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Possible Involvement of Different Connexin43 Domains in Plasma Membrane Permeabilization Induced by Ischemia-Reperfusion

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Abstract *In vitro* and *in vivo* studies support the involvement of connexin 43-based cell-cell channels and hemichannels in cell death propagation induced by ischemia-reperfusion. In this context, open connexin hemichannels in the plasma membrane have been proposed to act as accelerators of cell death. Progress on the mechanisms underlying the cell permeabilization induced by ischemia-reperfusion reveals the involvement of several factors leading to an augmented open probability and increased number of hemichannels on the cell surface. While open probability can be increased by a reduction in extracellular concentration of divalent cations and changes in covalent modifications of connexin 43 (oxidation and phosphorylation), increase in number of hemichannels requires an elevation of the intracellular free Ca^{2+} concentration. Reversal of connexin 43 redox changes and membrane permeabilization can be induced by intracellular, but not extracellular, reducing agents, suggesting a cytoplasmic localization of the redox sensor(s). In agreement, hemichannels formed by connexin 45, which lacks cytoplasmic cysteines, or by connexin 43 with its C-terminal domain truncated to remove its cysteines are insensitive to reducing agents. Although further studies are required for a precise localization of the redox sensor of connexin 43 hemichannels, modulation of the redox potential is proposed as a target for the design of pharmacological tools to reduce cell death induced by ischemia-reperfusion in connexin 43-expressing cells.

Keywords Hemichannel · Connexin · Connexin 43 · Pannexin · Ischemia · Phosphorylation · Redox potential

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Introduction

Vertebrate gap junction channels are formed by a family of transmembrane proteins, termed connexins (Cxs). In addition, most vertebrate cells, if not all, express other gap junction proteins without amino acid sequence homology to Cxs, termed pannexins (Pxs) because they are also expressed in invertebrates (Panchin, 2005; Bruzzone & Dermietzel, 2006). Both Cxs and Pxs are expressed in many cell types, including neurons and glia, and a single cell type can coexpress multiple Cxs and Pxs. For example, heart myocytes and cortical astrocytes are known to express more than one Cx (Kanter, Saffitz & Beyer, 1992; Yamamoto et al., 1990) and Px1 (Barbe, Monyer & Bruzzone, 2006), whereas erythrocytes do not express Cxs but do express Px1 (Locovei, Bao & Dahl, 2006a). In contrast, Cx-deficient HeLa cells do not express Px1 (Huang et al., 2007).

While the intracellular trafficking of Pxs has not been described in mammals, Cxs are known to oligomerize in the endoplasmic reticulum (ER)-Golgi network to form hexamers (Falk, Kumar & Gilula, 1994; Musil & Goodenough, 1993). Cx hexamers are termed "hemichannels," a name extended to Px oligomers corresponding to one-half of a Px-based gap junction channel. Alternatively, the hemichannels are termed "connexons" if the protein subunits are Cxs, and by analogy, they have been called "pannexons" if they are constituted of Pxs. After

oligomerization, Cx hemichannels may be transported to the plasma membrane along microtubules to sites of cell adhesion. Once inserted in the membrane, hemichannels, which generally have a low open probability, diffuse in the membrane to find and dock in series with hemichannels from an adjacent cell to form gap junction channels (Shaw et al., 2007). New channels are formed at the periphery of a gap junction plaque and removed intact from the central region by internalization into one of the cells, taking a little cytoplasm from the other cell (Gaietta et al., 2002). Internalized gap junctions (sometimes called "annular") are degraded in lysosomes. There is evidence of internalization of nonjunctional hemichannels and reinsertion (VanSlyke & Musil, 2005), but once formed into cell-cell channels, hemichannels seem not to be reused.

During the last two decades, the physiological importance of Cx-based channels has been partially unraveled in a number of multicellular systems, and active research is still in progress. At the cellular level, they provide pathways for direct signaling between the cytoplasms of adjacent cells (transfer through gap junction channels) or paracrine signaling (transfer between cytoplasm and the extracellular milieu through hemichannels) for coordinating numerous cellular responses. The relative importance of different Cxs at the tissue, organ and organism levels is inferred from transgenic and knockout mice and Cx mutations causing a number of human genetic diseases, such as X-linked Charcot-Marie-Tooth disease, nonsyndromic deafness, congenital cataracts, oculodentodigital dysplasia and erythrokeratodermia variabilis (White & Paul, 1999; Richard, 2003). A number of Cx mutations do not appear to affect electrical coupling when expressed in exogenous systems (Essenfelder et al., 2004; Abrams et al., 2002), and the pathogenesis may involve change in permeability, trafficking and open probability of hemichannels or other functions (Jiang & Gu, 2005; Stout, Goodenough & Paul, 2004). In addition, Cx43 has been detected in the mitochondria of ischemic heart, but its functional significance there remains speculative (Boengler et al., 2005).

The open probability of Cx43 hemichannels measured with whole-cell voltage clamp in cultured cells under resting conditions is very low (Contreras et al., 2003). However, increased uptake or release of small molecules can be observed after reduction of the extracellular Ca^{2+} concentration (Goodenough & Paul, 2003; Sáez et al., 2005; Evans, De Vuyst & Leybaert, 2006). Also, elevated plasma membrane permeability mediated by Cx hemichannels can occur in the presence of normal extracellular concentration of divalent cations in pathological conditions and with certain Cx mutations (Essenfelder et al., 2004; Liang et al., 2005; Abrams et al., 2002) and ischemic injury (John et al., 1999; Contreras et al., 2002; Vergara et al., 2003), one of the most common types of insult in clinical medicine. Frequently, ischemia happens as a consequence of a mechanical obstruction in the arterial system. There are also many other conditions associated to hypoxia and/or hypoglycemia that may increase opening of hemichannels, including severe anemia, hypotension, uncompensated diabetes, carbon monoxide poisoning, uncontrolled hemorrhage, venous collapse, trauma and shock.

In the present article, we review mechanisms proposed to control membrane permeability mediated by hemichannels in cells following ischemia-reperfusion or equivalent experimental conditions. Our focus is on actions at the different domains of the Cx subunits.

Hemichannels in Ischemia-Reperfusion

In contrast to hypoxia, during which anaerobic energy production proceeds, ischemia compromises delivery of energy substrates and removal of toxic metabolites. To facilitate understanding of mechanisms controlling hemichannel-mediated membrane permeabilization and its precise biological role(s), *in vitro* studies under well-defined conditions that mimic either the ischemia or reperfusion period have been useful.

As noted, Cxs, in particular Cx43 as well as Px1, are widely distributed and expressed in many cell types. Moreover, many cells express multiple Cxs, and the contributions of each type of hemichannel formed, both homomeric and heteromeric, need to be taken into account.

