Biochimica et Biophysica Acta 1838 (2014) 2578–2587

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

The P2X7 receptor: Shifting from a low- to a high-conductance channel — An enigmatic phenomenon?

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ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form 22 April 2014
Accepted 13 May 2014
Available online 21 May 2014

Keywords:
P2X7R
Ion channel activity
Patch-clamp
Calcium
Second messenger

ABSTRACT

The general structure of the P2X7 receptor (P2X7R) is similar to the structure of other P2X receptor family members, with the exception of its C terminus, which is the longest of this family. The P2X7R activates several intracellular signaling cascades, such as the calmodulin, mitogen-activated protein kinase and phospholipase D pathways. At low concentrations of ATP (micromolar range), P2X7R activation opens a cationic channel, similarly to other P2X receptors. However, in the presence of high concentrations of ATP (millimolar range), it opens a pathway that allows the passage of larger organic cations and anions. Here, we discuss both the structural characteristics of P2X7R related to its remarkable functions and the proposed mechanisms, including the dilation of the endogenous pore and the integration of another channel. In addition, we highlight the importance of P2X7R as a therapeutic target.

Contents

1. Introduction

The initial concept of purinergic extracellular actions was developed in the 1920s based on experiments using the cardiovascular system that demonstrated the effects of crude tissue extracts. The principal active compound in these extracts was identified to be adenosine-5′-monophosphate [1]. A study conducted in 1965 described the effects of caffeine on guinea pig atria and clearly demonstrated that adenosine receptors are inhibited by this alkaloid [2]. Nevertheless, more than half a century passed before purines were formally proposed as signaling molecules. Purinergic neurotransmission was formally proposed in a classical paper published in *Pharmacological Review* that described the identification of adenosine 5′-triphosphate (ATP) as the signal molecule in non-adrenergic, non-cholinergic inhibitory nerves in guinea pigs [3]. Three subclasses of purine and pyrimidine receptors have been identified to date: P1 adenosine receptors (A1, A2a, A2b and A3) and the P2 family, which is further divided into ionotropic (P2X) and metabotropic receptors (P2Y). The ionotropic P2X receptor subfamily is composed of

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http://dx.doi.org/10.1016/j.bbamem.2014.05.015
0005-2736/

ATP-gated ion channels and includes seven members (P2X1–7), while the P2Y G protein-coupled receptor (GPCR) subfamily contains eight subtypes (P2Y1, 2, 4, 6, 11, 12, 13 and 14) [4,5].

2. Overview of P2X receptors

The P2X family is composed of the excitatory ATP-gated P2X receptors (P2XRs). Seven genes (P2XR1–7) encode the seven P2XR subunits (P2X1–7) that are found in multiple species from unicellular organisms to humans [6]. However, prokaryotic P2XRs have not been reported [7]. The majority of P2XR subtypes are non-selective cation channels with high Ca^{2+} permeability. The exception is the P2X5Rs, which are permeable to Cl^{-} [8]. These receptors play important roles in cell–cell communication through modulating synaptic transmission, contracting smooth muscle, regulating immune responses, inducing rapid conformational changes in gates and triggering transmembrane fluxes of selective ions [7,9–11].

P2XR channels are composed of three subunits that assemble either as homo- or heterotrimeric complexes. Each subunit contains two transmembrane domains, including a large cysteine-rich extracellular domain (~280 residues) and intracellular C- and N-termini [18–21]. These subunits present a relatively simple architecture among ligand-gated ion channels (LGICs), in contrast to the tetrameric eukaryotic glutamate receptors (GluRs) [12] or the pentameric receptors of the Cys-loop family [13]. The trimeric purinergic structure more closely resembles epithelial Na^{+} channels (ENaC), including the recently described acid-sensing ion channels (ASICs) [14,15].

The second transmembrane domain (TM2) is a key structure in P2XR channel formation, although TM1 also plays a role in the integral function of these receptors [16]. Mutations in this domain cause minor changes in ATP-gated currents. In agreement with these findings, zebrafish P2X4 (zfP2X4) crystal structure models have shown that TM2 lines the ion pathway, while TM1 is positioned peripherally [17–24].

In general, the gating of P2XRs consists of three phases: a rapid phase involving an inward current induced by the application of an agonist (activation phase); a slow decay phase in the presence of the agonist (desensitization phase); and a rapid decay of the current following ATP depletion (deactivation phase). The main differences among P2XRs lie in their sensitivity to agonists and their desensitization rates [7,9].

Although all P2XRs open a cationic channel within milliseconds when activated by their native agonist, some channels (the P2X2R, P2X4R and P2X7R channels) provide not only a narrow conducting pathway that allows the passage of small ions but also a pathway for the passage of larger organic cations. The pore formation of P2X7R is prototypical for these channels. The mechanism by which this formation occurs is still unknown, but it may involve the dilation of the endogenous pore or via the participation of another channel through the activation of second messenger cascades [6,25–27].

