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Invited review

Gap junction channels and hemichannels in the CNS: Regulation by signaling molecules

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ABSTRACT

Coordinated interaction among cells is critical to develop the extremely complex and dynamic tasks performed by the central nervous system (CNS). Cell synchronization is in part mediated by connexins and pannexins; two different protein families that form gap junction channels and hemichannels. Whereas gap junction channels connect the cytoplasm of contacting cells and coordinate electric and metabolic activities, hemichannels communicate intra- and extra-cellular compartments and serve as diffusional pathways for ions and small molecules. Cells in the CNS depend on paracrine/autocrine communication via several extracellular signaling molecules, such as, cytokines, growth factors, transmitters and free radical species to sense changes in microenvironment as well as to adapt to them. These signaling molecules modulate crucial processes of the CNS, including, cellular migration and differentiation, synaptic transmission and plasticity, glial activation, cell viability and microvascular blood flow. Gap junction channels and hemichannels are affected by different signaling transduction pathways triggered by these paracrine/autocrine signaling molecules. Most of the modulatory effects induced by these signaling molecules are specific to the cell type and the connexin and pannexin subtype expressed in different brain areas. In this review, we summarized and discussed most of the relevant and recently published information on the effects of signaling molecules on connexin or pannexin based channels and their possible relevance in CNS physiology and pathology.

This article is part of the Special Issue Section entitled 'Current Pharmacology of Gap Junction Channels and Hemichannels'.

1. Introduction

Coordinated interaction among cells is critical to perform the extremely complex and dynamic tasks performed by the brain. Cell ability to sense local and neighboring microenvironments has evolved in different ways in more complex organisms. In vertebrates, cell interaction and synchronization is in part mediated by intercellular communication via connexin- and pannexin-based channels. Connexins and pannexins comprise two different gap junction protein families, which in mammals are composed of about 20 and 3 members, respectively (Abascal and Zardoya, 2012). Eumetazoans, with the only exception of echinoderms, express pannexins (called innexins in non-chordates), whereas the connexin family is exclusive to chordates (Abascal and Zardoya, 2012; Phelan and Starich, 2001; Shestopalov and Panchin, 2008). Despite the fact that connexins and pannexins do not share a relevantly homologous primary structure, they have similar secondary and tertiary structures with four α-helical transmembrane domains, connected by one cytoplasmic and two extracellular loops, where both N- and C-termini are intracellular (Fig. 1). Pannexins and connexins oligomerize into hexamers to constitute single hemichannels, except for pannexin2 (Panx2), which seems to form octamers (Ambrosi et al., 2010).

After assembly, connexin hemichannels are transported to the non-junctional plasma membrane and diffuse laterally to dock with connexin hemichannels from a neighboring cell to form gap junction channels (Sáez et al., 2003a) (Fig. 1). Gap junctions are aggregates of these intercellular channels and mediate an important form of direct intercellular communication in the animal kingdom. Gap junction channels favor the intercellular exchange of metabolites (e.g., ADP, glucose, glutamate and glutathione), second messengers (e.g., cAMP and IP_3) and ions, allowing the intercellular spread of electrotonic potentials in excitable and non-excitable tissues (Evans et al., 2006; Sáez et al., 2003a; Sohl et al., 2005). For a long time, the main function attributed to connexin

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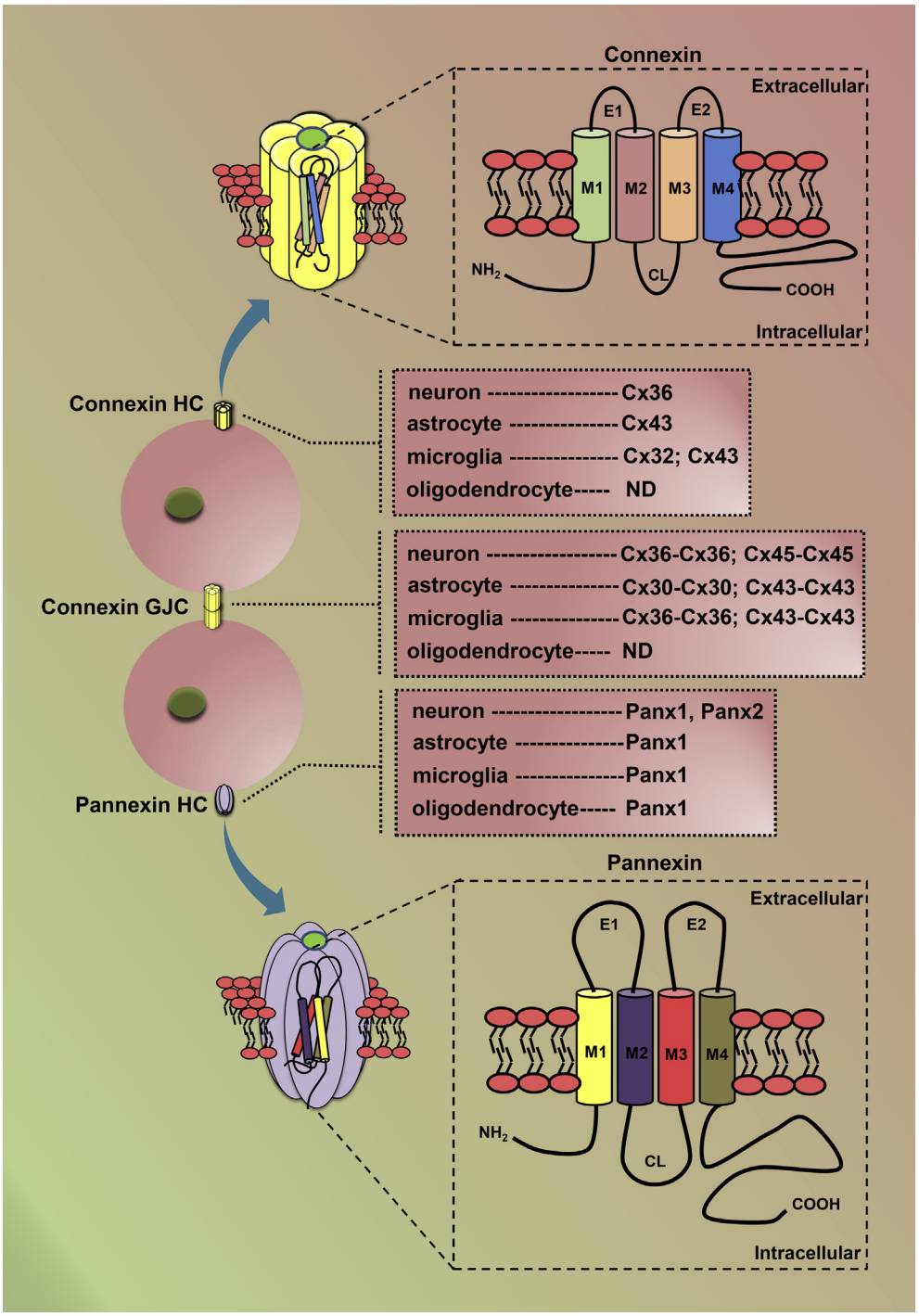

Fig. 1. Diagram illustrating basic structures of connexin- and pannexin-based channels and their patterns of expression in brain cells. Connexins and pannexins share similar membrane topology, with four α-helical transmembrane domains (M_1–M_4) connected by two extracellular loops (E_1 and E_2) and one cytoplasmic loop (CL), where both amino (NH_2)- and carboxy (COOH)-termini are intracellular. The top and bottom of the center show hemichannels formed by six connexin or pannexin subunits each, respectively. Recently, a banding pattern more consistent with an octamer than hexamer in pannexin hemichannels was observed by cross-linking and native gels of purified homomeric full-length and C-terminal Panx2 truncation mutants (Ambrosi et al., 2010). The middle center shows a connexin gap junction channel at close contact between two cells. A hemichannel is formed by connexins or pannexins that oligomerize laterally leaving a central pore in the activated state. Under resting conditions, hemichannels remain preferentially closed, but they can be activated by diverse physiological and pathological conditions, offering a diffusional transmembrane route between the intra and extracellular milieu. Note: Cellular distribution of hemichannels and gap junction channels is depicted in the respective brain cells as well. This description includes only the available information obtained under *in vivo* and/or *in vitro* studies using more than one experimental approach. Although there is no evidence that glial cells and neurons actually form heteromeric hemichannels or heterotypic gap junction channels, the evidence obtained from connexin and pannexin exogenously expressed in mammalian cell lines, support this possibility. Further studies will clarify this matter. ND: not determined.

