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The two faces of pannexins: new roles in inflammation and repair

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Abstract: Pannexins belong to a family of ATP-release channels expressed in almost all cell types. An increasing body of literature on pannexins suggests that these channels play dual and sometimes contradictory roles, contributing to normal cell function, as well as to the pathological progression of disease. In this review, we summarize our understanding of pannexin “protective” and “harmful” functions in inflammation, regeneration and mechanical signaling. We also suggest a possible basis for pannexin’s dual roles, related to extracellular ATP and K+ levels and the activation of various types of P2 receptors that are associated with pannexin. Finally, we speculate upon therapeutic strategies related to pannexin using eyes, lacrimal glands, and peripheral nerves as examples of interesting therapeutic targets.

Keywords: pannexin, Panx1, ATP, purinergic signaling, inflammation, regeneration

Introduction

Cell–cell and cell–matrix interactions are fundamental properties of multicellular organisms. Gap junctions, formed by connexins and innexins in vertebrate and invertebrate animals, respectively, allow direct passage of ions and small molecules (<2,000 Da) from cell to cell (Figure 1A).1–3 In addition to gap-junction channels, connexins may form hemichannels (HCs), termed “connexons” (Figure 1B),4,5 which are hexamers of connexin monomers (Figure 1A), each containing four transmembrane domains, two extracellular loops, and cytoplasmic N and C termini (Figure 1C).6 The vertebrate homologues of innexins, called “pannexins”, form mostly HCs, or pannexons (Figure 1B), due to the high level of glycosylation in their extracellular domains (Figure 1D).7–12 Similar to connexins (Figure 1C), pannexins have a cytosolic N-terminal domain, four transmembrane domains with two extracellular loops, and a cytosolic C-terminal domain (Figure 1D).13 However, pannexins have no homology to the vertebrate connexin gap-junction protein,8 and unlike connexins, which have multiple cysteine residues in both extracellular loops, pannexins have only two cysteine residues per loop (Figure 1C and D, black ovals).13

The pannexin family consists of three proteins, Panx1, Panx2, and Panx3, all of which have been shown to form a single-membrane channel.14,15 Panx1 is ubiquitously expressed in almost all cell types, including those in the nervous and immune systems, eye, muscle, olfactory epithelium, blood vessels, exocrine glands (eg, lacrimal and salivary glands), thyroid, prostate, kidney, and liver (Table 1).16–20 Panx2 transcripts are highly expressed in the central nervous system (CNS).21 Lower levels of Panx2 transcripts have been detected in nonneural tissues, including the testis, kidney, retina, and...
gastrointestinal tract, while Panx3 mainly localizes in the skin, osteoblasts, and chondrocytes (Table 1).15,20-24 Panx3 has also been found in skeletal muscle,25 lactating mammary glands, sebaceous glands, and the small intestine.15 Interestingly, Panx2 protein appears to be more ubiquitously expressed than initially predicted by mRNA expression21 (Table 1).

Endogenous Panx1 and Panx3 proteins are localized primarily at the plasma membrane,13,26 while Panx2 is highly

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**Table 1** Expression and localization of pannexin mRNA and protein

