has two hydrophobic transmembrane regions, which are sufficient long to cross the plasma membrane (Brake et al. 1994, North 1996, Valera et al. 1994). The presences of only two transmembrane segments distinguish P2X receptors from other ligand-gated cation channels. The first transmembrane segment (TM1) extends from residue 30 to 53, and the second transmembrane segment (TM2) from 328 to 353 (in the rat $P2X_2$ receptor) (Zhiyuan Li 2004). These two regions are both α-helix, form the central ion conduction pore and participate in the conformational changes during receptor activation. Three pairs of TM1 and TM2 domains are highly tilted to the cell membrane giving the pore

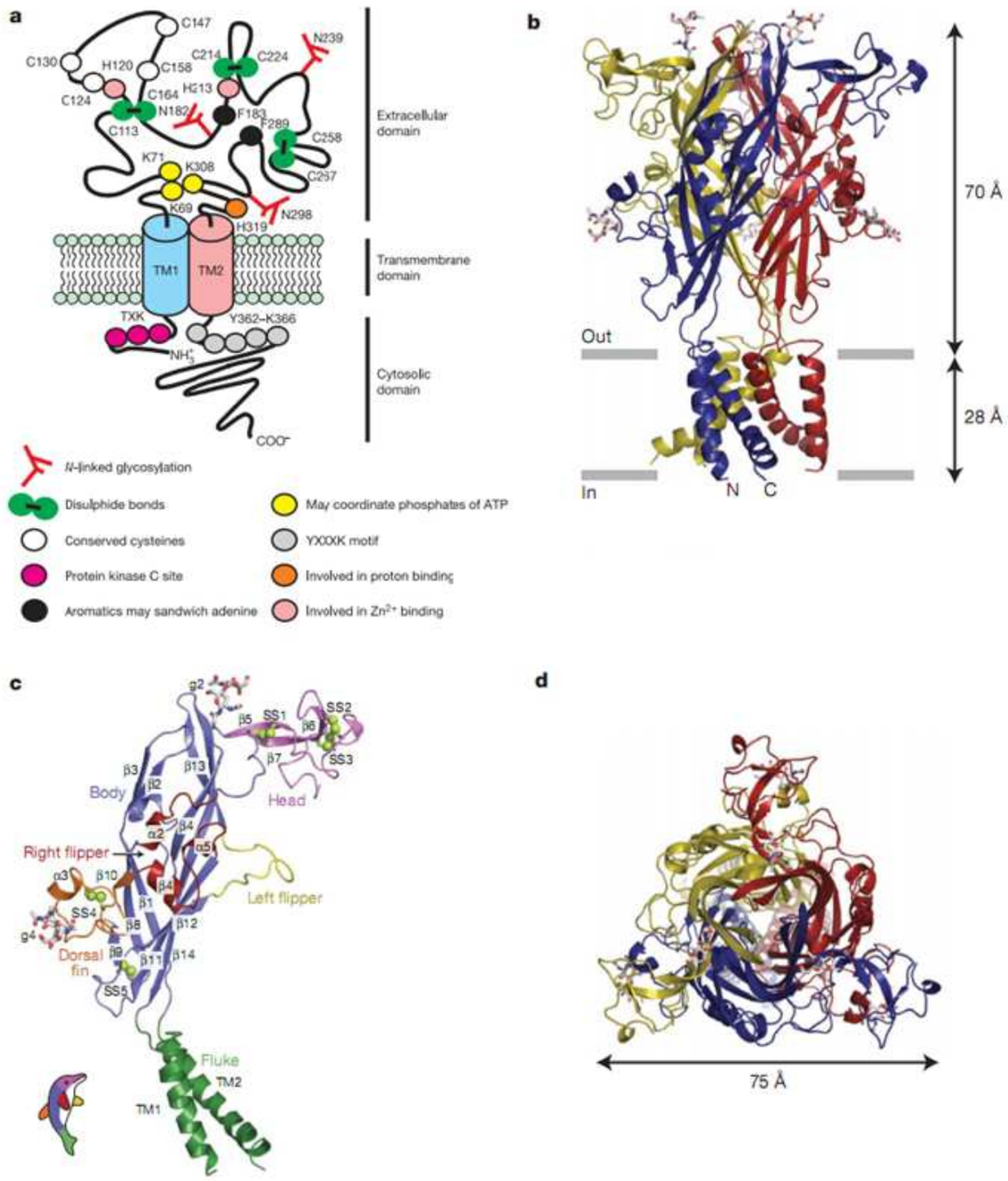

Fig. 2. Structure of P2X receptors. (a) Topology features of P2X receptors which based on the structure-function studies on $P2X_1$ and $P2X_2$ receptors. The numbers refers to rat $P2X_2$ receptors. (b) Stereoview of the homotrimeric $\Delta zfP2X_4$ structure. Three subunits are depicted in red, yellow and blue, respectively. NAG and glycosylated asparagines residues are represented in stick. This is a closed resting conformation. (c) Top view of the homotrimeric $\Delta zfP2X_4$ structure from extracellular side of the membrane. Fig. a is reproduced from reference (North 2006). Fig. b-d are reproduced from reference (Toshimitsu Kawate 2009).

an hourglass appearance (Toshimitsu Kawate 2009). The previous mutagenesis, functional work and 3D crystal structure give strong evidence that the TM2 domain plays major role in ion transduction and pore opening. TM2 domain is directly involved in the assembly of subunits into oligomeric complexes and is partly responsible for determining the rate and degree of desensitization (Evans 2010), interacts with the permeating ions and regulates specific properties of ion flow including conductance (Nakazawa et al. 1998), permeability (Migita et al. 2001), and Ca^{2+} flux (Egan et al. 2004) among P2X receptors. The diameter of the narrowest part of the open pore is thought to be about 8-20 Å (Toshimitsu Kawate 2009), which is from Thr 336 to Phe 346 (the number refers to rat $P2X_2$) (Mufeng Li 2008). Although the present data suggest that the TM1 domain involved in transducing agonist binding into channel gating, an explicit role for the TM1 has not been identified. The COOH- and NH_2-terminal regions are both in the cytoplasm.

2.4 Terminal regions

The N-terminus region is relatively short (about 25 amino acids) and contains a consensus site for protein kinase C-mediated phosphorylation; removal of this site leads to an accelerated fade in the amplitude of the ATP-gated current of the $P2X_2$ receptor. The C-terminus is longer (about 25–250 amino acids) and diverges in sequence considerably between seven subtypes and contains multiple sites that mediate subtype-specific effects. A YXXXK motif at the end of the TM2 is highly conserved among all P2X receptors, the Tyr and Lys of which have been proved to stabilize the P2X subunit in the plasma membrane, which was reported by Rassendren and his colleagues (Chaumont et al. 2004).

2.5 Extracellular domains

The extracellular loop also contains consensus sequences for the N-linked glycosylation involved in targeting the receptor to the cell surface membrane, for agonist and antagonist binding sites, and for intersubunit binding sites responsible for allosteric modulation of the ATP response by hydrogen ions and zinc.

The two transmembrane segments are connected by a long string of amino acids that form the extracellular of the receptor. This string contains ten conserved cysteines, which participate in disulfide bonds that help define the tertiary structure of the protein. From the N-terminus to the C-terminus, the cysteine pairs are 1-6 (Cys119 – Cys168), 2-4 (Cys129 – Cys152), 3-5 (Cys135 – Cys162), 7-8 (Cys220 – Cys230) and 9-10 (Cys264 – Cys273). These ten cysteine residues are very conserved in all mammalian P2X receptors (Clyne et al. 2002, Ennion et al. 2002). Two pairs of disulfide bonds which are pair 2-4 and 3-5, may exchange with each other during and after biosynthesis, because of the very close space.

