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Connexin and pannexin hemichannels in brain glial cells: properties, pharmacology, and roles

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Functional interaction between neurons and glia is an exciting field that has expanded tremendously during the past decade. Such partnership has multiple impacts on neuronal activity and survival. Indeed, numerous findings indicate that glial cells interact tightly with neurons in physiological as well as pathological situations. One typical feature of glial cells is their high expression level of gap junction protein subunits, named connexins (Cx), thus the membrane channels they form may contribute to neuroglial interaction that impacts neuronal activity and survival. While the participation of gap junction channels in neuroglial interactions has been regularly reviewed in the past, the other channel function of Cxs, i.e., hemichannels located at the cell surface, has only recently received attention. Gap junction channels provide the basis for a unique direct cell-to-cell communication, whereas Cx hemichannels allow the exchange of ions and signaling molecules between the cytoplasm and the extracellular medium, thus supporting autocrine and paracrine communication through a process referred to as “glutotransmission,” as well as uptake and release of metabolites. More recently, another family of proteins, termed pannexins (Panxs), has been identified. These proteins share similar membrane topology but no sequence homology with Cxs. They form multimeric membrane channels with pharmacology somewhat overlapping with that of Cx hemichannels. Such duality has led to several controversies in the literature concerning the identification of the molecular channel constituents (Cxs versus Panxs) in glia. In the present review, we update and discuss the knowledge of Cx hemichannels and Panx channels in glia, their properties and pharmacology, as well as the understanding of their contribution to neuroglial interactions in brain health and disease.

Keywords: astrocytes, oligodendrocytes, microglia, gap junctions, neuroglial interactions

INTRODUCTION

For a long time, it has been taken as dogma that connexin (Cx) proteins can only function as gap junction channels. Indeed, before the aggregation of Cxs at the junctional plaque and subsequent formation of gap junctions, hexameric rings of Cxs, termed connexons, were initially assumed to remain closed. An obvious reason for this occlusion was that, as gap junction channels are “poorly” selective for ions and permeable to low molecular weight molecules (<1 to 1.2 KDa), if once at the membrane connexons could open, the cell would lose its integrity or at least would have to spend tremendous energy to maintain this energetically unfavorable conformation. Such statements began to be challenged when evidence for “functional hemichannels,” a term proposed to substitute for “hemichannels,” was demonstrated in primary cultures of astrocytes in the absence of external Ca²⁺ (Hofer and Dermietzel, 1998). This observation was followed by the demonstration that metabolic inhibition performed in the presence of normal external Ca²⁺ concentrations (1–2 mM) induced cell permeabilization, due to Cx43 hemichannel opening, before loss of membrane integrity (Contreras et al., 2002). More recently, hemichannel opening in astrocytes was triggered either by treatment with pro-inflammatory cytokines or selective lipopolysaccharide (LPS) stimulation of microglia co-cultured with astrocytes, again in the presence of external Ca²⁺ (Retamal et al., 2007a). The opening of Cx43 hemichannels in astrocytes was also demonstrated to occur in experiments designed to decipher the mechanism of intercellular Ca²⁺ wave propagation in astrocytes to which both gap junction channels and hemichannels contribute (Scemes and Giaume, 2006; Leybaert and Sanderson, 2012). In this case, the release of ATP through Cx43 hemichannels.

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Connexin and Pannexin Expression in Glial Cells

Gap junctions provide a unique direct conduit between cells, influencing crucial cellular processes through activities including electrical conductance and metabolic cooperation, mediated by the passage of ions, amino acids, glucose, glutathione, ATP, and small signaling molecules (reviewed in Sohl and Willecke, 2012). Under pathological conditions, gap junctions have demonstrated changes in expression of Cxs and Panxs (Willecke et al., 2002) and Panxs (MacVicar and Thompson, 2010), this review will be limited to channel-related functions and properties.

While this review will focus on gap junction channels and hemichannels in brain glial cells, it is important to note that gap junction proteins have also been identified in brain neurons (Andrews et al., 1991; Söhl et al., 2005). These include Cx26, Cx30.2, Cx36, Cx43, and Cx57 (Rousch et al., 2002). Neurons also express Panx1 in vivo (Bruzzone et al., 2003), and recently it has been reported that neural progenitors in the adult rodent hippocampus express Panx2 (Swayne et al., 2010). It is thus possible, and has been reported in some situations, that neurons form gap junctions with astrocytes since some of the same Cxs are expressed in both cell types (Froes et al., 1999; Alvarez-Maureca et al., 2000). Astrocytes express multiple Cxs, including Cx43, Cx26, Cx30, Cx40, Cx45, and Cx46 (Giaume et al., 2012), as well as Panx1 (Iglesias et al., 2009) and Panx2 (Zappala et al., 2007). Non-treated microglia express Cx43 (Igescu et al., 2001; Gang et al., 2003), Cx36 (Dobrens et al., 2005), and Cx32 (Marzawa and Jin, 2010), as well as Panx1 (Orellana et al., 2011b). Finally, oligodendrocytes express Cx32, Cx47, and Cx29 in vivo (Altievogt et al., 2002; Wassef and Scherrer, 2011). The complexity of Cxs and Panxs functioning as gap junction channels/hemichannels and channels, respectively, in these various cell types can be viewed as providing a background level of activity to support and modulate the complex neural activity mediated via glutamatergic transmission.

The expression profiles noted have been associated with mature neurons and glial cell types. However, these various cell types arise developmentally from neuronal and glial precursors. Several studies have demonstrated changes in expression of Cxs and Panxs during brain development (reviewed in Vogt et al., 2005; Belousov and Fontes, 2013). Given that several reports have described disturbed neural development when expression of some Cxs is modified through the use of gene knockdown or knockout strategies, gap junction channels and hemichannels are likely to play significant roles in processes including cell proliferation, migration, and differentiation (Fushiki et al., 2003; Elas et al., 2007; Wienczker-Burger et al., 2007; Cina et al., 2009; Liu et al., 2012).

Initial studies on Cx functions in neural cells in culture and in brain slices focused on their expected role in forming gap junction channels, confirming their function via electrophysiological analysis or methods using low molecular weight dye tracers, which can pass from cell to cell through these intercellular channels (Giaume et al., 1991). Therefore, many of the phenotypic changes observed when enhancing or decreasing Cx expression were attributed to changes in gap junctional communication. With the recognition that Cxs can also form hemichannels, the consequences of Cx expression began to be considered in a new way (Contreras et al., 2004), and appropriate methods developed to assess their presence and activity (Giaume and Theis, 2010). Cx hemichannels and Panx channels are more typical of most membrane channels, like ion...
channels, in their direct communication between intracellular and extracellular space. However, they are unique in their pore size and conductance properties (Thompson and Macvicar, 2008).

The formation of gap junction channels and hemichannels by Cxs can help explain some contradictory effects of altering Cx expression. For example, enhanced Cx43 expression in brain glial cells, in culture and in vivo, has been shown to be both neuroprotective and neurodestructive in ischemic and excitotoxic situations (reviewed in Orellana et al., 2009; Kozoric and Naus, 2013). Recent studies have indicated that gap junction channels formed by Cx43 can be neuroprotective, while Cx43 hemichannels may be more detrimental to neuronal survival. The following sections of this review provide further insight into the distinct properties of hemichannels and how these impact the progression of neurological disorders.