Factors contributing to Cx43 hemichannel-mediated cell permeabilization during ischemia-like conditions are diverse and may include changes in transmembrane potential, intra- and extracellular free Ca^{2+} concentrations, cytosolic redox potential, intracellular adenosine triphosphate (ATP) levels and activity of intracellular transduction pathways mediating phosphorylation and dephosphorylation. The possible involvement of each of these mechanisms is described below. Moreover, several mechanisms that control the functional state of Px hemichannels have been recently described; opening of Px hemichannels can be enhanced by membrane depolarization, mechanical stimulation, increased intracellular free Ca^{2+} concentration, hypotonic stress and hypoxia (Bruzzone et al., 2003, 2005; Locovei, Bao & Dahl, 2006a; Locovei, Wang & Dahl, 2006b). Their unitary conductances may help to distinguish them from Cx hemichannels, which can be open under similar conditions (Bennett et al., 2003; L. Bao, Sachs & Dahl, 2004b). In addition, Px, but not Cx, hemichannels are insensitive to changes in extracellular Ca^{2+} concentration (Bruzzone et al., 2005; Pelegrin & Surprenant, 2006), a feature that together with their relative insensitivity to La^{3+}, heptanol, gp27 and flufenamic acid (Bruzzone et al.,

2005; Pelegrin & Surprenant, 2006) may help to determine the relative contribution of Cx and Px hemichannels during a membrane permeabilization response (Fig. 1).

Cultured hippocampal neurons express a cell surface channel activated by brief oxygen and glucose deprivation and with permeability and unitary conductance properties compatible with Px hemichannels (Thompson, Zhou & MacVicar, 2006). However, the putative Px hemichannels were sensitive to La^{3+} (Thompson et al., 2006), which is not a property of Px hemichannels expressed in HEK cells (Pelegrin & Surprenant, 2006). During oxygen-glucose deprivation, the ionic asymmetry is rapidly lost, in part due to the drop in intracellular ATP levels that limits Ca^{2+} extrusion, leading to a rise in intracellular free Ca^{2+} concentration. In addition, rises in intracellular Ca^{2+} concentrations could be achieved by inflow through several types of Ca^{2+}-permeable channel, such as voltage-sensitive Ca^{2+} channels and ligand-gated channels including P2X receptors activated by extracellular ATP and glutamate receptors of the *N*-methyl-D-aspartate (NMDA) and GluR2 lacking α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtypes. In addition, intracellular free Ca^{2+} concentration could be increased by Ca^{2+} release from intracellular stores. Although the activation mechanism(s) of putative Px hemichannels in ischemic hippocampal neurons was not identified, activation may result from the increase in intracellular Ca^{2+} concentration known to occur in hippocampal neurons in this condition (Zipfel, Lee & Choi, 1999). The marked reduction in extracellular Ca^{2+} concentration detected *in vivo* in the central nervous system during global ischemia would not increase Px hemichannel opening because Px1 hemichannels are insensitive to variations in extracellular levels of free Ca^{2+} (Bruzzone et al., 2005); however, the drop in extracellular Ca^{2+} would increase Cx hemichannel opening (Ye et al., 2003).

Metabolic inhibition or "chemical ischemia," an ischemia-like condition, increases membrane permeability through Cx43 hemichannels in several cell types (John et al., 1999; Li et al., 2001; Kondo et al., 2000; Contreras et al., 2002; Vergara et al., 2003). In the paradigms used, the inhibitors were irreversible or not removed, and no inferences can be made about the effects of reperfusion. Unpublished data from our laboratory revealed that 3-h hypoxia and glucose deprivation in a saline like cerebrospinal fluid does not significantly increase the membrane permeability of astrocytes, whereas a progressive increase in membrane permeability through Cx43 hemichannels occurs after reoxygenation; the degree of permeabilization and the response depend on the duration of hypoxia

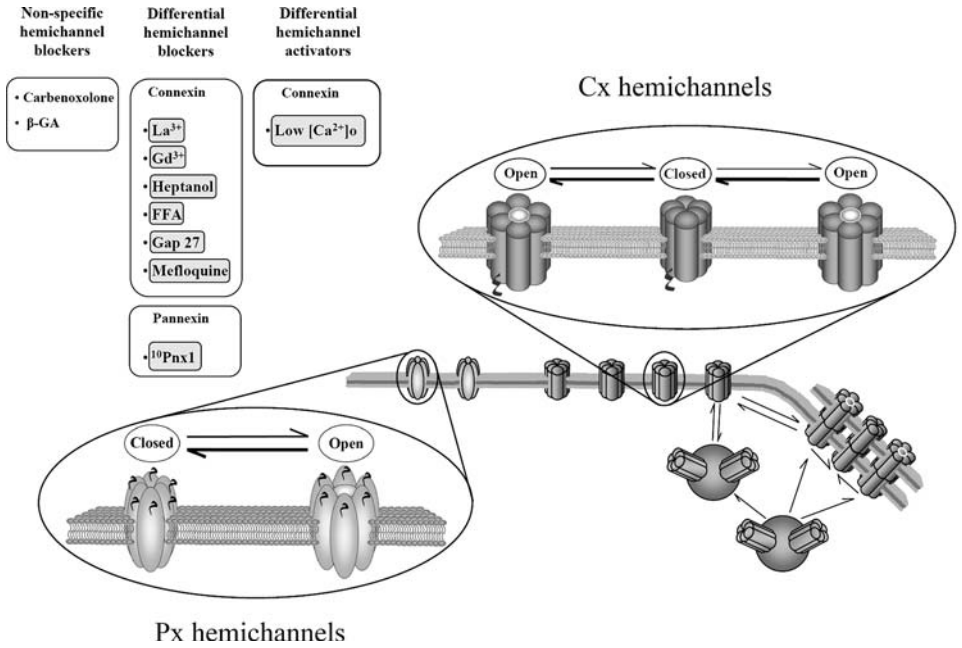

Fig. 1 Scheme showing regulatory and pharmacological differences between Cx and Px hemichannels. *Left top*, Cx hemichannel indicates covalent modifications at cytoplasmic sites (i.e., dephosphorylation/dephosphorylation, oxidation/reduction). Px1 can be glycosylated on its extracellular loops (S. Penuela, Q. Shao, X. Gong, C. S. Lounsbury, J. Manias, and D. Bai, *unpublished data*; D. Boassa, G. Gaietta, J. Hu, R. Bruzzone, G. Dahl and G .E. Sosinsky, *unpublished data*], but other covalent modifications have not been described. *Arrows* indicate changes in the amount, open probability or unitary conductance of hemichannels. *Ovals* at the center of the hemichannels indicate the open state. In the *left column*, two nonspecific hemichannel blockers are listed. In the *middle column*, ions or compounds encircled in gray show a differential inhibitory effect on Cx or Px hemichannels. In the *next column*, zero extracellular Ca^{2+} concentration opens only Cx hemichannels. (See Table 1 for nonspecific and specific hemichannel blockers.)