Based upon mutagenesis data and binding studies, eight highly conserved residues have been proved to be involved in ATP binding. In rat $P2X_2$ these residues are Lys74, Lys76, Phe188, Thr189, Asn293, Phe294, Arg295, and Lys313 (Jiang et al. 2000, Marquez-Klaka et al. 2007, Roberts et al. 2004, 2006). In the 3D crystal structure of $\Delta zfP2X_{4.1}$, these residues are lining a cavity which is surrounded by the head domain, and left flipper of one subunit, and dorsal fin of another (Toshimitsu Kawate 2009). The exact nature of these residues is not very clear so far. How do they interact with each other is unknown. Jiang and Roberts reported that the negatively charged triphosphate moiety may interact with positively charged residues Lys74, Lys76 and Lys313, whereas Phe188, Thr189, Asn293, and Phe294 might be interacted with the adenine ring and ribose moiety (Roberts et al. 2006).

All seven P2X receptor subtypes have consensus sequences for N-linked glycosylation (Asn-X-Ser/Thr) some of which is essential for protein to traffic to the cell surface. In rat $P2X_2$, Asp182, Asp239 and Asp298 all can be glycosylated in oocytes and HEK293 cell (Newbolt et al. 1998). If these glycosylation sites are removed or prevented by tunicamycin or mutagenesis, the full function of P2X receptors will be disturbed. Receptors give no response to ATP, if only one site is glycosylated. But if two of three sites are glycosylated, the cell can express full function receptors (North 2002). All P2X receptors have N-linked glycosylation, which are well conserved among species but different between receptor subtypes (North 2009., North 2002).

3. Physiological role of P2X receptor

Seven P2X receptor subunits ($P2X_{1-7}$) are widely expressed in tissues throughout body; for example, they are found in excitable and nonexcitable cells, such as neurons, myocytes, leukocytes and epithelial cells. And thus they play key roles in inter alia afferent signaling (including pain), regulation of renal blood flow, vascular endothelium, and inflammatory responses. Most P2X receptors are nonselective cation channels that discriminate poorly among monovalent cations, while exhibiting a stronger preference for calcium ions. The exception is $P2X_5$ receptor, which in some species such as human and chicken exhibits a modest permeability for chloride ions. The resulting rise in intracellular calcium evokes transmitter release from central and peripheral neurons and glia, promotes hormone release from endocrine glands, triggers contraction of muscle, regulates airway ciliary motility, and activates downstream signaling cascades in various of cells.

3.1 Central nervous system

ATP is a fast neurotransmitter, it can be released from the excitable and nonexcitable cells in normal physiological and pathophysiological conditions. As one member of the purinergic receptor family, P2X receptors are widely expressed on central nervous system at different mRNA and protein levels, for example, $P2X_2$, $P2X_4$ and $P2X_6$ are most abundant in neurons (North 2006). They play an important role in the regulation of neuronal and glial functions, which is participating in the synaptic process and mediating communications among them. In the central synapses, the significant function of P2X may be related to depolarizing neurons and considerable calcium permeability plays the key role (Pankratov et al. 2002), no matter it is at resting membrane potential or not.

3.1.1 Postsynaptic and presynaptic

Using post embedding immunocytochemistry, Rubio and his colleagues for the first time qualitatively and quantitatively described the precise location of $P2X_2$, $P2X_4$ and $P2X_6$ subunits in postsynaptic on the hippocampal CA1 pyramidal and cerebellar Purkinje cells (Rubio et al. 2001). Bouts of action potential firing are activated the synaptic P2X receptors. The function and densities of P2X receptors in the postsynaptic might be related to some specific proteins. Using the glutathione S-transferase pull-down experiment, it is proved that β-amyloid precursor protein-binding proteins Fe65 colocalizes and interacts with $P2X_2$ receptor in postsynaptic of excitatory synapses (Masin et al. 2006). Direct postsynaptic effects on neurons have been reported.

Khakh and Gu have reported that P2X receptors mediate presynaptic responses. Calcium ions play a key role in this responses, which are triggered and enter through the P2X

receptors or through Ca^{2+} channels (North 2006). Presynaptic P2X receptors may be active physiologically in some synaptics (Gu et al. 1997). Presynaptic actions form one component of ATP effects in hippocampus. A feed forward circuit forms among the CA3 pyramidal neurons, GABAnergic interneurons and output CA1 pyramidal neurons. The increased release of glutamate from presynaptic $P2X_2$ receptors works on the interneurons, which are depolarized by $P2Y_1$ receptors. The depolarization of interneurons is the concomitant reduction and activation of potassium and nonselective cationic conductance, respectively (Bowser et al. 2004, North 2006). ATP plays a special role in this feed-forward circuit, it might act as a physiological brake to runaway excitation.

3.1.2 Disorder

Because the ATP plays key roles in neurotransmission and neuromodulation, purine and pyrimidine receptor subfamilies have been involved in various pathological conditions. This pathophysiology of CNS disorders include brain trauma, ischaemia, neurodegenerative diseases and neuropsychiatric diseases. When injury happens, large amounts of ATP release into extracellular environment which are important for triggering cellular responses to trauma. The expression level of $P2X_4$ and $P2X_7$ has changed, which might stimulate the migration and chemotaxis of resting microglia to the site of damage (Ohsawa et al. 2007, Xiang et al. 2006), and $P2X_7$ have an important role in controlling microglia proliferation and death (Bianco et al. 2006, Franke et al. 2006). Cerebella lesions result in up-regulation of $P2X_1$ and $P2X_2$ receptors in precerebellar nuclei, and stab wound injury in the nucleus accumbens leads to increased expression of several subtypes of P2X and P2Y receptors.

Cerebral ischaemia can produce and exacerbate problems to the CNS, which include stroke and paralysis. This disease can increase the release of purines into cerebral cortical perdusates (Braun et al. 1998). In vitro studies of organotypic cultures from hippocampus, F. Cavaliere and their colleague found out that $P2X_2$ and $P2X_4$ were up-regulated by glucose/oxygen deprivation (Cavaliere et al. 2003), and this can be prevented by P2 receptor antagonists. On the other side, $P2X_4$ and $P2X_7$ receptors, which are on microglia, and may be involved in cortical damage, also produced by glucose/oxygen deprivation (Cavaliere et al. 2005).

Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS) are all belong to neurodegenerative diseases. $P2X_7$ receptor is related to Parkinson's disease and Alzheimer's disease, but the function of $P2X_7$ in the pathogenesis of the Parkinson's disease is not very clear. However, the animal model and patients with Alzheimer's disease showed that $P2X_7$ receptor is up regulated in the brain (McLarnon et al. 2006, Parvathenani et al. 2003). $P2X_7$ in microphages and microglia might enhance the degenerative (Rampe et al. 2004). Changes in P2X receptor-mediated neurotransmission in cortico-striatal projections have been found in two different transgenic models of Huntington's disease (Burnstock 2008, Burnstock et al. 2011). The density of $P2X_3$, $P2X_6$, and $P2X_7$ might be related to Diabetic neuropathy. On the diabetic rats, it is found out that they decreased in hippocampal nerve terminals.

Epilepsy seizures have devastating behavior. The pathogenesis is still unclear. P2X receptors play important roles in this disease, especially for $P2X_7$. The decrease of presynaptic P2X receptors in the hippocampus of rats might be related to the development of seizures and neurodegeneration during epilepsy (Burnstock 2008). Abnormal expression of $P2X_7$ might be associated with immunoreactivity in hippocampus and microglia (Rappold et al. 2006).