**Cx Hemichannel and Panx Channel Properties in Glia**

Today, most Cxs expressed in rodent glial cells, including Cx26, Cx30, Cx32, Cx43, and Cx45, have been shown to form functional hemichannels in endogenous and/or exogenous expression systems (Bennett et al., 2003). Nevertheless, it remains unknown if this is also the case for Cx29 and Cx47 found in oligodendrocytes. In exogenous expression systems, Cx30, Cx32, Cx43, and Cx45 hemichannels show a very low open probability at resting membrane potential and normal concentration of the extracellular divalent cations, Ca$^{2+}$ and Mg$^{2+}$. For Cx26 hemichannels, these properties are species-specific; human and sheep but not rat Cx26 forms hemichannels that at resting membrane potential and normal concentration of extracellular divalent cations show opening activity but the rat protein is characterized by a single evolutionary amino acid change (D139N) that limits this feature (González et al., 2006).

Opening of Cx hemichannels studied so far can be promoted by positive transmembrane voltages and reduced concentrations of extracellular divalent cations (Bennett et al., 2003). These conditions have been most frequently used to gain knowledge on biophysical features of hemichannels, including permeability properties (see below), identification of voltage sensitivity, unitary conductance, kinetic properties, and polarity of closure. However, other gating mechanisms can also promote opening of hemichannels, as described below (see Regulation). In general, hemichannels show a time- and voltage-dependent macroscopic current, activating with depolarization and inactivating with hyperpolarization. However, this feature is not equal in hemichannels formed by different Cxs. For example, the voltage dependency is strong...
hemichannels (Contreras et al., 2003). For Cx32 hemichannels, the conductance is determined by charges dispersed over the pore pathway (Oh et al., 2008). Hemichannels formed by human Cx26 or rat Cx43 show bipolar voltage behavior (Contreras et al., 2003; González et al., 2006); however, this appears to be less well established for Cx43, for which unitary events were found to monotonically rise in the voltage range of +30 to +90 mV (Wang et al., 2012). Hemichannels present two distinct voltage-gating mechanisms, which are called loop- (slow) and V(j)- (fast) gating, respectively (Contreras et al., 2003; Oh et al., 2008). For Cx32 hemichannels the loop-gate is mediated by a conformational change that reduces the pore diameter in a region located close to the first transmembrane domain and first extracellular loop (Bagiello et al., 2012). It was recently also proposed that the intracellular pore entrance narrows from 15 to 10 Å with loop-gated but not with V(j)-gated channel closure. Moreover, it was shown that the extracellular entrance does not undergo evident large conformational changes with either voltage-gating mechanisms (Kouon et al., 2013). The unitary conductance is characteristic for hemichannels with specific Cx composition; the fully open state for Cx26 hemichannels is characterized by ~320 pS unitary conductance (Contreras et al., 2003; Betamal et al., 2007a; Wang et al., 2012) and for Cx45 hemichannels ~57 pS (Valunus, 2002). They also present conductance substates as a result of an inactivation mechanism that hinders complete activation of hemichannels under prolonged depolarization (Valunus, 2002; Contreras et al., 2003; Gómez-Hernández et al., 2003).

The most general features mentioned above have been useful to demonstrate and understand the involvement of hemichannels in different physiologic functions and also to identify hemichannels with specific Cx composition in glial cells (Orellana et al., 2011). However, much more remains to be investigated since Cxs are post-translationally modified and thus, their biophysical features might be affected according to the metabolic state of the cell.

The biophysical properties of Panx channels are much less documented than those of Cx hemichannels, possibly as a result of their more recent discovery. Panx1 channels have been so far demonstrated to be present in microglia, astrocytes, and oligodendrocytes (Iglesias et al., 2009; Domercq et al., 2010; Orellana et al., 2011a), whereas Panx2 is expressed in post-ischemic astrocytes (Zappala et al., 2007). The open state of Panx1 channels is promoted by positive membrane voltages and elevation of intracellular free Ca2+ concentration. Voltage clamp experiments have indicated widely divergent unitary conductances for Panx1 channels, varying from 68 to 350 pS (Locovei et al., 2006; Kienitz et al., 2011; Ma et al., 2012). This controversy in the single channel conductance of Panx1 channels has not been resolved yet and might be explained in part by differences in recording conditions or solutions, cell type, phenotypic differences, and post-translational modifications. It could be partially explained by simultaneous opening of channels and thus, high conductance values might represent multiplets of a single channel with a lower conductance value. An issue to keep in mind is that biophysical properties of endogenously expressed Panx channels might be different from those expressed simultaneously with all membrane and intracellular proteins (e.g., P2X receptors) that might interact with them in endogenous expression systems.

**PERMEABILITY**

In addition to selective ion channels or transporters, other channels can alter the permeability of the cell membrane under physiological and pathological conditions (Schalper et al., 2009). The origin of these permeability changes could be Cx- and Panx-based channels located at the cell surface. However, other membrane channels might also be involved in drastic changes in membrane permeability (e.g., P2X receptors, transient receptor potential (TRP) channels and calcium homeostasis modulator 1 (CALHM1) ion channel) and their possible involvement should be proved or disproved when testing the permeability of Cx hemichannels or Panx channels in a particular cell type.

Net changes in membrane permeability due to hemichannels have been most frequently studied through the use of inhibitors or molecular biology approaches that induce or abolish the expression of Cxs or Panxs. With these experimental approaches it has been proposed that hemichannels (Panx1 and/or Cx26, Cx32, and Cx43) allow the release of precursor or signaling molecules [e.g., including NAD(+), ATP, adenosine, inositol trisphosphate (IP3), and glutamate; Bevans et al., 1998; Stout et al., 2002; Ye et al., 2003; Anderson et al., 2004; Bao et al., 2004a; Locovei et al., 2006; Gossman and Zhao, 2008; Kang et al., 2008; Song et al., 2011], uptake of second messengers [e.g., cyclic ADP-ribose (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP), nitric oxide (NO), and Ca2+]; Heidemann et al., 2005; Sánchez et al., 2009, 2010; Schalper et al., 2010; Song et al., 2011; De Bock et al., 2012; Figueroa et al., 2013] and uptake of various metabolites (e.g., glucose, glutathione, and ascorbate; Bruzzone et al., 2001; Ahmad and Evans, 2002; Rana and Dringen, 2007; Betamal et al., 2007a; see Table 1 for Cx43 hemichannels and Panx1 channels). In addition, it has been demonstrated that Cx hemichannels are permeable to synthetic molecules [e.g., Lucifer yellow, ethidium, calcin, propidium, 5(6)-carboxyfluorescein, and 2-(4-amidinophenyl)-1H-indole-6-carboxamide (DAPI)] for review, see Szé et al., 2010]. The fact that Cx26 and Cx32 hemichannels reconstituted in lipid membranes are permeable to Ca2+ and cyclic nucleotides, respectively (Harris, 2007; Schalper et al., 2010; Fiori et al., 2012), indicates that no additional binding partners or associated molecules are required for hemichannels to function as conduits for small molecules or ions across the cell membrane. Since the membrane permeability change due to transient hemichannel opening may allow release of enough molecules to reach effective concentrations in a confined extracellular space, astroglial hemichannels have been proposed as membrane pathways for paracrine/autocrine cell signaling with physiological and pathological implications for glial and neuronal cells (Orellana et al., 2011c; Stoberg et al., 2012).