(Fig. 2). The increase in membrane permeability is completely inhibited by application of La^{3+}, which blocks Cx, but not Px, hemichannels (Pelegrin & Surprenant, 2006; Retamal et al., 2006; J. A. Orellana, V. Velarde, M. V. L. Bennett and J. C. Sáez, *unpublished observation*) (Figs. 1 and 2). The permeabilization response of hippocampal neurons during oxygen and glucose deprivation is much faster, ~10 min (Thompson et al., 2006), suggesting that the mechanisms controlling Px hemichannels in hippocampal neurons differ from those controlling Cx hemichannels in astrocytes. Although this differential responsiveness could be attributed to differences in the hemichannel subunits, they could also be related to differences in the cell types.

The relative permeability of hemichannels formed by different Cx subunits varies widely. For example, Cx30.2 hemichannels expressed in HeLa cells are less permeable to 4′,6-diamidino-2-phenylindole than those formed by any other cardiac Cx, but their higher open probability makes them the major uptake pathway (Bukauskas et al., 2006). Moreover, the electrophysiological properties of Px1 hemichannels differ from those of Px1 coexpressed with Px2 (Bruzzone et al., 2003), suggesting that homomeric Px1 hemichannels and heteromeric Px1/Px2 hemichannels might have different permeability properties. Thus, the cellular response to ischemia-reperfusion could be due in part to differences in Cx and Px expression patterns. Related to this, it is interesting to note that hemichannels formed by different Cx types show a distinct sensitivity to inhibitors and activators (Table 1), features that might provide useful ways to modify the cellular outcome after ischemia-reperfusion.

The roles of different hemichannel types are likely to vary along the ischemia-reperfusion time course if their permeability properties change during the evolution of the ischemic episode. This possibility is at least conceivable for Cx43 hemichannels because they undergo progressive dephosphorylation during metabolic inhibition and hypoxia in *in vitro* ischemia models (Cotrina et al., 1998; Li & Nagy, 2000; Contreras et al., 2002, 2004; Retamal et al., 2006). Moreover, protein kinase C (PKC) phosphorylation of Cx43 can affect membrane permeability mediated by hemichannels and may reduce the permeability to larger molecules rather than cause total hemichannel closure (X. Bao et al., 2007).

Could the ischemia-reperfusion-induced, hemichannel-mediated membrane permeability be beneficial for the cells? In addition to its multimodal nature, postischemic or ischemia-induced cell death occurs with a delay after the insult period. This delay varies greatly, depending on the nature of the insult and the tissue affected. The process of ischemic cell death has at least three major stages: an early

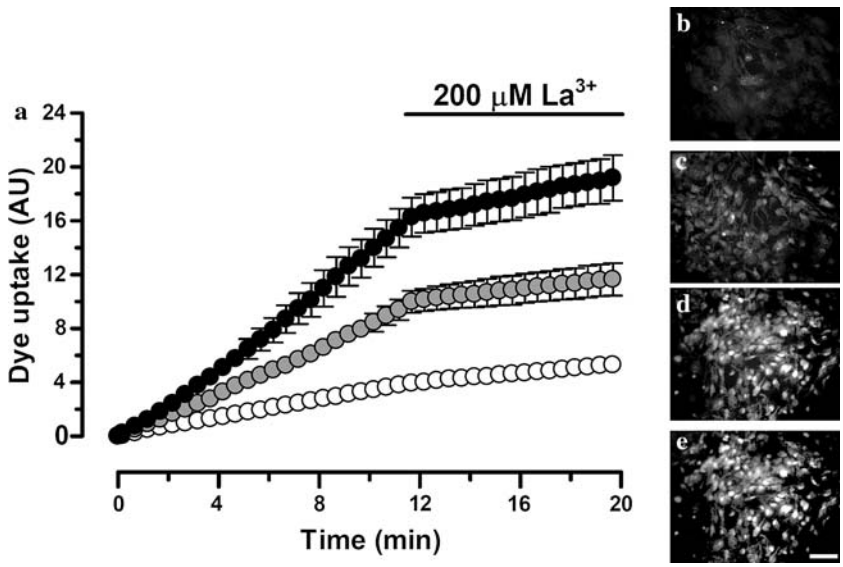

Fig. 2 Hypoxia-reoxygenation increases the plasma membrane permeability of astrocytes in a time-dependent manner. Rat cortical astrocytes in primary culture were placed in a sealed chamber without glucose and oxygen (95% N_2 plus 5% CO_2) for 3 or 6 h. **a** One hour after reoxygenation and addition of glucose, uptake of ethidium bromide (EtBr; determined by time-lapse recording in 5 μM EtBr in extracellular saline, Locke's solution) increased more after 6 h OGD (*black circles*) than after 3 h (*gray circles*, control; *white circles*, means of 20 cells in each of eight independent experiments). After ~12 min of recording, 200 μM La^{3+} was applied, which reduced uptake to a rate close to that in control. **b-d** Representative micrographs of EtBr uptake (~12 min application) 1 h after reoxygenation in astrocytes subjected to 3 (**c**) or 6 (**d**) h of hypoxia and control conditions (**b**). **e** Micrograph of the field shown in **d** 10 min after application of La^{3+} shows little further increase in fluorescence, indicating that this treatment blocked the hemichannels and greatly reduced further uptake. Bar = 100 μm

