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Mechanisms of pannexin1 channel gating and regulation☆

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ABSTRACT

Pannexins are a family of integral membrane proteins with distinct post-translational modifications, sub-cellular localization and tissue distribution. Panx1 is the most studied and best-characterized isoform of this gene family. The ubiquitous expression, as well as its function as a major ATP release and nucleotide permeation channel, makes Panx1 a primary candidate for participating in the pathophysiology of CNS disorders. While many investigations revolve around Panx1 functions in health and disease, more recently, details started emerging about mechanisms that control Panx1 channel activity. These advancements in Panx1 biology have revealed that beyond its classical role as an unopposed plasma membrane channel, it participates in alternative pathways involving multiple intracellular compartments, protein complexes and a myriad of extracellular participants. Here, we review recent progress in our understanding of Panx1 at the center of these pathways, highlighting its modulation in a context specific manner. This article is part of a Special Issue entitled: Gap Junction Proteins edited by Jean Claude Herve

1. Introduction

The pannexin (Panx) genes (Panx, Greek: *pan* = complete, everywhere and *nexus* = junction) were initially described as a second family of gap junction proteins in vertebrates, sharing a predicted topology similar to connexins [1]. However, the sequence homology to vertebrate connexins is negligible. As a result, pannexins soon became known as an 'independent' family of three glycosylated integral membrane proteins (Panx1, Panx2, and Panx3) [2,3].

Today, Panx1 is the best-characterized family member, and at the center of this review. It is widely accepted that Panx1 functions as an unopposed, large pore single membrane channel in vivo, regulated by post-translational modifications, sub-cellular localization and tissue distribution [4–7].

A hallmark of Panx1 is the almost ubiquitous expression in many organs as well as in several cell types of both the blood and immune system [8–14]. Initial reports suggested that in the CNS Panx1 largely colocalizes with Panx2 [3,15–17], where the expression is mainly neuronal [15,18,19], and coexpression of Panx1 and Panx3 have been found in skin, osteoblasts and specialized cartilage [7]. However, even though two or more pannexin members are coexpressed, it is unknown whether they intermix and whether channel functions alter as a result of intermixing. The initial evidence was suggesting that Panx2 cell surface distribution increases when it is coexpressed with Panx1, compromising channel function [6]. This view is challenged by more recent findings showing intracellular localization of Panx2 [20,21]. Additional evidence for glial expression of Panx1 was found in cultured astrocytes, oligodendrocytes, and microglia [22–24]. Further, Panx1 expression has also been described in major sensory systems including the eye, inner ear, taste buds, and the olfactory epithelium [3,25–29]. Meanwhile, Panx2, originally thought to be restricted to the central nervous system, is also expressed in the eye, thyroid, kidney, liver, and enteric nervous system, with highest expression levels in the brain and spinal cord [2,3,9,16,18,21]. Panx3 is mainly expressed in the skin and cartilage, including osteoblasts and synovial fibroblasts, but can also be found in heart ventricles, cochlea, lungs, kidney, thymus, liver, spleen and possibly astrocytes [3,7,8,28].

As the field continues to grow, different lines of evidence suggest that Panx1 is a major molecular hub with multiple functions. Panx1 function has been implicated in the regulation of the inflammasome, apoptosis, cellular migration, pain, epilepsy, ischemia, as well as learning and memory, to name a few. However, it has yet to be identified why Panx1 seemingly acts in physiological and pathophysiological contexts both harmfully and therapeutically. The objective of this review is to provide an overview of regulation of Panx1 channels. By doing this, we hope to address some of the existing conflicts in the field and provide a tool to understand and access context specific information about Panx1 channel regulation.