<table>
<thead>
<tr>
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<th>Panx1</th>
<th>Panx2</th>
<th>Panx3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mRNA-expression pattern and levels of expression</strong></td>
<td>Ubiquitously expressed in mammalian211 and chicken tissues212</td>
<td>Cerebral cortex, cerebellum: neurons and immature astrocytes21, hippocampus (high),27 kidney tubular cells (low), seminiferous duct cells in the testis (moderate), salivary glands (excretory), and striated ducts (weak)21</td>
<td>In humans, Panx3 mRNA found in the testis, stomach, spleen, salivary gland, lung, heart, duodenum, and adrenal tissue;213 in mice, Panx3 mRNA found in developing skeletal structures214</td>
</tr>
<tr>
<td><strong>Protein expression</strong></td>
<td>Ubiquitously expressed in many mammalian tissues,211,215 with lower levels in the lung, kidney, and heart ventricles13</td>
<td>Protein is expressed in many tissue types, including gastrointestinal tract glandular and epithelial cells, parietal cells, columnar epithelial cells of the human colon216 (strong), and mouse retina, lung, and skin</td>
<td>Extensive expression throughout the developing skeleton during chondrocyte and osteoblast differentiation,214 skeletal muscle,23 lactating mammary tissue, sebaceous glands, and the small intestine15</td>
</tr>
<tr>
<td><strong>Localization within the cell</strong></td>
<td>Mostly membrane,12,26,27 but may form permeable channels in the endoplasmic reticulum27</td>
<td>Membrane;14,28 predominantly cytoplasmic21</td>
<td>Membrane, may form channels in the endoplasmic reticulum and gap junctions after overexpression;28,219 diffuse cytoplasmic in the epidermis220</td>
</tr>
<tr>
<td><strong>Subunits</strong></td>
<td>6</td>
<td>6-8221</td>
<td>6</td>
</tr>
<tr>
<td><strong>Key domains (conserved in all isoforms)</strong></td>
<td>Transmembrane domains (&gt;4), extracellular loops (&gt;2), intracellular N-terminus, C-terminus, and intracellular loop</td>
<td>First extracellular loop</td>
<td>First extracellular loop</td>
</tr>
<tr>
<td><strong>Glycosylation site</strong></td>
<td>Second extracellular loop</td>
<td>First extracellular loop</td>
<td>First extracellular loop</td>
</tr>
</tbody>
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Figure 1 Connexins and pannexins.  
**Notes:** (A, B) Connexin and pannexin share a similar structure, despite the absence of sequence homology. Connexin and pannexin form functional connexon and pannexon hemichannels, respectively. (C, D) Connexins and pannexins are transmembrane proteins with four transmembrane domains, two extracellular loops, one cytoplasmic loop, and cytoplasmic N- and C-terminal domains. Connexin channels can assemble into a gap junction (A) that mediates intercellular communication, while pannexin’s extracellular loop has a high level of glycosylation in mammalian cells (D), which prevent the formation of gap junctions.  
**Abbreviation:** Glyc, glycosylation.

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Abbreviation: Glyc, glycosylation.
expressed in the cytoplasmic compartment, suggesting a unique intracellular function for Panx2. However, several studies have reported the cytoplasmic localization of Panx1 and Panx3 proteins when these proteins were overexpressed in cells. For example, Abeele et al demonstrated cytoplasmic localization of Panx1 transiently expressed in LNCaP cells, where it formed Ca²⁺-permeable channels in the endoplasmic reticulum (Table 1). It is quite possible that high levels of pannexin protein expression could lead to both membrane and endoplasmic reticulum-channel formation, thus contributing to sustained increases in intracellular Ca²⁺.

Pannexins are ATP-release channels that can be activated by caspase cleavage of their pore-associated C-terminal tail, the autoregulatory region controlling channel permeability. The regulated ATP (nucleotide) release through pannexin HCs is implicated in a number of normal physiological functions and in response to stressors or pathological states in cells and tissue. Well-characterized functions of pannexins include regulation of cell differentiation and migration, tissue development and regeneration, inflammation, wound healing and cell death. However, mechanistic explanations of how these proteins perform sometimes contradictory roles remain unclear.

In this review, we attempt to clarify existing controversies in the literature on the “protective” and “harmful” roles of pannexin HCs by addressing a question: How do pannexins acquire these different and often opposing roles? We seek to obtain deeper understanding of pannexin signaling, participants in which represent a potential source of novel and promising therapeutic targets in a variety of pathologies. Our focus is entirely on pannexins, with a full understanding that pannexin and connexin HCs have both distinct and complementary but often overlapping functions, particularly in ATP release and inflammation; therefore, we refer the reader to several excellent reviews comparing the roles of these channels.

**Pannexins, inflammation, and inflammasome activation**

The involvement of pannexins in the induction of inflammation has been reported in multiple publications. Inflammation is the major protective function maintained by the evolutionarily conserved innate immune system in response to harmful stimuli, such as pathogens, stress, injury, or cell death. Acute (short-term) inflammation stimulates a regenerative response, while persistent (chronic) inflammation can cause systemic inflammatory diseases. Activation of inflammasomes, facilitating the release of interleukin-1β (IL1β) and IL18 in response to pathogens and tissue injury, is a key function of the innate immune system. The inflammasomes, first characterized in monocytes in 2002 and in neural cells in 2008, are multi-protein complexes mediating proteolytic maturation of Casp1, Casp11, IL1β, and IL18. Proteolytic cleavage of IL1β and IL18 precursors is executed by active Casp1 (Figure 2); and the release of the mature cytokines occurs via megapores, formed by N-terminal domains of the Casp1/11-processed recently identified pore forming protein gadermin D. A large body of experimental evidence identifies Panx1 and its associated P2X receptors as essential upstream regulators of inflammasomes and proteolytic activation of Casp1 and Casp11. Panx1 has been reported to activate inflammasomes in many cell types, including macrophages, microglia, neurons, and astrocytes; however, the data on particular cell and inflammasome types remain controversial. Currently, the bulk of published data support a pivotal role for Panx1 in CNS/retinal inflammasome regulation. As such, strong suppression of its major components, including Casp1, Casp11, IL1β, and apoptosis-associate speck-like protein containing a caspase recruitment domain (ASC), is observed in both Panx1−/− mice and wild-type retinas after Panx1 blockade by probenecid.