3.2 Genitourinary system

The vitro and vivo experiments showed that ATP and its analogues alter renal vascular resistance and renal blood flow. Seven P2X receptors are expressed in renal vascular, glomerular, mesangial and tubular epithelial cells. For example, $P2X_1$ expressed in preglomerular arteries and afferent arteriole (Turner et al. 2003, Zhao et al. 2005), whereas $P2X_2$ are expressed in larger intrarenal arteries (Turner et al. 2003). $P2X_4$ and $P2X_7$ are all detected in medium arteries (Lewis et al. 2001).

The recent hypothesis is $P2X_1$ receptors play an important role in renal autoregulation via tubuloglomerular feedback. Using the immunohistological studies, it has been detected that $P2X_1$ expressed in afferent arterioles, if the glomerular filtration rate or the rate of reabsorption in the proximal tubule changes; the glomerular filtration rate will change correspondingly. Changes in glomerular filtration rate are sensed by macula dens cells which will release ATP to stimulate $P2X_1$ receptors on afferent arteriolar smooth muscle (North 2009). This process triggers an increase of afferent arteriolar resistance. This reduces pressure in the glomerular capillaries and decreases glomerular filtration (Guan et al. 2007).

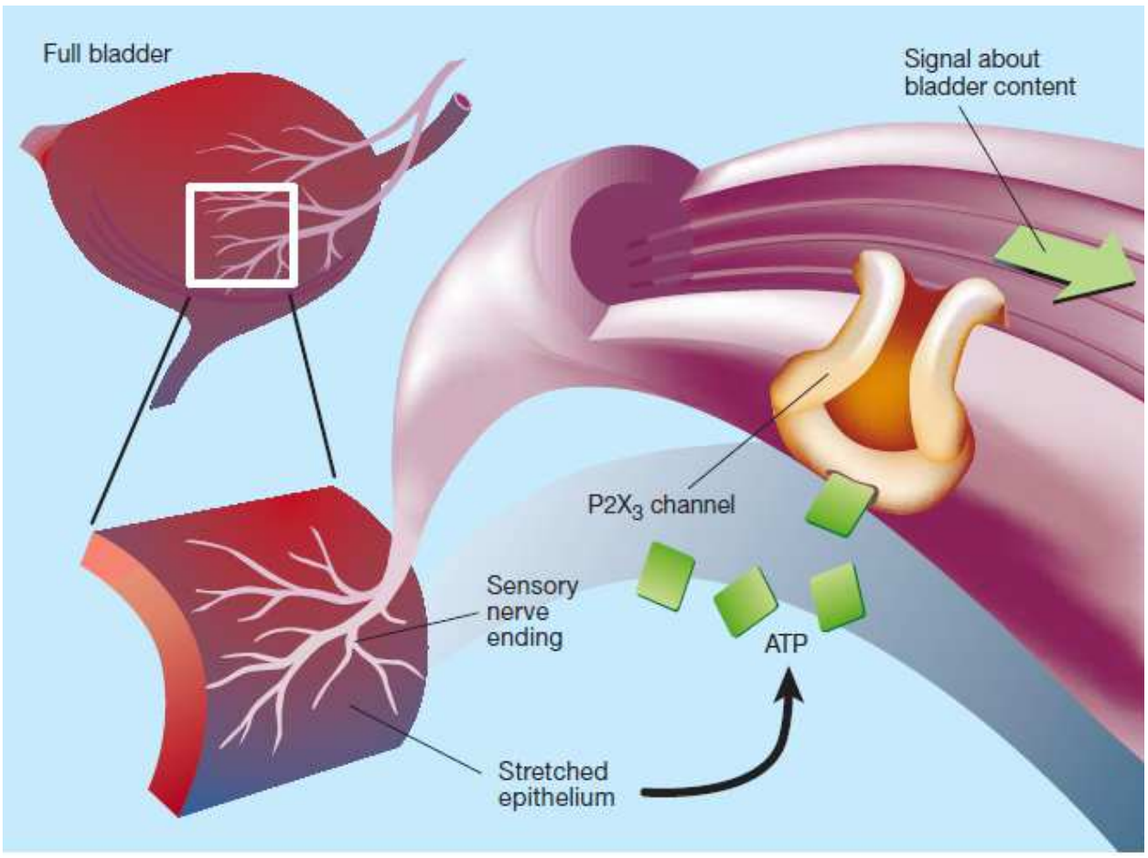

Fig. 3. Bladder urging, general view of bladder emptying. Stretching releases ATP from the inside of bladder epithelia cells, and channels made of $P2X_3$ receptors could detect ATP and trigger the neuronal pathway which responses for the bladder emptying. Fig.3 was reproduced from reference (McCleskey 2000)

Why we feel urge? The $P2X_3$ receptor knockout mice proved that $P2X_3$ receptors which are expressed only on sensor neurons, detect extracellular ATP, that is proposed to be released from the urothelium as a sensory mediator for the degree of the urinary bladder distention (Ferguson et al. 1997). If $P2X_3$ receptors are knocked out, the mice performed greatly decreased responses to the filling and stretching of urinary bladders, this means that they emptied their bladder less frequently than wild type. This urine storage disorders might be treated by antagonists of $P2X_3$ receptors.

Purinergic nerve-mediated contraction of the human bladder is increased to 40% in pathophysiological conditions such as interstitial cystitis (IC), outflow obstruction, idiopathic detrusor instability and probably also neurogenic bladder (Burnstock et al. 2011). Using bladder tissue sample of patients undergoing cystectomy or prostatectomy, Tempest et al found out that $P2X_2$ and $P2X_3$ receptors are all expressed in human bladder urothelium. Their protein expressing level did not correlate with the gene expression, which might be related to the pain with IC (Tempest et al. 2004).

3.3 Taste

A single taste bud contains three of four types of taste cells, including type I supporting cells, type II receptor cells, and III presynaptic cells, type IV basal cells. Type I cells on the outside of the type II and III. P2Y and P2X receptors are expressed on the type II cells, which are for the five tastes (umami, sour, salty, sweet and bitter), but type II cells do not synapse with afferent nerves within the taste buds. In taste system, ATP is a key neurotransmitter, which bridge between the taste bud and the nervous system. RT-PCR and immunohistochemical studies confirmed that $P2X_2$, $P2X_3$, $P2X_4$, $P2X_7$ receptor expressed on the taste bud cell. $P2X_2$ localized on the afferent nerve fibers and presynaptic cells, $P2X_3$ only localized afferent fibers, but the immunohistochemical studies showed no $P2X_4$ protein in taste bud cells and nerve fibers (Hayato et al. 2007, Kataoka et al. 2006). Homomeric and/or heteromeric $P2X_2$ and $P2X_3$ receptors play an important role in taste transduction. Finger and his colleagues found out that $P2X_2$ and $P2X_3$ knockout mice decreased the responses to sweet, glutamate and bitter substances (Finger et al. 2005). But single knockout mice recover some neural and behavioral responses to tastants (Finger et al. 2005). The exact role of $P2X_7$ is still not very clear, but it might be related to taste bud cells apoptosis (Hayato et al. 2007).

3.4 Hearing

Studies on the animal (rat, mouse) showed that $P2X_2$ and $P2X_3$ receptors might be in the inner and outer hear cells. This is only electrophysiological and immunolocalization data. There is no knockout animal model data to confirm the exact function of these P2X receptors.

3.5 Cardiovascular diseases

It has been reported that P2X receptors expressed throughout the cardiovascular system. In recent years, two tissues (the platelet and the endothelium) are studied in blood pressure and blood coagulation have advanced rapidly.