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2. Regulation of pannexin1

The study of Panx1 channels remains controversial, but protocols for both activation and inhibition have reached some agreement that elevated extracellular K^+, increased intracellular Ca^{2+}, ATP, stretch and mechanical stimulation, voltage, redox regulation, s-nitrosylation, nitric oxide (NO), NMDA stimulation, chemokines, changes in extracellular pH, interaction with purinergic and adenosine receptors, or caspases play a role in the process [11,30–40]. It is important to note that experimental stimulation protocols are widely variable. Not surprisingly, results and reproducibility are inconsistent between laboratories and can lack a mechanistic explanation of channel properties. Despite many challenges, the combination of molecular and cell biological techniques, along with electrophysiology and pharmacology, or modeling in transgenic animals, continue to provide the most compelling way to characterize Panx1 channel activity from cells to organisms.

3. Panx1 activation by voltage

Biophysical properties of Panx1 channels of human, rodent or fish origin demonstrate large-conductance channels with unitary conductance in the range of 300 pS to 500 pS, using *Xenopus laevis* oocytes [11,30,32,41–43]. One study suggests that it is the mode of stimulation that affects the open channel conformation: stimulation via potassium ions promotes a high-conductance (~500 pS) channel permeable to ATP (see Fig. 1, #9), whereas much lower conductance (~50 pS) conformations are present during voltage activation and are impermeable to ATP [44] (see Fig. 1, #3). The nature of certain channel conductance sizes remains uncertain. However, single channel property differences may be due to the use of different heterologous expression systems, although distinct properties have also been observed in the same cellular environment [43]. For instance, Ma et al. [45] recorded a 68 pS channel in mouse Panx1-transfected HEK293 cells that was anion selective and sensitive to the inhibitor carbenoxolone, a Panx1 blocker widely considered as having limited specificity. This result is difficult to reconcile with other work showing positively and negatively charged dyes permeating Panx1, in addition to the considerable lack of substantiating evidence with Panx1-specific blockers [11,32,35,43].

Recent work is addressing some of the conflicts noticed above and providing a framework for future work resolving the structure of the Panx1 channel and aligning structural changes with the gating mechanism [46]. Using human PANX1, Chiu et al. [46] demonstrate that a fully activated channel conformation is reached as a result of sequential removal of the carboxy-terminal (CT) tail from individual subunits in hexameric PANX1 channels. This process is causing a stepwise increase in both unitary conductance and channel activity (PO), which is accompanied by parallel increases in dye uptake and ATP release in both irreversible (caspase cleavage-mediated) and reversible (a1 adrenoceptor-mediated) forms of channel activation. This activation process is consistent with structural rearrangements of the PANX1 channel pore, suggesting that each step imparts distinct characteristics on the open conformation, controlling a common gate that coordinately regulates cell permeation of both small ions and large molecules to allow 'tunable' control of cell function and signaling [46].

4. Panx1 activation by potassium

Potassium (K^+) ions are a prime candidate of Panx1 channel gating. Constitutively activated K^+ conductances are a major determinant in setting resting membrane potentials [47]. Extracellular K^+ accumulation at concentrations near 100 mM, and accompanying changes to $[K^+]_e$ result in significant membrane depolarization, which is known to open Panx1 channels (see review) [48] (see Fig. 1, #9). However, K^+ induced pannexon activation is not an effect of the membrane deporalization as judged by the voltage dependence of pannexins [49]. Therefore, the opening mechanism of Panx1 after exposure to elevated concentrations of extracellular K^+ requires further investigation despite the experimental application of various K^+ constituents used to trigger Panx1 activation experimentally, including reports using ~50 mM KCl and potassium gluconate in the 100–150 mM range (see review) [50].