There are two major regulatory arms for inflammasome activation (Figure 2): signal 1 pathways sense environmental signals via surface TNF, Toll-like, and IL1 receptors and facilitate inflammasome “priming”, ie, transcriptional activation via MyD88–NFκB-mediated pathways; and signal 2 pathways regulate inflammasome assembly and processing of Casp1/11, IL1β, and IL18 precursors. This arm is regulated via the Panx1–P2X signalosome to facilitate ATP and K⁺ release, as well as uptake of extracellular Ca²⁺ and danger/pathogen-signaling patterns.

Though a role for Panx1 in the inflammasome regulatory cascade appears to be generally conserved across cell types, Qu et al suggested that pannexin is “dispensable” for inflammasome formation. In particular, LPS-primed bone marrow-derived macrophages were successfully able to activate Casp1 and secrete its associated inflammatory cytokines (IL1β and IL18) in response to a number of stimuli in the absence of Panx1. Moreover, the authors also concluded that P2X, and Panx1 can function independently and may be involved in distinct signaling pathways. These controversial views on Panx1 function could be explained by cell-type-specific differences and potential variation in culture conditions, and need to be resolved.

**Mechanisms of pannexin-channel activation**

Several diverse mechanisms regulating pannexin-channel function have been proposed to date. Pannexin channels...
have been posited to be activated by caspase-mediated channel cleavage in apoptotic immune cells, G-protein-coupled receptors in vascular smooth muscle, low oxygen tension in erythrocytes and neurons, high extracellular K+ in various cell types, and mechanical stretch. Progressive Panx1-channel opening is directly linked to ion- and large-molecule transport, and occurs during both irreversible (caspase-mediated cleavage) and reversible G-protein-coupled receptor (including α1-adrenoceptor-mediated) forms of channel activation. Panx1 activation by caspase-mediated cleavage enables the release of ATP as a “find me” signal that recruits phagocytizing macrophages to apoptotic T lymphocytes. This mechanism is critical for the fast clearance of apoptotic and dead cells during acute inflammation. Cleavage activation of Panx1 is also involved in pyroptotic cell death (Figure 2). A recent study employing electron microscopy and single-channel recordings of full-length and caspase-cleaved pannexin concatamers with defined numbers (0–6) of intact and truncated C termini revealed that Panx1 activation was increased in a sequential manner by stepwise removal of the autoinhibitory C termini. This also resulted in a graded increase in current and ATP/dye permeation. On the other hand, the reversible G-protein-coupled receptor-mediated mechanism is independent of caspase-mediated pannexin cleavage. Comparison of α1-adrenoceptor-activated with cleavage-activated Panx1 channels indicated that α1-adrenoceptor-activated Panx1 channels had a shorter mean open time, but progressively increasing conductance, suggesting that despite differences in gating kinetics, activation of Panx1 channels by both signaling mechanisms involves cumulative changes in open-channel properties.

**Pannexin signaling via ATP release**

Panx1 channels can release ATP under physiological conditions and play critical roles in many pathological processes. ATP is a prominent extracellular signaling molecule in both physiological and pathological conditions. For example, ATP release is important for muscle differentiation and function, and ATP-receptor activation plays a role in regulation of cell proliferation, DNA synthesis, cell differentiation, and cell survival during the course of CNS development. At the same time, ATP may also serve as a major danger signal for cells, despite it having a very short half-life due to rapid degradation by surface ecto-ATPases.
ATP is released from apoptotic, injured, and viable cells that are challenged by assorted cytokines, as well as mechanical or ischemic stress in the presence of elevated K⁺. ATP-mediated activation of Panx1, the ATP-release channel, typically ramps up in a vicious cycle only to a certain level, due to a retrograde feedback mechanism regulating activity of Panx1 HCs via a low-affinity ATP binding site. Therefore, the permeant (ATP) can inhibit the permeating channel when high extracellular ATP concentration is reached. Importantly, however, this inhibition is abrogated by an increased extracellular concentration of potassium ions (K⁺), suggesting a mechanism of toxicity of extracellular ATP in Panx1-expressing cells. In agreement with this mechanism are the findings that massive activation by Casp3/7 cleavage or expression of constitutively active Panx1 results in cell death. Therefore, the balance between ATP signaling and specific injury is influenced by the increase in intracellular Ca²⁺ and extracellular ATP and K⁺. An additional level of Panx1-channel regulation is achieved via interactions with purinergic P2 (eg, P2X and P2Y) receptors, which are activated by binding extracellular ATP at the plasma membrane. Several salient aspects of Panx–P2 interactions, including the mechanisms and significance of such interactions, as well as their sensitivity and specificity, are detailed in the following sections.