3. Structural aspects of the P2X7R

Because the focus of the present review is the P2X7R, we will use the available evidence to provide further details about the structural characteristics of this receptor. Different structural features are observed among the P2XRs, and consequently, these proteins have different functional behaviors. Compared with other P2XR subtypes, P2X7R requires a high concentration of ATP (>100 μM) to evoke a current, and upon activation by an agonist, it opens a high-conductance pore that allows the passage of high-molecular-weight molecules across the plasma membrane [28,29]. The processes by which this receptor undergoes this striking behavior remain unknown, and it is the most inexplicable phenomenon faced in P2X physiology (see hypothesis below).

There have been few studies addressing the structural binding site of P2X7R. However, the site appears to involve the same conserved lysine residues in the extracellular loop that are important for other P2X member subtypes. The conserved lysines have been proposed to interact with negatively charged phosphate groups because these residues have been shown to interact with ATP molecules in the P2X1 [29,30] and P2X2 [31] subtypes. The conserved lysine residues have also been found to interact with ATP in a crystal structure model of the P2X4 receptor [24]. Furthermore, the K193A and K311A mutations (P2X7 numbering) have been shown to impair P2X7 function; therefore, these residues could be directly involved in the binding of ATP to this site [32]. These data are consistent with the homologous residues found in the P2X1, P2X2 and P2X4 receptors [29,31,33–36]. Moreover, aromatic residues are necessary for interactions with the adenine base, which have been observed in other ATP-binding proteins [37], and these interactions confer base specificity. It has been hypothesized that this pattern also occurs in the P2X7R subtype, but further studies are needed to test this proposal.

One of the most intriguing domains of P2X7R is its C terminus (for a complete review of this topic, see Costa-Junior et al. [38]). P2X7R exhibits the longest C terminus of the P2X subtypes and bears structural motifs that suggest that it serves as a docking site for intracellular protein interactions [39]. Moreover, a lipopolysaccharide (LPS)-binding motif has been reported in P2X7R that might serve as a modulator of its function and could explain the role of P2X7R in response to intracellular pathogens [40]. In this context, it has been shown that point mutations in the C terminus can alter or abolish the function of P2X7R [41].

Similar to the other P2X subtypes, the second transmembrane domain of P2X7R appears to line the ion pathway across the membrane [42]. Data on other ion channels show that the selectivity characteristics of a channel can be altered via changes in the residues in the ion permeation pathway or in adjacent regions, as demonstrated for P2X2R [43], 5-HT(3a)R [44] and nicotinic AchR [45]. Recent data show that mutations in the second transmembrane domain of P2X7R alter its selectivity properties [42]. For example, the mutations T348K, D352N and D352K cause mutant receptors to become more permeable to anions, revealing the importance of these residues for ion selectivity. Moreover, in that same study, the authors used cysteine-accessibility scans to determine the accessibility of residues G345 and T348, both of which are likely to be located along the ion permeation pathway. When biotin-linked methanethiosulfonate ethylammonium (MTSEA), a methyl thiosulfonate reagent with molecular dimensions of $0.75 \times 0.80 \times 1.85$ nm, was used in these assays, the ATP-evoked currents were diminished only when the cells were treated with ATP prior to treatment with biotin-linked MTSEA. This finding reveals that the dimensions of the ion permeation pathway at these residue positions when the receptor is activated are greater than the dimensions of biotin-linked MTSEA.

As mentioned previously, P2X7R shows two distinct functions: a low-conductance channel that allows the passage of small ions across the membrane and a high-conductance channel (large-pore channel) that allows the uptake of high-molecular-weight dyes. However, the mechanism underlying the second state remains unknown. The literature presents two hypotheses to explain how P2X7R shifts from a low- to a high-conductance state: In the first hypothesis, the channel formed by P2X7R is gradually opened upon activation; thus, increasing its conductance from ions to large molecules (up to 900 Da in macrophages), it can pass rather than just ions or small molecules, i.e., the pore dilates [46,47]. In the second hypothesis, the P2X7R (high-conductance state) is formed upon activation through the involvement of second messengers that may activate an independent pore-forming membrane protein [25,27]. Evidence for both of these hypotheses is found in the literature, as illustrated in Table 1. These proposals will be discussed in the next sections.

4. P2X7R as an intriguing ion channel

In 1967, Diamant and Kruger [48] were the first investigators to clearly demonstrate the extracellular actions of ATP in mast cells, where it triggers histamine release. The receptor activated by ATP was formally defined by Cockcroft and Gomperts [49] and designated P2Z by Soltoff [50].

Table 1
Hypothesis for the mechanism of the pore activation associated with P2X7R.

