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Pannexins, distant relatives of the connexin family with specific cellular functions?

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Intercellular communication (IC) is mediated by gap junctions (GJs) and hemichannels, which consist of proteins. This has been particularly well documented for the connexin (Cx) family. Initially, Cxs were thought to be the only proteins capable of GJ formation in vertebrates. About 10 years ago, however, a new GJ-forming protein family related to invertebrate innexins (Inxs) was discovered in vertebrates, and named the pannexin (Panx) family. Panxs, which are structurally similar to Cxs, but evolutionarily distinct, have been shown to be co-expressed with Cxs in vertebrates. Both protein families show distinct properties and have their own particular function. Identification of the mechanisms that control Panx channel gating is a major challenge for future work. In this review, we focus on the specific properties and role of Panxs in normal and pathological conditions.

Keywords: calcium wave; connexin; gap junctions; hemichannels; intercellular communication; pannexin

Introduction

Intercellular communication (IC) is essential to coordinate cellular responses in tissues and organs, thereby fulfilling an essential role in the spreading of signaling, survival, and death processes. Gap junctions (GJs) mediate IC between cells. GJs are plaques of GJ channels, which are proteinaceous channels formed by the docking of two hemichannels of adjacent cells (Fig. 1).[1,2] It was thought that in vertebrates only connexins (Cxs) were able to form GJs. In invertebrates, another family of GJ proteins was identified, the innexins (Inxs). Orthologs for Inxs have been recently discovered in vertebrates, and named pannexins (Panxs).[3] Although Cxs and Inxs/Panxs evolved independently and display little sequence homology (reviewed in Ref.[4]), they possess many common structural and functional properties, including their ability to form GJs and hemichannels and to participate in IC processes.

Inxs, Cxs, and Panxs belong to one superfamily.[5,6] The invertebrate Inx family counts 25 genes in *Caenorhabditis elegans* and 8 *Drosophila melanogaster* genes.[6,7] Cx isoforms are members of the highly conserved multigenic family of transmembrane proteins consisting of 21 human[8,9] and 20 mouse[10] Cx genes, which are named on the basis of their predicted molecular mass (between 26 and 60 kDa).[10–12] The vertebrate Panx family counts only three members in mammals: Panx1 [426 amino acids (aa), 47.6 kDa], Panx2 (664 aa, 73.3 kDa), and Panx3 (392 aa, 44.7 kDa).

The potential physiological roles of GJs depend on their protein subunit composition, which defines their conductance and permeability properties, and are limited by the kind of signals and metabolites they allow to pass (Fig. 1). Some GJs are more permeable to anions, whereas others show preference for cations or exhibit little charge selectivity.[13,14]

While docked hemichannels form GJ channels, unpaired hemichannels can function as channels in membranes, similar to regular ion channels[15–31] (Fig. 1). Functional hemichannels were first described for Cx46,[15,16] but have now also been described for several other Cxs,[17–28] and more recently for Inxs[29] and Panxs.[30–33] Hemichannels for Cx, Inx, or Panx are called connexons,[34–36] innexons, or pannexons,[37,38] respectively.

Under basal physiological conditions, connexons and pannexons are closed.[39] However, changes in the extracellular and intracellular environment can lead to opening of these hemichannels and release of intracellular signaling molecules into the extracellular environment. It is important to note that the response to a certain extracellular or intracellular change or trigger may be very different for connexons and pannexons. Indeed, Cx43 hemichannels have mainly been reported to be opened under ischemic conditions[19,40–45] and by strong depolarization ($>+40$ mV),[20,46] although Cx32 hemichannels are also activated by low rises in free

Abbreviations: Panx, pannexin; Cx, connexin; Inx, innexin; GJ, gap junction; IP_3, inositol 1,4,5-trisphosphate; ER, endoplasmic reticulum; IC, intercellular communication; GJIC, gap junctional intercellular communication; PIC, paracrine intercellular communication; VRAC, volume-regulated anion channels.

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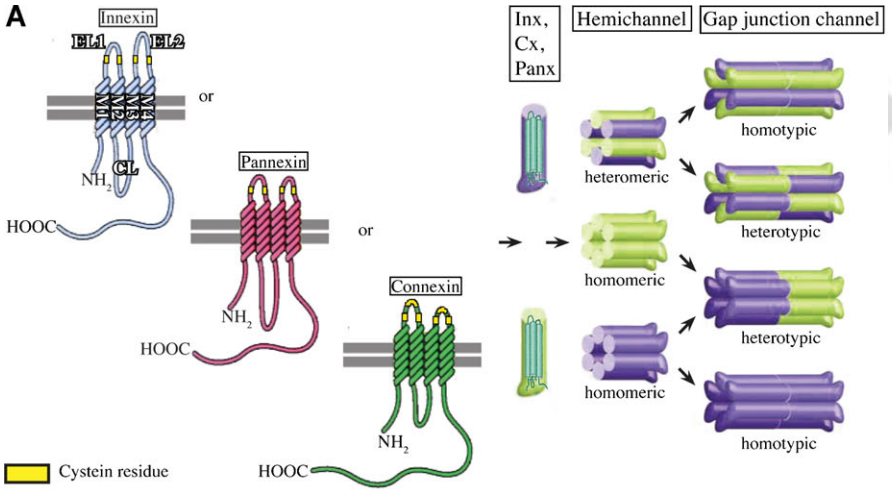

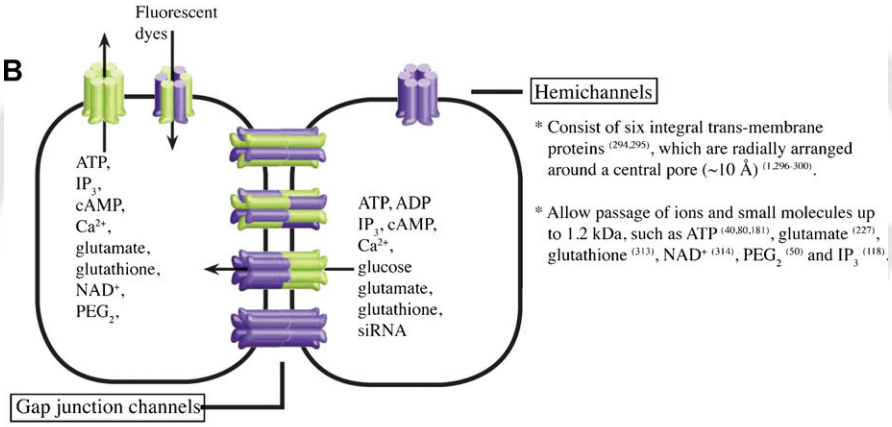

* Consist of six integral trans-membrane proteins [294,295], which are radially arranged around a central pore (~10 Å) [1,296-300].

* Allow passage of ions and small molecules up to 1.2 kDa, such as ATP [40,80,181], glutamate [227], glutathione [313], NAD^+ [314], PEG_2 [50] and IP_3 [118].

* Formed by the docking of two hemichannels, bridging a 2-4 nm gap between the cells [1,2].

* Allow intercellular exchange of metabolites, such as ATP, ADP, glucose, glutamate, glutathione, and second messengers including cAMP and inositol 1,4,5-trisphosphate (IP_3) [110,301-306] and other molecules including antigens [307,308] and small interference RNAs (siRNA) [309] GJs connecting electrically excitable cells permit the intercellular propagation of action potentials [310-312].