Functional interactions of Panx HCs with purinergic P2X and P2Y receptors

There are two major families of purinergic P2 receptors: ionotropic P2X and metabotropic P2Y receptors. Reciprocal interactions, whereby P2 receptors directly activate Panx1 channels, suggest that these proteins can form a signaling complex at the cell surface that mediates both paracrine and autocrine purinergic communication.

The P2X-receptor family contains 7 isoforms (P2X₁₋₇), and P2X receptors are classified as ligand-gated channels whose activation regulates cellular membrane potential and intracellular Ca²⁺ levels. More precisely, though, P2X family members are ATP-gated cation channels, selective for Na⁺, K⁺, and Ca²⁺ ions. In the nervous system, P2X receptors are pivotal transducers of ATP-mediated paracrine signals and have been implicated in physiological functions, such as chemotactic cell migration, intercellular calcium-wave propagation, as well as in nervous system dysfunction, leading to neuropathic pain or cell death. Five isoforms – P2X₁, P2X₂, P2X₃, P2X₄, and P2X₇ – have been shown to interact with Panx1, among which P2X₁ and P2X₃ are the most common interaction partners in different cell types. Both P2X₄ and P2X₇ isoforms are calcium channels known to dilate into larger pores upon activation. P2X₇-receptor activation results in the appearance of HC-like currents, reflecting the channel permeability for molecules up to 1 kDa and identified as Panx1 HC. Under pathological conditions, overactivation of the Panx1–P2X₇ signalosome complex has been implicated in inflammation, cell death, and neuropathic pain.

The P2Y-receptor family contains eight isoforms (P2Y₁₂₄₆₁₁₋₁₄). P2Y receptors are metabotropic G-protein-coupled receptors that couple to Gq, G₁₅, or G₁₃ in an isoform-specific manner, and their activation modulates intracellular inositol triphosphate, Ca²⁺, and cAMP levels. Different isoforms of purinergic P2Y receptors are activated by ATP and its degradation products ADP and UTP, and couple to distinct G proteins to induce cAMP production, activation of phospholipase C, or intracellular Ca²⁺ via inositol triphosphate second-messenger systems. Recent publications have implicated P2Y receptors and Panx1 signaling in the regulation of endothelial cell activation in vascular inflammation and cell-volume regulation. ATP release via Panx1 channels activates P2Y₁ receptors to amplify signaling in sensing chemotactic gradients in neutrophils. Polarization of surface expression by translocation of Panx1, P2Y₂, adenosine A₁ receptors, and ENTPD1 ectonucleotidase to the leading cell edge allows neutrophils to polarize within the gradients.

Sensitivity and specificity of Panx1–P2X signaling during normal tissue function and chronic inflammation

The activity and downstream consequences of several P2X- and P2Y-receptor isoforms are dependent on ATP binding, with varying sensitivity. P2X₇, expressed by microglia, astrocytes, and neurons, is the most studied isoform, and is implicated in cyto- and neurotoxicity via direct interaction with Panx1 channels. Due to its relatively low affinity to ATP (EC₅₀ 936 µM), it can only be activated by nonphysiological (≥1 mM) increases in extracellular ATP, which is locally achievable only at sites of injury or proximal to activated Panx1/Cx43 HCs. Conversely, the activation at low to medium (<900 µM) extracellular ATP levels can be mediated via an interaction between the high-affinity P2X₄ receptor (P2X₄; EC₅₀ 2.3 µM) and Panx1 (Figure 3A and B). This synergistic interaction was shown to coactivate P2X₄ receptor, resulting in massive local efflux of ATP via the Panx1 channel, a forward-feeding autocrine amplification loop (Figure 3C).
Functional synergy between P2X4 and cytotoxic P2X7 is known to be pivotal for Panx1-dependent extracellular ATP-induced cell death, which can be suppressed by a blockade of either component in the Panx1–P2X4/7 complex or by extracellular ATP removal with apyrase. Similarly, in the retina, genetic ablation or pharmacological inhibition of Panx1, P2X7, or P2X4 has protected retinal ganglion cells (RGCs) in both acute and chronic ocular hypertension (OHT)-injury models. In other studies, a similar blockade was shown to protect neurons and other cell types from death via a rise in ionized Ca2+ and the induction of the inflammasome in various injury paradigms.