hemichannels was the formation of gap junction channels. Nonetheless, it is currently widely recognized that connexin and pannexin hemichannels serve as aqueous pores permeable to ions and small molecules, allowing for diffusional exchange between the intra- and extracellular compartments. Thus, they grant the release of significant quantities of autocrine/paracrine signaling molecules (e.g., ATP, glutamate, NAD^+, adenosine and PGE_2) to the extracellular milieu (Bruzzone et al., 2001; Cherian et al., 2005; Lin et al., 2008; Stout et al., 2002), as well as the uptake and release of small molecules (e.g., glucose and reduced glutathione) (Retamal et al., 2007a; Stridh et al., 2008). Although most evidences indicate that only non-chordate pannexins (innexins) and connexins form gap junctions (Sosinsky et al., 2011), whether pannexins constitute gap junction channels still is matter of debate (Bruzzone et al., 2003; Ishikawa et al., 2011; Lai et al., 2007; Vanden Abeele et al., 2006).

Gap junction channels and hemichannels exhibit distinct unitary conductance, molecular permeability/selectivity and electric and chemical gating, depending on their protein subunit composition (Harris, 2007; Moreno and Lau, 2007; Sáez et al., 2005; Wang et al., 2012a). Based on their subunit composition, hemichannels can be homomeric, if they are composed of one connexin or pannexin isoform, or heteromeric if they are composed of more than one connexin or pannexin isoform. In turn, gap junction channels can be homotypic or heterotypic, if they are composed of hemichannels formed by the same or different connexin isoform, respectively.

2. Connexin and pannexin expression in the CNS

Connexins and pannexins are abundantly expressed in the CNS. Fig. 1 summarizes the expression pattern of functional channels formed by these proteins in different cell types as has been extensively reviewed in other studies (Nagy et al., 2004; Orellana et al., 2009; Rouach et al., 2002a; Theis et al., 2005). It has now become clear that a selective pattern of connexin expression in the CNS facilitates the arrangement of cell populations into distinctive functional networks (Giaume et al., 2010). For instance, Cx30 and Cx43 are differentially expressed within barrels compared with septa and other cortical layers. Studies performed by Houades and colleagues showed that both connexins are highly expressed in barrel astrocytes, but weakly expressed in septal astrocytes (Houades et al., 2008). Moreover, in the barrel cortex and layer IV outside the barrel field the shape of dye coupling is oval and circular, respectively. These data led the authors to propose that a preferential orientation of coupling inside the barrels, resulting from subpopulations of astrocytes with different coupling properties, contribute to determining astroglial networks (Houades et al., 2008). Few years ago, Kunze and colleagues reported that Cx43 and Cx30 are the main proteins mediating intercellular coupling between radial glial cells in the adult dentate gyrus (Kunze et al., 2009). In support to the role of these connexins maintaining networks in the CNS, mice lacking Cx43 and Cx30 in radial glial cells show almost complete inhibition of cell proliferation in the subgranular zone of adult dentate gyrus. Thus, gap junctional communication among radial glial cells is necessary for intact neurogenesis in the adult brain.

Connexin and/or pannexin-based channels are gated by several conditions and/or post-translational modifications, including pH, concentration of extracellular and intracellular cations, transmembrane and transjuctional potential, glycosylation, phosphorylation and S-nitrosylation (Johnstone et al., 2012; Sáez et al., 2005; Wang et al., 2012a). All these gating properties could be regulated differentially in diverse tissues and cell types of the CNS as a consequence of the numerous paracrine signals. In this review, we present up-to-date findings on the regulation of connexin- and pannexin-based channel activity by cytokines/growth factors, transmitters and oxidative/nitrative stress in the CNS.

3. Cytokines and growth factors

Cytokines and growth factors regulate a variety of physiological and pathological processes in the CNS. The effects of these factors on gap junction channels and connexin hemichannels have been analyzed in detail for glial cells (Chandross, 1998; Reuss and Unsicker, 1998), but neurons have received very little attention. Regarding to cytokines, the main focus of these studies has been placed within the context of inflammation. Therefore, in the following paragraphs we will discuss the modulatory mechanisms induced by pro- or anti-inflammatory cytokines as well as growth factors on connexin and pannexin-based channels of brain cells.

3.1. Regulation of gap junction channels by pro-inflammatory cytokines

An increasing number of studies have demonstrated that gap junction channel activity is drastically reduced upon treatment with pro-inflammatory cytokines (Table 1). For example, hippocampal multipotent progenitor cells under control conditions are strongly coupled via Cx43 gap junction channels (Rozental et al., 1998). Nonetheless, stimulation with IL-7 alone or with IL-7 plus bFGF/TGF-α to induce different stages of neuronal differentiation results in reduced electrical coupling and decreased levels of Cx43 (Rozental et al., 1998). Gap junctional intercellular communication (GJIC) is also regulated by IL-1β, which is known to be a pro-inflammatory cytokine produced by glial cells and neurons or by infiltrating immune cells (e.g., macrophages and T cells) during neuroinflammation. Indeed, this cytokine induces a prominent reduction of gap junctional conductance, dye coupling and loss of Cx43 protein as well as mRNA in primary cultures of human fetal astrocytes (John et al., 1999). In agreement, IL-1β potentiates the reduction on GJIC in astroglial cells induced by acute administration (10 min) of ATP, possibly by promoting the generation of arachidonic acid (AA) (Meme et al., 2004), which is known to down regulate astroglial gap junctions (Martínez and Sáez, 1999, 2000). In addition, IL-1β produces a robust decrease in astroglial coupling when combined with TNF-α, as measured by scrape-loading and dye transfer (SLDT) (Meme et al., 2006).

Astrocytes exhibit at least two types of calcium waves, one of them propagates through gap junction channels (Finkbeiner, 1992) and the other one depends on the sequential activation of P2X receptors (Hassinger et al., 1996; Scemes et al., 1998). The last one is an autocrine/paracrine signaling mechanism mediated by secreted phosphonucleotides, like ATP, first identified in mast cells (Osipchuk and Cahalan, 1992). Interestingly, calcium wave transmission via the purinergic (P2) receptor-mediated pathway is potentiated in IL-1β-treated fetal astrocyte cultures. On the contrary, calcium wave propagation via gap junctions between astrocytes is reduced by IL-1β treatment (John et al., 1999). Astroglial cell cultures treated with IL-1β show reduced expression of Cx43 protein and mRNA (John et al., 1999), which could explain the reduced gap junction component of the calcium wave propagation in these cells. However, suramin, a P2 receptor blocker, markedly reduces the efficacy of calcium wave propagation in IL-1β-treated astrocyte cultures to a level substantially below those observed after addition of the same concentration of suramin to control cultures (John et al., 1999). This phenomenon could suggest that a self-perpetuating propagation of calcium waves between astrocytes occurs under pro-inflammatory conditions (e.g., IL-1β stimulation), through the release of ATP via hemichannels and sequential activation of P2 receptors (Orellana et al., 2011a, 2011c). This ATP-dependent paracrine signaling could

Table 1
Regulation of gap junction channels and hemichannels by cytokines and growth factors in brain cells.