C

	Pannexin (3 subtypes)	Connexin (21 subtypes)
GJ/hemichannel	Hemichannel formation rather than GJ formation.	Hemichannel and GJ formation (Cx23 preferentially forms hemi-channels).
Channel type	Homomeric hemichannels rather than heteromeric hemichannels.	Homo- and heteromeric hemichan-nels and homo- and heterotypic GJ channels.
Extracellular loops	50-68 amino acids	~30 amino acids
Conserved cysteine residues	2	3 (except Cx23 with 2 conserved cysteine residues)
Unitary conductance	475 to 550 pS	15 to 300 pS
Half-life	>6 hours	1.5-5 hours

Figure 1. Schematic representation of the formation of gap junction (GJ) channels and hemichannels. **A**: Structure of innexin (Inx), connexin (Cx), and pannexin (Panx), which are folded in the membrane in the approximate shape of an "M" and which consist of four typical hydrophobic transmembrane domains (M1–M4) spaced by one cytoplasmic (CL) and two extracellular (EL1 and EL2) loops. Six transmembrane proteins (Inxs, Cxs, or Panxs), which are radially arranged around a central pore, form an innexon, connexon or pannexon, respectively. Innexons, connexons, or pannexons, which are located in the plasma membrane, are called hemichannels. When they consist of identical protein subtypes, they are called homomeric hemichannels, and when they consist of different protein subtypes, when two or more isoforms are expressed in the same cell, they are called heteromeric hemichannels. The docking of two identical homomeric or heteromeric hemichannels results in a homotypic GJ channel, while docking of two different homomeric or heteromeric channels forms a heterotypic GJ channel. **B**: Structure and properties of Cx and Panx channels. (Partially modified from Mese *et al.*[89]) **C**: A table summarizing the main differences between Cx and Panx channels.

intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In contrast, Panx1 hemichannels seem to be activated by different physiological stimuli, including mechanical stress during osmotic shock,[39,47–49] strong depolarizations (>+20 mV), and activation of purinergic receptors, including P2Y1, P2Y2, and P2X7, by ATP and other agonists.[50–58]

In non-excitable cells, two pathways for IC are important: gap junctional intercellular communication (GJIC) and paracrine intercellular communication (PIC).[22,59,60] In contrast to GJIC, PIC does not require cell–cell apposition but involves the release of diffusible extracellular messengers (Fig. 2). Cells produce and release different types of signaling molecules in the extracellular space. Released hydrophilic messengers, which are unable to cross the plasma membrane of the responding cell, bind as ligands to receptor proteins that are present in the plasma membrane. These receptors then relay the message across the membrane into the interior of the cell.

IC both *via* GJIC and PIC has been extensively documented for intercellular Ca^{2+} signaling. GJIC occurs *via* the diffusion of different signaling molecules, including Ca^{2+} or inositol 1,4,5-trisphosphate (IP_3) through GJs causing and modulating Ca^{2+} release from the intracellular stores of the neighboring cells[61] (Fig. 2). Upon reaching the cell boundaries, the intracellular Ca^{2+} wave propagates to the surrounding neighboring cells as an intercellular Ca^{2+} wave.[62,63]

A well-investigated paracrine factor in the propagation of intercellular Ca^{2+} waves in many cell types is the hydrophilic messenger ATP.[64–67] ATP can be released from healthy cells[68–71] during mechanical deformation in response to shear stress, stretch, or osmotic swelling, as well as during hypoxia, inflammation and stimulation by various agents.[71,72] *In vitro* evidence showed that ATP release can occur *via* multiple mechanisms including vesicular exocytosis[71] or *via* transport mechanisms, such as ATP-binding cassette (ABC) transporters, plasmalemmal voltage-dependent anion channels,[73] P2X7-receptor channels,[53,74–76] and also *via* Cx hemichannels[51,64,77–82] or Panx hemichannels[69–71,83] (for review, see Ref.[84]).

As the Cx family is very extensive with many members and a multiplicity of regulatory mechanisms, it seems remarkable that the Panxs are conserved in vertebrates and are expressed together with several members of the Cx family. This suggests that Panxs fulfill specialized functions under specific cellular conditions. The physiological function and subcellular localization of Panx channels have long been the subject of debate, and are still poorly documented. Recent evidence for Panx1 activity in hippocampal neurons[85,86] and new insights in the formation of Panx hemichannels[32,33]

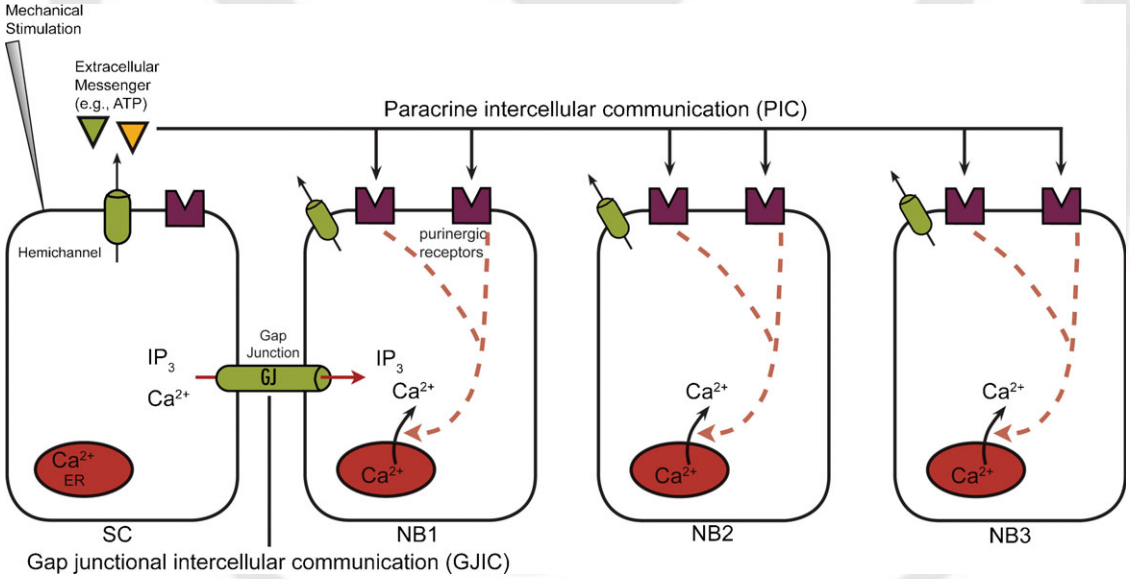

Figure 2. Intercellular Ca^{2+}-wave propagation in non-excitable cells involves both gap junctional intercellular communication (GJIC) and paracrine intercellular communication (PIC). Stimulation of a single cell results in a Ca^{2+} rise in the stimulated cell (SC) *via* Ca^{2+} influx and/or Ca^{2+} release. The Ca^{2+} rise spreads from the SC to neighboring cells (NB), resulting in intercellular Ca^{2+}-wave propagation. Two mechanisms, GJIC and PIC, are involved in the intercellular propagation. GJIC is a direct exchange of a mediator (IP_3 and/or Ca^{2+}) between the cytoplasm of adjacent cells. PIC involves release of a messenger (*e.g.*, ATP) into the extracellular space, which acts on receptors on neighboring cells. ATP can be released *via* hemichannels (Cx hemichannels, Panx hemichannels or a combination of P2X7 receptor channels with Panx hemichannels) or other mechanisms (see text). It is hydrolyzed by ectonucleotidases to ADP and AMP. ATP and ADP act on P2Y and/or P2X receptors on neighboring cells (NB1, neighboring cell 1; NB2, neighboring cell 2; NB3, neighboring cell 3).

suggest a specific cellular function of Panx hemichannels. In this review, we summarize the current view on the regulation and function of Panx channels in normal and pathological conditions. We focus particularly on specific mechanisms that discriminate Panxs from the broad Cx family.

How to discriminate between Cx and Panx channels?

It is very difficult to experimentally discriminate between Cx and Panx channel families, since (i) subtype-specific blockers for Cx and Panx channels are not available[31,87,88]; (ii) cells can express multiple Panx and Cx isoforms; and (iii) natural hemichannels can function as heterooligomers[89] with atypical sensitivity to blockers.

A myriad of chemical products used to block GJs are non-specific, and both Panxs and Cxs have a high sensitivity to drugs routinely used to block hemichannels (see Table 1). Great care must be exerted in using pharmacological blockers to identify Cx or Panx channels, since "cross-inhibition" of Cx/Panx channels and volume-regulated anion channels (VRACs), which share certain functions, by pharmacological agents has been reported[90] (see Table 1). Panx channels share some pharmacological properties with P2X7 receptors,[91] and P2X7 receptor inhibitors have also been shown to block Panx channels (see Table 1).