Cell type (P2X7R species)	Technique approaches	Reference	Results
Dilation hypothesis			
NTW8 mouse microglial (mouse)	Patch-clamp	[74]	1 mM ATP application reached a plateau — 300% increase over the current magnitude.
HEK-293 (rat)	Patch-clamp	[54]	The authors conclude that the maximum receptor activation causes an exponential dilation of the ion channel with a time constant of 25 s from an initial diameter of 0.8 nm to a final pore diameter of 3–5 nm.
HEK-293 (mouse, human and rat)	Large weight dye uptake and confocal imaging	[56]	Repeated 100 μM BzATP applications within 10 to 30 s stimulated increase in YO-PRO-1 accumulation suggesting a pore dilation.
THP-1 and HEK-293 (human)	Patch-clamp	[75]	ATP application promotes the dissociation of P2X7 from nonmuscle myosin in both cell types leading to large pore formation.
Hippocampal neuron–glia (rat)	Dye uptake and confocal imaging	[76]	BzATP application leads to cation channel function but it impairs the pore formations activity in mutant P2X7R (G345Y). It implicates that changes whithin P2X7R could alters directly the or formatin activity.
HEK-293 (rat)	Patch-clamp	[47]	After sustained application (40 s) of 100 μM BzATP the pore dilates reaching the permeability for organic cations independent of pannexin pharmacological block.
HEK-293 (human)	Patch-clamp	[60]	The authors developed a mathematical model suggesting that two agonist binding site occupancies lead to the channel activity while 3 occupied binding sites lead to the pore formation.
HEK-293 and GT1 (rat)	Patch-clamp	[77]	The authors also used a mathematical model that suggests that the number of agonist binding site occupancy determines the ion channel or pore function of P2X7 receptor as the same molecular entity.
HEK-293 (human)	Patch-clamp, cysteine accessibility and dye uptake	[42]	Some residues predicted to be in second transmembrane domain are accessible to large diameter methanosulfonate reagents and diminished the P2X7 dependent dye uptake
"Other protein" hypothesis			
Xenopus oocytes (rat)	Patch-clamp	[78]	Heterologously expressed P2X7 receptor did not produce pore forming activity.
HEK-293 (human)	Patch-clamp and dye uptake	[79]	C-terminal truncated P2X7 variant (P2X7B) fails to trigger membrane permeabilization although it keeps ATP-induced channel activity. It could suggest that some protein linked to C-terminal may be essential to non-selective pore formation.
Peritoneal macrophages (mouse)	Patch-clamp	[25,27]	Cationic channel and pore opening in the same patch in cell-attached and outside out approaches were not observed.
J774 (mouse), THP-1 (human) and HEK-293 (human)	Patch-clamp, and dye uptake assay	[81]	ATP induced dye uptake (not the ion channel activity) was inhibited in the absence or by the pharmacological block of pannexin. Pannexin co-immunoprecipitates with heterologously expressed P2X7.
2BH4 (mouse)	Dye uptake	[80]	In cell attached configuration, when ATP is applied in the bath, it may induce pore formation in the patch.
HEK-293 (mouse) and RAW 264.7 (mouse)	Dye uptake	[66]	The authors showed that there are two putative dye uptake pathways occurring in heterologously or naturally expressed P2X7 receptor. Negatively charged dyes were uptaken in a Ca^{2+} dependent manner and positively charged dyes were uptaken in a Ca^{++} independent manner.
HEK-293 (rat)	Patch-clamp	[58]	The authors showed that organic cation NMGD and the florescent dye YO-PRO use different mechanisms to enter the cell during prolonged ATP application in heterologously expressed rat P2X7 receptor.
Rat submandibular acinar cells	Dye uptake; measurement of the production of inositol phosphates; measurement of the concentration of protons	[155]	Propranolol inhibited the pore-forming activity of the P2X(7) receptor without impairing the opening of the small cation channel coupled to this receptor.
Xenopus oocytes (mouse)	Patch-clamp Ca^{2+} measurements	[156]	N,N-hexamethyleneamiloride (HMA) reduced the delayed component of the BzATP-induced influx ionic current. This delayed HMA-sensitive ionic current can be carried by large organic cations, such as NMDG+. In contrast, the rapidly activated HMA-insensitive current is carried by Na^+, Li′, and K′, but is poorly carried by NMDG+ and Tris+.
Xenopus oocytes (rat); fresh and cultured mice macrophages; HEK-293 (rat)	Patch-clamp; dye uptake; assay for nitric oxide (NO) receptor	[65]	Disrupting the microtubule network with colchicine did not affect currents generated by ATP in P2X2 and P2X7 receptor-expressing cells but inhibited uptake of the dye Yo-Pro-1 in *Xenopus* oocytes and HEK-293 cells expressing these channels. Peritoneal mouse macrophages showed less ATP-induced permeabilization to ethidium bromide in the presence of colchicine, and less reactive oxygen species (ROS) formation, nitric oxide (NO) and interleukin (IL)-1b release. Colchicine treatment did not affect ATP-evoked currents in macrophages.
HEK-293 cells (human)	Patch-clamp	[157]	HMA (5-(N,N-hexamethylene)-amiloride) was an effective antagonist at a concentration of 10 μM.
Chinese hamster ovary cells (K1 strain)	Dye uptake	[158]	Maitotoxin promoted ethidium bromide uptake and reduced by the calmodulin inhibitor W7, (N-(s-aminohexyl)-5-chloro-1-naphthalenesulfonamide) but unaffected by the ATP-P2X7 receptor antagonist oxidized ATP (adenosine 5-triphosphate periodate oxidized sodium salt) (oATP). BzATP induced ethidium bromide uptake and was inhibited by oATP, and unaffected by W7.