Agent	Cx/Panx type	Effect on GJCs	Effect on HCs	Used technique	Cell type	References
EGF	Cx43	n/a	↓	Dye leakage	Cortical astrocytes	Morita et al., 2007
	Cx43	N.E.	n/a	Dye coupling	Striatal astrocytes	Reuss et al., 1998
	Cx43	N.E.	n/a	Dye coupling	Cortical astrocytes	Reuss et al., 1998
	Cx43	N.E.	n/a	Dye coupling	Mesencephalic astrocytes	Reuss et al., 1998
bFGF	Cx43?	↓	n/a	Dual whole-cell patch-clamp	Neuroblast cells	Rozental et al., 1998
	Cx43	n/a	↓	Dye leakage	Cortical astrocytes	Morita et al., 2007
	Cx43	↓		SLDT	C6 glioma − Cx43	De Vuyst et al., 2007
	Cx43		↑	ATP release		
FGF-1	Cx43/Panx1		↑	Dye uptake	Spinal astrocytes	Garré et al., 2010
	Cx43	↓		SLDT/Dye coupling		
FGF-2	Cx43	N.E.	n/a	Dye coupling	Striatal astrocytes	Rufer et al., 1996
	Cx43	↓	n/a	Dye coupling	Striatal astrocytes	Reuss et al., 1998
	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Reuss et al., 1998
	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Reuss et al., 2000b
	Cx43	↓	n/a	Dye coupling	Mesencephalic astrocytes	Reuss et al., 1998
	Cx43	↓	n/a	Dye coupling	Mesencephalic astrocytes	Reuss et al., 2000b
	Cx43?	↑	n/a	Dye coupling	Midbrain neurons	SiuYi Leung et al., 2001
FGF-5	Cx43	↓	n/a	Dye coupling	Mesencephalic astrocytes	Reuss et al., 2000a
FGF-9	Cx43	↓	n/a	Dye coupling	Striatal astrocytes	Reuss et al., 2000a
	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Reuss et al., 2000a
	Cx43	↓	n/a	Dye coupling	Mesencephalic astrocytes	Reuss et al., 2000a
GDNF	n/a	↓	n/a	Dye coupling	Midbrain neurons	SiuYi Leung et al., 2001
IFN-β	Cx43	↑	n/a	Dye coupling	Glial co-culture	Hinkerohe et al., 2005
IFN-γ	Cx43	↓	n/a	Dye coupling	Glial co-culture	Hinkerohe et al., 2005
	Cx43	↑	n/a	Dye coupling	Cortical microglia	Eugenín et al., 2001
IGF	Cx43	↑	n/a	Dye coupling	Cortical astrocytes	Aberg et al., 2003
IL-1β	Cx43	↓	n/a	Dye coupling	Glial co-culture	Hinkerohe et al., 2005
	Cx43	↓	n/a	Dye coupling/Dual whole-cell patch-clamp	Human fetal astrocytes	John et al., 1999
	Cx43	↓	n/a	SLDT	Striatal astrocytes	Zvalova et al., 2004
	Cx43	↓	n/a	SLDT	Cortical astrocytes	Meme et al., 2004
	Cx43	↓		SLDT	Cortical astrocytes	Retamal et al., 2007a
	Cx43		↑	Dye uptake/Whole-cell patch-clamp		
	Cx43	↓		SLDT	Cortical astrocytes	Froger et al., 2009, 2010
	Cx43		↑	Dye uptake		
	Cx43	↓		SLDT	Neuron-glia co-culture	Froger et al., 2010
	Cx43		↑	Dye uptake		
	Cx43	↓	n/a	SLDT	Cortical astrocytes	Meme et al., 2006
IL-6	Cx43	↓	n/a	Dye coupling	Glial co-culture	Hinkerohe et al., 2005
NGF	Cx43/Cx32?	↑		Dye coupling		Belliveau et al., 2006
	Cx43/Cx32?		↑	ATP release	PC12 cells	
TNF-α	Cx43	↓	n/a	Dye coupling	Glial co-culture	Hinkerohe et al., 2005
	Cx43	↓		SLDT	Cortical astrocytes	Retamal et al., 2007a
	Cx43		↑	Dye uptake/Whole-cell patch-clamp		
	Cx43	↓		SLDT	Cortical astrocytes	Froger et al., 2009
	Cx43		↑	Dye uptake		
	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Haghikia et al., 2008
	Cx43	↓		SLDT	Neuron-glia co-culture	Froger et al., 2010
	Cx43		↑	Dye uptake		
	Cx32	n/a	↑	Glutamate release	Cortical microglia	Takeuchi et al., 2006
	Cx43	↓	n/a	SLDT	Cortical astrocytes	Meme et al., 2006
	Cx43	↑	n/a	Dye coupling	Cortical microglia	Eugenín et al., 2001
	Cx43	↓		SLDT	Brain endothelial cell line	Vandamme et al., 2004
	Cx43		↑	ATP release		
TGF-β1	Cx43	↑	n/a	Dye coupling	Glial co-culture	Hinkerohe et al., 2005
	Cx43	↑	n/a	Dye coupling	Cortical astrocytes	Robe et al., 2000
	Cx43	↓	n/a	Dye coupling	Glioma cells	Robe et al., 2000
TGF-β3	Cx43	↓	n/a	Dye coupling	Striatal astrocytes	Reuss et al., 1998
	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Reuss et al., 1998

Cx, connexin; Panx, pannexin; GJCs, gap junction channels; HCs, hemichannels; NE, no effects; n/a, data not available or unknown; ? = Data suggesting a direct effect on. SLDT, scrape-loading and dye transfer; FRAP, fluorescence recovery after photobleaching.

be related to the recruitment of microglia and/or the infiltration of immune cells during brain inflammation. At the same time, inhibition of GJIC by pro-inflammatory cytokines could reflect an attempt to reduce the spread of toxic substances or death signal molecules that may impair neuronal activity and survival (Farahani et al., 2005). In fact, astroglial cell GJIC is thought to contribute to death signal propagation following CNS injury. The activation of p38/stress-activated protein kinase 2 (p38/SAPK2) by IL-1β may be part of the signaling mechanism that regulates GIJC in astrocytes. IL-1β induces rapid p38/SAPK2 activation and the time course matches with the induction of dye coupling inhibition (Zvalova et al., 2004). The compound SB 203580, which specifically blocks p38/SAPK2 activation, reverts the IL-1β-induced inhibition of GJIC. Therefore, p38/SAPK2 is a central mediator of IL-1β-induced effects, including its inhibitory action on astroglial cell gap junctions (Zvalova et al., 2004).

It is clear that pro-inflammatory cytokines partially mediate the damage that occurs during several inflammatory diseases, including multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE) and meningitis (Toulmond et al., 1996). Notably, a loss of Cx43 immunoreactivity in infiltrated brain regions is observed in different models of these and other inflammatory-related pathologies (Brand-Schieber et al., 2005; Karpuk et al., 2011; Schalper et al., 2009; Takeuchi et al., 2011). Under such conditions, a wide spectrum of cytokines is possibly upregulated when affecting GJIC, rather than just one pro-inflammatory cytokine (e.g., IL-1β). Supporting this idea, other pro-inflammatory cytokines, such as TNF-α, IL-6, TGF-β3 and IFN-γ, reduce the levels of Cx43 and GJIC more effectively when applied in combination to cultured glial cells (Froger et al., 2009; Guo et al., 2007; Haghikia et al., 2008; Meme et al., 2006; Retamal et al., 2007a; Reuss and Unsicker, 1998). Importantly, most of these effects are prevented by anti-inflammatory cytokines, including TGF-β1 and IFN-β (Hinkerohe et al., 2005; Robe et al., 2000), and it has been proven that not only gap junctions from astrocytes are regulated by cytokines. In a resting surveillance state, microglia express very low or undetectable levels of Cx43 and Cx36 (Dobrenis et al., 2005; Eugenín et al., 2001; Garg et al., 2005; Meme et al., 2006; Parenti et al., 2002; Rouach et al., 2002b). Nevertheless, microglia activated by pro-inflammatory conditions exhibit an increased expression of Cx43 and form gap junction channels between themselves, as evaluated by dye-coupling. In fact, Cx43 expression and GJIC is induced in microglia by LPS or TNF-α plus IFN-γ (Eugenín et al., 2001) or Staphylococcus aureus-derived peptidoglycan (Garg et al., 2005).