Cx-mimetic peptides, corresponding to sequences of the extracellular loops of Cxs, are used to inhibit Cx GJ channels and hemichannels.[81,87,92–95] Surprisingly, ^{32}Gap24 and ^{43}Gap27 attenuate Panx currents,[87] questioning the specificity of Cx mimetic peptides (see Table 1). Panx mimetic peptides (^{10}Panx) inhibit Panx hemichannels,[74,86,87] but one study showed that ^{10}Panx also inhibited Cx46 hemichannels[87] (see Table 1). Antibodies against Panxs, such as Pannexin-1 K-20 (Santa Cruz),[5,47,96–101] or custom made antibodies 4512[86,97] and 4515,[85,97] ANT0027 (Diatheva)[30,102] were also used to block Panx channels.

Differences between Cx and Panx channels can also be studied by tagging tetracysteine and fluorophores to the N and C termini of recombinant Cx and Panx channels and performing patch-clamp experiments, a recently developed method.[103]

Finally, siRNA and shRNA have been used to specifically knockdown Panx channels.[52,74,86,101,104–106]

The use of Panx gene knockout animals would provide important insights in the physiological role of Panx channels. However, Panx1-deficient mice are viable without any obvious phenotype,[107] suggesting redundancy between different Panx isoforms or an overlap in function between Cx and Panx hemichannels. Nevertheless, the lack of phenotype in normal mice does not necessarily indicate that Panx hemichannels do not fulfill important roles under pathophysiological conditions.

Structural properties of Cx and Panx channels

Hemichannels can be homomeric (identical Cx/Panx subtypes) or heteromeric (different Cx/Panx subtypes)[13] (Fig. 1). The docking of two identical homomeric or heteromeric hemichannels results in a homotypic GJ channel, while docking of two different homomeric or heteromeric hemichannels forms a heterotypic GJ channel (Fig. 1).

Nearly all cells in the human body express at least one of the Cx genes and most vertebrate cell types express several different Cx isoforms in a temporal-, spatial-, and differentiation-specific manner.[108] Formation of functional Cx GJs (homotypic as well as heterotypic GJ channels) and Cx hemichannels (homomeric as well as heteromeric hemichannels) has been described in many cell types.[2,26,46,61,109] These channels differ from each other by their unitary conductance,[110] permeability,[111] and regulation,[108] which is crucial for maintaining proper embryonic development and sustaining tissue function in the adult organism. It has also become increasingly clear that Cxs have profound effects on gene expression (reviewed in Ref.[112]) and the presence of a Cx subtype can also influence the channel formation of other Cx subtypes.[113]

Panxs are expressed in many different cell types and abundantly in the vertebrate central nervous system,[114] and, like Cxs, the membrane expression of Panx1 might also be regulated by other Panx subtypes. While all Cx subtypes are able to form homomeric connexons, only Panx1[31] and Panx3 (albeit when overexpressed)[37] form homomeric pannexons. Very recently different studies have demonstrated the presence of functional Panx hemichannels by showing dye uptake *via* Panx hemichannels.[52,74,86,87,91,105,115–118] Panx1 hemichannel activities have been clearly demonstrated, but no active Panx2 hemichannels have been described yet.[119] Functional Panx1 (homotypic channels) and Panx1/Panx2 (heterotypic channels) GJ channels were demonstrated with patch clamp experiments in *Xenopus* oocytes.[119] The measured currents were, however, much smaller than Cx GJ currents in oocytes[33] and mouse neuroblastoma cells,[37] implying that these GJ currents are minimal and that the functional form of Panx1 channels is mainly a single membrane pannexon (hemichannel).[32,33,120] As yet, no evidence of canonical Panx GJs has been found in cultured neurons, and glia.[121] Until recently, Panxs had not been ultrastructurally identified as GJs or as any other membrane structure in vertebrate species. Morphological and ultrastructural studies in crayfish axons[122,123] and in rodent spinal cord,[124] both expressing Panxs, revealed small rosette-like GJ plaques that were completely different from the Cx GJ plaques, which have a bright punctate staining. Shestopalov and Panchin[4] hypothesized that these small rosette-like GJ plaques and the fine puncta observed in the

Table 1. Pharmacological inhibitors of large conductance channels

Drug	Cx GJ channels	Cx hemichannels	Panx channels	P2X7	VRAC
Carbenoxolone (CBX)	<50–100 μM[224,225] Cx50: EC_{50} = 118 μM[226]	Cx46: 50–100 μM[31] Cx43: EC_{50} = 3 μM[90] 10–100 μM[227] Cx32: 100 μM[82] Cx26: IC_{50} = 21 μM[228] Cx30: 100 μM[79]	IC_{50} = 5 μM[31] 50 μM[104] IC_{50} = 2–4 μM[74] Human Panx1: IC_{50} = 2 ± 1 μM[229] Mouse Panx1: IC_{50} = 4 ± 0.6 μM[229]	EC_{50} = 0.175 μM[53] IC_{50} = 2–4 μM[74] 5–10% increase[229]	EC_{50} = 3–10 μM[90]
Mefloquine	Cx50: EC_{50} = 34 μM[226] IC_{50} Cx36 = 0.3 μM[230] IC_{50} Cx50 = 1.1 μM[230] Cx43: >10 μM[231]	Cx50: 5 μM[232] Cx50: 10 μM[232] Cx30.2: IC_{50} = 5.5 μM[233]	50–100 nM[104] NE[74] Human/mouse Panx1: NE[229]	EC_{50} = 2.5 nM[53] NE[229]	IC_{50} = 1.19 ± 0.07 - μM[234]
Flufenamic acid (FFA)	20–60 μM[235] Cx50: 47 μM[235] Cx50: EC_{50} = 41 μM[226]	Cx46 and 50: 3 μM[31,236] Cx 43: >100 μM[227] Cx26: 200 μM[237] Cx43: 25–50 μM[80] Cx38: 50 μM[238]	30 μM[31] 0.3 mM[104]	EC_{50} = 0.655 μM[53] NE[229]	n/a
Niumic acid	Cx50: EC_{50} = 173 - μM[226] 300 μM[235]	Cx50 11 μM[236]	Human Panx1: >1 mM[229] Mouse Panx1: >1 mM[229]	NE[229]	200 μM[239]
NPPB [5-nitro-2-(3-phenylpropyl-amino)-benzoic acid]	Cx50: 100 μM[235]	Cx43: 30–100 μM[90] Cx46: IC_{50} of ~50 μM[115] Cx46 and 50: 15 μM[236]	IC_{50} of ~50 μM[115] Human Panx1: IC_{50} = 21 ± 4 μM[229] Mouse Panx1: IC_{50} = 15 ± 2 μM[229]	n/a	300 μM[90] IC_{50} = 14.6 μM[228] 123 μM[239] IC_{50} = 27 μM[240]
4,40-Diisothiocyanatostilbene-2,20-disulfonic acid (DIDS)	NE[235]	Cx46 and 50: NE[236] Cx43: NE[80]	Human Panx1: IC_{50} = 11 ± 2 μM[229] Mouse Panx1: IC_{50} = 11 ± 2 μM[229]	Human: 195 μM[229] Mouse: 130 μM[229] Rat: 90 μM[229] NE[241]	15% increase[229] 200 μM[239] IC_{50} = 256 μM[240]
pH_i	Cx26: 6.95 ± 0.02[242] Cx32: 6.47 ± 0.03[242] Cx37: 6.9 ± 0.02[242]	6[243]	Low pH[98]	$[H^+]$: IC_{50} = 0.4 μM = > pH = 6.4[244]	>8[245]

(Continues)

Table 1. (*Continued*)