In Brazil, the first studies targeting P2X7R (referred to as the P2Z receptor at that time) were conducted during the early 1990s by Albuquerque et al. [51], who were working with polykaryon macrophages and macrophages. They were surprised to observe that a channel allowed the passage of molecules up to 900 kD. This work was continued by Coutinho-Silva and Persechini [27], who performed patch-

clamp experiments in a cell-attached configuration. At this time, P2X7R was shown to be activated by ATP (at millimolar concentrations) and to activate a high-conductance channel with a unitary conductance of approximately 400 pS in mouse macrophages; this channel was referred to as termed a Z pore (high-conductance channel) [27,52]. These investigators demonstrated that this high-conductance channel was voltage dependent and allowed the passage of currents mediated by large molecules, such as N-methyl-D-glucamine and glutamate. These Z pores were found to be blocked by oxidized ATP and Mg^{2+} and to be functional at temperatures above 30 °C. However, these high-conductance channels did not appear in excised patches, providing the first clue regarding the possible participation of cytoplasmic elements, such as second messengers and cytoskeletal proteins. Thus, to our knowledge, this research group was the first to propose a theory other than pore dilation.

To date, most investigators have only reported a current of approximately 10 pS using a cell-attached configuration [53]. However, some groups have attempted to divide the macroscopic current induced by ATP in the whole-cell configuration into two distinct conductances (or components) [54–56]. The first component has been described as the opening of a small channel, while the second component is related to the high-conductance channel associated with P2X7R. However, there is no conclusive evidence because there are no recordings of single channels whose unitary conductance is consistent with the high-conductance channel of this second component.

In 2005, Faria et al. [25] observed a unitary conductance of approximately 400 pS, consistent with the Z pores found in murine macrophages and 2BH4 cells when stimulating P2X7R with ATP or benzyl ATP (BzATP) in a cell-attached configuration. These authors observed a linear response for positive and negative holding potentials. However, these high-conductance channels were never observed in excised patches, suggesting a dependence on intracellular signals, as previously suggested by Coutinho-Silva and Persechini [27]. An interesting finding of Faria et al. was the difference in the temperature dependence of the 2BH4 cells, in which the Z pores open below 30 °C. The unitary conductance value of pore associated with P2X7R remains controversial because other investigators have been unable to find any channels whose unitary conductance is consistent with the passage of molecules up to 900 Da [57,61].

A paper published by Schachter et al. [58] described the transition from a low-conductance channel to one with a higher conductance. Macrophages and HEK-293 cells transfected with P2X7R were studied using patch-clamp recordings to evaluate pore formation. High-conductance channels were not detected in the transfected HEK-293 cells, but they were observed in mouse peritoneal macrophages. Using dye-uptake experiments, these authors also observed a differential uptake of cations and anions during endogenous P2X7R pore formation in macrophages. The anionic pathways appeared to be associated with the high-conductance channel, while the cationic pathway remained unidentified. This channel was investigated in depth based on previous data that reported the effect of increased intracellular Ca^{2+} levels and the opening of a pore that is biophysically similar to the P2X7R pore. Micromolar concentrations of calcium ionophores induced dye uptake and ionic currents, resulting in a unitary conductance of 400 pS in a cell-attached configuration. Furthermore, the pore opening was unaffected by P2X7R blockers, but intracellular signaling components were modified, as was the high-conductance P2X7R channel. In addition, this effect was not observed in excised patches. However, the element responsible for the intracellular Ca^{2+}-induced pore opening in mouse macrophages and 2BH4 cells is still unknown [59].

In 2010, Yan et al. [60] performed experiments to clarify how the occupation of the three ATP-binding sites might affect P2X7R gating. They showed that ATP concentrations in the millimolar range biphasically activated and deactivated native receptors, whereas micromolar concentrations induced monophasic responses. Both phases of these responses were abolished following the addition of Az10606120, a P2X7R-specific antagonist. The slow secondary growth of the current in the biphasic response coincided temporally with pore dilation. This pore current was insensitive to Na^{+} and Ca^{2+} influxes, and the initial gating properties were fully re-established after 30 min of washout. The complex pattern of gating exhibited by wild-type channels can be accounted for by a Markov state model that includes the negative cooperativity of agonist binding to unsensitized receptors when one or two binding sites are occupied; when two sites are bound, the channel pore opens to a low-conductance state. When three sites are occupied, a high-conductance state (pore dilation) is triggered. In contrast, Flittiger et al. [61] investigated the participation of protons in the activation of human P2X7Rs (hP2X7Rs). They observed that hP2X7R expressed in *Xenopus laevis* oocytes was activated by ATP or BzATP at different pH levels. While the unitary currents were found to be blocked by protonation, the high-conductance channel was not recorded.

Recently, Roger et al. [62] characterized the functional properties of the inward current activated by ATP in human and rat P2X7Rs that were transiently expressed in HEK-293 cells. They found that this current was facilitated in humans, and it was independent of Ca^{2+}/calmodulin. In contrast, in rats, the current was Ca^{2+}/calmodulin dependent. Some authors suggest that the second component of this inward current is responsible for pore formation (high-conductance channel) [60,62,63].