Although it has a long history that $P2Y_1$ and $P2Y_{12}$ play a main role in thrombosis, the $P2X_1$ receptors might play an important role in thrombus formation under the condition of stenosis, in which shear stress is high. It has been proved by the $P2X_1$ knockout animal model which is platelet-dependent thrombotic occlusion of small arteries. In this model

blood flow is characterized by a high shear rate. In the model of systemic thromboembolism death rate of $P2X_1$ deficient mice is reduced and the size of mural thrombi was decreased compared to the wild type, mural thrombi was made by laser induced vessel wall injury (Hechler et al. 2003). It is also contribute to transient Ca^{2+} influx and platelet shape change in response to ATP or Alpha, beta-MeATP and responsible for the platelet activation induced by low concentrations of collagen (Gachet 2008, Rolf et al. 2001, Rolf et al. 2002). This makes the $P2X_1$ receptors represent the ideal target for an antithrombotic drug.

As we all know, ATP is a cotransmitter in the heart, which is released from ischemic and gypoxicmycytes. Using quantitative PCR and situ hybridization, Musa and colleagues measured expression of mRNA of the P2X receptors in rat left ventricle, right atrium, sinoatrial node (SAN), and human right atrium. It is found out that $P2X_5$ was expressed abundantly in all three regions of rat heart. Although in human right atrium and SAN mRNA of $P2X_{4\text{-}7}$ was expressed, the expression of $P2X_4$ and $P2X_7$ mRNA was highest in these two regions. $P2X_1$ mRNA was only detected in human SAN (Musa et al. 2009). The increased expression of $P2X_1$ in the atria might be contributed to suffer from dilated cardiomyopathy. That $P2X_4$ mRNA was up-regulated contribute to ligation-induced heart failure (Burnstock et al. 2011, Musa et al. 2009).

Endothelial $P2X_4$ receptors play a crucial role in flow-sensitive mechanisms, which regulate blood pressure and vascular remodeling. It has been proved by Yamamoto et.al on the $P2X_4$ deficient mice model. $P2X_4$ knockout mice didn't have normal endothelial cell responses to flow, for example, Ca^{2+} influx and production of the potent vasodilator nitric oxide (NO) which might lead to higher blood pressure (North 2009), and it is also found out that $P2X_4$ deficient mice excrete smaller amounts of NO in urine than wild type (Yamamoto et al. 2006).

3.6 Respiratory system

ATP is playing a crucial role in the central respiratory control, which might mediate changes in the activity of medullary respiratory neurons in hypercapnia. Gourine and colleagues found out the evidence that ATP acting on $P2X_2$ receptors, which are expressed in ventrolateral medulla that contains respiratory neurons responsible for generating and shaping the respiratory rhythm. Modulation of the function of $P2X_2$ receptors in ventrolateral medulla might be related to change in activities of ventrolateral medulla respiratory neurones (Gourine et al. 2003).

It has also been discovered that $P2X_4$ receptors expressed on lung epithelial cells can control ciliary beat frequency, which make the clearance of mucus from the airways (Zsembery et al. 2003). Cystic fibrosis might be benefit from manipulation of it. Some agonists, such as β2-adrenergic ($P2Y_2$ agonist) and ATP, can increase ciliary beating in trachea and airway epithelia via activation of P2Y and P2X receptors, which provide an key therapy in respiratory diseases (Leipziger 2003). Zhao et al found out that erythromycin can block P2X mediated Ca^{2+} influx, this action of which might contribute to the treatment of airway inflammation.

3.7 Exocrine glands

Exocrine glands can secrete hormones and other chemical messengers into ducts that lead directly into the external environment. ATP work on the purine receptors that are expressed on secretory epithelia with the consequent secretion of hormonal peptides, bicarbonate,

potassium and so on (Leipziger 2003). Pochet and colleagues found out that $P2X_7$ receptors are expressed in mice submandibularductal cells, and $P2X_4$ receptors are also involved. Using the wild type and $P2X_7$ knockout mice models, they found out strong evidence that $P2X_7$ salivary secretion through the modulation of phospholipid signaling processes, especially for phospholipase A2 and phospholipase D. In the knockout mice, ATP no longer active any phospholipid signaling and saliva showed a decreased potassium content (Garcia-Marcos et al. 2006). Using the RT-PCR and immunohistochemical staining, it is found out that $P2X_4$ receptors are in choangiocytes, which is likely the primary isoform involved, representing a functionally component which modulating biliary secretion (Doctor et al. 2005).

3.8 Intestinal motility

There is some evidence show that P2X receptors are expressed in the enteric nervous system, which underlies coordinated movements of the intestine. $P2X_2$, $P2X_3$ and $P2X_7$ receptors are all involved in the intestine system in health and gut disease. It has been detected that $P2X_2$, $P2X_3$ receptors are expressed on AH cells which is the principal intrinsic afferent neuron of the enteric nervous system (Furness et al. 2004, North 2009). In the $P2X_3$ knockout mice model, the gut peristalsis was inhibited. It has been proved that $P2X_3$ receptors might contribute to detection of distention or intraluminal pressure increases and initiation of reflex contractions (Bian et al. 2003). On the other side, $P2X_3$ receptors are involved in gut disorders. Irritable bowel syndrome (IBS) is characterized by chronic abdominal pain, bloating and discomfort. In the rat model of IBS-like visceral hyperalgesia, $P2X_3$ protein expression was significantly enhanced in colon-specific DRGs 8 weeks (Xu et al. 2008), which suggesting a potential role in dysmotility and pain (Yiangou et al. 2001). Wynn et al described that $P2X_3$ receptors signaling enhancement in colitis (Wynn et al. 2004). It is suggesting that $P2X_3$ receptors are potential targets for drug development of IBS.
$P2X_3$ receptors are expressed in intrinsic sensory neurones in the submucous plexus of gut and extrinsic sensory nerves (Xiang et al. 2004). It has been found out that in the substantial distension, ATP activates moderate distension higher threshold extrinsic sensory fibers and lower threshold intrinsic enteric sensory fibers that transmit the message to the CNS, via $P2X_3$ receptors from mucosal epithelia, respectively (Bian et al. 2003, Wynn et al. 2004, Wynn et al. 2003).

3.9 Immune system and inflammation

It is found out that $P2X_1$, $P2X_4$ and $P2X_7$ receptors are coexpressed in most immune cells in different level. $P2X_7$ receptors play a crucial role in inflammation and immunomodulation (Burnstock et al. 2011).
Inflammation is initiated by some pathogen constituents, injured or dying cells which can release intrinsic host molecular (Di Virgilio 2007). As the host endogenous pro-inflammatory Nucleotides especially ATP play a key role in inflammation in which human IL-1 family proteins are key mediators of the acute immune response to injury and infection. The $P2X_7$ receptor is the key player in interleukin (IL)-1β and IL- 18 maturation and release. In inflammation process, $P2X_7$ receptors induce the rapid activation of caspase-1 with subsequent release of the pro-inflammatory cytokine IL-1β from activated macrophage and microglia. That inhibition the $P2X_7$ receptor is directly decreased the levels of IL-1β in plasma or in the area of injury.