Table 1 Activators and inhibitors of connexin and pannexin hemichannels

Cx/Px	Cellular type	Hemichannel inhibitor	Hemichannel activator
Cx26	Oocytes *	(-) V_m [1,2,3] High $[Ca^{2+}]_o$ [1,3] Low pH [1,2] Co^{2+} (125μM) [1] CBX (10-100μM) [1,3]	(+)/(-) V_m [1,2,3] Low $[Ca^{2+}]_o$ [3]
	HEK293	High $[Ca^{2+}]_o$ [4] FFA (200μM) [4]	Low $[Ca^{2+}]_o$ [4]
	HeLa	CBX (25μM) [5] LPA(10μM) [5]	Low $[Ca^{2+}]_o$ [5] LPS (100ng/mL) [5] bFGF (10ng/mL) [5] AA [5]
	Cochlear supporting cells	High $[Ca^{2+}]_o$ [6] α-GA (35μM) [6]	Low $[Ca^{2+}]_o$ [6]
	Horizontal cells	Co^{2+} (25μM) [7] CBX [7,8]	
Cx30	HeLa	(-) V_m [9] High $[Ca^{2+}]_o$ [9] Heptanol (2mM) [9]	(+) V_m [9] Low $[Ca^{2+}]_o$ High Temperature [9]
Cx30.2	HeLa	Low pH [10] La^{3+} [10] MFQ (1-100μM) [10]	Low $[Ca^{2+}]_o$ [10]
Cx31.9	HeLa	MFQ (1-100μM) [10]	Low $[Ca^{2+}]_o$ [10]
Cx32	Oocytes *	(-) V_m [11] High $[Ca^{38}]_o$ [11] Mg^{2+} (1mM) [11] Ba^{2+} (1mM) [11] Co^{2+} (1mM) [11] Cd^{2+} (1mM) [11]	(+) V_m [11] Low $[Ca^{2+}]_o$ [11]
	C6	Gap 24 (0.25mg/L) [12] α-GA [12] CBX (100μM) [12]	$[Ca^{2+}]_i$ [12]
	Liposomes	2-APB (10-100μM) [13]	
Cx35	Oocytes *	(-) V_m [14,15] High $[Ca^{2+}]_o$ [15] Low pH [14] PKA [16]	(+) V_m [14,15] Low $[Ca^{2+}]_o$ [15] Quinine (100μM) [14]
	N2A	(-) V_m [15]	(+) V_m [15] Low $[Ca^{2+}]_o$ [15]
Cx37	Oocytes *	(-) V_m [16] High $[Ca^{2+}]_o$ [16] Gd^{3+} (100-200μM) [16] Mg^{2+} (1-10mM) [16]	(+) V_m [16] Low $[Ca^{2+}]_o$ [16]
	Macrophages	α-GA (10μM) [17] Gap 26 (160μM) [17] Gap 27 (160μM) [17]	
Cx38	Oocytes *	(-) V_m [18] High $[Ca^{2+}]_o$ [19,18,20] Low pH [21,18] Octanol (1.5-2mM) [20] FFA (50μM) [20] RA [18]	(+) V_m [18] Low $[Ca^{2+}]_o$ [18,19,20] Quinine [18] Osmolarity changes [20]
Cx43	Astrocytes	High $[Ca^{2+}]_o$ [22,23] La^{3+} (10μM-1mM) [26,25] Gd^{3+} (50μM) [22] Mg^{2+} (10μM-1mM) [25] Sr^{2+} (10μM-1mM) [25] Ba^{2+} (10μM-1mM) [25] Octanol (0.5-1 mM) [26,25] Heptanol (1 mM) [25] α-GA (40μM) [26,25] CBX (10-100μM) [25] FFA (50-100μM) [22,25] Radical scanvengers or reducing molecules [26,29,27]	Low $[Ca^{2+}]_o$ [22,23,24,25] Dephosphorylation [27] S-nitrosylation [27] Mechanical stimuli [28,22] Metabolic inhibition [26,29,27] Free radicals [27] Reducing molecules [27]
	Myocytes	La^{3+} (1-2mM) [30,31] Gd^{3+} (0.1mM) [31] Cl^- (150mM) [31] Halothane (1-9mM) [32] Heptanol (2-3mM) [32]	Low $[Ca^{2+}]_o$ [30,31] Metabolic inhibition [30,31,32]
	HeLa	(-) V_m [33] Low pH [36] PKC [34] La^{3+} (0.1 mM) [33] β-GA (35μM) [33] LPA (10μM) [5] LPS (100ng/mL) [5] bFGF (10ng/mL) [5] Gap 27 (0.25mg/L) [35]	(+) V_m [33] Low $[Ca^{2+}]_o$ [33,34,35] AA [5]
	NRK	CK1 [37]	Low $[Ca^{2+}]_o$ [34]
	Novikoff	Octanol (1mM) [34] Heptanol (1mM) [34] PKC [34]	Low $[Ca^{2+}]_o$ [34] ATP (5mM) [34] ADP (5mM) [34] GTP (5mM) [34] GMP (5mM) [34] Adenosine (5mM) [34]
	HEK293	La^{3+} (1-2mM) [30] Halothane [30]	Low $[Ca^{2+}]_o$ [30] Metabolic inhibition [30]
	HOBIT		Low $[Ca^{2+}]_o$ [38] Mechanical stimuli [39]

Table 1 continued

Cx/Px	Cellular type	Hemichannel inhibitor	Hemichannel activator
	Oocytes *	PKC [40, 41]	
	Liposomes	PKC [42, 43] MAPK [44]	Dephosphorylation [44]
	N2A	Oleamide (50μM) [45]	Osmolarity changes [45]
	Osteocytes		Mechanical stimuli [46]
	Fibroblast	PKC [47]	
	C6	Gd^{3+} (50μM) [22] CBX (25μM) [5] FFA (50μM) [22] Gap 26 (0.25mg/L) [5] Gap 27 (0.25mg/L) [5] PKC [5] MAPK [5] c-Src [5] LPA (10μM) [5]	Low $[Ca^{2+}]_o$ [22, 5] LPS (100ng/mL) [5] bFGF (10ng/mL) [5] AA [5]
	Renal hPT cells	Gd^{3+} (10μM) [48] PKC [48]	Metabolic inhibition [48] Dephosphorylation [48]
Cx44	Oocytes *	(-) V_m [49]	(+) V_m [49]
Cx45	HeLa	(-) V_m [50, 51] High $[Ca^{2+}]_o$ [50, 51] Low pH [50]	(+) V_m [50, 51] Low $[Ca^{2+}]_o$ [50, 51]
	RIN	(-) V_m [50] High $[Ca^{2+}]_o$ [50] Low pH [51]	(+) V_m [50] Low $[Ca^{2+}]_o$ [50]
Cx45.6	Oocytes *	(-) V_m [52] High $[Ca^{2+}]_o$ [52] Mg^{2+} (1mM) [52]	(+) V_m [52] Low $[Ca^{2+}]_o$ [52]
Cx46	Oocytes *	(-) V_m [53, 54] High $[Ca^{2+}]_o$ [53, 54, 55] Low pH [58] Co^{2+} (1mM) [53] Ni^{2+} [53] Mg^{2+} (5mM) [53, 55] Gd^{3+} (10-250μM) [57] β-GA (50-250μM) [57] FFA [57, 60] CBX [61] PKC [61]	(+) V_m [56, 53] Low $[Ca^{2+}]_o$ [57] Quinine [14] Mechanical stimuli [59]
	Lens cells	(-) V_m [62, 63] High $[Ca^{2+}]_o$ [62, 63] PKC [63]	
	HeLa	(-) V_m [9]	
Cx48.5	Oocytes *	(-) V_m [64]	(+) V_m [64]
Cx50	Oocytes *	(-) V_m [65]	Low $[Ca^{2+}]_o$ [65, 57]
		IIigh $[Ca^{2+}]_o$ [65] Low pH [65, 66, 57] Octanol (1mM) [65, 57] Gd^{3-} (10-250μM) [57] β-GA (50-250μM) [57] FFA (100-250μM) [57] NFA (25-250μM) [57] Co^{2+} (50μM) [66] Ni^{2+} (50μM) [66]	K^+ [66] Cs^+ [66] Rb^+ [65] NH_4^+ [66]
	HeLa	(-) V_m [9]	
Cx52.6	N2A	High $[Ca^{2+}]_o$ [67]	Low $[Ca^{2+}]_o$ [67] (+) V_m [67]
Cx56	Oocytes *	(-) V_m [68] High $[Ca^{2+}]_o$ [68] Co^{2+} (1mM) [68]	(+) V_m [68]
Px1	Oocytes *	(-) V_m [69, 70, 71] CBX (1-100μM) [69, 60] β-GA (50μM) [60] Low pH [71]	(+) V_m [69, 70, 71] Mechanical stimuli [70] $[Ca^{2+}]_i$ [71]
	Erythrocytes	(-) V_m [72] CBX (100μM) [72]	(+) V_m [72] Osmolarity changes [72] Hypoxia [72] Mechanical stimuli [72]
	HEK293, J774, Alveolar macrophages	^{10}Panx1 (30-200μM) [73] CBX (5-20mM) [73]	(+) V_m [73]
	Taste cells	CBX (5μM) [74]	
	CHO	CBX (5μM) [74]	
Px1/Px2	Oocytes *	(-) V_m [69] CBX (1-100μM) [60]	(+) V_m [69]
	Horizontal cells	High $[Ca^{2+}]_o$ [75] (-) V_m [75] Retinoic acid [77]	Low $[Ca^{2+}]_o$ [76, 77]
ND	Retinal cells		Quinine/quinidine [78]
	Corneal cells	Gap 26 [79]	Mechanical stimuli [79]
	ECV304	α-GA (50μM) [80] Gap 26 (0.25mg/L) [80] Gap 27 (0.25mg/L) [80]	Mechanical stimuli [80]
	GP8	High $[Ca^{2+}]_o$ [35] α-GA (50μM) [35] La^{3+} (100μM) [35] Gd^{3+} (30μM) [35] Gap 26 (0.25mg/L) [35] Gap 27 (0.25mg/L) [35]	Low $[Ca^{2+}]_o$ [35]
	Epithelial cells	Gap 26 [81]	