Two recent papers have used mutations to test the pore dilation theory; however, the results are not definitive, as the authors noted in the discussion sections of their papers. Sun and co-workers showed that the second transmembrane domain is necessary for pore formation because the dye uptake was reduced when the second transmembrane domain of P2X7R was replaced by the second transmembrane domain of P2X1R [64]. However, the surface expression was also reduced, suggesting that this reduction could be due to the decreased presence of the receptor at the membrane. In the other paper, Browne also proposed the direct involvement of the second transmembrane domain of P2X7R in the permeation pathway for high-molecular-weight dyes [42]. Using mutations in the second transmembrane domain and cysteine-accessibility experiments, the researchers showed that the mutant receptor could form (at least part of) the non-selective pore of P2X7R. Nevertheless, the previously mentioned issue of surface expression and reduced dye uptake could have affected the results. Moreover, the data require a more careful analysis. The dye-uptake curves appeared to be identical within the standard deviation. Similarly, we cannot discern a clear difference between the currents from the T348C mutant and the currents from the wild-type receptor in the presence of large methanethiosulfonate reagents. Thus, the entity responsible for the formation of the large pore remains unidentified despite the elegant experiments performed in these two papers.

5. Are other high-conductance channels associated with P2X7R?

As mentioned previously, there are some discrepancies in the literature regarding the high-conductance channel associated with P2X7R. The disagreements among several laboratories (see Table 1) regarding P2X7R pore formation (high-conductance channel) raise the following question: is there a single entity responsible for the P2X7R pore? The published data suggest the existence of more than one pore-formation mechanism, depending on the cell type, species or mode of cellular manipulation (for example, transfection) [58,65,66]. In addition, P2X7R may act through multiple “pore proteins” at the same time [67]. Our group [59] showed that calcium ionophores could greatly increase intracellular Ca^{2+} levels and induce dye uptake. Furthermore, we studied a pore with biophysical and pharmacological characteristics similar to the P2X7R pore. In addition, maitotoxin, which also triggers substantial intracellular Ca^{2+} entry, has been shown to induce pore opening and to enable the uptake of fluorescent dyes similarly to the P2XR pore [68]. Likewise, Cankurtaran-Sayar et al. recently demonstrated that RAW 264.7 cells and HEK-293 cells transfected with rat P2X7R take up cationic (propidium iodide, PI) and anionic (Lucifer yellow, LY) fluorescent

Table 2
Summary of membrane proteins sharing features that make them candidate P2X7 receptor associated pore by their biophysical properties.

Pore-forming protein	Unitary conductance (pS)	Agonists	Antagonists	Cutoff	Pore size (Å)	Activation in ischemic or hypoxic conditions	Compound Released or uptaken?	Reference
Maxi-anion	~400	ΔVm; Δ [NaCl]; tamoxifen, oremifene	Iberiotoxin; okadaic acid; 17beta-oestradiol; AMPc, Gd3Cl	~1000 Da	~30	Yes	Yes	[71,113,114,115]
Oncolitic cytolitic pore (COP)	N.D.	Maitotoxin; ionomycin	Glycine; L-alanine; general calcium channel blockers; calmidazolium	~900 Da	N.D.	N.D.	Yes	[116]
TRPV1 pore	N.D.	Capsaicin, resiniferatoxin, temperature >42 °C; pH < 6.0	Capsazepine; ruthenium red; SB366791	~900 Da	19	Yes	Yes	[119,151,153,154]
TRPA1 pore	N.D.	Allyl isothiocyanate	Ruthenium red; HC300031	~900 Da	16	Yes	Yes	[122,123]
Hemichannel	~90 (Cx32); ~220 (Cx43)	Low $[Ca^{2+}]_i$ and positive Vm; increase $[Ca^{2+}]_i$	High $[Ca^{2+}]_i$; negative Vm; carbenoxolone; heptanol; octanol	~1000 Da	10–15	Yes	Yes	[124,149,150]
Pannexin-1	~500	ΔVm	Carbenoxolone; flufenemic acid; RNAi	~1000 Da	17–21	Yes	Yes	[132,133,150]
Calcium homeostasis modulator 1	24	ΔVm and $[Ca^{2+}]_o$	Gd^{3+}; ruthenium red; Zn^{2+}; 2-APB	~1000 Da	14	Yes	N.D.	[134]
Maxi K^+ (BK_{Ca})	200–400	ΔVm and increase $[Ca^{2+}]_i$; NS-1619	Tetraethylammonium; iberiotoxin	N.D.	N.D.	Yes	Yes	[137]
Plasma membrane VDAC	~400	ΔVm	Polyanion Konig, okadaic acid	>1000 Da	25–30	N.D.	N.D.	[140–145,148]
Pore induced by raising intracellular Ca^{2+}	~400	ATP and calcium ionophores	Calmodulin; PLC and MAPK (p38) antagonists	~900 Da	N.D.	N.D.	N.D.	[80,146,147]
$P2X_7$	~400	ATP; BzATP; NAD; LL-37	Oxidized ATP; KN-62; BBG; HMA	~900 Da	8–40	Yes	Yes	[25,26,59,156]