3.2. Regulation of gap junction channels by growth factors

Fibroblast growth factor-2 (FGF-2) is a peptide that promotes the generation, differentiation, and survival of neurons and glial cells. The unilateral administration of FGF-2 (in striatum) in a piece of gelfoam caused a transient increase in Cx43-positive puncta, immediately adjacent to the implant. These changes were paralleled by a small increase in Cx43 protein determined by Western blot, but not by alterations in cellular coupling in the vicinity of the gelfoam implant (Rufer et al., 1996). However, in another study FGF-2 reduced Cx43-protein, -mRNA, and intercellular communication, as revealed by dye spreading. These changes occurred in cortical and striatal glial cells, but not in mesencephalic astroglial cells (Reuss et al., 1998). The downregulation of astroglial gap junctions by FGF-2 was also accompanied by upregulation of dopamine D_1 receptors and increased sensitivity to dopamine (Reuss et al., 2000b). Hence, FGF-2 produces broad changes in signaling pathways of particular brain areas. In cortical and striatal astrocytes, both FGF-9 and FGF-2 decrease astroglial connexin expression and functional coupling (Reuss et al., 2000a). But in mesencephalic astrocytes, FGF-5 (Reuss et al., 2000a), but not FGF-2 (Reuss et al., 1998), suppresses GJIC. Interestingly, most of these factors, especially FGF-2, are expressed ubiquitously in the CNS, implying that important regional differences occur during the regulation of astroglial cell gap junctions in different brain areas. FGF-2 also promotes survival of dopaminergic midbrain neurons, by means of a mechanism that depends on the upregulation of GJIC, since gap junction blockade with oleamide, abolishes the survival-promoting effects of FGF-2 on these cells (SiuYi Leung et al., 2001).

Although some studies indicate that gap junction channels are not affected by EGF (Reuss et al., 1998), other reports suggest that they are modulated by this growth factor. For instance, an inverse relationship between glioma migration rate and expression of Cx43 from different brain regions has been described (McDonough et al., 1999). Additionally, glioma cells stimulated to migrate by EGF, exhibit a concomitant reduction of Cx43 levels linked to phosphorylation in serine residues (McDonough et al., 1999). It was proposed that in order to locally invade brain parenchyma, glioma cells must first detach functionally, i.e. by reducing GJIC with neighboring cells. On the other hand, EGF decreases the Cx43 mRNA and protein levels in cortical astrocytes, a response prevented by MEK inhibitors (Ueki et al., 2001). Interestingly, activation of the EGF receptor pathway by high hydrostatic pressure decreased GJIC in astrocytes by a mechanism that involves phosphorylation of Cx43 in tyrosine residues (Malone et al., 2007). This mechanism may be involved in pathologic conditions, like glaucoma, in which an elevated intraocular pressure may reduced astroglial GJIC affecting tissue homeostasis (Malone et al., 2007).

Almost one decade ago, Aberg and colleagues evaluated the effects of recombinant human IGF-I on astroglial cell gap junctions. The application of 30 ng/ml IGF-I increased GJIC and Cx43 protein levels, whereas higher concentrations (150 ng/ml) did not affect both parameters (Aberg et al., 2003). Altogether, the information mentioned in the above paragraphs indicates that depending on the concentration and time of exposure, growth factors could differentially regulate gap junctions in diverse brain cells. An interesting case, although not directly related to functional regulation of gap junction channels, is the interaction between Cx43 and CCN proteins. The latter constitute a family of signaling molecules made up of extracellular matrix-associated proteins that participate in many cellular processes, including cell adhesion, migration and proliferation, as well as in pathological process such as inflammation and tumorigenesis (Holbourn et al., 2008). The CCN acronym comes from the names of the first discovered three members of this family: Cyr61 (cysteine-rich protein 61 or CCN1), CTGF (connective tissue growth factor or CCN2) and NOV (nephroblastoma overexpressed or CCN3). They share common structural modules, such as four conserved cysteine-rich domains like the insulin growth factor-binding domain and the Von Willebrand Factor Type C domain. The CCN proteins also possess an N-terminal secretory signal that lead to their secretion to the extracellular milieu. Therefore these proteins could have an extracellular and intracellular (cytoplasmic) localization. Members of CCN protein family have been shown to be associated to gap junction channels composed of Cx43. Fu and colleagues found that CCN3 co-localizes with Cx43 in gap junction plaques at the plasma membrane in glioma C6 cells transfected with Cx43 (Fu et al., 2004). In cells expressing a C-terminal truncation of Cx43 ($Cx43_{\Delta 244-382}$), CCN3 did not co-localize with the truncated Cx43. Moreover, glutathione S-transferase pull-down assays and co-immunoprecipitation demonstrated that CCN3 interact physically with Cx43, but not with $Cx43_{\Delta 244-382}$ (Fu et al., 2004), confirming the importance of the C-terminal domain of Cx43 for this interaction. Therefore, the interaction is specific for Cx43, which could play an important role in cellular growth suppression induced by the expression of this connexin. Interestingly, gliomas expressing high levels of Cx43 exhibited an increase expression of CCN3, which was associated to growth suppression (McLeod et al., 2001).

3.3. Brain hemichannels can be regulated by different growth factors and cytokines

Up to now, diverse research groups have described opening of hemichannels formed by connexins or pannexins in brain cells (Bennett et al., 2012; Orellana et al., 2009; Schalper et al., 2009). This indicates that under certain conditions these channels allow the release of ATP, glutamate and other paracrine signaling molecules necessary to ensure neuron–glia interactions. As in the case of gap junction channels, a series of studies show that hemichannels are also regulated by cytokines and growth factors. As

mentioned before, pro-inflammatory agents, such as TNF-α, IL-1β and amyloid-β peptide, induce ATP release through connexin and pannexin hemichannels in atrocytes and microglia (Bennett et al., 2012; Orellana et al., 2011b, 2011c), allowing calcium wave propagation by activation of P2 receptors in glial cells. Therefore, ATP as paracrine signal may be critical for the local propagation of calcium signaling leading to cell death during brain inflammation (Bennett et al., 2012; Orellana et al., 2011a). In fact, reduction of ATP release is associated with a reduction in secondary damage induced by a trauma model of spinal cord injury (Bennett et al., 2012; Garré et al., 2010). In these experiments the inhibition of FGF-1 receptors drastically reduced the ATP release and tissue damage (Bennett et al., 2012; Garré et al., 2010), suggesting that FGF-1 is a signaling molecule that triggers hemichannel opening. However, other growth factors may have the opposite effect, for example, in astrocytes exposed to extracellular divalent cation-free media, a condition that increases the open probability of connexin hemichannels, EGF and bFGF inhibited calcein leakage through hemichannels by a mechanism dependent on MAP kinase and without changes in total levels or electrophoretic mobility of Cx43 (Morita et al., 2007). Remarkably, IL-1β and inhibition of MAPK cascade reverted the inhibition of Cx43 hemichannels induced by EGF and bFGF, whereas IL-1β increased hemichannel opening in brain slices after exposure to a medium lacking divalent cations (Morita et al., 2007). These findings suggest a role for a MAPK-dependent pathway on the basal modulation of hemichannels in this tissue (Morita et al., 2007). Conditioned medium harvested from LPS-stimulated microglia, or a mixture of IL-1β and TNF-α, enhances cellular exchange with the extracellular milieu via Cx43 hemichannels (Retamal et al., 2007a). Both the reduction in GJIC and the increase in membrane permeability were mediated by a p38 MAPK-dependent pathway (Morita et al., 2007; Retamal et al., 2007a). However, the increase in membrane permeability, but not gap junction inhibition, was rapidly reversed by the sulfhydryl reducing agent dithiothreitol (DTT), indicating that final regulatory mechanisms are different (Retamal et al., 2007a). The increase in membrane permeability was attributable to a rise in hemichannel activity (Retamal et al., 2007a). Finally, the effect of pro-inflammatory cytokines enhanced uptake and reduced intercellular diffusion of glucose, which might explain changes in the metabolic status of astrocytes under inflammatory conditions. Accordingly, this opposite regulation may affect glucose trafficking and will certainly modify the metabolic status of astrocytes involved in brain inflammation (Retamal et al., 2007a). Recent evidences indicate that the opposite regulation of astroglial connexin-based channels is prevented by cannabinoids (CBs). These molecules have anti-inflammatory properties and their receptors are expressed by glial cells. Froger and colleagues found that CBs inhibited the LPS-induced release of IL-1β and TNF-α from microglia. Moreover, Cx43 dual regulation evoked by the pro-inflammatory treatments was prevented by CB applications. Pharmacological characterizations of CB effects on astroglial Cx43-based channels revealed that they are mainly mediated through CB_1 receptors (Froger et al., 2009).