Membrane permeability changes via Cx hemichannels are in part explained by variations in the number of hemichannels available at the cell surface. With regard to this, an increase in the number of surface Cx43 or Cx45 hemichannels explains the increase in membrane permeability induced by fibroblast growth...
factor-1 (FGF-1) in cells that express these proteins (Schalper et al., 2008b). However, in other conditions such as exposure to extracellular solution without divalent cations (Schalper et al., 2008a) or tlamyces treated with extracellular glucose (Orellana et al., 2012a), the increase in membrane permeability is explained by opening of hemichannels via a gating mechanism. In both cases there is a net increase in membrane permeability mediated by hemichannels, possibly without changes in intrinsic permeability properties of hemichannels themselves. Nonetheless, changes in membrane permeability could also result from alterations in permeability features of hemichannels. For instance, protein kinase C (PKC)-mediated phosphorylation of the six subunits of Cx43 hemichannels reconstituted in lipid membranes reduces the pore permeability allowing permeation of the small hydrophilic solute ethylene glycol (Mr 62) but not sucrose (Mr 342, Sko et al., 2007). Changes in other hemichannel permeability properties, such as affinity or charge selectivity induced by covalent modification including phosphorylation by other protein kinases or nitrosylation, cannot be ruled out yet.

The permeability properties depend on the size, shape, and net charge of each permeant ion or molecule while the concentration gradient across the cell membrane acts as the driving force. Since the study of permeability characteristics of a membrane transporter should be done with a rapid time scale (within a few seconds to a minute) it is important to have a library of permeant molecules that allow real time determination of their disappearance or appearance. Transport via Cx43 hemichannels has been shown to be cooperative and saturable with parameters (e.g., $V_{max}$, $K_m$, and Hill coefficient) depending on the permeating cationic species (Orellana et al., 2011a). These findings imply that opening of hemichannels does not necessarily lead to loss of important intracellular molecules because of competition effects with other, less important molecules or ions and this interpretation might be valid to transport in both directions across the cell membrane. Of note, the permeability properties of hemichannels to anionic molecules have not been reported yet. To the best of our knowledge, the permeability properties of Panx1 channels remain equally unknown. It is also not known whether channels formed by different Cxs or Panxs show different permeability properties such as size exclusion and charge selectivity. In general, quantitative kinetic properties of permeation of Cx hemichannels and Panx channels remain largely unexplored. Table 1 summarizes current evidence on the permeability of Cx43, Cx32 hemichannels, and Panx1 channels in brain glial cells.

### REGULATION

Hemichannels are regulated by diverse conditions of the extracellular and intracellular microenvironments. They include Ca$^{2+}$ concentration outside and inside the cell, monovalent cation concentration, pH, mechanical stress, extracellular ligands, protein kinases, protein phosphatases, as well as oxidant and reducing agents. Here, the most recent studies not included in previous reviews (Sáez et al., 2010; Wang et al., 2013), will be presented.

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**Table 1 | Overview of substances passing through glial Cx hemichannels and Panx channels and compounds that block these membrane pathways.**

| Models                  | Techniques                          | Permeant metabolic or signaling molecules | Hemichannel blocking condition used | Reference |
|-------------------------|-------------------------------------|-------------------------------------------|-------------------------------------|-----------|
| Astrocyte cultures      | Dye uptake                          | 2-NBDG (uptake)                          | Cx43, laranthanum (La$^{3+}$), Gap27 | Retamal et al. (2007a) |
| Astrocyte cultures      | Colorimetric Tietze method          | Glutathione (release)                    | Divalents, acids, fullenamic acid, carbonos tone | Rana and Dringen (2007) |
| Astrocyte cultures      | HPLC analysis                       | Glutamate, aspartate (release)           | Divalents, acids, fullenamic acid, carbonos tone | Ye et al. (2003) |
| Acute hippocampal slices, C6 transfected cells | Bioluminescence ATP imaging         | ATP (release)                            | Panx1, Panx1 knock out              | Costrina et al. (1998); Kang et al. (2008); Suadicani et al. (2012) |
| Astrocyte cultures      | Luciferin/luciferase assay          | ATP (release)                            | Panx1 and Cx43                      | Oreiliana et al. (2011a) |
| Microglia cultures treated with amyloid β | Luciferin/luciferase | ATP glutamate (release)                  | Panx1 and Cx43                      | Oreiliana et al. (2011a) |
| Microglia cultures treated with TNF-α | Colorimetric assay                  | Glutathione (release)                    | Cx32, Gap24, carbonos tone          | Takauchi et al. (2008) |

This table summarizes most if not all data available on signaling molecules passing through Cx or Panx channels (in bold, column 4) and conditions or blockers of the Cx hemichannels or Panx channels used in each particular study. Citations refer to the initial demonstration of the channel permeation property. The models, method of detection of the permeant compound, and reference where these findings were described are also included. 2-NBDG [N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-deoxyglucose is a fluorescent glucose analog.

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**Hemichannel opening and modulation by Ca**

Unsupervised Cx hemichannels of cells in culture are preferentially closed but can be opened by several trigger conditions (reviewed in Sáez et al., 2005, 2010). These include transmembrane voltage with activation threshold of ≈30 mV for Cx30 and +50 mV for Cx43 in the presence of normal extracellular Ca$^{2+}$ (Valleñas and Weingart, 2000; Wang et al., 2011), mechanical force/strain (Bao et al., 2004a, Luckprow et al., 2011), a decrease of extracellular Ca$^{2+}$ (Li et al., 1996; Stout et al., 2002; Ye et al., 2003; Ramachandran et al., 2007; Torres et al., 2012), an increase of intracellular free Ca$^{2+}$ (De Vyas et al., 2006; 2009; Wang et al., 2012), alterations in phosphorylation status (Bao et al., 2004c) and redox status (Betamal et al., 2007b), and ischemia-mimicking conditions (John et al., 1999; Contreras et al., 2002; Wang et al., 2013).

**Role of extracellular Ca$^{2+}$**. A decrease of extracellular divalent cation concentration was one of the first conditions reported to stimulate Cx43 hemichannel opening, as introduced above. Work in a hepatoma cell line expressing Cx43 demonstrated that a decrease of the extracellular Ca$^{2+}$ concentration triggered the uptake of lucifer yellow, a hemichannel-permeable fluorescent dye (Li et al., 1996). Similar observations were obtained in astrocytes, where hemichannel opening was demonstrated to trigger the release of glutamate and aspartate (Ye et al., 2003). Lowering extracellular Mg$^{2+}$ appeared to promote low Ca$^{2+}$-triggered hemichannel opening but Ca$^{2+}$ appeared to be the dominant factor. It is thought that normal physiological extracellular Ca$^{2+}$ concentrations (~1.8 mM) keep non-junctional Cx hemichannels closed in the plasma membrane, until they become incorporated into gap junction channels where they open by a process called “loop gating” as a consequence of extracellular loop interactions. In general, the half-maximal effective concentration for Ca$^{2+}$-related opening of Cx43 hemichannels is in the order of 100 μM extracellular Ca$^{2+}$. The proposed mechanism of millimolar Ca$^{2+}$ closure of Cx hemichannels involves an interaction of extracellular Ca$^{2+}$ with a Ca$^{2+}$ binding site that consists of a ring of aspartate residues at the extracellular vestibule of the hemichannel pore thereby closing the channel (Gómez-Hernández et al., 2003). Such a mechanism has been best documented for Cx32 but appears to be also plausible for the astroglial Cxs, Cx30 and Cx43. Recently, Torres et al. (2012) have elegantly exploited the extracellular Ca$^{2+}$ sensitivity of astroglial hemichannels to trigger their opening by photo-activating a Ca$^{2+}$-buffer (thereby increasing its Ca$^{2+}$-affinity) added to the interstitial fluid of a hippocampal acute brain slice preparation (discussed further below under Section “Impact of Hemichannel-Mediated Gliotransmission on Synaptic Activity and Behavior”).