Table 1 continued

This table was intended to show several examples and does not correspond to a compilation of all published evidence. * Xenopus leavis oocytes. ND: not determined; FFA: flufenamic acid; CBX: carbenoxolone; α-GA: 18α-glycyrrhetinic acid; β-GA: 18β-glycyrrhetinic acid; MFQ: mefloquine; RA: retinoic acid; 2-APB: 2-aminoethoxydiphenyl borate; Gap 26 and Gap 27: peptides correspond to Cx sequences; PKA: protein kinase A; PKC; protein kinase C; LPA: lysophosphatidic acic; LPS: lipopolysaccharide; bFGF: basic fibroblast growth factor; CK1: casein kinase 1; MAPK: mitogen activated protein kinase; NFA: niflumic acid; 10Pnx1: pannexin 1 mimetic peptide; AA: arachidonic acid. 1 (Ripps, Qian & Zakevicius, 2004); 2 (Gonzalez, Gomez-Hernandez & Barrio, 2006); 3 (Gerido et al., 2007); 4 (Stong et al., 2006); 5 (De Vuyst et al., 2007); 6 (Zhao, 2005); 7 (Fahrenfort et al., 2004); 8 (Pottek et al., 2003); 9 (Valiunas & Weingart, 2000); 10 (Bukauskas et al., 2006); 11 (Gomez-Hernandez et al., 2003); 12 (De Vuyst et al., 2006); 13 (Tao & Harris, 2007); 14 (White et al., 1999); 15 (Valiunas et al., 2004); 16 (Puljung et al., 2004); 17 (Wong et al., 2006); 18 (Ripps, Qian & Zakevicius, 2002); 19 (Ebihara, 1996); 20 (Bahima et al., 2006); 21 (Francis et al., 1999); 22 (Stout et al., 2002); 23(Stout & Charles, 2003); 24 (Hofer & Dermietzel, 1998); 25 (Ye et al., 2003); 26(Contreras et al., 2002); 27 (Retamal et al., 2006); 28 (Arcuino et al., 2002); 29 (Contreras et al., 2004); 30 (John et al., 1999); 31 (Kondo et al., 2000); 32 (Li et al., 2001); 33 (Contreras et al., 2003); 34(Li et al., 1996); 35 (Braet et al., 2003)36 (Basilio et al., 2004); 37 (Cooper & Lampe, 2002); 38 (Romanello & D'Andrea, 2001); 39 (Romanello, Veronesi & D'Andrea, 2003); 40 (Bao et al., 2004); 41 (Bao, Altenberg & Reuss, 2004); 42 (Bao et al., 2004); 43 (Bao et al., 2007); 44 (Kim et al., 1999); 45 (Quist et al., 2000); 46 (Cherian et al., 2005); 47 (Liu & Johnson, 1999); 48 (Vergara et al., 2003); 49 (Gupta et al., 1994); 50 (Valiunas, 2002); 51 (Bader & Weingart, 2004)Pearson; 52 (Tong & Ebihara, 2006); 53 (Ebihara & Steiner, 1993); 54 (Trexler et al., 1996); 55 (Ebihara, Liu & Pal, 2003); 56 (Paul et al., 1991); 57 (Eskandari et al., 2002); 58 (Trexler et al., 1999); 59 (Bao et al., 2004); 60 (Bruzzone et al., 2005); 61(Ngezahayo et al., 1998); 62 (Pfahnl & Dahl, 1999); 63 (Jedamzik et al., 2000); 64 (Cheng et al., 2004); 65 (Zampighi et al., 1999); 66 (Beahm & Hall, 2002); 67 (Zoidl et al., 2004); 68 (Ebihara, Berthoud & Beyer, 1995); 69 (Bruzzone et al., 2003); 70 (Bao et al., 2004); 71 (Locovei et al., 2006); 72 (Locovei et al., 2006); 73 (Pelegrin & Surprenant, 2006); 74 (Huang et al., 2007); 75 (DeVries & Schwartz, 1992); 76 (Malchow, Qian & Ripps, 1993); 77 (Zhang & McMahon, 2001); 78 (Malchow, Qian & Ripps, 1994); 79 (Gomes et al., 2005); 80 (Braet et al., 2003); 81 (Pearson et al., 2005)

development of ionic and chemical changes, a resulting activation of effectors and a subsequent change in critical functions and structures that lead to cell death (Lipton, 1999). It must therefore be considered that, as for other adaptive and nonadaptive cell responses, the relative role of hemichannels in ischemia may vary with the course and severity of noxious stimuli, passing from a beneficial event early after sublethal insults to a later cell death accelerator in severely injured cells or tissues. Therefore, it is conceivable that the opening of a fast diffusion-mediated uptake of energy substrates and/or release of toxic metabolites to the extracellular milieu might be beneficial for injured cells. The putative beneficial role of Cx43 hemichannels during ischemia might be supported by the demonstration that astrocytes treated with proinflammatory cytokines are permeabilized through Cx43 hemichannels and take up more fluorescent glucose (M. A. Retamal, N. Froger, P. Ezan, J. C. Sáez & C. Giaume, *unpublished observation*) and that the targeted reduction or absence of Cx43 increases apoptosis and inflammation after focal ischemia in mice (Nakase et al., 2004). Moreover, release of toxic by-products such as ammonia (molecular weight [MW] = 17.03 Da), lysophosphatidic acid (MW = 436.52 Da) and oxidized glutathione (MW = 612.63 Da) might help to detoxify injured cells.