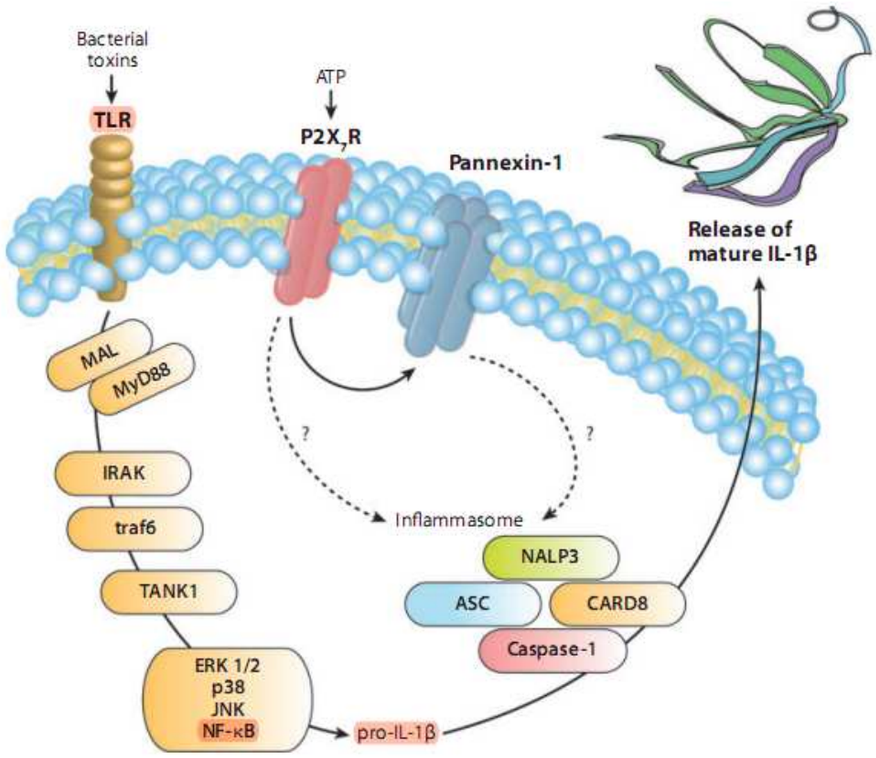

Fig. 3. $P2X_7$ receptor and the NALP3 inflammasome. The $P2X_7$ receptor is the key player in interleukin (IL)-1β and IL-18 maturation and release. High concentrations of extracellular ATP, present at sites of inflammation, activate a P2X7R/pannexin-1 protein complex which induces the rapid activation of caspase-1 with subsequent release of the pro-inflammatory cytokine IL-1β from activated macrophage and microglia. This figure is reproduced from reference (North 2009).

Mycobacterium tuberculosis (MTB) is a monocyte/macrophage (M/M) parasite. Lammas and colleagues proved that $P2X_7$ receptors associated with ATP induced apoptosis in macrophages that result in killing of the mycobacteria within them (Lammas et al. 1997). Roberta et al also proved that mycobacterial infection leads to an increased expression of $P2X_7$, and at the same time infected macrophages induced the extracellular release of ATP. Cell death can't be induced by MTB after using the oxidized ATP (oATP), which is the $P2X_7$ receptor antagonist (Placido et al. 2006). Taken together, the death of intracellular bacilli directly related to $P2X_7$ mediated macrophage apoptosis, but the mechanisms underlying the IL-1 cytokines (IL-1α, IL-1β and IL-18) cellular release are still unclear. Pelegrin and colleagues investigated the release patterns in LPS-primed peritoneal macrophage, RAW264.7 macrophage, and J774A.1 macrophage. They found out that IL-1β is the direct cause of macrophage apoptosis, and two release pathways are proved (Pelegrin et al. 2008). One is the caspase-1 mediating release of processed IL-1β that is selectively blocked by inhibition of caspase-1 or panx1, the other is a calcium independent, caspase-1/ panx1-independent release of pro-IL-1β that is selectively blocked by glycine (Pelegrin et al. 2008). Although it is proved that $P2X_7$ receptors are highly expressed in these macrophage, apoptosis of which is related to its prolonged activation, and concomitant killing of intracellular pathogens (North 2009, Pelegrin et al. 2008), it is unclear that whether the macrophage apoptosis is necessary for killing of intracellular mycobacteria.

Receptors	Distribution	Antagonist	Diseases and potential therapeutic strategies
P2X$_1$	Sympathetic neurons, Sensory neurons, Smooth muscle, Cardiac muscle, Endothelial cells, Inner ear, Osteoblasts, Platelets, Cochlea Oligodendrocytes, Exocrine secretary cells, Kupffer cells	Ip5I, MRS 2220, NF023	
P2X$_2$	Sympathetic neurons, Sensory neurons, Eye, Tongue, Parasympathetic neurons, Enteric neurons, Central nervous system, Retinal neurons, Endothelial cells, Osteoblasts, Keratinocytes, Sperm, Erythrocytes, macrophages, Endocrine secretory cells, Cholangiocytes, Inner ear, Olfactory organ, Cochlea		
P2X$_3$	Sympathetic neurons，Tongue, Parasympathetic neurons, Keratinocytes, Müller cells, Sensory neurons, Enteric neurons, Retinal neurons, Smooth muscle, Cardiac muscle, Endothelial cells, Inner ear, Cholangiocytes	A-317491, TNP-ATP,	neuropathic and inflammatory animal pain (Jarvis et al. 2002) thermahyperalgesia and mechanical allodynia, ATP-induced allodynia (Nakagawa et al. 2007)
P2X$_4$	Sympathetic neurons, Parasympathetic neurons, Enteric neurons, Central nervous system, Retinal neurons, Cardiac muscle, Osteoclasts, Epithelial cells, Microglia, Müller cells, Endothelial cells, Erythrocytes, Immune cells, Exocrine/Endocrine secretory cells,	Suramin, PPADS, Reactive Blue 2, CORMs	
P2X$_5$	Sympathetic neurons, Parasympathetic neurons, Sensory neurons, Retinal neurons, Smooth muscle, Cardiac muscle, Osteoblasts, Keratinocytes, Epithelial cells, Müller cells		
P2X$_6$	Sympathetic neurons, Central nervous system, Smooth muscle, Cardiac muscle, Epithelial cells, Cholangiocytes		
P2X$_7$	Sympathetic neurons, Sensory neurons, Enteric neurons, Central nervous system, Retinal neurons, Smooth muscle, Eye, Osteoblasts, Keratinocytes, Fibroblasts, Epithelial cells, Microglia, Cochlea, Müller cells, Enteric glial cells, Sperm, Erythrocytes, Immune cells(predominantly), Inner ear, Kupffer cells, Exocrine/Endocrine secretory cells	AZD9056 (clinic trial Ⅱ), KN62, Brilliant Blue, oATP, A-438079, PPADS, Suramim, Decavanadate	rheumatoid arthritis (Elsby et al. 2011) Parkinson disease (Marcellino et al. 2010) antinociception (McGaraughty et al. 2007)

Table 2. Characteristics of P2X receptors

Osteoclasts function in concert with osteoblasts, fibroblast lineage cells which deposit new bone. Normal bone remodeling and the maintenance of skeletal integrity need osteoblasts and osteoclasts coordinate their activity. Using extracellular monoclonal antibody, Gartland et al. performed an experiment in vivo and in vitro, which demonstrated that $P2X_7$ receptors are expressed in various stages of osteoclast differentiation (Gartland et al. 2003). Osteoclastresorptive activity was inhibited by its antibody. Comparing the $P2X_7$ knockout mice model with wild type, it is indicating that $P2X_7$ does not regulate longitudinal bone growth, but $P2X_7$ knockout mice significantly reduced in total and cortical bone content and periosteal circumference in femurs (Ke et al. 2003). Thus, the $P2X_7$ receptor represents a novel therapeutic target for skeletal disorders.