**Role of intracellular Ca$^{2+}$**. Besides extracellular Ca$^{2+}$, intracellular cytoplasmic Ca$^{2+}$ also influences hemichannel function. In fact, it was found that Cx channel opening triggered by a decrease of extracellular Ca$^{2+}$ could be induced by extruding the cells with the Ca$^{2+}$ buffer 1,2-bis(o-aminophenyl)ethane-N,N,N′,N′-tetra-acetic acid (BAPTA), added to dampen intracellular Ca$^{2+}$ changes (De Vyas et al., 2006). Further investigations demonstrated that Cx32 hemichannels are activated by moderate (below 500 nM) changes in intracellular Ca$^{2+}$ and inhibited by Ca$^{2+}$ changes above 500 nM. Subsequent work showed that Cx43 hemichannels are also characterized by such a biphasic, bell-shaped, response to changes in intracellular Ca$^{2+}$ concentration, in ATP release hemichannel studies as well as in dye uptake studies in gloma cells and other cell types (De Vyas et al., 2009; De Bock et al., 2012). Recently, single channel studies of Cx43 hemichannels robustly confirmed these observations (Wang et al., 2012). Cx hemichannels are Ca$^{2+}$ permeable (Sánchez et al., 2009, 2010; Schalper et al., 2010; Foirot et al., 2012) and the biphasic dependency on intracellular Ca$^{2+}$ thus confers a positive feedback below 500 nM intracellular Ca$^{2+}$ concentration (Ca$^{2+}$-induced Ca$^{2+}$ entry via Cx43 hemichannels) and negative feedback at higher concentrations. Interestingly, IP$_3$ receptor channels present in the endoplasmic reticulum (ER) are Ca$^{2+}$ release channels that have a similar bell-shaped Ca$^{2+}$ dependency (Benitezvanni et al., 1991). Positive and negative Ca$^{2+}$ feedback at IP$_3$ receptor channels forms the basis of Ca$^{2+}$ oscillations; thus, it was hypothesized that Ca$^{2+}$ feedback at Cx hemichannels could also play a role in Ca$^{2+}$ oscillations (De Bock et al., 2012). Experimental work with various peptides targeting Cx hemichannels indeed demonstrated that inhibiting hemichannel opening, or preventing their closure in response to high intracellular Ca$^{2+}$, blocked Ca$^{2+}$ oscillations triggered by bradykinin. Thus, Cx hemichannels constitute a putative gliotransmitter release pathway. Also, they may contribute to Ca$^{2+}$ oscillations in astrocytes and thereby potentially lead to gliotransmitter release via other Ca$^{2+}$-dependent pathways (Ollert and Mother, 2006; Zorec et al., 2012). In brain endothelial cells, it was found that Cx hemichannel blocking peptides prevented the opening of the blood–brain barrier, triggered by bradykinin, by inhibiting Ca$^{2+}$ oscillations in the endothelial cells (De Bock et al., 2011). Below, we discuss in more detail some peptide-based hemichannel inhibitors.

**Influence of extracellular Ca**

Elevations in extracellular K$^+$ shift the activation potential of Panx1 hemichannels to a more physiological range. Panx1 hemichannels expressed in astrocytes might serve as K$^+$ sensors for changes in the extracellular milieu such as those occurring under pathological conditions (Sudicani et al., 2012). This type of regulation might also occur under physiological conditions with high neuronal activity. Thus, elevations in extracellular K$^+$ of the magnitude occurring during periods of high neuronal activity have been proposed to affect the extracellular signaling among astrocytes (Necres and Spray, 2012). Accordingly, the gliotransmitter release in the hippocampus in response to high neuronal activity is sensitive to P2 receptor and Panx1 channel blockers (Henrich et al., 2012). Cx30 gap junction channels have recently been demonstrated to be regulated by the concentration of external K$^+$ (Roux et al., 2011); whether astroglial Cx hemichannels are influenced by the extracellular K$^+$ concentration is currently not known.

**Influence of pH**

Under pathological conditions, the extracellular and intracellular pH may display abrupt changes. Related to this issue and in the presence of extracellular divalent cations, Cx43 hemichannels are opened by alkaline pH applied at the extracellular side

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*Frontiers in Pharmacology* | Pharmacology of ion Channels and Channelopathies | July 2013 | Volume 4 | Article 88 | 6
The activity of Cx43 hemichannels recorded at positive membrane potentials and in the absence of extracellular Ca\(^{2+}\) is drastically reduced by acidification (Valiunas, 2002). In addition, the pH sensitivity might be potentiated by protonated aminosulfonates (Bevans and Harris, 1999), such as taunine that could be in millimolar concentration in the cytoplasm of several cell types including astrocytes (Olson, 1999). This effect is Cx specific; Cx26 but not Cx32 hemichannels are closed by aminosulfonates at constant pH (Bevans and Harris, 1999). At the ultrastructural level (high-resolution atomic force microscopy), Cx26 hemichannels are closed at pH \(<\) 6.5 (4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) buffer) and opened reversibly by increasing the pH to 7.6 (Yu et al., 2007). Moreover, molecular studies have revealed that Cx26 hemichannel closure induced by taunine requires the interaction between the cytoplasmic loop and the C-terminal (CT, Locke et al., 2011).

### Influence of mechanical forces

Mechanical stress is another condition that might be relevant under pathological situations. For example, after brain trauma the extracellular medium becomes hypertonic due to the release of intracellular solutes to the extracellular microenvironment (Somjen, 2002). In astrocytes, hypertonicity induces glutamate release through Cx43 hemichannels (Jiang et al., 2011). This response could be mediated by integrin α5β1 (Batra et al., 2012), a RhoA GTase and the contractile system (Ponsaerts et al., 2010). This sensitivity is likely to result from the interaction between negatively charged amino acid residues of the CT end (Asp278 and Asp279) and a domain of the intracellular loop (D’Hondt et al., 2013). Activation of Panx1 channels by mechanical stress is not present in all cell types (Bao et al., 2014a; Reyes et al., 2009), suggesting that it should be tested in glial cells in order to validate its possible relevance in these cells.

### Influence of extracellular factors and signals

The functional state of glial Cx hemichannels and Panx1 channels is actively regulated by extracellular signals. In primary cultures of mouse astrocytes, stomatins inhibits Panx1-mediated whole cell currents by interacting with its CT (Zhan et al., 2012). In contrast, pro-inflammatory agents such as the β-amyloid peptide increase the surface expression and activity of Cx43 hemichannels and Panx1 channels in microglia and Cx43 hemichannels in astrocytes (Orellana et al., 2011c). Moreover, in brain astrocytes, epidermal growth factor (EGF) and FGF-2 inhibit Cx hemichannel activity via the mitogen-activated protein kinase cascade, and the effect of the growth factors is reversed by interleukin-1β (IL-1β; Monta et al., 2007). In contrast, pro-inflammatory cytokines (tumor necrosis factor alpha (TNF-α) and IL-1β) released by LPS-activated microglia increase the activity of astroglial Cx43 hemichannels via p38 kinase (Retamal et al., 2007b; Orellana et al., 2011c) and this response is inhibited by activation of CB1 receptors by synthetic cannabinoid agonists (Froser et al., 2009). The orchestrated involvement of Cx hemichannels and Panx1 channels has also been found in other experimental preparations. In spinal cord astrocytes, FGF-1 leads to vesicular ATP release followed by sequential activation of Panx1 and Cx43 hemichannels (Garré et al., 2010). Moreover, up-regulation of astroglial Panx1 channels and Cx43 hemichannels has been found in brain abscess (Karpuk et al., 2011).

