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Review

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

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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.

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 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

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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 P2X7 receptor

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 × 0.80 × 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. P2X7 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].

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,

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