In contrast to P2X7, interactions between P2X4 receptor and Panx1 and their link to RGC loss and inflammasome in the retina still require exploration. However, strong evidence of a key cellular role of P2X4 in response to sublethal levels of ATP has been suggested in experiments on channel blockade with the 5-BDBD antagonist in macrophages, as well as on inflammasome activation in various tissue types. In contrast to P2X7, P2X4 blockade has been shown selectively to suppress IL1β but not IL18 cytokine levels, which was reported as potentially neuroprotective.

More recently, an interesting phenomenon in acute wound healing following the use of intracellular ATP delivery was described. In this study, ATP application was accompanied by a massive increase in macrophage trafficking, in situ proliferation, and direct collagen production within the wound. Although the signaling mechanism of this phenomenon has not been determined, other research has demonstrated that the recognition and clearance of dying cells and debris from focal points of inflammation is critical in both the induction and resolution of inflammation. Moreover, Panx1-mediated vesicular nucleotide transporters (responsible for ATP accumulation in secretory vesicles)-mediated ATP release have been shown to recruit neutrophils/macrophages to injury sites. It is quite possible that in some types of acute injury, an increase in or acceleration of postinjury inflammation may lead to more rapid resolution of inflammation through ADP or other signaling mechanisms.
Other mediators of Panx signaling and inflammation

In addition to Panx HCs, significant amounts of ATP can be released by bacteria, which trigger Panx1/P2X activity (Figure 3C). Bacterial ATP may affect different types of cells and lead to the production of proinflammatory cytokines and growth factors. A recent study showed that commensal bacteria-derived ATP activates CD70^{high}CD11e^{low} cells in the intestinal lamina propria, induce IL6 and IL23 production, as well as TGF-β pathway activation. This then led to local differentiation of IL17-producing CD4^+ T lymphocytes (T-helper T_t17, cells involved in host defense and several immune disorders). Moreover, systemic or rectal administration of ATP into germ-free mice resulted in a marked increase in the number of lamina propria T_{t17} cells. The specific effect of ATP on T_{t17} differentiation was mediated by P2X and P2Y receptors, and ATP-induced T_{t17} differentiation was inhibited by P2X- and P2Y-receptor blockade. Interestingly, this mechanism commonly operates during the differentiation of both “naturally occurring” and “pathogenic” T_{t17} cells.

Although ATP-gated unselective cation P2X channels are induced mainly by ATP, some studies report that they also may be activated by other molecules. β-Toxin produced by Clostridium perfringens is a key virulence factor in fatal hemorrhagic enterocolitis and enterotoxemia. This toxin belongs to a family of β-pore-forming toxins. The results of a recent study suggested that Panx1 opening is achieved through the interaction of β-toxin with the P2X, receptor. Then, ATP released by Panx1-channel opening promotes oligomer formation of the toxin, leading to cell death. These studies suggest that Panx1 HC is an important contributor to P2X_β-receptor signaling and provides a mechanistic link among bacterial stimuli, P2X_β-Panx1 signaling, and inflammation.

Pannexins, mechanical signaling, and the cytoskeleton

A mechanosensitive role for connexin HCs in the propagation of intracellular calcium, initiated by the extracellular binding of ATP, was first noted in 1990. Numerous reports since then have demonstrated the sensitivity of connexin HCs to extracellular Ca^{2+}, which are believed to keep connexin HCs in a closed state at physiological Ca^{2+} levels. In contrast, Panx HCs are not gated by external Ca^{2+} and the mechanical sensitivity of pannexin HCs was not noted until 2004, when single-channel currents were elicited by changes in pressure imposed pneumatically upon membrane patches of Xenopus oocytes expressing Panx1. Since then, mechanosensitive purinergic signaling pathways, including pannexin-mediated ATP release, have been demonstrated in many cell types in response to mechanical stimuli. For example, inhibition of pannexin function suppressed hypertonic stress-induced ATP release and reduced downstream transcriptional activation induced by hypertonicity, and inhibition of Panx1 and several P2X receptors reduced downstream transcriptional activation induced by hypertonicity. Similarly, pannexin- and/or P2-receptor-dependent ATP release has been observed in RGCs, lens epithelial cells, fibrosarcoma cells, urothelial cells, and astrocytes that were subjected to hypoosmotic conditions. In addition to altered tonicity, shear stress has been shown to activate mechanosensitive pannexin channels. Indeed, bone cells and red blood cells have demonstrated robust pannexin-mediated ATP release in response to oscillatory fluid shear stress. Consistent with this function, it was recently suggested that pannexin activity induced by transient fluid shear during media changes and manipulation of tissue-culture containers could confound the interpretation of cell-culture experiments.