dyes following stimulation with 1 mM ATP [66]. Moreover, HEK-293 cells exhibit a pathway that is permeable to cationic dyes in a Ca^{2+}-independent manner, while another pathway is permeable to anionic dyes in a Ca^{2+}-dependent manner. In contrast, RAW 264.7 cells are permeable to both dyes, and intracellular Ca^{2+} chelation only slightly reduces the effect induced by ATP. However, the addition of an extracellular saline solution without Ca^{2+} reduces LY uptake without interfering with PI uptake. Taken together, these results suggest the possibility that different channels are involved in P2X7R pore formation (high-conductance channel). We hypothesized that these other high-conductance channels may contribute to P2X7R pore formation in certain conditions depending on various factors, including the level of endogenous expression in a particular cell type, the species or the activation of the intracellular signaling pathways that regulate these channels. Moreover, there are several candidates for channels that allow the passage of low-molecular-weight dyes, such as LY and YO-PRO-1, through the formation of high-conductance channels whose conductances are generally greater than 200 pS and whose pore diameters range from 8 to 40 Å [7,25,59]. In mammalian cells, these candidates are the connexin hemichannels (Cx43 and Cx32), the pannexins (pannexin-1), the maxi-anion channel, the voltage-dependent anionic channel (VDAC), the maxi-K channel, the maitotoxin pore, the transient receptor potential vanilloid type-1 (TRPV1) pore, the transient receptor potential ankyrin type-1 (TRPA1) pore, the ATP-activated P2X pores (P2X2, P2X4 and P2X7R) and the calcium homeostasis modulator 1 (CALHM1) pore. The general characteristics of these channels are summarized in Table 2 and illustrated in Fig. 1. In addition, it is a well-established fact that the activation of P2X7R causes cellular swelling [69], a common event that activates most of the channels mentioned above [70–72]. This second possibility is illustrated in Fig. 2. At pharmacological concentrations (millimolar levels) in vitro, ATP can activate P2X7R, causing cellular swelling that can open the channel, as shown in Fig. 2. This process can initiate a positive feedback loop, increasing ATP secretion [152] as well as the intracellular passage of high-molecular-weight dyes from the extracellular side. Other researchers in the P2X7R field have also begun to notice the potential participation of other channels as conduits for dyes such as Yo-Pro-1, LY and PI [59,66,67,73,74].

6. Therapeutic potential of agonists and antagonists of P2X7R in different diseases

Since the seminal paper by Burnstock in 1972 [3] that established the class of purinergic receptors, only one medication, a P2Y12R antagonist, has been approved for clinical use, and this medication has shown great success at lowering the death rate associated with acute coronary syndrome [82]. Currently, this compound is third-generation acting through inhibition of platelet aggregation. For P2X7R, no such compound is used in clinical practice. Nevertheless, there are several phase I and II clinical trials aimed at evaluating the efficacy of P2X7R antagonists in treating various diseases, including chronic pulmonary conditions, rheumatoid arthritis and inflammatory bowel diseases (reviewed by Arulkumaran) [83]. Further research on P2X7R and its actions may reveal novel therapeutic targets for combating neurodegenerative conditions, and pre-clinical studies indicate that P2X7R antagonists may be used to treat neuro-inflammatory diseases, such as stroke, brain trauma, amyotrophic lateral sclerosis and multiple sclerosis. In initial studies, P2X7R knockout mice showed no general damage from excitotoxic or ischemic insults compared with wild-type mice [84] but did show decreased inflammatory and neuropathic pain with unaltered, normal nociception [11,85]. More recent data suggest an important role for P2X7R in calcium-dependent microglial cell death induced by stroke-like conditions during oxygen–glucose deprivation [86].

The inhibition of ATP receptors (P2X7R or P2X4R), downstream signaling pathways, chemokines and proinflammatory cytokines should