It is predictable that in cells permeabilized by ischemia-reperfusion, the intracellular concentration of H^+ buffers such as bicarbonate (MW = 60.98 Da) and phosphate (MW = 94.93 Da) would decrease. Consequently, the acidosis of ischemic cells could result from deficient buffer capacity in addition to enhanced generation of organic acids, e.g., lactic and arachidonic acids. Changes in intra- and extracellular electrolytes as a consequence of open hemichannels of ischemic cells could also induce and/or affect cell volume regulation responses. Alternatively, hyperosmotic stress could increase the membrane permeability through hemichannels, as has been proposed to occur in myocardiocytes and Cx43-transfected HeLa cells (John, Cesario & Weiss, 2003).

The available data support that in permeabilized cells the ionic membrane potential collapses and that cells are depleted of metabolically relevant compounds such as ATP, NAD^+ and free radical scavengers, e.g., reduced glutathione and ascorbic acid (Cotrina et al., 1998; Li et al., 2001; Bruzzone et al., 2001; Stout et al., 2002; Braet et al., 2003b; Stout & Charles, 2003; Gomes et al., 2005; Rana & Dringen, 2007). Membrane permeabilization might also affect the viability of other cells through a paracrine mechanism. The latter might frequently occur in nervous tissue, where the release of glutamate and K^+ through astrocytic and microglial hemichannels would enhance neuronal depolarization and excitotoxicity (Ye et al., 2003; Takeuchi et al., 2006). Hemichannel-mediated release of arachidonic acid by-products such as prostaglandin E_2 could also spread cell death in a paracrine manner (Mergenthaler, Dirnagl & Meisel, 2004; Ahmad et al., 2006).

In the brain, astrocytes form functional syncytia through intercellular communication mediated by gap junctions. This coupling supports several astrocytic functions, including homeostasis of the extracellular medium (e.g., spatial buffering). Under normal conditions, the low open probability of hemichannels present in nonjunctional domains prevents leakage of "buffered" ions and small molecules. In contrast, during *in vitro* ischemia-reperfusion, the membrane permeability mediated by hemichannels is increased during the reperfusion period (Fig. 2), and therefore, astrocytic functions are impaired. Consequently, neurons as well as astrocytes are more vulnerable to the insult.

The effect of ischemia-reperfusion could be altered by variations in the cellular microenvironment which might act as conditioning factors to enhance or reduce the cellular

permeabilization response. For example, high levels of extracellular glucose during hypoxia enhance permeabilization of cultured rat cortical astrocytes (J. A. Orellana, V. Velarde, M. V. L. Bennett & J. C. Sáez, *unpublished observation*). This finding might be related to the worse stroke outcome of hyperglycemic patients compared to normoglycemic patients (Bruno et al., 1999; Capes et al., 2001). While hyperglycemia leads to preferential neuronal death, hypoglycemia causes massive death of astrocytes as well as neurons (Muranyi et al., 2006).

Mechanisms Mediating the Increase in Membrane Permeability through Hemichannels in Ischemia-Reperfusion

Postischemic cell death is initiated in part by metabolic changes that result in inhibition of oxidative phosphorylation and the consequent chain of events. These changes may be mechanistically or temporally related and include decreased ATP levels; acidosis; ion pump dysfunction (e.g., Na^+, K^+ and Ca^{2+} pumps) with influx of water, Na^+ and Ca^{2+} and efflux of K^+; membrane depolarization; and initiation of free radical production at different subcellular levels (Lipton, 1999; Nieminen, 2003). The overall process is extremely complex due to the large number of interactions between pathways, and there are many features that may affect directly or indirectly the number of hemichannels in the membrane and their open probability.

Role of Intra- and Extracellular Free Ca^{2+} and Mg^{2+} Concentrations

Although Ca^{2+} signals are necessary for cell communication and survival, abnormal cellular Ca^{2+} load can trigger different cell death programs. After an ischemic insult, different plasma membrane channels such as voltage-dependent channels, ligand-gated channels including NMDA receptors and acid-sensing ion channels, contribute to cell depolarization and intracellular Ca^{2+} accumulation in the central nervous system (Xiong et al., 2004; Bano & Nicotera, 2007). In parallel, the extracellular Ca^{2+} concentration is drastically reduced (Ohta et al., 1997), which could potentially enhance the Cx hemichannel activity (Ye et al., 2003).

Low extracellular Ca^{2+} concentration activates hemichannels formed by a number of Cxs but not Px1 (Evans et al., 2006; Barbe et al., 2006; Bruzzone et al., 2005). A ring of 12 Asp residues located within the external vestibule of the pore have been shown to be responsible for the binding of Ca^{2+} that accounts for pore occlusion of Cx32 hemichannels (Gómez-Hernández et al., 2003). In contrast, Cx32, Cx43 and Px1 hemichannels have been shown to mediate increases in membrane permeability in response to rises in free intracellular Ca^{2+} concentrations; Cx32 hemichannels respond over a narrow and low level in free intracellular Ca^{2+} concentration (Braet et al., 2003a; De Vuyst et al., 2006; Locovei et al., 2006b). The mechanism of opening by intracellular Ca^{2+} is unknown for Cx43 and Px1 hemichannels. For Cx32 hemichannels, a calmodulin-dependent pathway is thought to increase the open probability (De Vuyst et al., 2006). In astrocytes, ATP release is enhanced in low-Ca^{2+} medium, and this response is completely abrogated by application of Ga^{3+} or flufenamic acid (Stout et al., 2002), two Cx hemichannel blockers to which Px1 hemichannels are relatively insensitive (Bruzzone et al., 2005; Pelegrin & Surprenant, 2006), indicating that response of cultured astrocytes to low extracellular divalent cations is mediated exclusively by Cx hemichannels despite their expression of Px1 (Huang et al., 2007).