5. Panx1 activation by calcium

The initiation of intracellular Ca^{2+} release from ER stores occurs when extracellular ATP increases and binds to purinergic receptors, allowing the increase in inositol 1,4,5-triphosphate (IP_3) and Ca^{2+} release [51]. When intracellular Ca^{2+} levels increase, Panx1 channels open and release ATP, which acts as the diffusible agent that mediates the spread of locally raised levels of Ca^{2+} between cells that are not in physical contact (see Fig. 1, #7). In astrocytes, the feedback mechanism involving Ca^{2+} and ATP plays a key role in intracellular Ca^{2+} propagation to neighboring cells [52] (see Fig. 1, #4). Research by Pelegrin and Surprenant [35] supports Panx1 playing an integral part in the Ca^{2+} signaling, based upon its main function as an ATP release channel and the fact that it is a key interacting partner with P2XRs. Also, Kurtenbach et al. [39] conducted dye uptake experiments in neuroblastoma cells providing compelling evidence of Panx1 acting as a channel responsive to intracellular Ca^{2+} changes. Although Panx1 does not have known calcium-binding sites, this [Ca2 +]i mediated opening of Panx1 has been described in the CNS, specifically in microglia and astrocytes [53].

6. Activation by mechanical stimulation

At the cellular level, translating mechanical information is relevant in the context of many physiological and pathological conditions, including, but not limited to changes in osmolarity as well as physiological ATP release used to transduce mechanical into chemical signals, or as a result of traumatic brain injury [54]. The mechanosensitive release of ATP from astrocytes and neurons [55], due to mechanical prodding [56,57], swelling [58] or shear stress uses vesicular and non-vesicular release mechanisms [59]. Using oocytes, Bao et al. [30] demonstrated with negative pressure during electrophysiological recordings that Panx1 channels are sensitive to mechanical stimulation. Since then, Panx1 mechanosensitive responses have been shown in neurons and retinal ganglion cells [54]. Other cell types expressing Panx1 are likely to use this property as a mode for signaling processes. Such cells include erythrocytes [11], cells of the capillary endothelium [60], cardiomyocytes and cardiac fibroblasts [61], afferent and efferent neurons for pain perception, and skeletal [62,63] or smooth muscle cells [64]. Furthermore, mechanical stress can activate programmed cell death, but the link between mechanical strain and neuronal cell loss is not fully understood. Typically, the relevance of Panx1's mechanosensitivity has been characterized in the context of ATP release. Furthermore, using electrophysiology, mechanical forces have also been demonstrated in promoting the large conductance state of Panx1 [44] (see Fig. 1, #8).

However, all of the above-summarized evidence for activation by mechanical stimulation has to be critically weighted against the possible involvement of other channels. Potential candidates are LRRC8/SWELL channels, which are considered sharing a common ancestor with Panx1 [65]. These channels are part of the volume-regulated anion channel (VRAC) and can play a role in maintaining cell volume in response to osmotic challenges [66]. Interestingly, LRRC8/SWELL channels share significant properties with Panx1, including subcellular localization, expression in both the nervous and immune system, as well as carbenoxolone-sensitivity and possibly release of ATP [65,67].

7. Activation by the carboxy-terminal tail and interactions with caspase proteins

In non-apoptotic cells, Panx1 activation can occur in the absence of a carboxy-terminal (CT) cleavage. In contrast, caspase-mediated