In contrast to the bulk of the literature, only one study in HEK293 cells subjected to hypotonic media has suggested that pannexin HCs are not directly mechanosensitive. It is likely that this controversial observation may have reflected a unique feature of the examined cell type and/or methodological differences. The authors’ choice to use ethidium bromide internalization as an indicator of pannexin HC activity may have led to different outcomes compared with more conventional and commonly used indicators of pannexin activity, such as dye uptake or ATP release.

While mechanical activation of pannexin HCs has been studied primarily in a general context, there is increasing evidence that mechanical signaling can facilitate pathological states, such as edema that stretches the plasma membrane. Mechanical strain was recently reported to trigger a robust inflammatory response, transcriptional priming of NLRP3 inflammasome formation, and IL1β production via activation of Panx1–P2X, signaling.

The eye has emerged as an important model in understanding pathological mechanotransductive roles for Panx1. When the retina is exposed to mechanical stress, ATP is released physiologically by glia and neurons via Panx1 channels. In OHT-injured retina, synergistic effects of mechanical stress induced by elevated pressure and massive ATP release facilitate sustained extracellular ATP elevation and prolonged activation of the Panx1–P2X pathway, a combination that is particularly toxic to RGCs, which are
Experimental data generated in the murine eye indicate that RGC loss and axonal damage strongly correlate with mechanical deformation and repetitive intraocular pressure spikes. An increase in extracellular ATP has been reported in eyes exposed to acute or chronic OHT in animal models, as well as in human primary open-angle glaucoma. Conversely, strain-activated, pannexin-regulated release of cytokines may also serve a protective role, as demonstrated by increased IL3 and IL6 expression in RGCs subjected to a 4% chronic strain in vitro or increased intraocular pressure in vivo. Furthermore, the release of IL18 via inflammasome activation has also been reported to be neuroprotective.

Concurrent with Panx1-mediated ATP release, Cx43 HCs have been demonstrated as another key pathway of ATP release. Although the contribution of Cx43 vs Panx1 to ATP release has been heavily debated recently, the current consensus indicates that Panx1 channels initiate and Cx43 HCs facilitate the bulk of ATP release from macroglia, especially in the presence of TNFα and IL1β. Consistent with this view, upon their exposure to TNFα and IL1β cytokines, glial cells become activated and release ATP via Cx43 HCs.

Given its structural role, the cytoskeleton is a leading candidate to participate in mechanical signaling. Actin dynamics, particularly those mediated by ARP2/3, have been reported to be regulated by pannexin activity, and pannexin interacts physically with actin (through the C terminus of Panx1), but not tubulin/microtubules. In a recent publication, it was reported that the Panx1–P2X, autocrine loop induced by ATP increased the migration speed of dendritic cells by promoting reorganization of the actin cytoskeleton. In addition, pannexin influences a number of cellular changes that require cytoskeletal plasticity, including migration, differentiation, and proliferation. A role for actin is emphasized by evidence that Rho-kinase pathways, which have long been implicated in regulating actin dynamics, are dependent on pannexin-channel activity. The importance of pannexins in regulating cytoskeletal dynamics has also been suggested by the localization of pannexin to the actin-rich filopodia of directionally migrating or path-finding cells. We have shown that loss or inhibition of Panx1 increases neurite extension and branching in sensory neurons ex vivo. This finding suggests that pannexins may play a suppressive role in neuronal growth. Finally, pannexin channels may also somehow play a role in the mechanical sensitivity of other mechanosensitive channels, such as TRPV4 and TRPV1. It is not yet clear how the activity of these channels is coupled to pannexin activity. Such regulation may also be mediated by the cytoskeleton, the rigidity of which likely influences mechanosensitive-channel response and the stability of which may be regulated by pannexin–P2X-mediated pathways, such as those already noted. Therefore, mechanosignaling through Panx HCs may play a role in normal cell migration, growth, and differentiation. However, persistent exposure to mechanical stress may facilitate sustained activation of a Panx1–P2X-signaling loop, contributing to chronic inflammation.