Contrary to the expectations regarding Cx43 as the protein with the highest probability to form hemichannels in microglia, TNF-α treatment was shown to induce glutamate release through a pathway inhibited by a Cx32 (^{32}Gap27), but not a Cx43 (^{43}Gap27) mimetic peptide (Takeuchi et al., 2006). Moreover, surface levels of Cx32 increase in microglia treated with TNF-α. Noteworthy, the increased neuronal death associated with the release of glutamate was inhibited completely with the ^{32}Gap27 mimetic peptide (Takeuchi et al., 2006). Later on, the same group proposed that glutamate released via Cx32 hemichannels play a key role in neuronal damage promoted by brain ischemia (Takeuchi et al., 2008) and experimental autoimmune encephalomyelitis (Shijie et al., 2009). The use of cell cultures derived from microglia specific Cx32 null mice and/or knockdown of Cx32 in microglia might ensure the involvement of Cx32 hemichannels in neuronal damage induced by these pathological conditions. It is relevant to keep in mind that connexin mimetic peptides are highly homologous to extracellular domains of Cx32 and Cx43, but their effects on hemichannel activity have not been well documented, which has caused to question their specificity (Dahl, 2007; Evans and Leybaert, 2007; Wang et al., 2007).

Connexin hemichannels may also play a role during neuronal differentiation and/or maturation. Connexin overexpression enhances neurite outgrowth in PC12 cells treated with nerve growth factor (NGF) to initiate neuritogenesis. The neurite outgrowth induced by NGF was promoted by a mechanism that required functional hemichannels and ATP release into the media (Belliveau et al., 2006). Such studies showed that activation of purinergic receptors enhances neurite outgrowth. When ATP receptor antagonists, suramin or PPADS, were added to PC12 cells transfected with Cx32, Cx32-EGFP, Cx43, or Cx43-EGFP, the increase in neurite length induced by NGF stopped. In addition, the purigenic receptor agonist 2-MeSATP induced formation of neurites in EGFP-expressing PC12 cells, mimicking the effects of connexin-EGFP expression in PC12 cells. Interestingly, ATP released to the extracellular medium via connexin hemichannels in radial glia promotes proliferation of cortical neural progenitor cells (Weissman et al., 2004). Radial glial cells are neuronal progenitors that are transiently expressed during brain development, serving as pathways for neuronal migration and cortical pyramidal neuron generation. Weissman and coworkers found that calcium waves propagate through radial glial cells and require connexin hemichannels, extracellular ATP, $P2Y_1$ receptors and intracellular IP_3-mediated calcium release (Weissman et al., 2004). Moreover, disruption of calcium wave signaling with suramin, MRS2179 ($P2Y_1$ receptor antagonist), thapsigargin or 2-APB (IP_3 receptor blocker) decreased proliferation in the ventricular zone during the peak of embryonic neurogenesis (Weissman et al., 2004).

Pannexin hemichannels are also regulated by growth factors and cytokines. Spinal astrocytes exposed to FGF-1 or ATP for 2 h show increase membrane permeability first by activation of pannexin1 (Panx1) hemichannels via $P2X_7$ receptors and then followed by activation of connexin hemichannels (Garré et al., 2010). At least in an early phase of astrocyte treatment with FGF-1, the release of ATP occurs through vesicles, because it was inhibited by botulinum neurotoxin A, a blocker of vesicular release. Subsequently, ATP release continues by activation of Panx1 and Cx43 hemichannel opening (Garré et al., 2010). Thus, it was proposed that during an inflammatory process, hemichannel opening reduces astroglial-mediated protection in spinal cord trauma and neurodegenerative disease.

3.4. Transmitters

Brain transmitters can be critical players in the regulation of connexin- and pannexin-based communication, ensuring synchronized responses to several stimuli. Here, we review only part (due to space limitations) of available information, regarding the regulation of gap junction channels and hemichannels by different gliotransmitters and neurotransmitters in brain cells (Table 2).

3.5. Acetylcholine

To date, only a few studies have addressed the possible role of acetylcholine and related agonists in the regulation of GJIC in the CNS, and to our knowledge, no evidence regarding regulation of

hemichannels by acetylcholine has yet been described. Pioneering studies performed by Velasquez and colleagues, showed that carbachol, a cholinergic agonist, reduces Lucifer yellow (LY) coupling of CA1 neurons in hippocampal slices by 50% (Velazquez et al., 1997). Moreover, electrotonic coupling potentials or spikelets, which represent electrical interactions via gap junctions (Valiante et al., 1995), were also inhibited by carbachol. Additionally, the co-application of carbachol and atropine resulted in LY coupling and spikelet measurements similar to those observed in control conditions. This suggests that carbachol blocked neuronal gap junctions through muscarinic receptors (Velazquez et al., 1997). In agreement, blockade of α-3 and α-7 subunit-containing nicotinic acetylcholine receptors (nAChRs) increased LY and electrical coupling in adrenal chromaffin cells (Martin et al., 2003). Noteworthy, newborn rats, in which cholinergic synaptic transmission had not yet fully matured in the adrenal medulla, exhibited upregulated GJIC in chromaffin cells (Martin et al., 2003). Thus, it seems that nAChRs exert a tonic inhibitory control on GJIC among chromaffin cells. Similar results were recently reported by Colomer and colleagues, but in this case α9-containing nAChRs were involved in the tonic inhibitory control of GJIC exerted by cholinergic signaling in the adrenal medulla (Colomer et al., 2010). In contrast, Subcoeruleus neurons showed a reversible increase in spikelet-like events after treatment with carbachol (Heister et al., 2007).

Table 2
Regulation of gap junction channels and hemichannels by transmitters in brain cells.