Drug	Cx GJ channels	Cx hemichannels	Panx channels	P2X7	VRAC
	Cx40: 6.67 ± 0.04[242] Cx43: 6.71 ± 0.03[242] Cx45: 6.98 ± 0.03[242] Cx46: 7.03 ± 0.08[242] Cx50: 7.17 ± 0.03[242]				
Tamoxifen	Cx43: 3–25 μM[246]	Cx43 5–10 μM[90] Cx46 and 50: NE[236]	n/a	NE[247]	EC_{50}: >100 μM[248] 10 μM[90] $IC_{50} = 2.6$ μM[240]
Octanol	0.1 mM[249] Cx43: $EC_{50} \approx$ 120 μM[250] Cx 37 and 40: 4 mM[251]	Cx50: 177 μM[236] Cx43: 10 μM–1 mM[227] Cx43: 1 mM[252] Cx43: 1 mM Cx38: 1.5 mM[238]	n/a	<1.5 mM[53]	NE[227]
Heptanol	1 mM[249] Cx43: 1 mM[254,255] Cx43: 2 mM[256] Cx 37 and 40: 4 mM[251]	Cx43: ≥1 mM[227] Cx43: 1 mM[252] Cx43: 0.2–2 mM[257] Cx30: 2 mM[258]	NE[74]	<1.5 mM[53]	NE[227] NE227
Halothane	Cx43: 1.6 mM[259] Cx43: 2–4 mM[260]	Cx43: 2 mM[253] [41] Cx43: $EC_{50} = 0.92$ - 0.92 mM[257]	n/a	NE[261]	n/a
18α-Glycyrrhetinic acid	1.5 μM[262] 2 μM[224]	Cx43: 10 μM[227] Cx26: 35 μM[263] Cx37: 10 μM[264] Cx45: 40 μM[265]	n/a	n/a	NE[227] NE[265]
18β-Glycyrrhetinic acid (βGA)	2 μM[262] 25 μM[226]	Cx46 and 50: 2 μM[236] Cx43: 35 μM[46] Cx43: 10 μM[39] Cx43: 20 mM[253]	n/a	n/a	50 μM[90]
2-Aminoethoxydiphenyl borate (2-APB)	Cx36 and 50: $IC_{50} =$ 3.0 μM[266] Cx50: $IC_{50} =$ 3.4 μM[266] Cx45: $IC_{50} =$ 18.1 μM[266] Cx46: $IC_{50} =$ 29.4 μM[266] Cx43: $IC_{50} =$ 51.6 μM[266]	Cx32 (homomeric): $IC_{50} \sim 47$ μM[267] Cx32/26 (heteromeric): $IC_{50} \sim 47$ μM[267]	n/a	n/a	$IC_{50} = 122.8$ μM[268]
Proadifen hydrochloride (SKF-525A)	Cx26:[269] Cx43: 75 μM[270]	Cx26: 100 μM[263]	n/a	n/a	n/a

(Continues)

Table 1. (*Continued*)

Drug	Cx GJ channels	Cx hemichannels	Panx channels	P2X7	VRAC
Oleamide	Cx 37 and 40: 200 μM[251]	Cx43: 50 μM[39]	n/a	n/a	n/a
Mg^{2+}	10 mM[271]	Cx43: 10 μM–1 mM[227] Cx32: IC_{50} = 1.30 mM[272] Cx32: 1 mM[273] Cx46: 5 mM[16,274]	n/a	IC_{50} = 0.5 mM[244]	1 mM[275]
Ba^{2+}	NE[276]	Cx43: 10 μM–1 mM[227] Cx32: 1 mM[273]	n/a	n/a	n/a
Gd^{3+}	n/a	Cx43: 50 μM[80] Cx43: 10 μM[253] Cx46 and 50: 3 μM[236] 2 mM[227] Cx32: 100–200 μM[272]	NE (0.1–1 mM) [74]	n/a	n/a
La^{3+}	NE[40] NE[79] Cx37 and 40: 5 μM[251]	Cx43: 0.1 mM[40] Cx43: 10 μM–1 mM[227] Cx43: 1 mM[41] 100 μM[177] Cx30: 100 μM[79] Cx30.2: 5–100 μM[233]	NE[74]	n/a	n/a
Sr^{2+}	n/a	Cx43: 10 μM–1 mM[227]	n/a	n/a	n/a
Zn^{2+}	n/a	Cx46: 10 μM[232] 2 mM[227] 30 μM[277] 2 mM[227] Cx26: 3.5 mM[228] Cx26: 2–4 mM[215] Cx32: IC_{50} = 107 μM[272] Cx32: EC_{50} = 1.3 mM[273] Cx38: 3 mM[280] Cx46: 0.1 mM[274] Cx26, Cx34.7, Cx35, Cx43, Cx27.5, Cx44.1, and Cx55.5: 2 mM[281] Cx50: 5 mM[243]	n/a	$IC_{50} \approx 5$ μM[278] IC_{50} = 11 μM[244] NE[279]	n/a
Extracellular Ca^{2+}	NE[227]		NE[227]	IC_{50} = 2.9 mM[244]	n/a
Intracellular Ca^{2+}	Cx43: IC_{50} = 310 nM[282]	Cx32: Opened by ~500 nM[82] Opened by limited range of $[Ca^{2+}]_i$[227]	Opened by high $[Ca^{2+}]_i$[31]	IC_{50} = 2.9 mM[244]	Opened by high $[Ca^{2+}]_i$[283]
Retinoic acid	n/a	Cx38: 1–10 μM[280] Cx26, Cx34.7, Cx35, Cx43, Cx27.5, Cx44.1, and Cx55.5: EC_{50} = 0.44 mM[281]	n/a	n/a	n/a

(Continues)

Table 1. (*Continued*)

Drug	Cx GJ channels	Cx hemichannels	Panx channels	P2X7	VRAC
IAA-94	n/a	Cx43: 50–200 μM[90] Cx46 and 50: NE[236]	Mouse Panx1: $IC_{50} = 95 \pm 6$ μM[229]	n/a	200 μM[90]
4-Acetamido-40-isothiocyanostilbene-2,20-disulfonic acid (SITS)	n/a	Cx46 and 50: NE[236]	Human Panx1: $IC_{50} = 13 \pm 3$ μM[229] Mouse Panx1: $IC_{50} = 11 \pm 3$ μM[229]	n/a	0.5 mM[284]
Cx mimetic peptide Gap24 ([32]Gap24: GHGDPLHLEEVKC)	n/a	Cx32: 0.25 mg/L[82]	200 μM[87]	n/a	n/a
Cx mimetic peptide Gap26 ([43]Gap26: VCYDKSFPISHVR; [37,40]Gap 26: VCYDQAFPISHIR)	Cx43: 200 μM[87] Cx37 and 40: 300 μM[285]	Cx43: 300 μM[286,287] Cx43: 0.25 mg/L[82] Cx43: 160 μM[288] Cx37: 160 μM[264] Cx43: 200 μM[87]	n/a	n/a	n/a
Cx mimetic peptide Gap27 ([43]Gap 27: SRPTEKTIFII; [40]Gap 27: SRPTEKNVFIV)	Cx43: 300 μM[131] Cx43: 200 μM[87] Cx43: 600 μM[289] Cx37, 40, and 43: 300 μM[285]	Cx43: 0.25 mg/L[290] Cx43: 0.25 mg/L[82] Cx43: 190 μM[288] Cx43: 1 mg/mL[53] Cx37: 160 μM[264]	NE[74] 200 μM[87]	n/a	n/a
Cx mimetic peptide Gap36	n/a	Cx43: 130 μM[288]	n/a	n/a	n/a
Panx mimetic peptide ([10]Panx1: WRQAAFVDSY)	n/a	Cx46: 200 μM[87]	$IC_{50} = 30$–50 μM[74] 200 μM[87] 400 μM[291] 200 μM[74] 100 μM[86] 500 μM[105]	n/a	n/a
Pannexin1 peptide E1b, SSFSWRQAAFVDS	n/a	n/a	200 μM[87]	n/a	n/a
Probenecid	n/a	n/a	$IC_{50} = \sim 150$ μM[115] Human Panx1: $IC_{50} = 360 \pm 21$ μM[229] Mouse Panx1: $IC_{50} = 352 \pm 31$ μM[229]	NE[229]	n/a

(Continues)

Table 1. (*Continued*)