**Pannexin and receptor plasticity**

The magnitude and persistence of an activating signal have been noted to influence the functional plasticity of pannexin and pannexin-associated purinergic receptors (Figure 4A and B). In addition to the low-affinity binding already mentioned, another negative-feedback mechanism response to increased extracellular ATP involves rapid internalization of Panx1 into endosomes (Figure 4C) in as little as 15 minutes, possibly through signaling initiated by P2X receptors. Positive feedback mechanisms include coregulation of pannexins with purinergic signaling proteins, detected in experiments with hypertonic saline treatment, which triggered both pannexin-channel activity and expression levels of P2X receptors in Jurkat T cells. Similar coregulation was observed in chronic mechanical strain in astrocytes that resulted in an increased expression of Panx1, -2, and -3 both in vitro and in vivo.

**Therapeutic implications of Panx1 inhibition**

Pannexin has been implicated in regulating normal and pathological cellular function in a wide range of tissue...
types. In normal physiology, pannexin has been shown to modulate vascular tone,\textsuperscript{170} brain development,\textsuperscript{9} memory, sleep,\textsuperscript{166,171} skeletal muscle homeostasis,\textsuperscript{172,173} red blood-cell biomechanics,\textsuperscript{149} retinal signaling, response to ischemia,\textsuperscript{117,174} and leukocyte emigration.\textsuperscript{101} In pathology, pannexin-mediated signaling has been implicated in brain ischemia,\textsuperscript{64,175} ischemic stroke,\textsuperscript{176} pain,\textsuperscript{177,178} cardiomyocyte fibrosis,\textsuperscript{179} microbial infection,\textsuperscript{59,180} cancer,\textsuperscript{181} brain inflammation (autoimmune encephalomyelitis/multiple sclerosis),\textsuperscript{182–185} and immunogenic cell-death-inducing antineoplastic agents.\textsuperscript{96,186} On one hand, this potential breadth of functions renders pannexin a powerful and widely applicable therapeutic target. On other hand, this same breadth suggests potentially significant side effects of anti-pannexin therapy, manifested within the same cell type, within the same tissue, or systemically. In addition, as demonstrated by varying results of different commonly used pharmacological inhibitors of pannexin or P2 receptors, including probenecid, Panx1-blocking peptide (\textsuperscript{10}Panx),\textsuperscript{105} carbenoxolone, P2-receptor-inhibiting peptides, and the extracellular ATP scavenger apyrase, the specificity of inhibition can appreciably impact a phenotypic response. In the following sections, we briefly describe possible outcomes related to the authors’ expertise, in which the diverse functions of Panx1 must be considered within translational therapeutic strategies.

Panx1 inhibition in the eye

Panx1 forms an ATP-, K\textsuperscript{+}-, and Ca\textsuperscript{2+}-permeable membrane channel that is highly expressed in the retina, making this easily accessible neural tissue a good model system for delineation of Panx1 function. In the retina, Panx1 has been shown to be activated by mechanical stress,\textsuperscript{29,69,111,130} intracellular Ca\textsuperscript{2+},\textsuperscript{117} extracellular K\textsuperscript{+},\textsuperscript{117} interactions with transient-receptor-potential channels,\textsuperscript{142,188} N-methyl-D-aspartate receptors,\textsuperscript{189} activation of C\textsuperscript{2+}-dependent caspsases 1/11 and NLRP1/3 inflammasomes,\textsuperscript{29,117,190,191} and purinergic receptors upon binding extracellular ATP.\textsuperscript{189} Several of these stressors and agonists are activated in the retina challenged by ischemia, OHT stress, or glaucoma, which can synergize to sustain prolonged Panx1 opening. Consistently with this observation, therapeutic Panx1 blockade protects RGCs and other neurons against mechanical stress and ischemia.\textsuperscript{29,174} However, due to the physiological significance of Panx1, only transient blockade and partial suppression represent therapeutically feasible options, as they are sufficient to block inflammasome and ionized Ca\textsuperscript{2+} influx without affecting global retina functionality.

Panx1 inhibition increases lacrimal-gland repair

Recent studies have proposed distinct roles for both Panx1 and P2X\textsubscript{7}, receptors in the control of inflammasome activation, leading to the release of mature IL1\alpha and IL1\beta. These data support the model in which Panx1–P2X\textsubscript{7}, signaling is the key regulator of inflammatory response.\textsuperscript{52,192} Probenecid, a well-studied inhibitor of Panx1 and P2X\textsubscript{7}, receptors\textsuperscript{93,194} and organic anion transporters, has been traditionally used to treat an inflammatory gout disease.\textsuperscript{193} Treatment with probenecid has been found to affect ATP release\textsuperscript{195} and suppress neuronal death in ischemic stroke\textsuperscript{176,196} and cerebral edema.\textsuperscript{197} This suggests that modulation of Panx1 signaling may prevent inflammatory damage of brain tissue. Another study reports that in vivo administration of the P2X7R antagonist A438079 in the mouse model of salivary gland exocrinopathy could ameliorate salivary gland inflammation and enhance saliva secretion.\textsuperscript{198}