Agent	Cx/Panx type	Effect on GJCs	Effect on HCs	Used technique	Cell type	References
Acetylcholine	Cx43?	↑	n/a	SLDT	Hippocampal mix culture	Wang et al., 2004
	Cx43	N.E.	n/a	SLDT	Striatal astrocytes	Rouach et al., 2002c
(Carbachol)	n/a	↓	n/a	Electrotonic coupling/Dye coupling	Hippocampal neurons	Velazquez et al., 1997
	Cx36?	↑	n/a	Electrotonic coupling	SubCoeruleus neurons	Heister et al., 2007
	Cx43	N.E.	n/a	SLDT	Striatal astrocytes	Rouach et al., 2002c
ATP	Cx43	↓	n/a	SLDT/Dual whole-cell patch-clamp	Cortical astrocytes	Meme et al., 2004
	Panx1	n/a	↑	Dye uptake	Cortical neurons	Orellana et al., 2011a
	Panx1	n/a	↑	Dye uptake	BV-2 microglial cells	Bernier et al., 2012
(BzATP)	Panx1	n/a	↑	Dye uptake/Whole-cell patch-clamp	Cortical astrocytes	Iglesias et al., 2009
	Panx1/Panx2	N.E.	N.E.	Dye leakage/Whole-cell patch-clamp	Cortical astrocytes	Bargiotas et al., 2011
(2-MeS-ATP)	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Enkvist and McCarthy, 1992
	Cx43	↓	n/a	SLDT	Cortical astrocytes	Meme et al., 2004
Dopamine	Cx35?	↑	n/a	Electrotonic coupling	Mauthner cells	Pereda et al., 1992
	n/a	↓	n/a	Dye coupling	Cortical neurons	Rorig et al., 1995
	Cx57/50?	↓	n/a	Dye coupling	Retinal horizontal cells	Teranishi et al., 1983
	Cx57/50?	↓	n/a	Dye coupling	Retinal horizontal cells	Piccolino et al., 1984
	Cx57/50?	↓	n/a	Dual whole-cell patch-clamp	Retinal horizontal cells	Lasater and Dowling, 1985
(SKF38393)	n/a	↓	n/a	Electrotonic coupling/Dye coupling	Hippocampal neurons	Velazquez et al., 1997
	n/a	↓	n/a	Electrotonic coupling/Dye coupling	Accumbens core neurons	O'Donnell and Grace, 1993
	Cx36	↓	n/a	Dye coupling	Retinal amacrine cells	Kothmann et al., 2009
(Quinpirole)	n/a	↑	n/a	Electrotonic coupling/Dye coupling	Striatal neurons	Onn and Grace, 1994
	n/a	↑	n/a	Electrotonic coupling/Dye coupling	Accumbens shell neurons	O'Donnell and Grace, 1993
(Apomorphine)	n/a	↑	n/a	Electrotonic coupling/Dye coupling	Striatal neurons	Onn and Grace, 1994
	n/a	↑	n/a	Electrotonic coupling/Dye coupling	Accumbens shell neurons	O'Donnell and Grace, 1993
	n/a	↓	n/a	Electrotonic coupling/Dye coupling	Hippocampal neurons	Velazquez et al., 1997
Endothelins	Cx43	↓	n/a	SLDT	Cortical astrocytes	Giaume et al., 1992
	Cx43	↓	n/a	SLDT	Striatal astrocytes	Blomstrand et al., 1999a
	Cx43	↓	n/a	Dye coupling	Hippocampal astrocytes	Blomstrand et al., 2004
	Cx43	↓	n/a	Dual whole-cell patch-clamp	Hippocampal astrocytes	Meme et al., 2009
GABA	Cx43	N.E.	n/a	SLDT	Cortical astrocytes	Rouach et al., 2000
	Cx36	↓	n/a	Electrotonic coupling/Dye coupling	Hypothalamic neurons	Park et al., 2011
(Muscimol)	Cx43	N.E.	n/a	SLDT	Cortical astrocytes	Rouach et al., 2000
	n/a	↓	n/a	Dye coupling	Suprachiasmatic neurons	Shinohara et al., 2000
	Cx32?	↓	n/a	Dye coupling	Suprachiasmatic neurons	Colwell, 2000
	Cx36	↓	n/a	Electrotonic coupling/Dye coupling	Hypothalamic neurons	Park et al., 2011
(Baclofen)	Cx43	N.E.	n/a	SLDT	Cortical astrocytes	Rouach et al., 2000
Glutamate	Cx43	↑	n/a	Dye coupling	Cortical astrocytes	Enkvist and McCarthy, 1994
	Cx43	↑	n/a	SLDT	Cortical astrocytes	Blomstrand et al., 1999b
	Cx43	n/a	↑	ATP release	Cortical astrocytes	De Vuyst et al., 2009
	Cx43	n/a	↑	ATP release	C6 glioma − Cx43	De Vuyst et al., 2009
	Cx43	↑	n/a	SLDT	Cortical astrocytes	Rouach et al., 2000
	Panx1	n/a	↑	Dye uptake	Cortical neurons	Orellana et al., 2011a
	Cx43	n/a	↑	ATP release	Hippocampal astrocytes	Torres et al., 2012
(LY379268)	Cx36	↑	n/a	Electrotonic coupling/Dye coupling	Hypothalamic neurons	Park et al., 2011
(Kainate)	Cx43	↑	n/a	Dye coupling	Cortical astrocytes	Enkvist and McCarthy, 1994
	Cx43	↓	n/a	Dual whole-cell patch-clamp	Bergmann glial cells	Muller et al., 1996
	Cx43	↑	n/a	Dye coupling	Hippocampal astrocytes	Takahashi et al., 2010
	Cx43	↑	n/a	SLDT	Cortical astrocytes	Rouach et al., 2000
	Cx43	↓	n/a	FRAP	Cortical astrocytes	Jiang et al., 2011
(Quisqualate)	Cx43	↑	n/a	Dye coupling	Cortical astrocytes	Enkvist and McCarthy, 1994
(NMDA)	Cx43	N.E.	n/a	SLDT	Striatal astrocytes	Rouach et al., 2002c
	Panx1	n/a	↑	Dye uptake/Whole-cell patch-clamp	Hippocampal neurons	Thompson and Macvicar, 2008
Serotonin	Cx43	↓	n/a	SLDT	Cortical astrocytes	Blomstrand et al., 1999b
	Cx43	↑	n/a	SLDT	Brain stem astrocytes	Blomstrand et al., 1999b
	n/a	↓	n/a	Dye coupling	Cortical neurons	Rorig and Sutor, 1996
	n/a	↓	n/a	Dye coupling	Cortical neurons	Szabo et al., 2010

Cx, connexin; Panx, pannexin; GJCs, gap junction channels; HCs, hemichannels; NE, no effects; n/a, data not available or unknown; ? = Data suggesting a direct effect on. SLDT, scrape-loading and dye transfer; FRAP, fluorescence recovery after photobleaching.

Discrepancies between this study and others might depend on distinct brain regions and/or cell types analyzed, or different expression of receptor subtypes, or connexin and pannexin types (Fig. 1 and Table 2).

Regarding glial cells, almost a decade ago it was suggested that acetylcholine increases GJIC in hippocampal astrocytes co-cultured with neurons (Wang et al., 2004). Although it is known that astrocytes express nicotinic receptors (Sharma and Vijayaraghavan, 2001), the use of co-cultures difficult to discern if increases in GJIC are triggered directly in astrocytes or indirectly through the intervention of neurons and/or microglia. However, the latter was clarified by Rouach and colleagues who showed that acetylcholine and carbachol do not change gap junctional activity in pure striatal astrocytes (Rouach et al., 2002c).

3.6. ATP

Activation of metabotropic purinergic (P2Y) and ionotropic purinergic (P2X) receptors by extracellular ATP regulates intercellular communication between glial cells and neurons (Fields and Stevens, 2000). Almost two decades ago, 2-methylthio-ATP (2-MeS-ATP), a specific agonist of P2Y receptors, was shown to reduce dye coupling in astroglial cell cultures (Enkvist and McCarthy, 1992). Similar results were further obtained by Même and colleagues by using SLDT or dual whole-cell patch-clamp techniques. They demonstrated that 2-MeS-ATP or ATP decreased GJIC between cortical astrocytes (Meme et al., 2004). Moreover, MRS2179, a $P2Y_1$ receptor antagonist, but not P2X receptor blockers, totally prevented the ATP-induced reduction in GJIC. Interestingly, the ATP-induced reduction of GJIC was potentiated by IL-1β, which could be related to an increased expression level of P2Y receptors, as it was observed previously upon IL-1β treatment (John et al., 1999). Alternatively, IL-1β could enhance the ATP-evoked release of arachidonic acid from astrocytes (Stella et al., 1997); arachidonic acid is a byproduct known to drastically reduce GJIC in astroglial cell cultures (Giaume et al., 1991; Martínez and Sáez, 1999).