4. Novel therapeutic drugs

Since seven P2X receptors ($P2X_{1-7}$) are widely distributed in excitable and non-excitable cells of vertebrates, and play a crucial role in the pathology of several disease states, such as neuropathic pain, chronic inflammation, rheumatoid arthritis and so on. Thus P2X receptors are the potential targets for drug development. Considerable efforts have been made to synthesize therapeutic antagonists. Besides being important pharmacological tools for characterization of the pathophysiological roles of P2X receptors in native systems, such ligands may represent new therapeutic entities of potential interests in various human diseases. The most prospective areas for drug discovery at present are for the treatment of visceral pain with $P2X_3/P2X_{2/3}$ receptor antagonists and neuropathic and inflammatory pain with $P2X_7$ antagonists up to present.

4.1 $P2X_7$ receptors

$P2X_7$ receptors is a unique family have been extensively studied in immune cells where they are related to rapid release of pro-inflammatory cytokines and the initiation of the inflammatory cascade. Until now there are some therapeutic antagonists have been synthesized. They are evaluated in different levels from animal model experiment to clinical trial. Some of them are still evaluated in cell test as a tool for pathophysiological study. The $P2X_7$ receptors antagonists are nonselective or selective, and they show different affinity and IC_{50}.

The most promising $P2X_7$ receptor antagonist is AZD9056, which was developed for oral treatment of rheumatoid arthritis. AZD9056, a weakly basic secondary amine (pKa 9.77) with a hydrophobic adamantine moiety that is highly protein bound (97%), is in clinic trial Ⅱ (Elsby et al. 2011). Efficacy and drug-drug interaction (DDI) studies performed as part of the clinical development program for a new candidate drug. This study is prior to patient studies. Since patients with rheumatoid arthritis receive multiple co-medications, that might be taken a risk of the co-morbid conditions, it is necessary to evaluate the potential of RA drug candidates to perpetrate a drug-drug interaction (DDI) with methotrexate which is a most commonly drug treating RA (van Roon et al. 2009). Methotrexate is a substrate of the human transporters OAT1, OAT3, MRP2 and BCRP which all-mediate active renal elimination. AZD9056 can't inhibit OAT1 and OAT3 transporting methotrexate, but have a weakly inhibition effect on BCRP mediated transporting (IC_{50}=92 μM) (Elsby et al. 2011). Recently, Keystone and colleagues have reported their work on phase Ⅱ studies, which assess the effects of orally administered AZD9056 on the signs or symptoms of rheumatoid arthritis (RA). They used randomized, double blind, placebo-controlled, and parallel-group

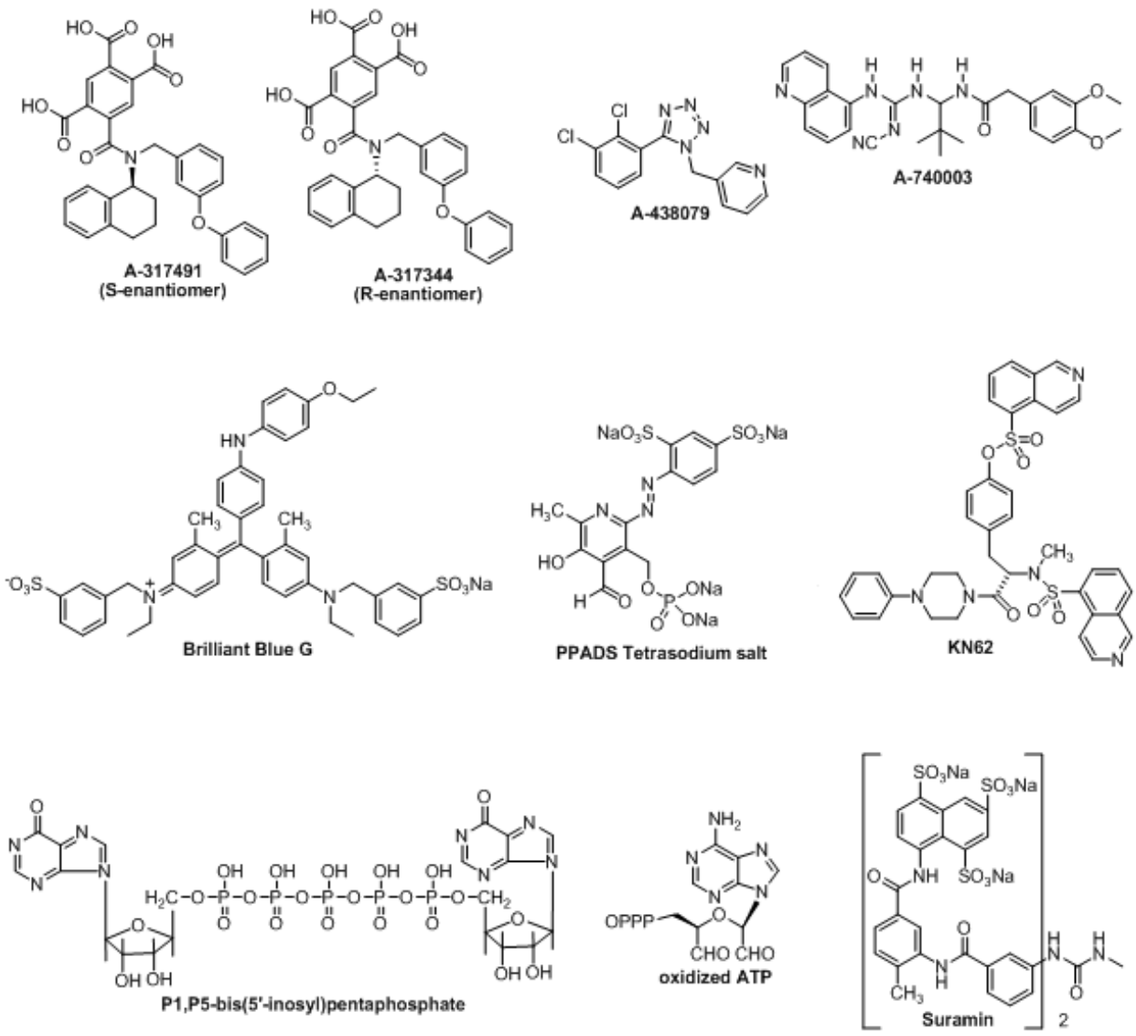

Fig. 4. Structures of P2X receptors antagonists. A-317491 which is the first non-nucleotide antagonist, has high selectivity (IC_{50} > 10 μM) to $P2X_3$ and $P2X_{2/3}$ receptors; the R-enantiomer of A-317491, A-317344, was ineffective (ED_{50} > 100 μmol/kg s.c.) in neuropathic and inflammatory animal pain models; 3-((5-(2,3-dichlorophenyl)-1H-tetrazole-1-yl)methyl pyridine (A-438079) effectively inhibit Bz-ATP stimulation for $P2X_7$ receptors; A-740003 ((N-(1-{[(cyanoimino) (5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2- (3,4-dimetho- xyphenyl)acetamide) shows highly specific and potent for $rP2X_7$ and $hP2X_7$ of which potency is 18-40 nM (Honore et al. 2006); Brilliant Blue G is a more potent and selective antagonist, IC_{50} for rat and human $P2X_7$ receptor is 10 nM and 200 nM, respectively (North 2002); KN62 (1-[N,O-bis(5-isoquinoline-sulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine) which is isoquinoline derivative (Humphreys et al. 1998), inhibit ATP – stimulated Ca^{2+} influx and selectively inhibit calcium/calmodulin-dependent protein kinase II. TNP-ATP, PPADS and suramin is non-selective antagonist at P2X receptors; ATP 2′,3′-dialdehyde (oxidized-ATP, oATP) is a irreversible antagonist at $P2X_7$, $P2X_1$ and $P2X_2$ receptor (Di Virgilio 2003); Ip5I is a potent and selectivity antagonist for recombinant rat $P2X_1$ receptors at nanomolar concentration (pIC_{50} = 5.6) (King et al. 1999).

in the phase Ⅱa and phase Ⅱb studies. Through months studies, they found out that 65% of patients who received 400 mg/day which is a tolerated dose responded at the ACR 20 level that is American College of Rheumtology 20% response criteria, comparing with 27% of placebo-treated recipients. In Ⅱb studies 383 randomized patients who received AZD9056 had no clinically or statistically significant effects on RA compared with placebo group. But in both studies, AZD9056 was used in tolerated dose (400 mg/day) so that the proportion of patients meet the ACR20 criteria. The results showed that AZD9056 does not have significant efficacy for RA (Keystone et al. 2011).