Drug	Cx GJ channels	Cx hemichannels	Panx channels	P2X7	VRAC
Benzoyl-benzoyl-ATP (BzATP)		Cx46: NE[91]	20 μM[91]		
BBG (Coomassie brilliant blue G)	n/a	n/a	0.1 μM[91]	0.1 μM[53]	n/a
ATP	n/a	Cx46: NE[91]	Human Panx1: $IC_{50} = 825 \pm 56$ μM[229] Mouse Panx1: $IC_{50} = 752 \pm 42$ μM[229] 200 μM[91]	n/a	n/a
UTP	n/a	n/a	Human Panx1: $IC_{50} = 1350 \pm 60$ μM[229] Mouse Panx1: $IC_{50} = 1256 \pm 56$ μM[229]	NE[229]	n/a
GTP	n/a	n/a	Human Panx1: $IC_{50} = 1420 \pm 108$ μM[229] Mouse Panx1: $IC_{50} = 1290 \pm 87$ μM[229]	NE[229]	n/a
Polyethylene glycol (PEG)	n/a	n/a	PEG1500: 200 μM[87]	n/a	n/a
Primaquine-1 (PQ1)	Cx43: 10 μM[231]	n/a	PQ1 protects neuro-retinal cells from ischemic apoptosis[231], this can suggest Panx hemichannel involvement.[86,177]	n/a	n/a
PQ4	Cx43: 10 μM[231]	n/a	n/a	n/a	n/a
Meclofenamic acid (MFA)	Cx50: $EC_{50} =$ 21 μM[226] Cx50: 100 μM[235]	NE[45]	n/a	n/a	n/a
Arachidonic acid	Cx43: 20 μM[259] Cx43: $EC_{50} \approx$ 32 μM[250]	n/a	n/a	n/a	4–5 μM[292]
Oleic acid	Cx43: 20 μM[259] Cx43: $EC_{50} \approx$ 35 μM[250]	n/a	n/a	n/a	n/a
Oleyl alcohol	Cx43: $EC_{50} \approx$ 35 μM[250]	n/a	n/a	n/a	n/a
Palmitoleic acid	Cx43: $EC_{50} \approx$ 60 μM[250] Cx43: 50 μM[276] Cx 37 and 40: 50 μM[251]	Cx45: 50 μM[265]	n/a	n/a	NE[265]
Stearic acid	Cx43: $EC_{50} \approx$ 102 μM[250]	n/a	n/a	n/a	n/a
Caprylic acid	Cx43: $EC_{50} \approx$ 185 μM[250]	n/a	n/a	n/a	n/a

(Continues)

Table 1. (*Continued*)

Drug	Cx GJ channels	Cx hemichannels	Panx channels	P2X7	VRAC
Palmitic acid	Cx43: $EC_{50} \approx 243$ - μM[250]	n/a	n/a	n/a	n/a
Methyl-oleyl ester	Cx43: $EC_{50} \approx 690$ - μM[250]	n/a	n/a	n/a	n/a
Ouabain	Cx43: 0.1 mM[276]	n/a	n/a	n/a	NE[293]

NE, no effects; n/a, data not available or unknown; all ions are for extracellularly applied except intracellular Ca^{2+}.

cell junctional area of HeLa and LNCaP cells might represent GJs formed by Panxs.

Recently, different arguments were raised as to why the formation of Panx GJ channels is less likely. First, Boassa *et al.*[32,33] showed that Panx1 is glycosylated in the lumen of the endoplasmic reticulum (ER) at its second extracellular loop at Asn254. This glycosylation[32] and the bulky carbohydrate moieties of the extracellular domain of Panx1 interfere with intercellular channel formation.[33] Similar conclusions were obtained for Panx3.[37] Moreover, upon deglycosylation, GJ formation increased significantly in pairs of oocytes expressing Panx1,[32] indicating that glycosylation hinders GJ formation. Secondly, the preference of Panxs for hemichannel formation may also be an intrinsic property of these proteins. While Cxs and Panxs share a number of structural similarities, there are also important differences particularly in the extracellular loops, the number of cysteine residues, the cytoplasmic loop, and the N and C termini, which can influence the formation and properties of GJ channels and/or hemichannels.[125] In GJ channels, the cysteine residues form intramolecular disulfide bonds between the two extracellular loops of each protein, resulting in the formation of anti-parallel beta sheets, which resemble the beta-barrel structure of porin channels. Upon docking, the beta-barrel structures of each opposing hemichannel interdigitates and hydrogen bonds stabilize this structure.[126] In Panxs, each extracellular loop contains 50–68 aa but only two conserved cysteine residues, while Cxs harbor three cysteines in their relatively small (~30 aa) loops[3,5,96] except for Cx23, which has only two conserved cysteine residues.[17] Panxs[32,33] and Cx23,[127] having only two cysteines, preferentially form hemichannels, suggesting that the number of conserved cysteine residues may play role in the determination of hemichannels. However, Cx23 is also capable of forming functional GJs and hemichannels in zebrafish,[17] suggesting that also the length of the extracellular loops plays an important role in the docking process. In addition, the N and C termini show great variation in terms of sequence and length between Cxs and Panxs and between different Cx subtypes.[128–131] N-terminal additions result in non-functional GJ channels or hemichannels.[20] Accordingly, an intact N terminus is required for hemichannel gating and IC,[117] and the C terminus also plays an important role in channel gating. Moreover, the cytoplasmic carboxyl-tail and loop are susceptible to various post-translational modifications (*e.g.*, phosphorylation), which have regulatory roles.[132] Phosphorylation of Cxs regulates the assembly and modulation of the physiological properties of these channels.[132–134] Post-translational modifications may also alter the gating mechanisms of Panxs and the regulation of channel formation and channel permeability.[133,135–137]

Properties in trafficking and turnover of Cx and Panx channels

Cxs are known to have a short half-life, estimated at 1.5–5 h depending on the cell type,[59] whereas the half-life of Panxs is more than 6 h.[37] This points to a different regulation of Panx trafficking and expression levels.

Previous studies have shown that Panxs are not only localized in the plasma membrane, but that Panx1 is abundantly detected in intracellular organelles and Golgi apparatus.[30,101,138,139] Accordingly, Panx1 overexpression in LNCaP (human prostate cancer epithelial cells) showed accumulation in both the plasma membrane and in the ER,[101] implying that post-translational modification and assembly of pannexons share the same route demonstrated for Cxs.[140,141] The ER-bound Panx1 could be either a pool of unprocessed precursor proteins[121,138] or assembled functional pannexons that serve as ER-Ca^{2+}-release channels, thereby facilitating Ca^{2+} leakage from the ER.[101]

Panx trafficking has been investigated by differences between tagged and untagged Panxs and by treating the cells with brefeldin A (BFA),[32,33] which promotes Golgi breakdown.[142,143] Both wild-type and Myc or tetracysteine-tagged Panx1 are *N*-glycosylated and properly trafficked to the plasma membrane. In contrast to tagged Cxs, Myc or tetracysteine-tagged Panxs are degraded at a faster rate than wild-type oligomers, suggesting that the tags might interfere with some molecular chaperones important for stabilizing Panxs at the cell surface.[32] They concluded that Panx1 is initially glycosylated in the ER and modified later in the Golgi apparatus where it resides en route to the plasma membrane.

Glycosylation of membrane proteins can affect their folding, stability, trafficking, and function.[144–148] In oocytes,

both glycosylated and non-glycosylated forms of Panx1 are present,[33,37,121] indicating that glycosylation does not influence the folding of Panx1. Glycosylated Panx1 is targeted to the plasma membrane and the non-glycosylated Panx1 is retained in intracellular compartments. *N*-Glycosylation of Panx1 could be a significant mechanism for regulating Panx1 trafficking to the cell surface, hereby possibly affecting its function in different tissues.[32] In *Xenopus* oocytes pairs, localization of Panx1 at the cell surface is rescued when glycosylation-deficient mutant proteins are co-expressed with Panx1 wild-type proteins.[32] Glycosylated Panx1 would be predominantly expressed at the plasma membrane to form hemichannels and non-glycosylated Panx1, if localized at the cell surface, could potentially form intercellular channels. However, it remains unclear whether this non-glycosylated form can be targeted to the mammalian cell surface *in vivo*, but its very existence suggested an impact on cell–cell communication mechanisms.