An alternative mechanism involved in the modulation of hemichannel-mediated cell permeability is alteration in the number of functional hemichannels in the cell surface. In astrocytes subjected to metabolic inhibition, surface expression is increased (Retamal et al., 2006); and in cells subjected to mild hyperthermia or oxidative stress, internalization and degradation of Cx43 are reduced (VanSlyke & Musil, 2005). Unpublished data from our laboratory reveal that metabolically inhibited Cx43-transfected HeLa cells show a progressive increase in their intracellular free Ca^{2+} concentration, which is paralleled by increased hemichannel-mediated dye uptake and levels of surface Cx43 (M. A. Retamal, K. Schalper, K. Shoji, M. V. L. Bennett & J. C. Sáez, *unpublished data*). Interestingly, the increases in both dye uptake and the number of surface hemichannels are almost completely prevented with 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid tetra-acetoxymethyl ester (BAPTA-AM), an intracellular Ca^{2+} chelator, a result suggesting that intracellular Ca^{2+} mediates the increase of Cx43 hemichannels in the plasma membrane. Moreover, increased hemichannel-mediated dye uptake and surface levels of Cx43 are also observed in Cx43-transfected HeLa cells treated with a Ca^{2+} ionophore (K. A. Schalper, M. A. Retamal, K. F. Shoji, A. D. Martínez and J. C Sáez, *unpublished data*), a response that is prevented by pharmacologically inhibiting p38 mitogen-activated protein (MAP) kinase (K. A. Schalper and J. C. Sáez, *unpublished observation*). In HeLa cells, the permeability response can be attributed to the transfected Cx because these cells do not express Px1 (Huang et al., 2007). The possible sources of the increased intracellular Ca^{2+} concentration during metabolic inhibition include release from intracellular stores, Ca^{2+} pump failure and entry mediated by ischemia-activated channels as well as uptake through hemichannels (Li et al., 2001; De Vuyst et al., 2006). It is important to

note that both $[Ca^{2+}]_i$ rises and ischemia itself activate signaling pathways known to affect the functional state of hemichannels, such as phospholipase A_2 that generates arachidonic acid (Mancuso et al., 2004; Adibhatla, Hatcher & Dempsey, 2006).

Physiological extracellular Mg^{2+} concentrations also reduce hemichannel open probability, and the increase in membrane permeability induced by reductions in extracellular Ca^{2+} and/or Mg^{2+} might underlie the old belief that reductions in extracellular concentrations of divalent cations destabilize the plasma membrane. Conversely, the inhibitory effect of extracellular Mg^{2+} on Cx hemichannels might provide a rationale for the reduction in brain damage by $MgSO_4$ administered to patients after stroke (Muir, 2002; Sacco et al., 2007).

Protein Dephosphorylation

Most Cxs are phosphoproteins (Sáez et al., 1998; Lampe & Lau, 2004), and Px1 presents several putative phosphorylation sites in its C-terminal domain (Barbe et al., 2006). Studies on the Px phosphorylation state are not available, and the possible effects of ischemia remain unknown. Because Cx43 is the most ubiquitously expressed Cx type and most studies have been performed on cells expressing this protein, data obtained in cells expressing only Cx43 are discussed below.

Under normal conditions, Cx43 is phosphorylated at multiple residues in its carboxy terminus, and activation of PKC induces phosphorylation of serine 368 and closure of Cx43 hemichannels (Li et al., 1996; Liu & Johnson, 1999; X. Bao, Altenberg & Reuss, 2004a). Other protein kinases known to phosphorylate Cx43 are MAP kinase and cdc2 kinase (Sáez et al., 1997; Kanemitsu, Jiang & Eckhart, 1998; Warn-Cramer et al., 1998; Kim et al., 1999), which might be relevant in regulating Cx43-based channels under cell growth conditions.

The lack of glucose and oxygen together with mitochondrial failure induced by free radicals generated by the increase in intracellular Ca^{2+} concentration (Brookes et al., 2004) causes a drastic reduction in intracellular ATP levels, considered to be a key step in ischemic cell death (Nieminen, 2003).

The phosphorylation state of phosphoproteins depends on the activity of protein phosphatases and protein kinases. The latter reaction is limited by the ATP availability; thus, under ischemia the phosphorylation of many phosphoproteins will tend to be reduced. Moreover, activation of Ca^{2+}-dependent phosphoprotein phosphatases, such as calcineurin, could accelerate dephosphorylation of phosphoproteins. Different groups have reported reduction in Cx43 phosphorylation in cells subjected to ischemia (Beardslee et al., 2000; Li & Nagy, 2000) or chemical metabolic inhibition (Contreras et al., 2002; Retamal et al., 2006). Moreover, reversible dephosphorylation of Cx43 during hypoxia and reoxygenation has been directly linked to cellular levels of ATP (Contreras et al., 2002; Turner et al., 2004). Dephosphorylation of Cx subunits was identified as an important covalent modification controlling the opening of Cx43 hemichannels and therefore proposed as the main mechanism mediating cell permeabilization through hemichannels in response to metabolic stress (Vergara et al., 2003; John et al., 2003). This idea was supported by data obtained from Cx43 hemichannels reconstituted in liposomes, in which dephosphorylated Cx43 formed hemichannels permeable to small molecules and phosphorylation of Cx43 by MAP kinase decreased hemichannel-mediated uptake (Kim et al., 1999). Similarly, Cx43 hemichannels phosphorylated at serine 368, a consensus site for PKC phosphorylation, remain preferentially closed (X. Bao et al., 2004a), and hemichannels formed by a mutated Cx43 lacking serine 368 (Cx43-S368A) remain preferentially in the open state (X. Bao et al., 2004b).

In vitro studies showed that cortical astrocytes subjected to metabolic inhibition increase their membrane permeability through hemichannels, which is associated with dephosphorylation of total Cx43 protein as evaluated by its electrophoretic mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Martínez & Sáez, 2000; Contreras et al., 2002). Further studies by our group using surface protein biotinylation revealed the progressive dephosphorylation of Cx43-forming hemichannels at the plasma membrane of cortical astrocytes subjected to metabolic inhibition that is paralleled by increase in dye uptake (Retamal et al., 2006). Treatment with cyclosporin A, a calcineurin inhibitor, partially prevents the dephosphorylation of Cx43 but does not affect the increase in membrane permeability induced by metabolic inhibition (Contreras et al., 2004), suggesting that dephosphorylation is not the main mechanism that mediates the increase in plasma membrane permeability. Even though all three Pxs have consensus sites for phosphorylation by serine/threonine or tyrosine kinases, there is no evidence of functional modulation of Px hemichannels by this covalent modification.

Recently, reconstituted Cx43 hemichannels phosphorylated by PKC were shown to be permeable to smaller molecules than the unphosphorylated Cx43 hemichannels (X. Bao et al., 2007). Whether phosphorylation by MAP kinase also changes the permeability cut-off of Cx43 hemichannels is unknown.

