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Review

Interactions of pannexin 1 with NMDA and P2X7 receptors in central nervous system pathologies: Possible role on chronic pain

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ABSTRACT

Pannexin 1 (Panx1) is a glycoprotein that acts as a membrane channel in a wide variety of tissues in mammals. In the central nervous system (CNS) Panx1 is expressed in neurons, astrocytes and microglia, participating in the pathophysiology of some CNS diseases, such as epilepsy, anoxic depolarization after stroke and neuroinflammation. In these conditions Panx1 acts as an important modulator of the neuroinflammatory response, by secreting ATP, by interacting with the P2X7 receptor (P2X7R), and as an amplifier of NMDA receptor (NMDAR) currents, particularly in conditions of pathological neuronal hyperexcitability. Here, we briefly reviewed the current evidences that support the interaction of Panx1 with NMDAR and P2X7R in pathological contexts of the CNS, with special focus in recent data supporting that Panx1 is involved in chronic pain signaling by interacting with NMDAR in neurons and with P2X7R in glia. The participation of Panx1 in chronic pain constitutes a novel topic for research in the field of clinical neurosciences and a potential target for pharmacological interventions in chronic pain.

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1. Introduction

Pannexin 1 (Panx1) is a membrane channel, abundantly expressed in the central nervous system (CNS) of mammals in all cell types (microglia, astrocytes, oligodendrocytes and neurons) [1]. Specifically, Panx1 transcript has been found in cerebellum, cortex, retina and cerebral cortex; in hippocampus, amygdala, substantia nigra, olfactory bulb and spinal cord, among other neural structures [2]. This protein functions as a *bona fide* membrane channel, with high non selective conductance to small molecules (1.5 kDa) [3], high permeability to ATP, Ca^{+2}, glutamate and some inflammatory mediators, and can be activated by several mechanisms, such as mechanical stimulation, increases of extracellular K^+, proteolytic cleavage of its C-terminus, raising of the intracellular Ca^{+2}, and several intracellular signaling [4].

Understanding the physiological and pathological role of Panx1 is fundamental, because this high conductance channel has the particular property of potentiating the activity of some ligand-gated receptors of the CNS in pathological conditions, such as neuroinflammation, anoxic depolarization, stroke, neuronal death, seizure, among others [5]. Thus, in this review we highlighted diverse aspects of Panx1 in CNS, and its interactions with *N*-methyl-*D*-aspartate receptor (NMDAR) and the ionotropic purinergic P2X7 receptor (P2X7R), two of the main components involved in a wide variety of CNS diseases. Furthermore, we review the available evidence of the participation of Panx1 in chronic pain, focusing the discussion toward the potential role of Panx1 in chronic pain signaling via interactions with NMDAR and/or P2X7R.

1.1. Panx1 characteristics

Panx1 is a glycoprotein that belongs to the family of the pannexin membrane channels expressed in diverse cell types in chordates [2]. This protein has three isoforms, Panx1, Panx2 and Panx3, with different structure and function [6]. In the year 2000, Panchin and colleagues identified in the mammalian genome a homolog sequence of innexins, the gap junction protein in invertebrates, by performing BLASTP and PSI-BLAST searches against GenBank using Inx sequences [7], being initially classified as part of the family of the gap junctions [8]. Due to this phylogenic argument, many authors currently keep referring to pannexins as "hemichannels", arguing the possibility of this protein to bond to another pannexin and form gap junctions. However, although several studies have shown structural similarity of the innexins with pannexins (reviewed by Dahl and Muller [9]), confirming that both proteins belong to the same family [8,11], there is consistent evidence that demonstrate that these proteins are functional membranes channels and do not act as an intercellular channel in appositional membranes like connexins does [11,12]. Several arguments support this idea: (a) Panx1 channels expressed in individual cells, such as erythrocytes, do not form gap junctions [12]; (b) Panx1 are located exclusively at the apical membrane of polarized cells, such as epithelial cells of the airways [13]; (c) in neurons, Panx1 are distributed asymmetrically in synapses only at the postsynaptic density, in co-localization and co-expression with the postsynaptic membrane protein PSD95 [14]; (d) pannexins, including Panx1, are glycoproteins and they have a N-glycosylation site which would prevent the coupling of these channels by steric hindrance [15,16].

1.2. Structure

The three-dimensional structure of Panx1 remains unclear. However, due to the gross structural similarities with connexin and innexins, it is predicted that Panx1 has a hexameric conformation, with four transmembrane segments, two extracellular loops and one intracellular, with its N and C terminal at the intracellular space [7,10,15,17]. Particularly interesting is the N-glycosylation site at cysteine 254 of the second extracellular loop, which has a role in the trafficking of the protein to the membrane, and would prevent the binding of a Panx1 to another to form a gap junction [11]. Using substituted-cysteine accessibility method and electron microscopy of Panx1 pore structure, it has been found that residues from the N-terminus, from the first transmembrane domain, and from the extreme of the C-terminus, constitute the hydrophilic pore lining [18], with a pore diameter estimated at a size of ~7–21 Å [19].

1.3. Expression

In humans, Panx1 is expressed in a wide variety of tissues: virtually at the entire digestive system, in skeletal muscle, heart, endothelium, skin, among others, initially identified by Northern blotting [8]. In central nervous system, Panx1 has been localized in microglia, astrocytes, oligodendrocytes and neurons [20,21] in regions such as cerebellum, cortex, retina, cerebral cortex; in hippocampus, amygdala, substantia nigra, olfactory bulb, and spinal cord [6,21–24].

1.4. Biophysics

Using patch clamp single channel recording in hippocampal neurons, the conductance of Panx1 was 527pS in a protocol of oxygen-glucose deprivation [25,26]. In isolated hippocampal neurons, current–voltage (IV) relationship is linear in ramps of ±80 mV [27]. During ischemia Panx1 looses its rectification leading to an increase of the time-dependent currents [25], thus supporting that Panx1 is in fact a membrane channel with a variable voltage-dependent conductance.

1.5. Mechanisms of Panx1 channel activation in the CNS

Under resting conditions, Panx1 remains closed to prevent loss of the electrochemical gradients across the plasma membrane and responds to changes in voltage, as previously mentioned. At resting potentials, the channel open probability is very low. However, at positive potential, Panx1 shows high conductance in neurons [28]. Besides the voltage dependence to open the channel, there are at least five physiological Panx1 opening mechanisms in the CNS.

1.5.1. Mechanical stimulation

While there are descriptions in the literature about activation of Panx1 in the CNS by the action of mechanical forces [26], this feature has been described in different cell types (erythrocytes, capillary endothelium, etc.) as a channel opening mechanism on hypotonic stress conditions or during stretching the cell. Although it has not been demonstrated in the CNS, it could potentially be a mechanism of response in traumatic injury of the CNS [29].

1.5.2. Increases in the extracellular concentration of K^+

This opening mechanism has been poorly researched despite that it is an interesting approach to investigate the role of