Cx43 hemichannels expressed by tanyocytes, glial cells present in the hypothalamus, open in seconds after exposure to physiological concentrations of extracellular glucose. This response is mediated by the sequential activation of glucosensing proteins (Orellana et al., 2012a), a response that is absent in cortical astrocytes (Orellana et al., 2010). However, high glucose levels enhance the increase in astroglial Cx43 hemichannel activity induced by hypoxia-reoxygenation (Orellana et al., 2010).

As a result of cell interaction with extracellular signals or conditions that affect the energy supply, intracellular metabolic pathways involving levels of free Ca\(^{2+}\) concentration, activity of protein kinases and phosphatases, as well as levels or activity of intracellular redox molecules, are affected. Therefore, drastic reduction in ATP levels are expected to favor the dephosphorylated state of Cx43 hemichannels and thus, they could present an increased activity (Bao et al., 2007). Moreover, the lack of energy leads to a rise in intracellular free Ca\(^{2+}\) that favors the generation of reactive oxygen and nitrogen species including NO. The latter induce rapid nitrosylation of Cx43 and opening of hemichannels (Retamal et al., 2007b; Figureas et al., 2013). This mechanism might partially explain the increase in Cx43 hemichannel activity observed in astrocytes under ischemia-like conditions since free radical scavengers, diethiothreitol, a sulfhydryl (SH) reducing reagent, and high intracellular levels of reduced glutathione revert the hemichannel activity to levels found in normal cells (Contreras et al., 2002; Retamal et al., 2007b; Orellana et al., 2010). Finally, extracellular ATP mediates the ischemic damage to oligodendrocytes and is partially explained by the activation of Panx1 channels (Domercq et al., 2010). In contrast to Cx43 hemichannels, S-nitrosylation of Panx1 channels reduces their activity (Loehman et al., 2012), suggesting that their opening might be relevant only under conditions where NO is overcome by –SH reducing agents such as reduced glutathione.

### HEMICHANNEL BLOCKERS – IMITATIVE PEPTIDES AND OTHERS

Specifically targeting unapoposed hemichannels is difficult because they are composed of the same Cx building blocks as gap junction channels. As a consequence, most gap junction blockers also inhibit hemichannels (see Gaume and Theis, 2010). Moreover, many Cx hemichannel/gap junction channel blockers also block Panx channels. Gap junction blockers include glycerethetic acid and its derivative carbeneoxolone, long-chain alcohols like heptanol or octanol, halogenated general volatile anesthetics like halothane, fatty acids like arachidonic acid and oleic acid, fatty acid amides like oleamide and anandamide, and fenamates like flufenamic acid, niflumic acid, or meclofenamic acid (reviewed in Bodniewski and Raman, 2010). Most of these substances have been used in the past to block hemichannels but the results obtained can only be unequivocally interpreted in terms of hemichannels when the contribution of gap junction channels is negligible or absent. Substances like the trivalent ions gadolinium (Gd\(^{3+}\)) and lanthanum (La\(^{3+}\)) block hemichannels without inhibiting gap junction channels or Panx1 channels, but these ions also inhibit
other channels including maxi-anion channels (Liu et al., 2006) and Ca2+ channels (Mlinar and Enyeart, 1993). A possible way to differentiate between Cx hemichannels and Panx channels is the use of long-chain alcohols that block Cx hemichannels but have only a very small effect of Panx channels. In contrast, low concentrations (3–10 μM) of carbamoylphosphonic blocks Panx1 channels and only has a minor inhibitory effect on Cx hemichannels (Schalper et al., 2008a). However, all these compounds are not specific and in addition to their inhibitory effects on Cx and Panx channels (including gap junction channels and hemichannels) they also affect other neuronal and glial membrane properties which limits their use (see Giaume and Theis, 2010).

A more specific targeting of Cx channels is to be expected, at least in principle, from mimetic peptides of Cx proteins. Peptides identical to certain sequences on the Cx protein have been extensively used to interfere with the Cx channel function (Figure 2). The first Cx mimetic peptides that were introduced in the 1990s were identical to specific domains on the extracellular loops (first or second extracellular loop) of the Cx protein. The sequences mimicked were located in domains thought to be involved in the docking of two hemichannels during formation of a full gap junction channel (Warner et al., 1995). Exogenous addition of peptides mimicking parts of these domains were hypothesized to interact with yet unknown extracellular loop domains, thereby preventing extracellular loop interactions of apposed hemichannels during docking and thus hindering gap junction channel formation.

FIGURE 2 | Peptide tools to interfere with Cx hemichannel function. Gap26 and Gap27 are composed of sequence located on the extracellular loops 1 (EL1) and 2 (EL2), respectively (amino acids 64–76 and 201–210, respectively) of Cx43 mouse. These Cx mimetic peptides first inhibit hemichannels and with some delay also gap junction channels. The sequences they mimic are well conserved between different Cxs and peptides based on Cx43 sequences could also inhibit channels composed of other Cxs (e.g., Cx37). L2 peptide is identical to the L2 domain on the rat gap junction (CT, amino acids 119–144). Gap19 is a nonapeptide located within the L2 domain (amino acids 128–136). L2 and Gap19 peptides block Cx43 hemichannels without blocking gap junctions and without blocking Cx40 hemichannels or Panx1 channels (other Cxs still need to be tested). CT8 and CT10 are the last 10 and last 9 amino acids of the C-terminal (CT, amino acids 373–382 and 374–382, respectively) CT tail with the cytoplasmic loop of Cx43 where the L2 domain is located (Seki et al., 2004; Hirst-Jensen et al., 2007 and references therein). CT9/CT10 peptides remove the closure of Cx43 hemichannels with high micromolar cytoplasmic Ca2+ concentration and thus stimulate the opening of hemichannels. This particular effect of the CT peptides is independent of the last amino acid residue 382 that is involved in blocking Cx43 to scaffolding proteins involving ZO-1 interactions (amino acid sequence 384–381) has the same effect as CT8 (Desplantez et al., 2013). These domains, known as Gap26 and Gap27, are present on the first and second extracellular loops, respectively. Accordingly, Gap26 and Gap27 peptides were indeed found to inhibit gap junctional coupling (Evans and Boitano, 2001). Interestingly, subsequent work demonstrated that Gap26 and Gap27 peptides also inhibited unapposed hemichannels with which these peptides are supposed to interact (Brecht et al., 2003; Evans et al., 2006, 2012; Desplantez et al., 2012; Wang et al., 2012). Currently, clear evidence that these peptides indeed interact with the extracellular loops is only available for Gap26 (Liu et al., 2006). Nevertheless, both Gap26 and Gap27 rapidly inhibit hemichannels within minutes, followed by a somewhat delayed inhibition of gap junctional channels, often in the range of hours, with some exceptions (Matchkov et al., 2006). The exact mechanism of hemichannel block is currently not known. It is known that Gap26/27 influence voltage-dependent gating (Wang et al., 2012) but this does not explain their inhibitory action on hemichannels—opened by triggers other than voltage. It has been suggested that these peptides inhibit hemichannels by just blocking the pore because of steric hindrance effects (Wang et al., 2007). However, this has been carefully checked and it was found that this only occurs at very high (1 μM and above) concentrations (Wang et al., 2012). Although Gap26/27 peptides proved to be interesting tools to inhibit Cx hemichannels, their delayed inhibition of gap junction channels remains a serious drawback. Moreover, Gap26/27 peptides also show rather limited specificity toward different Cx types. Indeed, the extracellular loop domains mimic sequences that show pronounced homology between different Cx proteins. Thus, Gap27 directed against Cx43 in astrocytes is also known to inhibit channels or hemichannels composed of Cx37, a vascular Cx present in brain blood vessels (Martin et al., 2003).