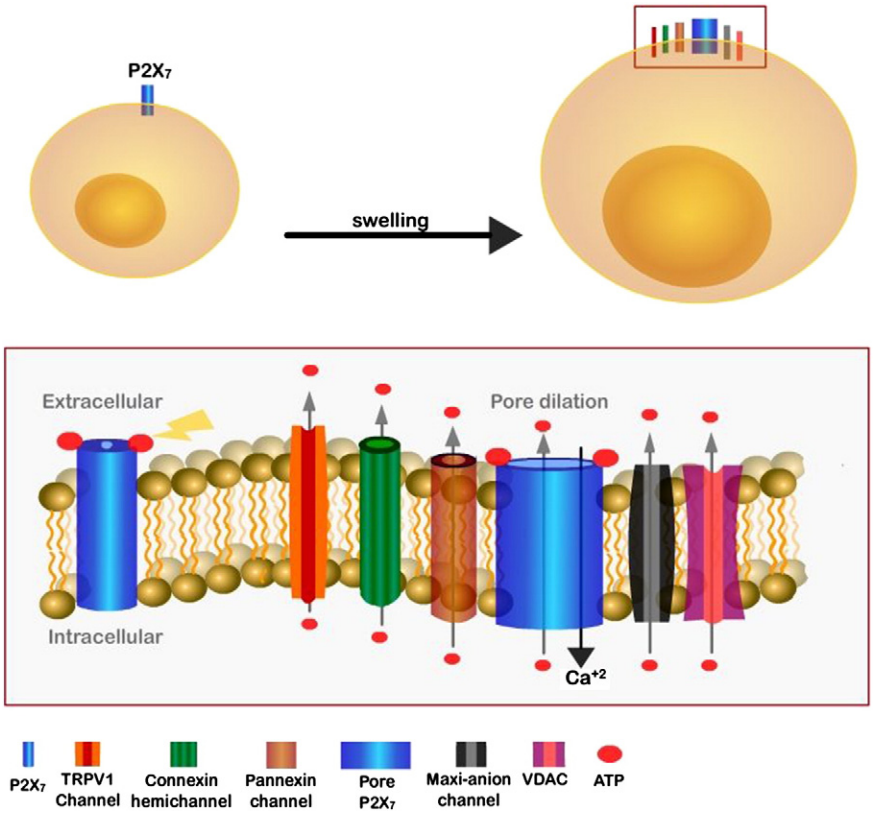

Fig. 1. Cellular swelling after P2X7R activation at pharmacological concentrations (millimolar range) of ATP. This scheme shows channels that are opened by cellular swelling. Some channels, such as maxi-anion channels, are selective for anions; TRPR-V1 is selective for cations, and connexins and pannexins show low selectivity.

contribute to novel therapies for chronic pain [87,88]. Microglial P2X7R and its downstream signaling pathways play a pivotal role in the induction of spinal long-term potentiation (LTP) and persistent pain [89]. It has been shown that P2X7R has a role in the onset and persistence of neuropathic pain and that spinal P2X7R mediates microglia activation [90].

A commonality among all of these diseases is an inflammatory component. In this regard, P2X7R is a key element because it activates the inflammasome, a multiprotein oligomeric complex composed of NALP3 (a type of NOD-like receptor), caspase 1 and apoptosis-associated speck-like protein containing a carboxy-terminal caspase activation and recruitment domain (CARD) (ASC, an adaptor protein),

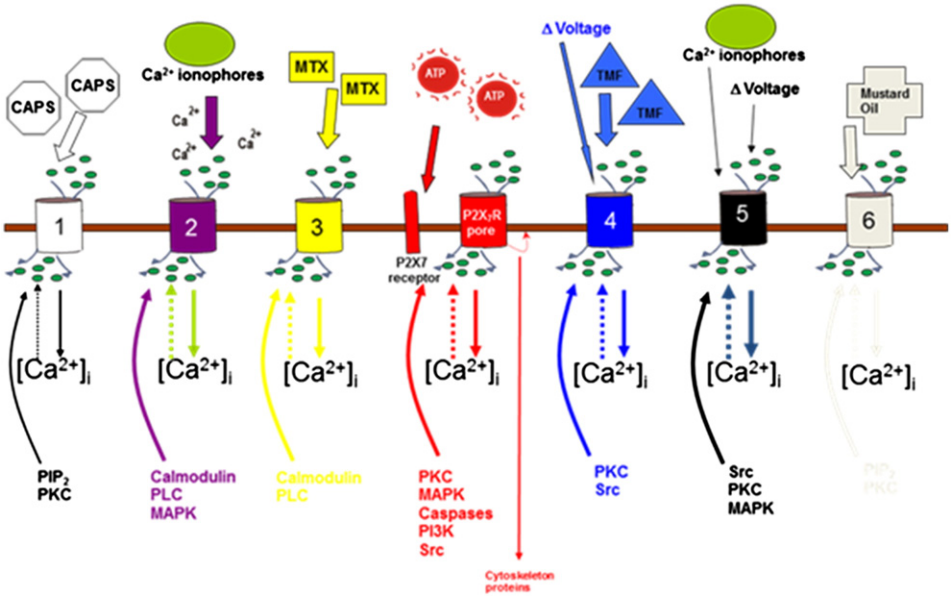

Fig. 2. Signaling pathways of high-conductance channels. This scheme shows the intracellular pathways related to regulation of the high-conductance channels along with the biophysical, pharmacological or functional properties of the P2X7R-associated pore. The scheme illustrates the following types of channels: TRPV (1); the pore induced by increased intracellular Ca^{2+} levels (2); the maitotoxin-induced pore (3); the maxi-anion channel (4); connexin and pannexin-1 hemichannels or the calcium homeostasis modulator 1 (CALHM1) (5); and the TRPA1 pore (6).

in association with other signaling pathways. These components are key players in the release or regulation of several pro-inflammatory mediators, including interleukin-1 (IL-1)-beta, IL-8, tumor necrosis factor (TNF)-α, nitric oxide (NO) and reactive oxygen species (ROS) [91]. Thus, P2X7R antagonists have become a pharmacological target for several pharmaceutical companies. Recently, several groups have provided evidence that P2X7R antagonists could be used to treat gouty arthritis. In support of this idea, the inflammasome activation by uric acid particles has been shown to depend on P2X7R activation [92]. Furthermore, it has been demonstrated that colchicine may also act through P2X7R inhibition [65]. An elegant discussion of this hypothesis for a P2X7R-based treatment of gouty arthritis has been published by Tao and coworkers [93].