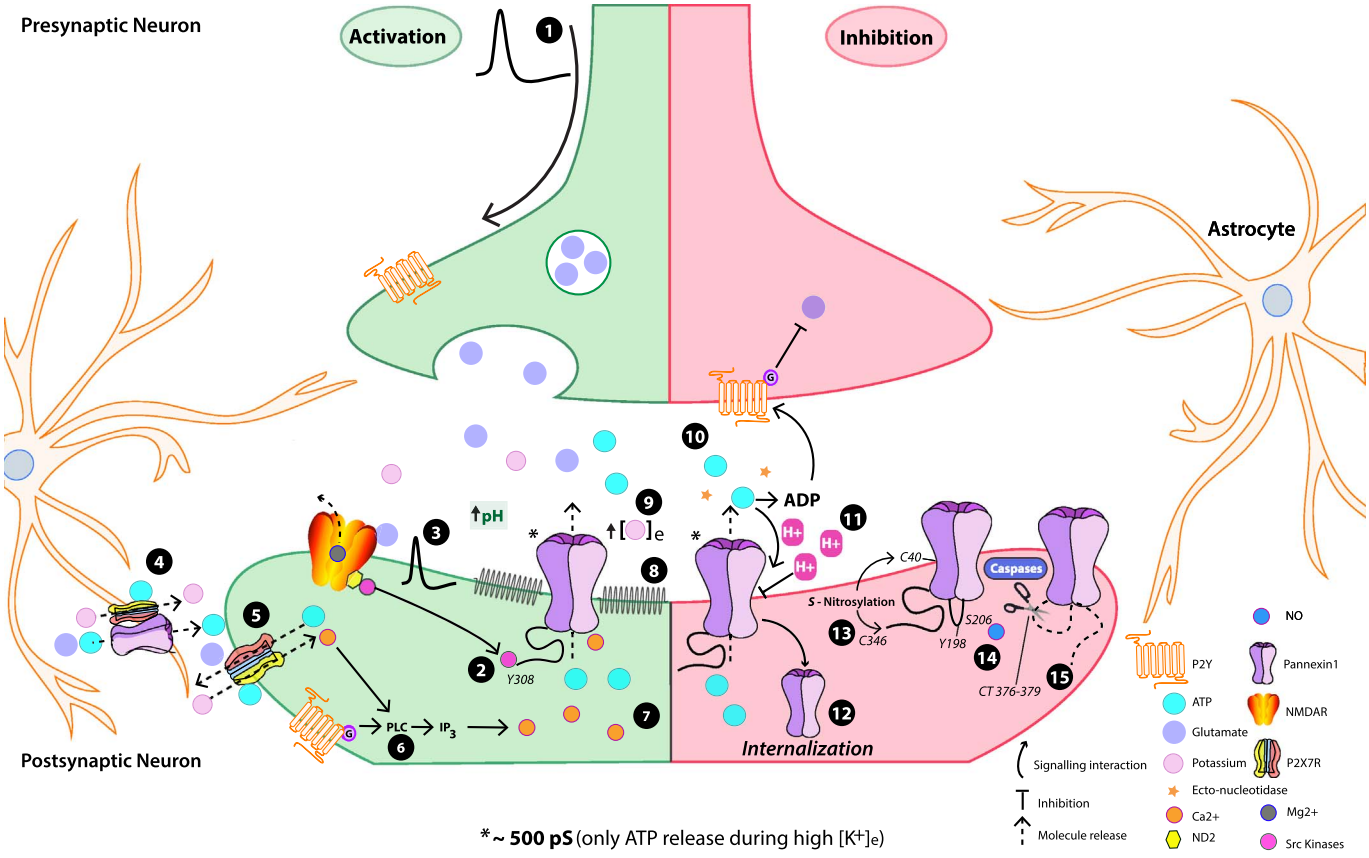

Fig. 1. Schematic diagram of the complex gating dynamics involved in inhibition and activation of the pannexin1 channel. Numbers 1–15 refer to modes of Panx1 channel activities described in the text. Modes of Panx1 activation are indicated in the green portion of the figure. (1) Presynaptic depolarization leading to vesicular glutamate release, activation of NMDARs and interactions with Src Family Kinases. (2) Src kinases phosphorylate Panx1 at residue Y308 on the CT-tail. (3) Voltage activation of Panx1. (4) Glutamate, Ca^{2+} and ATP released from P2X7 receptors on astrocytes can activate Panx1, P2X7 and Panx1 coupling has been suggested. (5) P2X7-mediated Panx1 activation is identified, however, the exact mechanism remains to be elucidated. (6) ATP activates metabotropic P2Y receptors and ionotropic P2X receptors to evoke a calcium wave or ATP release respectively. (7) Extracellular ATP increases and binds to purinergic receptors, increasing inositol 1,4,5-triphosphate (IP_3) and intracellular Ca^{2+} release, opening Panx1 channels. (8) Panx1 is sensitive to mechanical stimulation and stretch. (9) Extracellular K^+ accumulation at concentrations near 100 mM, and experimental modes of K^+ stimulation, can activate Panx1. Modes of Panx1 inhibition are highlighted within the red portion of the figure. (10) Millimolar concentrations of ATP inhibit Panx1 channels. (11) Metabolic ATP break down to ADP inhibits presynaptic glutamate release, and generates protons to decrease pH and inhibit Panx1. Conversely, increased pH levels can activate the channel. (12) Prolonged release and high levels of ATP can stimulate Panx1 to internalize to endosomal compartments. (13) S-nitrosylation of C40 and C346 can inhibit Panx1. (14) Phosphorylation of Ser-206 can inhibit Panx1 channel function in the context of NO rich tissues. (15) The CT-tail of Panx1 has been implicated in both the activation and inhibition of Panx1. A 'ball-and-chain' model has been proposed to inhibit Panx1 opening, however, caspase mediated cleavage at residues 376–379 causes sustained, powerful opening of Panx1. In addition, increased number of cleaved CT-tails can lead to increased Panx1 conductance.

proteolytic cleavage of the C-terminus allows dissociation of the auto-inhibitory region from the pore to activate the channel in apoptotic T-lymphocytes [68] and macrophages [37]. Using a CT-truncated human Panx1, which rendered the channel constitutively active, substantiated the relevance of the CT region in Panx1 gating. This line of investigation also brought to light the importance of the interaction with the caspases 3, 7 and 11 [69,70]. Typically, caspase 3, along with caspase 7, has been found to irreversibly activate the channel during apoptosis [68,71], binding to amino acid positions 376–380 (DVVDG) or 375–379 (DIIDG) in human and mouse Panx1 (see Fig. 1, #15). More recently, cleavage-based Panx1 activation of the same region via caspase-11 has been observed during lipopolysaccharide-induced pyroptosis, indicating that the catalytic activity of caspase-11 is required for cleavage of Panx1 to induce ATP release and P2X7-mediated pyroptosis [72].