Besides peptides, antibodies have also been used to target the free extracellular loops of unapposed Cx hemichannels resulting in their inhibition. These tools offer additional advantage because they allow both functional blocking of the hemichannels and visualizing their distribution (Hofer and Dermietzel, 1998; Clair et al., 2008; Riquelme et al., 2013). However, currently single channel electrophysiological data showing hemichannel block by an antibody directed against an extracellular epitope is only demonstrated for Cx43 hemichannel activity induced in fibrillar-treated astrocytes (Orellana et al., 2011c). Additionally, the specificity toward effects on other Cxs or Panx still needs to be determined.

To improve specificity toward different Cxs, peptides have been devised that should target sequences on the intracellular domains of the Cx protein. The intracellular sequences are poorly conserved and very different between different Cxs. The L2 peptide is identical to a sequence on the cytoplasmic loop of Cx43. This peptide is known to prevent the closure of gap junction channels by binding to the CT tail, thereby preventing interaction of the CT tail with the cytoplasmic loop of Cx43 where the L2 domain is located (Seki et al., 2004; Hiroi-Jensen et al., 2007 and references therein). Conceptually, interaction between the CT tail and the L2 domain is thought to block the gap junction channel pore upon exposure to acidification according to a particle-receptor model, and exogenous addition of L2 peptide prevents this interaction and its associated channel closure. Unexpectedly, it was found...
that the L2 peptide inhibited Cx43 hemichannels, based on ATP release studies, indicating that this peptide has distinct effects on unopposed hemichannels and those incorporated into gap junctions (Ponsaerts et al., 2010). Further work with Gap19, a mimetic nonapeptide located within the Cx43 L2 domain, demonstrated a similar action; it inhibited single channel hemichannel currents and ATP release via Cx43 hemichannels while it did not influence gap junctional couplings after 30 min and stimulated coupling after 24 h (Wang et al., 2013). Moreover, the effect appeared to be specific for Cx43 as Gap19 had no effect on Cx40 hemichannels or Panx1 channels. While the effects of L2 and Gap19 are understood at a molecular operational scale, the full details of why preventing interactions between the CT tail and the cytoplasmic loop have such different effects on hemichannels as compared to gap junction channels still needs further clarification. However, distinct effects on hemichannels and gap junction channels have been reported for other Cx channel influencing molecules or conditions; for example, basic FGF (bFGF) and cytokines like TNF-α or IL-1β have all been demonstrated to promote Cx43 hemichannel function while inhibiting gap junctions (De Vuyst et al., 2007; Betanu et al., 2007a). Given the specific actions of L2 and Gap19 peptides at the level of both Cx types and channel types (hemichannels versus gap junction channels), these peptides offer promising possibilities to explore the role of astroglial Cx43 hemichannels in the complex environment of intact neural tissues, cvivo or in vitro. Recent work has, for example, demonstrated that injection of L2 peptide in the rat amygdala strongly suppresses fear memory (Stebberg et al., 2012; discussed further below under Section “Impact of Hemichannel-Mediated Gliotransmission on Synaptic Activity and Behaviour”). Equally interesting with respect to identifying the role and functions of Cx43 hemichannels is the G138R mutation of Cx43 in which a Gly at position 138 is replaced by an Arg. This is one of the several mutants found in the inherited human disease sculodentodigital dysplasia (DIDDD). Importantly, G138R mutant Cx43 is characterized by a loss-of-function of gap junctional coupling and a gain-of-function of hemichannels (Dobrowolski et al., 2007, 2008). Thus, this particular mutant Cx43 can be used to stimulate Cx43 hemichannel function as a complementary experimental approach to hemichannel inhibition. Recently, Torres et al. (2012) have exploited such an approach to investigate the role of hemichannels in glial-neuronal communication.

**ROLES OF GLIAL HEMICHANNELS IN HEALTH AND DISEASE**

**ASTROGLIAL Ca2+ WAVES**

Initially, gap junction channels were demonstrated to constitute the pathway allowing the propagation of intercellular Ca2+ signaling between astrocytes, termed as Ca2+ waves (Finkbeiner, 1992; Venance et al., 1993). Then, after the discovery of an external component that also contributes to this interglial signaling process, a Gly at position 138 is replaced by an Arg. This is one of the several mutants found in the inherited human disease sculodentodigital dysplasia (DIDDD). Importantly, G138R mutant Cx43 is characterized by a loss-of-function of gap junctional coupling and a gain-of-function of hemichannels (Dobrowolski et al., 2007, 2008). This glycoprotein has been shown to form channels in the ER perinuclear cisternae (Vanden Abeele et al., 2006; D’Hondt et al., 2011). There is a consensus on the interpretation of these two sets of in vitro studies and it is admitted that both Cx channel functions are contributing. Indeed, they likely participate in various proportions to Ca2+ waves depending on the brain structure, the mode of stimulation to trigger the waves, the age and the in vitro conditions (Scemes and Giaume, 2006; Leybaert and Sanderson, 2012). In addition, several of these points could also account for the alternative proposal claiming that in astrocytes the external component is not supported by Cx43 hemichannels but by Panx1 channels (Suadicani et al., 2006; Scemes et al., 2007). This point of controversy remains to be clarified in the future although it is likely that both might participate in various proportions under different conditions.

More recently, several reports have confirmed that Ca2+ waves in astrocytes are present in vivo in the normal brain (Hoogland et al., 2009; Kuga et al., 2011; Mathiesen et al., 2013) as well as in pathological situations (Kuchibhotla et al., 2009; Mathiesen et al., 2013). As expected, the occurrence, amplitude and extent of in vivo Ca2+ waves are more similar compared to the initial in vitro observations. However, as Ca2+ signaling is considered as the mode of cellular excitability in astrocytes, such waves certainly play a role in the spatial and temporal features of neuronal interactions. As a consequence, this means that in many cases dynamic neuronal interactions are not limited to the sole tri-partite synapse but also occur at a more integrated level involving larger cerebral areas (Giaume et al., 2010). This situation is also likely true at the glial interface where there is a high level of Cx expression between astroglial perivascular astrocytes and neuronal tissues, especially in healthy brain parenchyma.