Panx1 and P2 receptors are strongly upregulated during acute and chronic inflammation of the lacrimal gland,\textsuperscript{18} the primary contributor to the aqueous layer of tear film in humans. Moreover, lacrimal-gland injury due to inflammation leads to aqueous tear-deficiency dry eye. Most current therapies to treat lacrimal-gland disorders suggest topical treatments, including usage of artificial tears and autologous serum eyedrops, but they do not treat the cause of the disease and lead to limited success. Cell-based regenerative therapies may provide better and longer relief to dry-eye patients; however, survival of transplanted cells strictly depends on the degree of inflammation.\textsuperscript{199,200} We recently have shown that the best cell engraftment is observed when Panx1 has been blocked with specific Panx1 inhibitors, including \textsuperscript{10}Panx and self-deliverable RNAi (sdRNAi) specific to Panx1.\textsuperscript{200} Moreover, lacrimal-gland treatment with Panx1 sdRNAi resulted in significant reduction in IL1\beta and Nlrp3 expression in TSP1\textsuperscript{–/–} mice, a mouse model of aqueous tear-deficiency dry eye.\textsuperscript{200} These findings have implications for therapeutic strategies targeting Panx1-signaling pathways for suppression of inflammation and/or increasing donor lacrimal-gland progenitor-cell engraftment.

The role of Panx1 in peripheral nerve disease and repair

Pannexins have been implicated in a number of pain-sensitization pathways, in both the peripheral nervous system and the CNS, through activity within and communication between neurons and their supporting cells.\textsuperscript{99,100,201,202} Zhang et al provided initial evidence that cell bodies of sensory neurons release ATP in response to electrical stimulation.
Further, this release stimulated activation of P2X7 receptors and subsequent release of the inflammatory cytokine TNF-α in neighboring glial cells. Pannexin interactions with various isoforms of P2 receptors may be localization-specific, as P2X4 receptors have also been implicated in purinergic pain pathways: in this case, through activity at sensory endings in the skin. Interestingly, P2X7 receptors have also been noted to play a prominent role in pannexin-mediated signaling in DRG neurons. Consistently with a role for pannexin-P2X7 activity in pain pathways, the neurotoxin BomoTx, from the Brazilian lancehead pit viper, activates ATP release through pannexin HCs and downstream P2X3 receptor activation, resulting in inflammatory pain, thermal hyperalgesia, and mechanical allodynia. Further, nerve injury results in increased Panx1 gene and protein expression due to epigenetic mechanisms. In contrast, a recent study revealed that Panx1 inhibition, genetically or through pharmacological reagents, reduced hypersensitivity induced by nerve injury.

Although studied less comprehensively, pannexins also likely play an important role in peripheral nerve development and regeneration. For example, a role in myelination has been suggested for both P2X7 receptors and pannexin, likely due to communication between stimulated neurons and their flanking Schwann cells. In addition, a recent study by our research team indicated that pannexin negatively regulated developmental and regenerative growth of peripheral neurons, as suggested by the increased caliber of axons in the sciatic nerves of Panx1−/− mice and increased regenerative outgrowth and branching of cultured DRG explants harvested from Panx1−/− mice, as well as in wild-type DRG explants treated with inhibitors of the Panx1-signaling pathway, including apyrase, probenecid, and 10Panx. Based on its physiological properties and roles in inflammasome activation, it is feasible to suggest that pannexin activity can modulate the neuroregenerative environment, which is enriched in inflammatory cytokines, as well as immune and activated glial cells that respond to inflammatory cues. As such, pannexin reduction may be an effective strategy to reduce pain and promote regeneration after nerve injury.

Conclusion

The switch between normal (minor) and pathological (massive) ATP release from Panx HCs and downstream P2 receptors and other channels can determine whether an outcome will be “good” or “bad”. Low levels of extracellular ATP and K+ produced by physiological pannexin activity is required for homeostatic cell function (Figure 5A). Conversely, high levels of extracellular ATP and K+ and overload of the intracellular compartment with Ca2+ synergistically lead to sustained activation of Panx1–P2X7, signaling and inflammasome pathways, inducing Casp1/11-dependent pyroptotic cell death (Figure 5B). Therefore, therapeutic modulation of Panx1 channels represents a feasible new strategy to reduce inflammation and promote regeneration (Figure 5C).

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Disclosure

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