These glycosylation mechanisms show that the regulation mechanisms of Panx hemichannel expression are different from to those of Cx hemichannels, which are mainly regulated by their rapid turnover, resulting in a low number of active channels.[149,150]

Cx and Panx hemichannels: gating and regulation

The fact that in vertebrates Cxs and Panxs are co-expressed and evolutionarily distinct implies that both protein families have their own specific function. However, the exact physiological difference between the two channel-forming protein families is still unknown. Cxs and Panxs have different primary sequence and properties, suggesting a different regulation.[97,151] To sustain ionic gradients and avoid lethal effects of prolonged channel opening, Cx/Panx-hemichannel gating must be regulated very carefully in time. It is now clear that different extracellular and intracellular stimuli can influence the gating-state and gating-kinetics of hemichannels.

Voltage-sensitive gating

Not only trafficking and expression, but also channel gating is a highly regulated and finely tuned process. Voltage-patch clamp studies showed that positive transmembrane potentials open Panx1 channels.[98] Recordings of single Panx- or Cx-channel currents show the presence of multiple substates with variable transition rates. Panx1 exhibits at least five open states: the fully open state and no less than four subconductance states with 5, 25, 30, and 90% of the maximal conductance.[47,119] Panx channels rarely remain in fully open or closed states, residing mainly in the subconductance states. The unitary conductance of Panx hemichannels is larger ($\sim$500 pS[47]), compared to a unitary conductance of 15–300 pS in Cx channels.[152]

Information on gating of Cx hemichannels is reviewed in more detail by Saez *et al.*[23] Cx hemichannels appear to have two types of voltage-dependent gating mechanisms (for review, see Ref.[153]). A first type, called "loop gating" is slow and closes the channels at negative membrane potentials. This type of gating is controlled by pore-lining residues in the first extracellular loop of Cxs[154] and is modulated by extracellular Ca^{2+} and by docking of hemichannels. A second type of gating, called "fast V_j gating" can close the channel to a substate, either at positive potentials (*e.g.*, in Cx26, Cx30, Cx46, and Cx50 hemichannels) or at negative potentials (*e.g.*, in Cx31, Cx43, and Cx45 hemichannels). Fast V_j gating is thought to be due to a "ball and chain" interaction of either the C terminus (*e.g.*, in Cx43) or the N terminus (*e.g.*, in Cx26 and Cx32) with the intracellular loop.[155–158] Charged residues in the first positions of the N-terminal domain of Cx26 and Cx32 have been reported to be involved in sensing voltage.[159] Within the intracellular loop of Cx43, H142 in the L2 region of the cytoplasmic loop has been identified as a voltage-sensor for fast V_j gating.[160]

Panx hemichannels slowly close upon hyperpolarization ($V_m < -20$ mV), probably *via* loop gating, and partially close to a substate after depolarizations to positive membrane potentials of about $+20$ mV.[31,47,97,119] Similar to heteromeric Cx hemichannels, heteromeric Panx1/Panx2 hemichannels exhibited modified voltage-gating kinetics with respect to homomeric Panx1 channels.[119]

Mechanical stress

Cx46 hemichannels are mechanosensitive, but the mechanosensitivity of other Cx subtypes is not clear yet. The opening of these channels could be triggered by mechanical stress at negative transmembrane potentials. At positive voltages, mechanical stress closes the channel.[48] Although still speculative, Cx26 was proposed to be involved in mechano-transduction of sound waves in the cochlea[161] and a putative role for Cx43 hemichannels and P2 receptors has been proposed as a mechanoreceptor complex involving the primary cilium of bovine chondrocytes.[162] For Panx1, the probability of channel opening is highly increased during mechanical stretch, which illustrates its mechanosensitivity.[39,47,49]

Extracellular Ca^{2+}

The regulation of hemichannels by extracellular Ca^{2+} is strikingly different between Panxs and Cxs. Under normal physiological conditions (extracellular Ca^{2+} concentration ($[Ca^{2+}]_0$) = 1–2 mM) connexons are closed,[39] likely due to

loop gating (slow V_j gating). However, removal of extracellular Ca^{2+} leads to Cx hemichannel opening. Accordingly, the pore diameter of Cx43 hemichannels is increased by lowering the $[Ca^{2+}]_0$, indicating that the probability of Cx43 hemichannels opening is controlled by extracellular Ca^{2+}. These Ca^{2+}-dependent conformational changes are regulated by the hydrophobic extracellular domains of Cxs.[163] As low $[Ca^{2+}]_0$ favors the opening of Cx hemichannels, it is likely that Cx hemichannels are open under conditions in which $[Ca^{2+}]_0$ and $[Mg^{2+}]_0$ is reduced, as in ischemic brain.[164,165] In contrast, Panx hemichannel activation is unaffected by changes in $[Ca^{2+}]_0$ levels.[31]

Intracellular Ca^{2+}

$[Ca^{2+}]_i$ also regulates the opening and closure of Cxs and Panxs hemichannels in a different way. The opening of Cxs hemichannels, like Cx32 display a biphasic bell-shaped Ca^{2+} dependence, indicating that rises in $[Ca^{2+}]_i$ below 500 nM (submicromolar range) promote hemichannel opening, whereas rises in $[Ca^{2+}]_i$ above 500 nM (micromolar range) inhibit hemichannel opening.[82] In contrast, patch-clamp experiments on Panx1 expressed in *Xenopus* oocytes revealed that these channels have a linear dependence on Ca^{2+}, which means that higher $[Ca^{2+}]_i$ leads to larger Panx1 currents.[98] $[Ca^{2+}]_i$ above the resting levels (>100 nM) seem to be sufficient to activate Panx1 opening. However, increase in $[Ca^{2+}]_i$ does not appear to be a requisite for Panx1 hemichannel activation in hippocampal neurons, since the activation of Panx1 channels through *N*-methyl-D-aspartate receptors (NMDARs) was independent of the increase in $[Ca^{2+}]_i$, demonstrated by Panx1 hemichannel opening in the presence of intracellular Ca^{2+} buffers.[86] Nevertheless, it is likely that Panx1 hemichannels are opened during agonist-induced Ca^{2+} signaling, thereby playing an important role in mediating ATP release and IC.

The effect of $[Ca^{2+}]_i$ may be a direct effect of Ca^{2+} on the Panx1 protein, since Panx1 hemichannels are activated by submicromolar concentrations of Ca^{2+}. However, a role for calmodulin on Panx1 hemichannels cannot be ruled out, since calmodulin is an important regulator of Cx50[166] and Cx32[167] channel gating, and interacts with other Cx isoforms.

Not only does $[Ca^{2+}]_i$ regulate Panx1 hemichannel activity, Panx1 may also control $[Ca^{2+}]_i$, since Panx1 has been implicated in regulating the passive Ca^{2+} leak from the ER. Accumulation of ectopic eGFP-Panx1 in the ER leads to an increased Ca^{2+} leak rate from the ER, whereas Panx1 knockdown decreased the efflux rate of Ca^{2+} from the ER.[101] However, how Panx1 channels in the ER are regulated is not known, *e.g.*, by the ER-Ca^{2+} content and/or by cytosolic Ca^{2+}. Nevertheless, the role of pannexons as passive Ca^{2+} leak channels in the ER regulating the ER-Ca^{2+} content should be further elucidated, and may open important perspectives for the role of pannexons in processes that are highly dependent on the ER-Ca^{2+} content, like store-operated Ca^{2+} influx, protein folding and apoptosis.