The demonstration of a caspase-based cleavage mechanism leading to activation raised the question, how many Panx1 CT-tails must be cleaved to achieve full activation, and whether stepwise cleavage might explain sub-conductance states of Panx1 channels. Recent research demonstrated that progressive CT-tail cleavage leads to stepwise increases in Panx1 channel conductance, with full activation of the channel demonstrating an outward ~100 pS conductance [46]. A similar mode of stepwise, quantized progression activation has also been observed with alpha-1 adrenoreceptor mediated Panx1 activation. However, this mode is reversible and has a shorter mean open time [46].

How do conformational changes correspond with channel activity? Regarding mechanistic insight, the CT-tail has been proposed to block and inhibit Panx1 via a 'ball-and-chain' type mechanism, similar to what occurs in voltage-gated channels [73] and connexins [74,75]. This mechanism has been proposed based upon studies demonstrating cysteine-mediated cross-linking of the CT-tail interfering with cleavage-mediated activation, as well as inhibiting cleavage activated channels with the purified CT domain [68]. Amino acid residues between positions 379 to 391 appear to be required for maintaining a basal inhibition state of Panx1 [76]. Together, this highlights a particular functional role of the intrinsic Panx1 CT-tail as a region targeted for inhibition or activation of the Panx1 channel that restrains channel gating (see Fig. 1, #15).

8. Posttranslational modifications and regulation of Panx1 channels

Post-translational modifications have proven to be vital in the function of Panx1. N-glycosylation in the second extracellular loop of

Panx1 (Arg-254) is important for trafficking to the membrane, intermixing with other pannexins, preventing docking as gap junction-like structures, and allowing functional Panx1 channel expression in the membrane [5–7,77]. While important, an impact of glycosylation on Panx1 channel regulation is indirect. However, it is worth noting that Panx1 was initially thought to be a gap junction protein based on biophysical properties recorded after expression in the *X. laevis* oocyte model [3,41]. More recently, Sahu et al. [78] reported gap junction formation in HeLa cells, but not in Neuro2a or PC-12 cells. This suggests that Panx1 is capable of forming functional gap junctions in a cell-specific manner and only in its non-glycosylated form.