Redox Potential

An imbalance between free radical generation and activity of free radical scavengers could result in oxidative stress, a

condition that has been associated with ischemic and ischemia-reperfusion cell injury (Granger & Korthuis, 1995; Dirnagl, Iadecola & Moskowitz, 1999; Mergenthaler et al., 2004; Elahi & Matata, 2006). Ion fluxes are critical for normal cell functioning, and free radicals can alter ion fluxes through channel and pumps in different biological systems (Giordano, 2005). In focal ischemia, free radicals are important mediators of the infarction process (Siesjo et al., 1995). The first suggestion that Cx43 hemichannels may be affected by redox potential came from the observation that trolox, a free radical scavenger, blocked the hemichannel-mediated dye uptake induced by metabolic inhibition in cortical astrocytes (Contreras et al., 2002). Confirmation of this hypothesis came from experiments showing that dithiothreitol (DTT), a cysteine-reducing agent, markedly reduced hemichannel-mediated dye uptake by metabolically inhibited astrocytes without changing Cx43 hemichannel phosphorylation as inferred from electrophoretic mobility (Retamal et al., 2006). The effect of DTT could be mimicked by cell-permeant reduced glutathione ethyl ester (GSH-EE) but not by the impermeant GSH, suggesting that one or more of the three intracellular cysteines of Cx43 is oxidized in metabolically inhibited cells and that these sites may be important in sensing the intracellular redox potential (Retamal et al., 2006). Nitric oxide (NO) production is also increased in cells under metabolic stress (Kader et al., 1993; Globus, Prado & Busto, 1995; Zhang et al., 1995). NO can oxidize free cysteine residues to yield nitrosylated cysteines (Stamler, 1994; Broillet, 1999; Hess et al., 2005), which could be a mechanism to control hemichannel activation in ischemic cells. Consistent with this idea, we observed S-nitrosylation of Cx43 hemichannels in cells subjected to metabolic inhibition. Moreover, application of NO donors to control cells not only induced S-nitrosylation of Cx43 but also caused a rapid increase in hemichannel-mediated membrane permeabilization (Retamal et al., 2006). In contrast, under normoxic conditions DTT increases the open probability of hemichannels in astrocytes and in Cx43-transfected HeLa cells (Retamal et al., 2007). A possible explanation for this apparent contradiction is modulation of the redox potential sensitivity of Cx43-forming hemichannels by progressive change in phosphorylation status. Thus, application of DTT to astrocytes subjected to metabolic inhibition for different periods had different effects: after 20 min (little Cx43 dephosphorylation), DTT increased the hemichannel-mediated dye uptake as in control conditions; after 30 min (moderate Cx43 dephosphorylation), the response to DTT was small and irregular; and after 40 min (marked Cx43 dephosphorylation), DTT reversed the hemichannel-induced membrane permeabilization (Retamal et al., 2007). We cannot rule out other cysteine posttranslational modifications that in addition to S-nitrosylation may be important in the modulation of Cx43 hemichannel function, such as S-glutathionylation and S-hydroxylation. Notably, S-glutathionylation can also be reversed by DTT (Borges et al., 2002; Wang et al., 2005), and there may be other oxidation reactions that control the function of hemichannels. Studies with Cxs containing specific amino acid substitutions will help to clarify these issues.

Other Possible Mechanisms

There are other physiopathological elements of ischemia that could affect the function of hemichannels.

Accelerated phospholipid catabolism has been implicated as an important biochemical mechanism underlying electrophysiological alterations and membrane dysfunction in ischemic myocardium (Katz & Messineo, 1981). Rise in $[Ca^{2+}]_i$ is known to activate Ca^{2+}-dependent phospholipase A_2, and Ca^{2+}-independent phospholipase A_2 activity is increased in cardiac ischemia (Ford et al., 1991; Mancuso et al., 2004). Therefore, the augmented activity of phospholipase A_2 might change the lipid environment, inducing conformational changes of the protein subunits forming the hemichannels that might affect their functional state (Munaron, 2002). Alternatively, activation of phospholipase-dependent pathways can elevate levels of arachidonic acid, which increases the cell permeability mediated by Cx43 hemichannels (De Vuyst et al., 2007) and is implicated in hemichannel-mediated increase in cell permeability of cortical astrocytes subjected to metabolic inhibition (Contreras et al., 2002). On the other hand, arachidonic acid causes increase in $[Ca^{2+}]_i$ in different cell types (Munaron, 2002), which could again enhance (or reduce) the function of Cx and Px hemichannels. Moreover, arachidonic acid increases the noncapacitative entry of Ca^{2+} in endothelial cells through an unknown route, and endothelial cells express Cxs (Mottola et al., 2005). Some eicosanoids have also been shown to activate Ca^{2+} influx in different cell types (Munaron, 2002) and thus could indirectly affect membrane permeability through action on hemichannels.

Changes in the concentrations of monovalent cations such as Na^+ and K^+ on each side of the plasma membrane, which are known to occur in ischemic cells, can also alter the function of hemichannels formed by Cx46 or Cx50 (Srinivas et al., 2005).

Another factor that should be considered is the reduction in both intra- and extracellular pH during ischemia, which has been shown to drastically reduce the activity of Cx hemichannels (Francis et al., 1999; Trexler et al., 1999; Yu et al., 2007). The effect of pH on the functional modulation of Px gap junctions remains unknown, although Px

hemichannels are blocked by low pH (Locovei et al., 2006b). Hemichannel regulation is complex, and the relative importance of the different controlling mechanisms is still being established.

Perspectives and Concluding Remarks

Hemichannels are thought to constitute an important pathway for cellular release or uptake of physiologically relevant molecules, but mechanisms that control their opening in normal cells in the presence of ordinary concentrations of extracellular divalent cations remain unclear. In most systems, hemichannels coexist with gap junction channels and both hemichannels and cell-cell channels are believed to be important in cell-cell communication. Nevertheless, clean dissection of the contributions of each pathway can be difficult. Our current knowledge of hemichannel blockers that do not affect gap junction channels is limited to agents that also affect other plasma membrane channels (e.g., lanthanides), and a similar lack of specificity is also characteristic of most gap junction blockers. Therefore, use of these agents to study hemichannels and the mechanisms that control them requires controls for the effects on other ion channels. As discussed here, the relative contribution of hemichannels constituted of Cxs or Pxs can be demonstrated using selective blockers.

The contribution of hemichannels formed of different Cx compositions remains problematic. Clearly, some hemichannels formed by different protein subunits show different pharmacological sensitivities, suggesting that molecular pharmacology may in the future provide specific compounds to either activate or inhibit specific hemichannels. Similar studies may elucidate the extent to which hemichannels can be activated or inhibited by endogenous compounds. Identification of such compounds will facilitate advances in our knowledge of the regulation and function of hemichannels in normal as well as injured tissues.

In injured cells, enhanced hemichannel activity can lead to acceleration of cell death. Control mechanisms include sensitivity to changes in ionic concentrations and covalent modification of hemichannel subunits (e.g., proteolysis, phosphorylation and oxidation). Moreover, hemichannels can be inserted or removed from the surface membrane. It will be important to elucidate the mechanisms that control hemichannel pathways, opening of which may be protective or deleterious in promoting the spread of cell damage or death.

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