In 2006, Abbott Laboratories disclosed two series $P2X_7$ antagonists: disubsituted tetrazaled and cyanoguanidines, which show enhanced potency and selectivity to $rP2X_7$ and $hP2X_7$ (Honore et al. 2006, Nelson et al. 2006). 1-Benzyl-5-aryltetrazoles were discovered to be novel $P2X_7$ antagonists by Nelson et al (Nelson et al. 2006). 3-((5-(2, 3-dichlorophenyl)-1H-tetrazole-1-yl) methyl pyridine (A-438079) effectively inhibits Bz-ATP stimulation. The compound can inhibit calcium flux in human and rat $P2X_7$ cell lines. It's IC_{50} for human and rat $P2X_7$ receptors are 100 and 300 nM respectively. It has devoid activity to other P2 receptors (IC_{60}>> 10 μM) and its analogues can also have the ability to inhibit IL-1β release and $P2X_7$ mediated pore formation in human THP-1 cells (Nelson et al. 2006). Daniel Marcellino et al using $P2X_7$ receptor antagonist A-438079 as a tool found out that blockade of $P2X_7$ might be a novel protection strategy for striatal DA terminals in Parkinson's disease (Marcellino et al. 2010). Mcgaraughty et al utilized A-438079 investigate $P2X_7$ related antinociception mechanism in vivo and vitro. Three different rat models of neuropathic pain showed attenuated formalin-induced nocifensive behaviors after injecting 10-300 μmol/kg (i.p.) of A-438079 (McGaraughty et al. 2007). A-438079 dose dependently (0.3-3μM) decreased the quantity of cytokine and IL-1β (McGaraughty et al. 2007). Comparing other P2 receptors, A-740003 ((N-(1-{[(cyanoimino) (5-quinolinylamino) methyl] amino}-2, 2-dimethylpropyl)-2-(3, 4-dimetho- xyphenyl) acetamide) shows highly specific and potent effects on $rP2X_7$ and $hP2X_7$ of which potency is 18-40 nM (Honore et al. 2006). It also changes intracellular calcium concentration in a competitive way. Both compounds' sufficient bioavailability in intraperitoneal administration allows for in vivo investigation on disease models of $P2X_7$ receptors (Honore et al. 2006, Nelson et al. 2006).

Suramin (IC_{50}> 300 μM for rat $P2X_7$) and PPADS (IC_{50}～50 μM) (pyridoxalphosphate- 6-azopheny2', 4'-disulfonate) are both prototypic nonselective P2X receptor antagonists (North 2002). They show noncompetitive antagonism and low affinity (Ki>10 μM). KN62 (1-[N,O-bis(5-isoquinoline-sulfonyl) -N-methyl-L-tyrosyl]-4-phe-nylpiperazine) which is isoquinoline derivative (Humphreys et al. 1998), and being a most potent compound with 13.4 nM IC_{50}, can inhibit ATP–stimulated Ca^{2+} influx and selectively inhibit calcium/calmodulin-dependent protein kinase II. But it shows significantly species differences. It is more potent to human $P2X_7$ versus rat $P2X_7$ (Humphreys et al. 1998). Brilliant Blue Gis a more potent and selective antagonist, IC_{50} for rat and human $P2X_7$ receptor is 10 nM and 200 nM, respectively (North 2002).

Michel et al reported the decavanadate, which is a polymeric form of vanadate, is a reversible and competitive $P2X_7$ receptor antagonist. But it also displays non-selectivity because it not only inhibits the $P2X_2$ and $P2X_4$ mediated response, but also interacts with IP3 binding and inhibition of ribonuclease A (Michel et al. 2006).

ATP 2', 3'-dialdehyde (oxidized-ATP, oATP), ATP with the 2'- and 3'-hydroxyl moieties oxidized to aldehydes by periodate treatment, is a irreversible antagonist required 1 or 2 h incubation to inhibit $P2X_7$ receptor (Di Virgilio 2003). oATP is used in mouse macrophage to

lock $P2X_7$ initiated responses. But except that, oATP can also inhibit $P2X_1$ and $P2X_2$ mediated responses (Evans et al. 1995). It also inhibits nuclear factor-κB (NF-κB) and cytokine release. So these limit its role as a pharmacological tool like decavanadate.

4.2 $P2X_3$ and $P2X_{2/3}$ receptors

$P2X_3$ and $P2X_{2/3}$ receptors localize on peripheral and central processes of sensory afferent nerves (Jarvis et al. 2002). Experiment with antisense oligonucleotides and $P2X_3$ knockout mice proved that $P2X_3$ receptors play a crucial role in the signaling of chronic inflammatory pain and some features of neuropathic pain.

Virginio et al. identified 2'3'-O-(2,4,6-trinitrophenyl)-ATP (TNP-ATP) can block $P2X_1$, $P2X_3$ and $P2X_{2/3}$ receptors, but it is ineffective in blocking currents produced by activated $P2X_2$, $P2X_4$ and $P2X_7$ receptors. It can completely block fast component of ATP mediated and αβmeATP mediated sustained current in neurones of dorsal root and nodose ganglia (Bradbury et al. 1998). It is a competitive antagonist for $P2X_3$ and $P2X_{2/3}$ receptors with pA2 values of -8.7 and -8.2 (Burgard et al. 2000). The IC_{50} values for $hP2X_{2/3}$ and $rP2X_{2/3}$ are 3 to 6 nM. It displays a rapid inhibition in onset, reversible, and use independence. The $P2X_{2/3}$ receptors can fully recover from TNP-ATP after removal it for more than 5 s (Burgard et al. 2000).

A-317491 has high selectivity (IC_{50}> 10 μM) to $P2X_3$ and $P2X_{2/3}$ receptors, which can potently block human and rat $P2X_3$ and $P2X_{2/3}$ mediated calcium flux (Ki=22 – 92 nM) (Jarvis et al. 2002). Using neuropathic and inflammatory animal pain models, A-317491 can reduce nociception. They use s.c. administration found out that A-317491 dose-dependently (ED_{50} = 30 μmol/kg s.c.) reduced complete Freund's adjuvant-induced thermal hyperalgesia in the rat pain models, but ineffective in acute pain, postoperative and visceral pain (Jarvis et al. 2002). However, it is most effective (ED_{50} = 10 - 15μmol/kg) in attenuating both thermahyperalgesia and mechanical allodynia after chronic nerbe constriction injury. On the contrary, the R-enantiomer of A-317491, A-317344, was ineffective (ED_{50} > 100 μmol/kg s.c.)in neuropathic and inflammatory animal pain models. Nakagawa et al use the pain animal models found out that i.t. co-administration of ATP and A-317491 (30 nM) significantly prevented the ATP-induced allodynia (Nakagawa et al. 2007). In other drug treatment group, co-administration of suramin and PPADS significantly prevented the induction of longlasting allodynia (Nakagawa et al. 2007).