**HEMICANNEHS AS Ca2+ CHANNELS**

As indicated above, glial cells express several Cxs and out of these at least four of them (Cx26, Cx30, Cx32, and Cx43) have been demonstrated to form functional hemichannels (Li et al., 1996; Ihree et al., 1996; Valinus and Weingart, 2000; Ripps et al., 2002), and at least Cx26, Cx32, and Cx43 serve as a membrane pathway for Ca2+ waves and A TP release studies, indicating that this peptide has distinct effects on unopposed hemichannels and those incorporated into gap junctions (Ponsaerts et al., 2010). Further work with Gap19, a mimetic nonapeptide located within the Cx43 L2 domain, demonstrated a similar action; it inhibited single channel hemichannel currents and ATP release via Cx43 hemichannels while it did not influence gap junctional couplings after 30 min and stimulated coupling after 24 h (Wang et al., 2013). Moreover, the effect appeared to be specific for Cx43 as Gap19 had no effect on Cx40 hemichannels or Panx1 channels. While the effects of L2 and Gap19 are understood at a molecular operational scale, the full details of why preventing interactions between the CT tail and the cytoplasmic loop have such different effects on hemichannels as compared to gap junction channels still needs further clarification. However, distinct effects on hemichannels and gap junction channels have been reported for other Cx channel influencing molecules or conditions; for example, basic FGF (bFGF) and cytokines like TNF-α or IL-1β have all been demonstrated to promote Cx43 hemichannel function while inhibiting gap junctions (De Vuyst et al., 2007; Betanu et al., 2007a). Given the specific actions of L2 and Gap19 peptides at the level of both Cx types and channel types (hemichannels versus gap junction channels), these peptides offer promising possibilities to explore the role of astroglial Cx43 hemichannels in the complex environment of intact neural tissues, cvivo or in vitro. Recent work has, for example, demonstrated that injection of L2 peptide in the rat amygdala strongly suppresses fear memory (Stebberg et al., 2012; discussed further below under Section “Impact of Hemichannel-Mediated Gliotransmission on Synaptic Activity and Behaviour”). Equally interesting with respect to identifying the role and functions of Cx43 hemichannels is the G138R mutation of Cx43 in which a Gly at position 138 is replaced by an Arg. This is one of the several mutants found in the inherited human disease sculodentodigital dysplasia (DIDDD). Importantly, G138R mutant Cx43 is characterized by a loss-of-function of gap junctional coupling and a gain-of-function of hemichannels (Dobrowolski et al., 2007, 2008). This glycoprotein has been shown to form channels in the ER perinuclear cisternae (Vanden Abeele et al., 2006; D’Hondt et al., 2011). There is a consensus on the interpretation of these two sets of in vitro studies and it is admitted that both Cx channel functions are contributing. Indeed, they likely participate in various proportions to Ca2+ waves depending on the brain structure, the mode of stimulation to trigger the waves, the age and the in vitro conditions (Scemes and Giaume, 2006; Leybaert and Sanderson, 2012). In addition, several of these points could also account for the alternative proposal claiming that in astrocytes the external component is not supported by Cx43 hemichannels but by Panx1 channels (Suadicani et al., 2006; Scemes et al., 2007). This point of controversy remains to be clarified in the future although it is likely that both might participate in various proportions under different conditions.

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Glial cell type remains to be demonstrated.

**ALTERNATIVE PATHWAY FOR GLUCOSE ENTRY IN INFLAMMATORY ASTROCYTES**

Through the so-called astrocyte-neuron lactate shuttle, astrocytes participate in an activity-dependent manner to the uptake of glucose from the blood supply and the delivery of lactate to neurons (Prada and Magistretti, 2012). In normal conditions, glucose and lactate transporters are the membrane elements that support this astroglial contribution to brain metabolism. While glucose is well known to be essential for correct brain function, its homeostasis is altered by inflammatory conditions (Allaman et al., 2011). In vitro, an inflammatory situation for astrocytes can be mimicked either by co-culturing them with microglia selectively activated by the endotoxin LPS or by directly treating them with pro-inflammatory cytokines, such as TNF-α and IL-1β. Interestingly, in both cases these treatments induce an opposite regulation of Cx43 channels at least in cultured astrocytes: a decrease in gap junctional communication and an activation of hemichannels (Retamal et al., 2007a). In these conditions Cx43 hemichannels were shown to allow the influx of 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG), a glucose fluorescent analog, demonstrating that reactive or inflamed astrocytes can take up more glucose via Cx43 hemichannels when their metabolic coupling through gap junction channels is reduced (Rousch et al., 2008). Such opening of Cx43 hemichannels may represent an alternative metabolic pathway for glucose entry in astrocytes during pathological conditions associated with an inflammation status (Sofroniew and Vinters, 2010). Interestingly, this observation identifies a pathway that could contribute to the increase of glucose uptake found in several uncoupling situations (Taberner et al., 2006), this was correlated with the up-regulation of GLUT-1 (glucose transporter 1) expression and to the induction of the expression of GLUT-3, an isoform that is not normally expressed in astrocytes, as well for type I and type II hexokinases, respectively (Sánchez-Alvarez et al., 2004). However, the respective contribution of hemichannels and glucose transporters in inflammatory conditions remains to be established. Because the permeability of the blood–brain barrier is increased during the inflammatory response (Schnell et al., 1999), more glucose coming from the circulation could become available to the brain. Thus, in addition to transporters, the entry of glucose through Cx43 hemichannels could contribute to an increase of lactate formation by astrocytes that are adapted for anaerobic metabolism. Finally, the opposite regulation of Cx43 channels (Retamal et al., 2007a) may lead to a failure in intercellular glucose trafficking (Rousch et al., 2008) that could be compensated for by the increase in glucose influx through Cx43 hemichannels in individual astrocytes. Moreover, although not demonstrated yet, Cx43 hemichannels could also support lactate efflux to feed neurons since Cx43 gap junction channels in astrocytes are permeable to lactate (Taberner et al., 1996; Rousch et al., 2008). As a whole, Cx43 hemichannel activation should certainly contribute to modify the metabolic status of reactive astrocytes, a situation that should be taken into account when considering the role of astroglia in brain inflammation.

**IMPACT OF HEMICHLANNEL-MEDIATED GLIOTRANSMISSION ON SYNAPTIC ACTIVITY AND BEHAVIOR**

By analogy with the concept of a neurotransmitter, the term “gliotransmitter” has been proposed to name active molecules that are released by astrocytes and regulate neuronal activity as well as synaptic strength and plasticity (Haydon and Carmignoto, 2006). There are mainly three candidates that are proposed to play this role: glutamate, ATP, and β-serine. All of them can be released by a Ca2+-dependent vesicular release mechanism in astrocytes (Zorec et al., 2012). However, at least glutamate and ATP have been demonstrated to permeate Cx43 hemichannels in astrocytes (Ye et al., 2003; Kang et al., 2008; Orellana et al., 2011b; Iglesias and Spray, 2012) as well as Panx1 channels in microglia (Orellana et al., 2011a), thus Cx43 and Panx1 channels could also play a role in gliotransmission. Recently, the activation of Cx43 hemichannels in astrocytes was shown to impact synaptic activity and plasticity (Stehberg et al., 2012; Torres et al., 2012). The first evidence comes from hippocampal acute slices in which a decrease in extracellular Ca2+ concentration is generated by the local photolysis of a photolabile Ca2+ buffer. This opens Cx43 hemichannels in astrocytes through which ATP is released and activates P2Y1 purinergic receptors on a subset of inhibitory interneurons, initiating the generation of spikes by interneurons that in turn enhances synaptic inhibition at glutamatergic synapses (Torres et al., 2012). A similar neural loop of interaction acting as a negative feedback mechanism is expected to occur during excessive glutamatergic activity that is associated with a decrease in extracellular Ca2+ concentration (Rusakov and Fine, 2003). The second set of evidence comes from in vivo experiments in which Cx43 hemichannels were inhibited (Stehberg et al., 2012). In this study, the rat basolateral amygdala was microinfused with the TAT-L2 peptide that selectively inhibits Cx43 hemichannels assumed to be only present in astrocytes. Such in vivo blockade of Cx43 hemichannels during memory consolidation induces amnesia for auditory fear conditioning, as assessed 24 h after training, without affecting short-term memory, locomotion, or shock reactivity. Moreover, the amnestic effect is transitory, specific for memory consolidation. Learning capacity recovers after co-infusion of the Cx43 hemichannel blocker and a mixture of gliotransmitters including glutamate and ATP. These observations suggest that gliotransmission mediated by Cx43 hemichannels is necessary for fear memory consolidation at the basolateral amygdala. Thus, the study of Stehberg et al. (2012) represents the first in vivo demonstration of a physiological role for astroglial Cx43 hemichannels in brain cognitive function. Finally, an in vivo contribution of Panx1 channels to cognition has recently been addressed by using a Panx1 knockout mouse (Prochnow et al., 2012). This constitutive knockout mouse was found to have increased excitability and potently enhanced early and persistent long-term potentiation (LTP) responses in the CA1 hippocampal region with additionally impaired spatial memory and object recognition memory. However, while the expression of Panx1 is well documented in hippocampal neurons (MacVicar and Thompson, 2010), its detection at the membrane of astrocytes is still debated (Iglesias et al., 2009; but see Orellana et al., 2010, 2011c). Consequently, it is premature to state that these Panx1-dependent changes in hippocampal models of learning and memory have an astroglial origin.
NEUROLOGICAL DEATH AND BRAIN PATHOLOGIES