Intracellular pH

Intracellular acidification negatively influences the probability of many Cx channels[168,169] being open. In addition, for Panx1, induction of low intracellular pH by CO_2 perfusion abolishes the conductance.[98] Interestingly, while regions involved in pH gating have been located in intracellular Cx domains,[170] pH-induced conformational changes in Cx43 hemichannels could be detected extracellularly by atomic force microscopy.[163] These observations[170] suggested that the underlying mechanism is different from that for Ca^{2+}-induced closure.[171]

Phosphorylation status

Differences in phosphorylation/dephosphorylation state of Cx-serine/threonine and tyrosine residues within Cxs are known to change the permeability of Cx channels (*e.g.*, Cx43 channels[172,173]); therefore, it is likely that phosphorylation also affects the properties of Panx channels, which are predicted to have multiple phosphorylation sites.[37] Opening of hemichannels induced by metabolic inhibition has been proposed to imply dephosphorylation-induced dilation of Cx43 hemichannels.[172] A role in metabolic inhibition for pannexons is suggested, but still remains to be tested.

Regulation by oxidative mechanisms

Cx43 hemichannels are regulated by redox potential and oxidative stress. On the one hand, reducing the intracellular redox potential, either by chemical reducing agents (*e.g.*, DTT) or by intracellular physiological reducing molecules (*e.g.*, GSH), results in enhanced Cx43-hemichannel activity.[174] This effect of reducing agents on the opening of Cx43 hemichannels is likely mediated by reduction of intracellular cysteines that are located in the C-terminal tail of Cx43. On the other hand, opening of Cx43 hemichannels is induced by metabolic inhibition or ischemic conditions, which leads to intracellular accumulation of NO and *S*-nitrosylation of the three intracellular cysteines located in the C-terminal tail of Cx43 hemichannels.[175] It is not clear how reducing agents inhibit the increase in hemichannel permeability caused by oxidative stress during metabolic inhibition, but yet enhance hemichannel opening under normoxic conditions. This may suggest that the same cysteine residues are substrates of

different redox reactions, including formation and reduction of disulfide bonds, cysteine *S*-nitrosylation, and/or glutathionation.[176] Alternatively, the same modifications may lead to different conformational changes or modulation of different cysteine residues. Therefore, it will be essential to identify the physiological function for each of the three cysteine residues in the intracellular C-terminal tail of Cx43 by site-directed mutagenesis approaches.

Also Panx1 hemichannels may open during oxygen and glucose deprivation, thereby contributing to the anoxic depolarization, a process often observed during ischemic insults, which results in neuronal death.[177] As stated above, Panx1 seems to regulate Ca^{2+} leakage from the ER, another event promoting neuronal necrosis during ischemia.[101] However, further investigations on the role of oxidative stress, reactive oxygen species and *S*-nitrosylation of pannexons should provide important mechanistic insights in these processes.

ATP release *via* Cx and Panx hemichannels

There is increasing evidence that Cx hemichannels are involved in ATP release.[42,51,64,73,78,80,81,107,178–181] However, ATP release *via* Cx hemichannels has only been demonstrated under non-physiological conditions. Since it was demonstrated that ATP release occurs *via* Panx hemichannels under physiological conditions, it was claimed that ATP release, previously believed to occur through Cx hemichannels, may actually occur *via* Panx hemichannels.[30,38,107,151]

Panx1 hemichannel opening caused by an increase in $[Ca^{2+}]_i$ leads to a rapid ATP release, and generation of an ATP-specific induced current across the membrane.[47,98] Depolarization-induced ATP release occurs in Panx1-expressing oocytes.[47] Panx hemichannels contribute to ATP release in astrocytes[38] and neurons.[86] In mouse taste buds, ATP release *via* Panx1 hemichannels has been suggested,[30] but Cx hemichannels have also been shown to be involved.[73] Overall, it remains difficult to unequivocally decide about the contribution of Cx and Px hemichannels in different cell types and conditions.

Recent studies[99,116,182–184] suggested an interaction between Panx1 and the P2X7 receptor. P2X7 receptors are non-selective cation channels or form large pores that allow ATP passage and can mediate apoptotic cell death.[75] Pore formation is observed in some cell types, while other cell types exhibit only the cation channel activity.[185] The observation that P2X7 pore formation in oocytes occurred in response to injection of macrophage mRNA[186] suggested that an additional component is necessary for P2X7 pore formation. Panx1 is the molecular counterpart of the permeabilization pore (or death receptor channel) recruited into the P2X7 receptor signaling complex, and ATP-induced activation of P2X7 induces prolonged activation of Panx1 channels resulting in cell death.[52] Exposure to exogenous ATP of cells coexpressing Panx1 with either P2Y or P2X7 receptors results only in a transient activation of Panx1 channels. Qiu and Dahl[91] described a negative feedback loop controlling Panx1 channel activity. Activation of P2X7 by ATP leads to activation of Panx1 channels, but a significant inhibition of Panx1 channels was prominent at ATP concentrations slightly higher than required for activation of purinergic receptors, including P2X7 and P2Y2. ATP-binding to extracellular parts of the Panx1 channel resulted in specific inhibition of Panx1-mediated currents, and Arg75 was shown to be critical for this ATP-induced inhibition of Panx1-mediated currents.[91]

Cx and Panx hemichannels: role in cellular malfunction

Pannexons, with their ability for ATP-induced ATP release, their activation upon elevation of $[Ca^{2+}]_i$ and their insensitivity to physiological (1–2 mM) levels of $[Ca^{2+}]_0$, are able to open under both physiological and pathological conditions.[86,177,187] Panx1 has been implicated in long-range Ca^{2+}-wave propagation and in cell response to several pathological insults, including initiation of inflammatory response,[74,105,188] different paradigms of cell death such as ATP-induced cell death,[189] ischemic death of neurons[177] and in tumor suppression[139] (Fig. 3).

Besides the role of GJs and hemichannels in the regulation of the cell cycle (cell growth, proliferation, differentiation, migration, and injury repair) exchange of molecules *via* channels could play a role in cell death, *e.g.*, by transferring toxic factors or stimuli of apoptosis to adjacent cells. This has been called the "bystander effect" or "kiss of death."[109,190–192] GJIC, mediated by Cxs,[191,193,194] is demonstrated to be involved in the bystander effect.

Panx1 is involved in the release of muramyl dipeptide (MDP) from acidified vesicles into the cytosol.[195] MDP is the microbial activator of nucleotide-binding oligomerization domain 2 (Nod2), which induces NF-κB and MAPK activation, resulting in the production of multiple anti-bacterial and proinflammatory molecules. This role for Panx1 hemichannels is similar to the recently described role for Cx43 hemichannels in the strategies exploited by bacterial pathogens to invade non-phagocytic cells.[196] It was also shown that the Panx1 expression is elevated by a factor of 3–7 upon exposure to diverse pro-inflammatory stimuli (*e.g.*, TNF-α, IFN-α, IFN-γ, lipopolysaccharide, cold and systemic inflammation),[4] suggesting that Panx1 is an essential component of the acute inflammatory response at the cellular level. The