Recent evidence points to the importance of the reversible post-translational modification on cysteines by S-nitrosylation for protein function regulation in Panx1 expressing HEK 293T cells and mouse aortic endothelial cells [33]. S-nitrosylation of Panx1 was found initially in hippocampal neurons after oxygen-glucose deprivation, causing activation of the channel [79]. Conflicting evidence emerged when inhibitory roles of S-nitrosylation on Panx1 channels were found. Early studies, utilizing site-directed mutagenesis for investigating specific cysteine residues involved in S-nitrosylation, identified a zebrafish Panx1 mutation at Cys-282 that reduced channel function [80]. Further investigations identified the role of intracellular Cys-40 and Cys-346 for murine Panx1 channel function [81,82]. In the context of S-nitrosylation, a single mutation of these two cysteines did not prevent the inhibition of the Panx1 current by S-nitrosylation. However, mutations of both residues imply S-nitrosylation at Cys-40 and Cys-346 in Panx1 channel inhibition via S-nitrosylation regulation by NO, which is relevant in NO-rich tissues of the nervous system and vasculature [33] (see Fig. 1, #13). However, it is important to note that NO may have a variation of effects based on its concentration, exposure time and physiological context [33]. For instance, NO can regulate the inhibition of Panx1 channel function by phosphorylation through a cGMP-PKG dependent pathway in HEK-293 cells [83]. A likely target for phosphorylation is Ser-206, as its mutation to alanine completely abolished NO-mediated inhibition (see Fig. 1, #14).

Phosphorylation of Panx1 by other protein kinases as a means to modulate channel properties has not yet been studied in depth. However, phosphorylation of Tyr-308 in hippocampal brain slices has been linked to activation of the Panx1 channel mechanistically via NMDA receptor and Src Family kinase (SFK) signaling [40,84,85] (see Fig. 1, #2). SFK-dependent phosphorylation of Tyr-198 has also been implicated in activation of the Panx1 channel downstream of endothelial cell activation of TNF-alpha receptors [86] (see Fig. 1, #14).

9. ATP

An overarching theme in the pannexin field is the relation between Panx1 and ATP. Bao et al. [30] were the first to describe Panx1 channels as mechanosensitive conduits for ATP. In rapid succession, other reports followed showing that Panx1 can mediate the release of ATP in most, but not all, cell types and tissues (for review see: [49]. Another exciting finding was that Panx1 channel activity is subject to control by its permeant [43], which led to a plethora of studies correlating ATP and Panx1 channel activity with important functional outcomes. For instance, ATP-induced ATP release activates metabotropic P2Y receptors to evoke a calcium wave [11], or ionotropic P2X receptors, propagating additional ATP release in a positive feedback loop on the cell itself and neighboring cells nearby, which can, in turn, stimulate Panx1 opening [87] (see Fig. 1). As a result, it was determined that micromolar concentrations ATP activates Panx1 channels [32] (see Fig. 1, #6). However, this feedback loop eventually derails when ATP binds to the extracellular loops of Panx1. Qiu and Dahl [43] found a cellular mechanism for Panx1 self-regulation, discovering an important arginine located in the first extracellular loop of the protein for ATP binding. This area seemingly plays a role in preventing persistent excitatory signaling of Panx1 mediated ATP release, causing a reduction in channel conductance and preventing further effects from ATP – both its permeability and release [87]. This finding is supported by previous work by Iwabuchi and Kawahara [88], who found that P2X7 receptors are responsible for closing the Panx1 channel. Additional inhibitory effects of ATP on Panx1 is demonstrated by recent evidence linking increased extracellular levels of ATP with Panx1 internalization to endosomal compartments, removing the channel from the cell surface and preventing further excitatory ATP release [89] (see Fig. 1, #12). Together, this suggests that once ATP reaches millimolar concentrations, it can inhibit Panx1 channels [43] (see Fig. 1, #10).