While there are now a number of reports indicating that the expression of glial Cxs changes in many brain pathologies and injuries (Giaume et al., 2010), this remains to be investigated in detail for Panxs. On this basis, the question of Cx channels contributing to neuronal death and brain diseases has been debated for many years. Indeed, opposite perspectives regarding the roles of glial Cx in neuronal death have been discussed and reviewed (see Kirchhoff et al., 2001; Rouach et al., 2002; Perez Velazquez et al., 2003; Nakase and Naus, 2004; Farahani et al., 2005; Giaume et al., 2007; Orellana et al., 2009). In general, this duality was attributed to differences in (i) the experimental models (in vitro, ex vivo, and in vivo) or the protocols used to induce neuronal death, (ii) the mode (pharmacology versus transgenic animals) of Cx channel inhibition selected, (iii) the type of neuronal death or pathology investigated, and (iv) the time points considered. However, retrospectively the opposite roles attributed to Cx channels could be simply due to the fact that for a long time this question was solely taking into account the gap junctional function of Cxs. Indeed, in most cases, interpretation of the data was not considering the hemichannel and channel functions of Cxs and Panxs, respectively. Nevertheless, the development of compounds and transgenic animals that discriminate between Cx gap junction channels, Cx hemichannels and Panx channels (see above) has allowed identification of their respective contribution in various in vitro and ex vivo models of brain pathologies (see Kielian, 2008; Orellana et al., 2009; Bennett et al., 2012). Although hemichannels have been reported to be involved in several brain pathologies or injuries (reviewed in Bennett et al., 2012) as well as infection (Karpuk et al., 2011; Xiong et al., 2012), we will illustrate this situation by two recent examples that demonstrate the impact of glial Cx hemichannel and Panx channel activation in cells on neuronal death.

Modifications in the pattern of Cx and Panx expression in glia are associated with phenotypic changes observed during neuroinflammation and the “reactive gliosis” that is a common feature of most brain diseases (see Sofroniew and Vinters, 2010). Indeed, activated microglia and reactive astrocytes exhibit changes in their respective pattern of expression for Panx1 and Cx43 as well as in their hemichannel function leading to neuronal damage and death (Bennett et al., 2012; Orellana et al., 2012b). When an inflammatory context is mimicked in vitro by using LPS treatment, Cx43 hemichannel activity is triggered in astrocytes through the production of TNF-α and IL-1β by activated microglia. These events are not observed when astrocytes are cultured from Cx43 knock-out mice (Retamal et al., 2007a). Furthermore, in neuron–astrocyte co-cultures the N-methyl-D-aspartate (NMDA)-induced neuronal excitotoxicity is reinforced by TNF-α + IL-1β treatment, while such a potentiating effect is not observed in cultures of neurons alone treated with the two cytokines (Froger et al., 2010). This observation suggests that the well-known protective role of astrocytes against excitotoxicity (Chen and Swanson, 2003) is impaired under pro-inflammatory conditions when astroglial Cx43 hemichannels are activated. Neuron–astrocyte co-cultures, made with astrocytes from Cx43 knock-out and neurons from wild-type mice, revealed that the NMDA excitotoxicity is not reinforced by the pro-inflammatory treatment applied on these co-cultures made with Cx43-deficient astrocytes (Froger et al., 2010). This result suggests that astroglial Cx43 is involved in the potentiated excitotoxicity induced by pro-inflammatory treatments. Interestingly, application (<30 min) of mimetic peptides (Gap26, Gap27) that inhibit Cx43 hemichannels and have no effect on Cx43 gap junctional communication (Retamal et al., 2007a), prevents the potentiated excitotoxicity response induced by pro-inflammatory cytokines (Froger et al., 2010; Orellana et al., 2012b). Connexin hemichannels might also play a role in the patho-

CONCLUDING REMARKS

During the last decade our view and understanding of the structure (Macca and Tsukihara, 2011), mutations (Pfenninger et al., 2011), properties (Rackauskas et al., 2010), and biological roles (Kar et al., 2012) of Cx channels has been incredibly enlarged, and to some extent this is also true for Panx channels (MacVicar and Thome-

In fine these gliaotrans-
biophysics and their regulation have provided a better understanding of the roles that glial Cxs and Panxs play in healthy and diseased brains. Indeed, as reported above, there is now strong evidence to state that Cx and Panx channels in glia have an impact on neuronal activity and survival. However, there are at least two aims that remain to be achieved in order to determine whether and how the contribution of glial Cxs and Panxs is causal or secondary in normal and pathological situations. One is to distinguish what is due to gap junction channels versus hemichannels, the other is to discriminate between Cx hemichannels and Panx channels. To achieve these goals it is essential to have available pharmacological and genetic tools with glial cell type, Cx type, and Panx type specificities (see Giaume and Thes, 2010). Through a simple pharmacological approach this is unlikely to be achieved since Cx and Panx channels are sensitive to the same agents so far described. However, strategies using short-term treatment with mimetic peptides (Evans et al., 2012) or antibodies targeting extra-cellular domains (Riquelme et al., 2013) could be developed for all the glial Cxs/Panxs and their specificity demonstrated. In contrast, due to the cytoplasmic location of their two channel ends, gap junction channels are likely less easy to target by a pharmacological strategy. Another more accurate approach consists in the development of transgenic mice with appropriate mutations that prevent channel pairing without affecting hemichannel activity or that block one but not the other channel function. Alternatively, small interfering RNA (siRNA) and transgenic/viral techniques have been used with some success (see Giaume and Thes, 2010). But here again, the expression of multiple Cxs in glial cells makes the task difficult with genetic and molecular approaches. A recent report by Nederpelt's group of an enhanced synaptic plasticity (LTP) due to the engraftment of human glial progenitors into immunodeficient mice (Han et al., 2013) gives weight to search such difficult challenges. Indeed, upon maturation in these grafted mice, human astrocytes were shown to be coupled to host astrocytes, suggesting a contribution of Cx channels in neuronal interactions during synaptic plasticity. In line with such contribution, double Cx43/Cx30 knock out is associated with reduced LTP (Pannasch et al., 2011). Also, the observation of an up-regulation of astrocyte Cxs in reactive astrocytes contacting amyloid plaques in a transgenic murine model of Alzheimer’s disease has been validated in human patient brain samples (Nagy et al., 1996; Koválski et al., 2012), giving another example of the value in searching for strategies that block Cx channels.

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