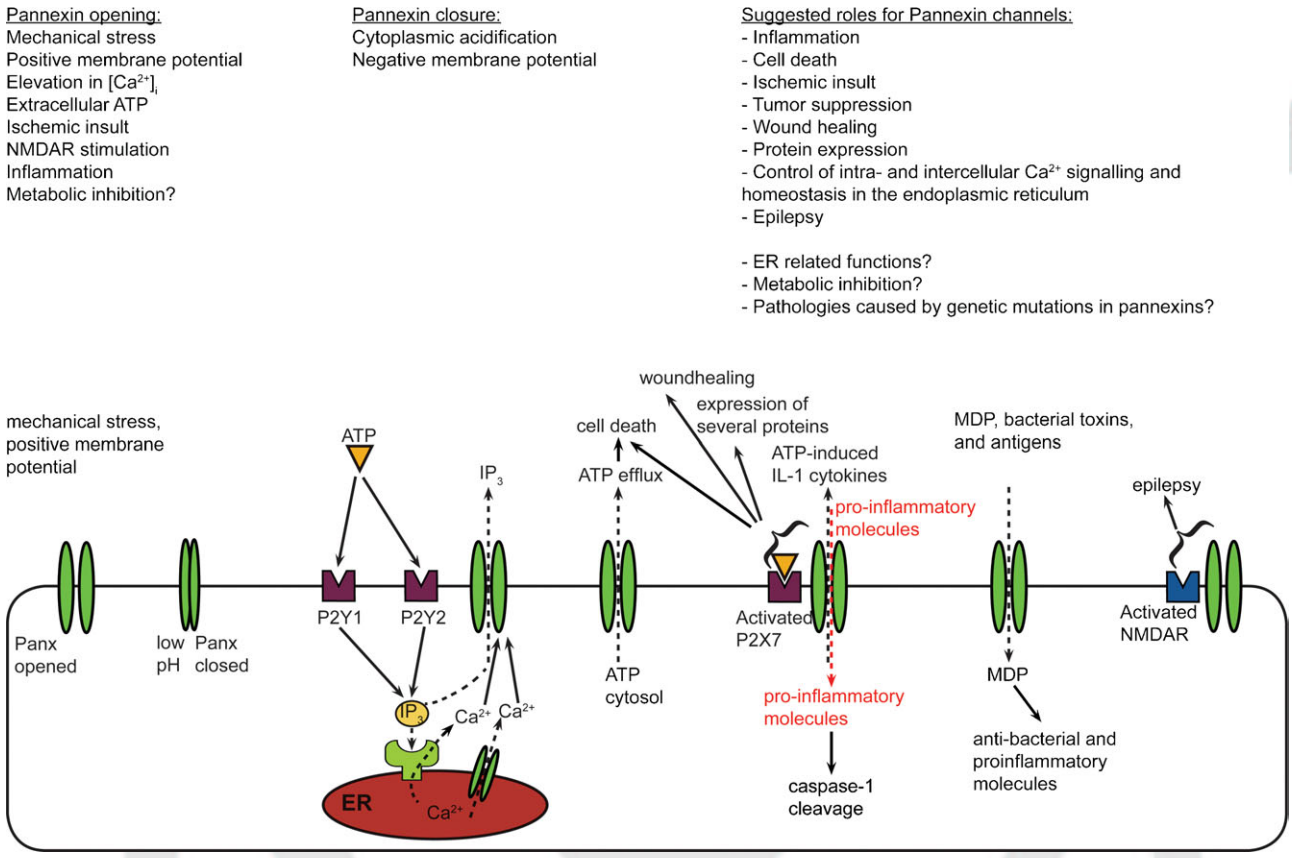

Figure 3. Regulation and role of Panx hemichannels. Panx hemichannels are activated *via* mechanical stimulation, positive membrane potential, stimulation of purinergic P2Y and P2X receptors, increase in cytosolic Ca^{2+} and some pathological stressors. When Panx channels are open, they are known to pass dye, Ca^{2+}, ATP and other small molecules, including pro-inflammatory molecules, muramyl dipeptide (MDP), bacterial toxins and antigens. Low intracellular pH closes Panx hemichannels. Panxs are suggested to play a role in long-range Ca^{2+}-wave propagation, vasodilation, initiation of inflammatory responses, wound healing, ischemic death of neurons and tumor suppression. Panxs also might play a role in ER-related functions and epilepsy (dashed lines: transport).

opening of large numbers of hemichannels following ischemia or inflammatory injury has also been suggested to be a trigger for the pathophysiological cascade, leading to cell depolarization, collapse of ionic gradients, loss of small metabolites, and elevation of intracellular Ca^{2+}.[4]

As stated above, Panx1 hemichannels have been shown to represent the non-selective pore that opens upon P2X7 activation, hereby facilitating the entry of pro-inflammatory molecules into the cytosol,[74,105,188] which are required for activation of cryopyrin-dependent inflammosome and caspase-1 cleavage.[188,197–199] Panx1 may play a role in processing and secretion of cytokines. Although distinct mechanisms of IL-1 release exist and some appear to be Panx1 dependent, the exact role of Panx1 in IL-1 release remains to be elucidated.[74,105] Acid-sphingomyelinase, as an effector of P2X7-receptor-dependent p38 MAPK phosphorylation, is necessary and sufficient for release of IL-1-containing microparticles, but it does not seem to interfere with Panx1 pore functioning.[200] The interaction between P2X7 and Panx1 was suggested to control the expression of several proteins[99] and to play a role in wound healing. Panx1 expression was lacking in P2X7 knockout mice, resulting in a delayed corneal reepithelialization. In addition, the expression of proteins in the corneal epithelium was altered, resulting in morphological changes in the stroma and compromised wound healing.

The link between Panx1 hemichannel opening and ATP-induced stimulation of P2X7 was also shown to contribute to ATP-induced cell death.[52] A possible role for Panxs in cell death has been suggested in the retina, where Panx hemichannels mediate an increased ATP release during elevation in pressure across the retina, leading to the death of ganglion cells in acute glaucoma.[189] An intracellular role for Panx channels was proposed[101] and a role was suggested in the control of intra- and intercellular Ca^{2+} signaling and homeostasis in the ER, which contribute to the Ca^{2+} leakage of the ER and thereby affect the Ca^{2+} load of the ER.

Panx1 activation was shown to cause neuronal excitotoxicity during stroke, leading to swelling, Ca^{2+} dysregulation and ischemic neuronal death in pyramidal neurons.[177] A recent study by the same authors[86] demonstrated that opening of Panx1 hemichannels, which are expressed at postsynaptic sites,[85] is triggered by NMDAR stimulation and can contribute to postsynaptic responses in the hippocampus during epileptiform seizure activity.

Cxs also function as tumor suppressors and numerous studies have explored restoration of GJIC as a potential therapy against cancer.[201] GJs are down-regulated in many types of cancer, including gliomas, breast carcinoma, and prostate cancers.[202,203] Restoring GJIC through ectopic expression of Cx43 inhibited tumor growth.[204,205] Restoring Panx1 expression also plays a tumor-suppressive role in C6 glioma cells.[139]

In a number of pathologies,[206] genetic mutations in Cx genes have been shown to lead to alterations in important biological functions of GJ channels and hemichannels. These mutations lead to intracellular aggregation of specific isoforms,[59,207] disturbed IC,[59] and/or altered hemichannel activity,[208,209] hereby causing symptoms of hereditary human disorders. These diseases can be divided into six major classes: neuropathic[210] or myelin disorders,[211] non-syndromic[207,212–215] and syndromic deafness,[207,215] skin diseases,[216,217] cataracts,[218,219] oculodentodigital dysplasia,[220] and idiopathic atrial fibrillation[221,222] (for review, see Ref.[223]). While genetic defects in Cxs are known to affect different organs, no such defects have been attributed to Panxs as yet, but it is very likely that they may be detected in the future, and it can be anticipated that they may play a role in different pathologies.

Conclusions

Panxs, structurally similar to but evolutionarily distinct from Cxs, are co-expressed with Cxs in vertebrates. Basic Panx channel properties as well as their regulation are distinct from those of Cxs, suggesting that both protein families have specific cellular functions. Panxs mainly form hemichannels that are important in paracrine signaling, and mediate transmembrane transport of Ca^{2+} and ATP in response to physiological and pathological stimuli. Panxs are suggested to play a role in long-range Ca^{2+}-wave propagation, vasodilation, initiation of inflammatory responses, ischemic death of neurons, epilepsy, tumor suppression, and ER-related functions. The increasing evidence on the role of hemichannels in IC, and the novel insights in the formation of Panx hemichannels emphasizes the importance of investigating Panx function particularly in comparison to Cx hemichannels. Up to now, few studies allow a direct side-by-side comparison of Panx and Cx hemichannels in the same cellular context, which would reveal functional differences. In addition, the role and regulation of Panx hemichannels in physiological and pathophysiological conditions remains largely unexplored. Identification of the mechanisms that control opening and closing of these channels is a major challenge for future work. The question about a unique physiological function of Panx hemichannels is as yet unanswered, as a Panx1-knockout mouse was viable with no obvious phenotype. Nevertheless, the availability of such animal and/or cellular models is required to further assess the significance of Panxs for normal cell physiology, IC, and responses to pathological conditions.

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