10. pH-regulation

Kurtenbach et al. [39] determined that both Panx1 channels from zebrafish functioned as pH-sensitive channels. The response to environmental pH changes was comparable, with increased pH causing an increase in dye uptake (see Fig. 1, #11). It is important to note that cells expressing human [90], mouse or fish Panx1 [39] take up dye at the physiological pH range, suggesting that channels regularly undergo open and closed states in different species. However, the mechanism of sensing pH and how dye is carried across the cell membrane in human, rodent or fish Panx1 requires further investigation to reveal the molecular, cellular and structural details of this process.

Gating by pH correlates Panx1 channels to physiological processes during development, vision or the circadian clock. Work by Vroman et al. [91] demonstrated how Panx1 channels could engage in such operations in the outer retina, where ATP is released through Panx1 channels and subsequent metabolic degradation to adenosine releases protons. The local acidification of the synaptic cleft between photoreceptors, bipolar cells, and horizontal cells closes Panx1 channels in a pH-dependent manner, modulating photoreceptor output [91]. While the exact mechanism behind pH sensing remains to be determined, Ardiles et al. [92] proposed the following synaptic cleft pH regulation hypothesis. In their model extracellular ATP hydrolysis also generates protons and phosphates causing extracellular pH to decrease [91]. They propose that this acidification could inhibit both, voltage gated calcium channels (VGCC) present in the presynaptic terminal, and NMDAR on postsynaptic membrane, reducing glutamate release and NMDA receptor activation. The absence of Panx1 channels or their inhibition prevents the release of ATP, stops the production of phosphate buffer and produces the alkalization of the synaptic cleft, which increase the release probability and the activation of NMDARs. This would explain how the absence of Panx1 in adult knock out mice leads to increased excitability in the adult hippocampus [92,93]. In conclusion it is reasonable to speculate that similar processes may occur in other conditions, including pathophysiological conditions of the CNS with acidification (decreased pH) or alkalization (increased pH). In such conditions, pH-dependent Panx1 channel gating can have serious biological effects on cell functions and could be detrimental to internal ATP and glucose stores.

11. Receptor-mediated gating: purinergic and NMDA receptors

Panx1 interactions with both NMDA and P2X7 receptors in the CNS have been investigated, and thoroughly reviewed on potential roles in chronic pain (reviewed in [94]), stroke [95], and anoxia [96]. However, the mechanistic details involved in these interactions have yet to be characterized. Glutamate signaling dynamics involving NMDARs present in neurons, or glutamate release from astrocytes [97,98] (see Fig. 1, #4), could imply that NMDAR-Panx1 complexes affect Panx1 gating. In this context, glutamate may activate NMDARs, remove the Mg^{2+} block and further enhance receptor activity by direct interactions with Src Family Kinases (SFKs) [99] (see Fig. 1, #1). Interestingly, Src-specific phosphorylation in the Panx1 CT-tail at residue Y308 has recently been linked to activation of Panx1 channels [40,85] (see Fig. 1, #2). In addition, a decoy peptide representing Panx1 residues 305–318

acts on SFK, antagonizing NMDAR-dependent Panx1 activation during anoxic depolarization [40]. Together, interactions with SFKs need to be considered as evidence for a possible NMDAR-Panx1 complex and feedback loop responsible for propagating Panx1 gating activity.