Recent evidence indicates that ATP, via P2X and P2Y receptor activation, increases hemichannel activity in several cell types (Baroja-Mazo et al., 2013) (Table 2). The mechanism underlying the uptake of small molecules upon $P2X_7$ receptor activation remains unknown. However, two different theories have been proposed to explain this phenomenon. One suggests a progressive dilation of $P2X_7$ receptor pores, allowing membrane permeabilization to small molecules. The other theory proposes that $P2X_7$ receptors activate a second non-selective permeabilization pathway that depends on another channel (Pelegrin, 2011). So far, there is no consensus regarding which mechanism actually takes place, but it is possible that both may coexist. Just a few years ago, Panx1 hemichannels were proposed to be partial mediators of the second large-conductance pore of $P2X_7$ receptors (Locovei et al., 2007; Pelegrin and Surprenant, 2006). Since then, other reports on different cell types have described a similar involvement of Panx1 hemichannels in this phenomenon. Recently, this conception was challenged by studies showing that permeation of large molecules upon $P2X_7$ receptor activation is independent of Panx1 (Baroja-Mazo et al., 2013). These data suggest that large pore formation induced by ATP may use different permeation pathways (Cankurtaran-Sayar et al., 2009; Schachter et al., 2008). Nevertheless, activation of Panx1 hemichannels by ATP has been observed in astrocytes, neurons and microglia (Bernier et al., 2012; Iglesias et al., 2009; Orellana et al., 2011c). Indeed, performing electrophysiological and dye uptake experiments, Iglesias and colleagues showed that activation of $P2X_7$ receptors with BzATP in astrocytes increase the activity of Panx1 hemichannels, but not Cx43 hemichannels (Iglesias et al., 2009). Interestingly, they quantified the BzATP-induced ATP release from wild-type, Cx43-null and Panx1-knock/down astrocytes, and observed that downregulation of Panx1, but not of Cx43, prevented ATP release from these cells. Further studies carried out in Panx1-null astrocytes showed similar results (Suadicani et al., 2012). In contrast, a recent study demonstrated that BzATP-induced ATP release and dye leakage remain unaltered in Panx1/Panx2-null astrocytes when compared with wild type astrocytes (Bargiotas et al., 2011). Moreover, outward currents induced by a voltage ramp did not differ between wild type and Panx1/Panx2-null astrocytes (Bargiotas et al., 2011). Hence, future studies are needed to clarify these contrasting findings and to elucidate the truly role of Panx1 hemichannels in the above phenomena.

In cortical neurons, concentrations ranging from 5 to 100 μM ATP increased ethidium uptake, which was entirely suppressed upon by Panx1 hemichannel blockers (Orellana et al., 2011b). In contrast, concentrations above 100 μM ATP inhibited Panx1 hemichannel activity in a concentration dependent-manner. This is consistent with the proposed ATP-induced negative feedback loop, which controls these channels (Qiu and Dahl, 2009). It is interesting to note that ATP-induced Panx1 hemichannel activity was associated with increased neuronal death, which was prevented by $P2X_7$ receptor inhibitors (Orellana et al., 2011b). Similarly, in BV-2 murine microglial cells, permeation of small molecules via $P2X_7$-dependent pore formation required Panx1 hemichannels, whereas formation of $P2X_4$ pore did not (Bernier et al., 2012). The authors concluded that permeation of small molecules via $P2X_4$ receptors occurs through pore dilation, as it was observed in Xenopus oocytes, which is a system lacking endogenous pannexins and other possible channels permeable to small molecules (Bruzzone et al., 2005).

3.7. Dopamine

Dopamine is a widely known neurotransmitter that regulates electrical synapse in different brain zones. Early studies focused on dopamine and neuronal gap junctions and were related to its modulatory effect on retinal networks, which has been extensively reviewed (Bloomfield and Volgyi, 2009). Normally, upon light stimulation, dopamine released from amacrine cells binds to D_1 receptors, triggering the cAMP-dependent activation of PKA in horizontal and AII amacrine cells (DeVries and Schwartz, 1989; Kothmann et al., 2009; Lasater and Dowling, 1985; Mills and Massey, 1995; Mills et al., 2007; Piccolino et al., 1984; Teranishi et al., 1983). Then, PKA phosphorylates connexins [e.g., Cx36 in AII amacrine cells (Urschel et al., 2006)] producing further reductions in gap junction conductance. Nevertheless, it seems that other mechanisms may be involved as well. One of which, indicates that dephosphorylation of Cx36 via PKA-dependent phosphatase 2A, mediates the uncoupling effect of dopamine in AII amacrine cells (Kothmann et al., 2009). Dopamine could also act on D_2/D_4 receptors, reducing the activity of adenylate cyclase and cAMP levels, which results in the consecutive increase of gap junction conductance by diminishing the activity of PKA (Mills et al., 2007; Ribelayga et al., 2008).

In addition to its regulatory role in the retina, dopamine also affects electrotonic interactions in other areas of the CNS. Studies performed in goldfish Mauthner cells showed that dopamine increases electrotonic coupling via D_1 receptors and production of cAMP (Pereda et al., 1992). Similarly, O'Donnell and Grace (1993) demonstrated that activation of D_1/D_2 and D_2/D_3 receptors with apomorphine and quinpirole, respectively, increases dye coupling and electrotonic coupling in accumbens shell neurons. Interestingly, neurons from the core region of accumbens exhibited decreased dye coupling upon D_1 receptor stimulation, which was blocked by the SCH 23390 and SCH 39166 D_1-receptor antagonists. It is also worth noting that striatal neurons seem to behave

similarly to neurons from the shell region of accumbens, because apomorphine and quinpirole increased electronic coupling and dye coupling in these cells (Onn and Grace, 1994). In contrast, both hippocampal and cortical neurons exhibit reduced GJIC when dopamine or dopamine receptor agonists are applied (Rorig et al., 1995; Velazquez et al., 1997). For example, apomorphine and the specific D_1 receptor agonist SKF 38393 reduced dye coupling and spikelet frequency among cortical pyramidal cells, an effect that was reversed by the D_1 receptor antagonist SCH 23390 (Velazquez et al., 1997). The contrasting data mentioned here may reflect differences in expression of different connexins involved or in the domain-specific modulation of the same connexin.

It is yet to be elucidated whether dopamine can modify hemichannel activity in neurons or glial cells. However, Cx35, the perch ortholog of mammalian Cx36, has been detected in AII amacrine cells and processes of the inner plexiform layer (O'Brien et al., 1998). Noteworthy, Cx35 forms functional hemichannels in Xenopus oocytes that are regulated by a cAMP/PKA-dependent pathway associated to a consensus PKA phosphorylation site required for channel gating (Mitropoulou and Bruzzone, 2003). It remains unknown whether dopamine regulates Cx36 hemichannels in retinal cells.

3.8. Endothelins

Endothelins comprise a family of three members (ET_1, ET_2, and ET_3), often associated with a wide variety of physiological functions in several tissue types, including the CNS (Kohan et al., 2011). In the early 1990s, Giaume and colleagues described for the first time that ET_1 and ET_3 strongly inhibit the extent of dye coupling among astrocytes (Giaume et al., 1992). Later, these findings were extended by demonstrating that both ET_1 and ET_3 inhibit propagation of intercellular calcium waves in astrocytes as well (Blomstrand et al., 1999b). Although it has been proposed that the endothelin-induced effect requires activation of both ET_B and ET_A receptors (Blomstrand et al., 1999b, 2004; Meme et al., 2009), others have suggested that only activation of ET_B receptors is necessary (Rozyczka et al., 2005). In the latter study, ET_1 and ET_3 inhibited the expression of Cx43 in astrocytes, but blockade of only ET_B receptors (not ET_A) partially attenuated this response (Rozyczka et al., 2005). Nevertheless, it was not evaluated whether this effect explains the inhibitory effect of endothelins on GJIC. In contrast, although the inhibitory effect of ET_3 on astroglial cell GJIC is abolished by ET_B receptor blockade, a synergistic action of ET_B and ET_A antagonists is required to prevent the inhibitory effect of ET_1 (Blomstrand et al., 2004). Moreover, endothelin-induced changes in Cx43 protein expression exhibit similar pharmacological profiles for GJIC reduction. The mechanism underlying the endothelin-induced reduction in astroglial cell GJIC is not fully understood. However, some evidence indicates that Cx43 desphophorylation could be involved (Blomstrand et al., 2004; Rozyczka et al., 2005).