The role of P2X7R in transplantation has also been studied; preclinical studies with mice and human samples have revealed that an antagonist of P2X7R, oxidized ATP, increases islet cell and heart transplantation survival. These studies have demonstrated an upregulation of P2X7R expression in graft-infiltrating T cells [94,95].

Cancer also provides a possible target for P2X7R agonists (or in some cases, antagonists) [99]. Several types of tumor cells express high levels of P2X7R [96,97]. There has been some debate regarding whether P2X7R activation would induce apoptosis in cancer cells or behave as a growth factor [75,98,100]. As suggested by Di Virgilio, this discrepancy could be related to the ATP concentration used, i.e., a low concentration might induce cellular growth, while high concentrations (mM range) would induce cellular death [97]. Indeed, P2X7R expression has been correlated with the progression of glioma and particularly with resistance to ATP-induced cell death. Human U-138 MG and U-251 MG glioma cells have been shown to be resistant to death when treated with either ATP (5 mM) or BzATP (100 μM), whereas the cell viability of radiosensitive M059J glioma cells significantly decreases under these conditions [100]. In addition, stimulation of P2X7R can increase the antigen presentation and secretion of IL-1-beta by dendritic cells; this result suggests a role for P2X7R in the immune response against tumor cells [101]. A link has also been demonstrated between statins, cholesterol-lowering drugs and cancer protection in different types of cells (lung, pancreatic and prostatic). Statins, or extracellular ATP, induce a complex response in insulin-stimulated A549 cells, leading to depletion of nuclear pAkt [102]. Recently, extracellular ATP-induced invasive growth in PTEN-positive cells was counteracted by statins via P2X7R [103]. This work reported that P2X7 signaling involves Eps15 homology (EH) domain-binding protein 1 (EHBP1), which has previously been shown to be associated with aggressive prostate cancer and insulin-stimulated trafficking and cell migration. A complex is formed between EHBP1 and P-Rex1, a guanine nucleotide exchange factor, and this complex is involved in the anti-invasive effect of atorvastatin [103]; thus, the study showed that statins decrease the levels of phosphorylated Akt. Another possible target for P2X7R agonists is tuberculosis (TB), which is a chronic infectious condition caused by *Mycobacterium tuberculosis* (MTB), an intracellular pathogen. This disease constitutes a global health problem, and it is estimated that one-third of the world's population is infected with MTB. Only approximately 5–10% of infected individuals will develop the clinical disease [104,105]. Macrophages act as the major host cells for intracellular mycobacterial replication and are also responsible for regulating the growth and viability of this pathogen [106]. In 1997, Lammas et al. showed that ATP could induce the killing of mycobacteria by human macrophages, suggesting that the P2X7R pathway might mediate the control of *M. tuberculosis* infection in humans [107]. Since the publication of that milestone paper, several groups have shown that P2X7R activation induces apoptosis and that infected macrophages interfere with certain MTB escape mechanisms, leading to mycobacterial death [112]. In addition, several polymorphisms in P2X7R have been associated with tuberculosis in various ethnic groups [108]. A recent meta-analysis found that 1513A/C mutations in P2X7R are associated with increased susceptibility to tuberculosis [111]. Thus, ATP agonists may function as chemotherapeutic drugs in association with the common antibiotics used to treat tuberculosis. Other intracellular pathogens might also be potential targets for P2X7R agonists [100].

One possibility that appears to have been overlooked by pharmaceutical companies is the potential for drug delivery using the high-conductance channel of P2X7R to increase the passage of hydrophilic drugs into the cytoplasm of cells expressing the P2X7R pore. Thus, the generation of a P2X7R agonist that would selectively stimulate the high-conductance channel and not the other signaling pathways associated with this receptor could lead to beneficial results. Indeed, different structural ligands for many specific G protein-coupled receptors are able to bind to different conformational states that are responsible for the differential activation of signaling cascades [113].

7. Concluding remarks

Since the first formal definition of P2X7R as an ATP receptor in mast cells by Cockcroft and Gomperts [49], several articles have been published on the role of P2X7R in different cell types in an attempt to elucidate the mechanisms associated with the high-conductance channel (pore) that is responsible for the passage of dyes. Evidence has been presented that supports two distinct theories of pore formation, leading to disagreements among several laboratories. The divergence of these findings may be related to the differences in the particular cell type studied and/or the different signaling mechanisms associated with pore formation. The activation of other channels with the same cut-off as the P2X7R-associated pore should also be considered, as illustrated in Table 1. Despite the great efforts exerted in this exciting research area, the pore-formation mechanism continues to be an enigmatic phenomenon that must be deciphered to permit the rational use and synthesis of novel agonists and antagonists of P2X7R.

Acknowledgements

The authors would like to thank the groups that have been involved in the projects related to this review as well as their collaborators in this field. The projects related to this review are supported by the Instituto Oswaldo Cruz-Fundação Oswaldo Cruz, Rio de Janeiro, Brasil, the Conselho Nacional de Desenvolvimento Científico e Tecnológico and the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, Brasil. RAMR is supported by CNPq (INNT) and FAPERJ.

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