A less direct mechanism involving glutamate signaling may be mediated by ionotropic P2X purinergic receptors, in particular, P2X7. It is possible to speculate that these receptors can be activated by ATP release [100], possibly via Panx1 channels in a process initiated by increases in extracellular glutamate [101], and in turn potentiating Panx1 activation. Other mechanisms stimulating P2X7 receptors can lead to activation of Panx1 by intracellular mechanisms, namely by Ca^{2+} [35,102,103] (see Fig. 1, #5). Other modes of activation of indirect activation of Panx1 channels use mitogen-activated protein kinases (MAPK) [12], Ca^{2+}-calmodulin kinase II [104], as well as neurotransmitters such as acetylcholine [105], and GABA [106]. Although a physical Panx1 and P2X7 interaction complex have yet to be resolved, the first evidence for a functional association was reported in 2006 by Pelegrin and Surprenant using pharmacology and siRNA-mediated knockdown [35]. In this study, an ATP-stimulated "large pore formation" and IL-1 beta release from macrophages was shown. One model explains the observed large-pore conductance as an intrinsic property of the ionotropic receptors, which allow pore dilation with continued stimulation [107]. However, another study suggests that there may be a Src kinase-dependent coupling between P2X7 and Panx1 based upon P2X7-mediated Panx1 activation being sensitive to Src Kinase inhibitors and a peptide that contains a Src homology 3 domain of P2X7 [84] (see Fig. 1, #5). Also, genetic analysis implies that the carboxy-terminal region of P2X7 could be efficient for coupling to Panx1 [108]. A lot of controversies reside around purinergic receptor-mediated Panx1 activation. While the exact mechanism of Panx1 activation remains to be elucidated, this research supports the idea of direct coupling, suggesting that the activation of both Panx1 and P2X7Rs is required for full functionalities.

Although there have been multiple claims of Panx1 activation via P2X receptors [84,109,110], some recent studies have demonstrated dye uptake or intact ATP release following P2X7 activation despite genetic deletion or pharmacological block of Panx1 [37,111,112]. This conflict has yet to be resolved since opposite conclusions are possible due to Panx1-P2X coupling occurring only under specific conditions [113], or P2X7 receptors activate more than one pathway [111,114]. Further, P2X3 receptors showed downstream activation of Panx1 [115], and some evidence suggests that interaction with P2Y receptors can activate Panx1 intracellularly [32]. Interestingly, investigations into a multiprotein complex involving both P2X and P2Y receptors, a dihydropyridine receptor voltage sensor, Panx1 channels, nucleotide receptors and other signaling molecules is currently underway, suggesting finely coordinated events involving multiple players playing a role in Panx1 dynamics [116].

12. Concluding remarks

The field of Panx1 research is developing rapidly with a growing number of Panx1 functions recognized in the central nervous system and beyond. More interactions of Panx1 with cell signaling pathways will be identified, which will reinforce prominent roles of Panx1 as a central molecular hub in purinergic signaling and through ATP release. Beyond this progress, it is becoming increasingly evident that basic channel and mechanical gating properties of Panx1 need to be fully elucidated to shed some light on both the functionality of the protein physiologically, and the consequences of the dysfunction of the protein pathophysiologically in various contexts. This also brings to light the growing need to solve the bizarre Panx1 channel pharmacology [117], which has not been addressed in this review. Further, the pannexin field needs high-resolution structures of the Panx1 channels in the open and closed state, to complement research currently emerging in the field by revealing the sequential conformational changes that accompany channel activation or inhibition. Future studies need to address the myriad of factors influencing the interpretation of Panx1 gating at the cellular and molecular level, which complicate the understanding of pannexin channel dynamics and functional roles. Also, there are many caveats associated with various models of investigations into functions of Panx1. For example, transgenic animal modeling raises questions regarding compensation mechanisms in knock out populations, cell-based models yield varying results depending on their ability to allow glycosylation or not, and in vitro investigations may introduce a multitude of other consequences due to the disruption in neural networks. To finish, although explanations for conflicting Panx1 reports do not appear immediately obvious, they reinforce the concept that Panx1 is a versatile membrane channel that attracts significant attention as a potential therapeutic target for human disorders.

Transparency document

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