AF-353, which is synthesized by Roche Palo Alto are novel, selective and highly potent $P2X_3$ and $P2X_{2/3}$ receptor antagonist. It has been proved that AF-353 is orally bioavailable (%F = 32.9) and stable in vivo for the treatment of pain which is in clinical trial (Gever et al. 2010). The antagonistic potencies (pIC(50)) for rat and human $P2X_3$ and human $P2X_{2/3}$ receptors ranged from 7.3 to 8.5 which had little or no effect on other P2X receptors (Gever et al. 2010). Comparing with A-317491 and TNP-ATP, AF-353 inhibits activation by ATP in non-competitive fashion. It is observed that A-317491 has reasonable half-life (t(1/2) = 1.63 h) and plasma-free fraction (98.2% protein bound) (Gever et al. 2010). This compound is good for studying $P2X_3$ and $P2X_{2/3}$ receptors in animal models and optimize compound for clinic trial.

4.3 $P2X_4$ receptors

Finding antagonists for the $P2X_4$ receptor has been more problematic. Suramin, PPADS and reactive Blue 2 are conventional non-selective antagonists for $P2X_4$ receptors up to present. PPADS is a reversible antagonist required 20 to 30 min washing can partially reverse (North

2002). The $hP2X_4$ receptor is more sensitive to PPADS than $rP2X_4$. Jones et al reported that IC_{50} for mouse $P2X_4$ receptor is 10 mM (Jarvis et al. 2002, Jones et al. 2000). Recently Wilkinson and Kemp proved that CORMs which is co-releasing molecules is an effective antagonist at human $P2X_4$ receptors (Jarvis et al. 2002).

4.4 $P2X_1$ receptors

Diinosinepentaphosphate (IpnI, n is the number of phosphates), comprise two ribosylatedinosine molecules bridged by a phosphate chain. These dinucleotides are synthesized by deaminating diadenosine polyphosphates with non-specific AMP-deaminase of Aspergillus sp. (Pintor et al. 1997). P1,P5-bis(5'-inosyl) pentaphosphate (Ip5I), the member of this family, has been showing a potent antagonist at a specific dinucleotide receptor in rat brain synaptosomes (IC_{50} = 4 nM) and P2X receptor (IC_{50} = 30 µM) (Pintor et al. 1997). King et al. reported that Ip5I is a potent and selectivity antagonist for recombinant rat $P2X_1$ receptors at nanomolar concentration (pIC_{50} = 5.6). Non-linear of Schild plot proved that Ip5I is a noncompetitive antagonist (King et al. 1999). It represents a different manner in low and high concentrations. At low (≤ 100 nM) concentrations Ip5I represents a high affinity antagonist for $P2X_1$ receptors, however, in higher (> 100 nM) concentrations it represent a more complex actions.

Jacobson et al. synthesize two antagonists which are analogues of PPADS. MRS 2220 is selective antagonist. Comparing with PPADS (IC_{50} = 98.5 +/- 5.5 nM), MRS 2220 represents a lower IC_{50} (IC_{50} = 10.2 +/- 2.6 mM) at recombinant $P2X_1$ receptor, But unlike PPADS its effect was reversible with washout and surmountable (Jacobson et al. 1998).

NF023 (8, 8'-(carbonylbis (imino-3, 1-phenylene carbonylimino) bis (1, 3, 5-naphthalenetrisulf onic acid)) is suramin analogue, which represent a competitive fashion at P2X receptor-mediated responses in certain vascular and visceral smooth muscles. $P2X_1$ receptors represent a most sensitive manners with IC_{50} values of 0.24 and 0.21 µM for rat and human homologues, respectively (Soto et al. 1999). $P2X_2$ (IC_{50}> 50 µM), $P2X_3$ (IC_{50} = 8.5 and 28.9 µM for rat and human), $P2X_4$ (up to 100 µM) shown different sensitivity (Soto et al. 1999). TNP-ATP is potent antagonist at $P2X_1$ and $P2X_3$ receptors at nanomolar concentrations.

4.5 Other P2X receptors

So far, there is no selective and potency antagonists that is orally bioavailable and stable in vivo for $P2X_2$, $P2X_5$ and $P2X_6$. Suramin, PPADS and TNP-ATP are non-selective antagonists at P2X receptors. Seven P2X receptors represent different sensitivity.

5. Conclusion and future issues

P2X receptor, which is a novel family of ligand-gated cation channels, is non-selective cation channel that gated in the presence of ATP. Seven P2X receptor subunits ($P2X_{1-7}$) are widely distributed in excitable and non-excitable cells of vertebrates and play a crucial role in inter alia afferent signaling (including neuropathic pain), regulation of renal blood low, vascular endothelium, and chronic inflammation. Thus P2X receptors are potential targets for drug development. The field is still limited by the availability of agonist, antagonist and modulator which could accelerate our understanding of the physiological roles of P2X receptors. However, there are some notable antagonists of $P2X_1$, $P2X_3$ and $P2X_7$ receptors

with highly potency, selectivity and nanomolar affinity, such as AF-353, A-317491 and AZD9056. There are some important issues for the antagonist application. The first is actions at receptors other than P2X receptors. If the antagonist is developed to be a potential drug, it has to overcome the actions on other receptors or other subtype of P2X receptors. Selectivity is important for drug development. For example, TNP-ATP, PPADS and suramin are non-selective antagonists of P2X receptors. Seven subtypes of P2X receptors represent different sensitivity. But it would be welcomed to study the physiological and functional role for developing such compounds. The second is the species specificity. Human and rat P2X receptors represent different sensitivity to a compound which means that it is difficult to do animal model experiments before the clinic trial. So far, there has been promising development of clinical $P2X_7$ antagonists, notably Abbott compounds – A-438079 and A-740003. Both compounds represent highly specificity and potency for $rP2X_7$ and $hP2X_7$. Sufficient bioavailability is likely to allow for in vivo investigation on disease models of $P2X_7$ receptors. Another promising $P2X_7$ receptor antagonist is AZD9056, which was developed for oral treatment of rheumatoid arthritis. But recent clinical trail II experiments showed that AZD9056 did not have significant efficacy for RA. They concluded that $P2X_7$ receptors do not appear to be a therapeutically useful target in RA. However, it is proved that AZD9056 is a selective and potent antagonist of $P2X_7$ receptors before clinical trial II. So it might be efficacious for other diseases which are related to $P2X_7$ receptors. AF-353 is most promising compound that may reach the market introduction. The good orally bio-availability and stability of AF-353 in vivo may bring the biggest advantage for patients in pain treatment. Antagonists for some of the other P2X receptors remain to be developed. P2X receptors are likely to have widespread therapeutic usage in the future.

6. References

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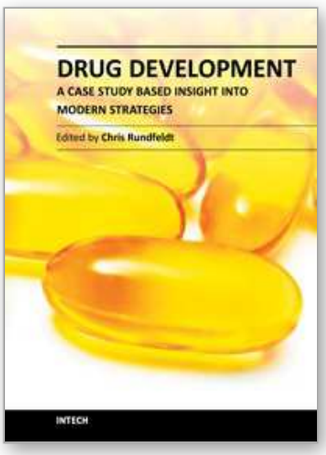

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This book represents a case study based overview of many different aspects of drug development, ranging from target identification and characterization to chemical optimization for efficacy and safety, as well as bioproduction of natural products utilizing for example lichen. In the last section, special aspects of the formal drug development process are discussed. Since drug development is a highly complex multidisciplinary process, case studies are an excellent tool to obtain insight in this field. While each chapter gives specific insight and may be read as an independent source of information, the whole book represents a unique collection of different facets giving insight in the complexity of drug development.

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