3.9. GABA

The role of GABA in the modulation of gap junctions has been examined in neurons by using muscimol, a selective agonist of $GABA_A$ receptors, which are widely distributed in the brain (Vithlani et al., 2011). Muscimol inhibited dye coupling between cultured neurons from the suprachiasmatic nucleus in a dose-dependent fashion, and this effect was reversed by bicuculline, a $GABA_A$ receptor antagonist (Colwell, 2000; Shinohara et al., 2000). Moreover, *in vitro* or *in vivo* experiments performed on hypothalamic neurons have shown that muscimol or GABA reduces neuronal dye coupling and Cx36 protein levels, whereas bicuculline alone or in combination with picrotoxin induces the opposite effect (Park et al., 2011). Furthermore, two $GABA_B$ receptor antagonists, baclofen or phaclofen, did not increase Cx36 protein levels, suggesting that $GABA_A$ (but not $GABA_B$) receptors are involved in the above response. The mechanism associated with regulation of neuronal Cx36 via $GABA_A$ receptors seems to occur through L-type voltage-gated Ca^{2+} channels and PKC-dependent signaling (Park et al., 2011). In contrast to data obtained in neurons, GABA, muscimol or baclofen do not change astrogial GJIC in astrocytes (Rouach et al., 2000).

3.10. Glutamate

Glutamate is a crucial gliotransmitter and neurotransmitter that plays a key role in chemical and electrical synapses. Current evidences indicate that glutamate or diverse glutamate receptor agonists increase GJIC and hemichannel activity in astrocytes and neurons. Activation of AMPA or kainate receptors with quisqualate or kainate results in increased dye coupling among astrocytes (Enkvist and McCarthy, 1994). Similar results have been found in astrocytes stimulated with glutamate (Blomstrand et al., 1999a; Enkvist and McCarthy, 1994; Rouach et al., 2000) (Table 2). In fact, short application (10–20 min) of glutamate or kainate is enough to increase GJIC in cortical, hippocampal and brain stem astrocytes, but not in hypothalamic astrocytes (Blomstrand et al., 1999a; Rouach et al., 2000). Accordingly, increased GJIC has been recorded between astrocytes from brain slices of kainate-treated rats. Furthermore, an important direct correlation of the latter with protein levels of Cx30, and indirect correlation with levels of Cx43 was observed (Takahashi et al., 2010). Ionotropic glutamate receptors are not the only ones that have been linked to the effects described above. In hypothalamic and somatosensory cortical neurons, activation of group II metabotropic glutamate receptors (mGluRs) enhances electrotonic and dye coupling, whereas an increase in protein levels of Cx36 is observed (Park et al., 2011; Wang et al., 2012b). In support to the role of Cx36 in this response, the mGluRs-induced increase in GJIC does not occur in Cx36-null neurons (Park et al., 2011).

In contrast to the abovementioned data, few studies suggest that activation of glutamate receptors may downregulate gap junctions. A recent report indicated that 1 h of exposure to kainate decreases GJIC in cortical astrocytes, whereas exposure for 6 days reduced Cx43 protein levels (Jiang et al., 2011). Interestingly, both effects were prevented when mitochondrial ATP-sensitive K^+ channels (K_{ATP} channels) were opened with diazoxide, indicating that crosstalk between both channels could regulate GJIC among astrocytes. In contrast to these data, blockade of surface K_{ATP} channels was shown to increase coupling among astrocytes (Velasco et al., 2000). Jiang and collaborators proposed that activation of mitochondrial K_{ATP} channels may enhance the metabolic machinery (Riess et al., 2008) and the consequent increase in ATP would inhibit the surface K_{ATP} channels, resulting in an increase in GJIC.

Kainate can rapidly reduce electrical and dye coupling in Bergmann glial cells, when applied at a 1 mM concentration (Muller et al., 1996). The reduction in GJIC was potentiated at high extracellular Ca^{2+} concentration, whereas kainate only slightly diminished it at low extracellular Ca^{2+} concentration. The first piece of evidence involving glutamate receptor activation in relation to enhanced hemichannel activity came from studies performed in hippocampal neurons. Neurons exposed to NMDA exhibit nonselective currents and calcein leakage, which can be abolished with ^{10}Panx1 mimetic peptide (homologous to a domain of Panx1 extracellular loop 1 or by downregulation of Panx1 (Thompson and Macvicar, 2008). Moreover, in hippocampal slices subjected to NMDA receptor stimulation, pyramidal neurons exhibit an increase in sulforhodamine 101 uptake, indicating that NMDA receptors could also activate neuronal Panx1 hemichannels *ex vivo*. In

agreement with these data, glutamate increases Panx1 hemichannel activity in cortical neurons both in a concentration- and NMDA-dependent manner (Orellana et al., 2011b). Notably, Panx1 hemichannel opening decreased neuronal survival, which can be reduced upon blockade of NMDA receptors and/or Panx1 hemichannels. Moreover, glutamate can activate hemichannels in glial cells as well. Few years ago, it was described that glutamate increases ATP release via Cx43 hemichannels in a concentration-dependent manner, both in C6-Cx43 cells and glial cells (De Vuyst et al., 2009). According to these findings, the glutamate-induced ATP release was prevented upon treatment with several antioxidants, BAPTA or inhibitors of p38- MAP kinase, suggesting the involvement of a complex signaling cascade. In support of the above data, Torres and colleagues recently demonstrated, by means of an elegant study, that ATP released through Cx43 and Cx30 hemichannels is critical to propagate glutamate-induced calcium waves in astrocytes (Torres et al., 2012).

3.11. Serotonin

The first studies linking serotonin action to gap junctions in the CNS were performed in rats during early postnatal development of the neocortex. By using injections of the gap junction permeability tracer neurobiotin into single neurons, Rorig and Sutor (1996) demonstrated that serotonin reduced dye-coupling between pyramidal cells of lamina II/III in a concentration-dependent and reversible manner (Rorig and Sutor, 1996). The uncoupling effect was totally suppressed upon blockade of IP_3 and 5-HT_2 receptors, but mimicked by 5-HT_2 receptor agonists. This suggests the involvement of 5-HT_2 receptors and the release of Ca^{2+} from intracellular stores (Rorig and Sutor, 1996). Electrophysiological recordings performed in motoneurons from snails have confirmed that serotonin could inhibit neuronal gap junctions (Szabo et al., 2010). In contrast, short stimulation periods (10 min) with serotonin suppress dye coupling in hippocampal astrocytes, but not in hypothalamic astrocytes, suggesting that a specific arrangement of 5-HT receptors on different glial cells could play an important role in the fate of serotonin-dependent gap junction regulation (Blomstrand et al., 1999a).

4. Oxidative and nitrosative agents

Free radicals are species containing one or more unpaired electron with the ability to independently exist and react with other molecules. Most common free radicals in living systems are reactive oxygen and nitrogen species (ROS/RNS), which constitute a family of molecules derived from oxygen or nitrogen, respectively. Under physiological conditions, ROS/RNS concentrations are maintained at low levels by the action of antioxidant molecules, such as reduced glutathione. However, under pathological conditions, they may cause massive protein, lipid, and DNA oxidation when there is a misbalance between reduced glutathione and free radicals.

Extensive evidence indicates that despite their low concentration, ROS/RNS are involved in several physiological responses, mainly inducing changes in ion channel properties (Gonzalez et al., 2009; Wilkinson and Kemp, 2011) and/or activating intracellular signaling pathways, such as the cGMP-PKG cascade (Cataldi, 2010; Smolenski, 2012). Among the ion channels modulated by ROS/RNS, connexin and pannexin-based channels are included. As mentioned before, glial cells and neurons express several different types of connexins and pannexins in the CNS (Fig. 1), some of which are modulated by ROS/RNS. In the following paragraphs, we will discuss the effect of ROS/RNS on main connexins and pannexins expressed in CNS cells (Table 3).

Cx30. An important connexin expressed by astrocytes in the CNS is Cx30 (Gosejacob et al., 2011; Koulakoff et al., 2008). Despite the high expression level and important functions of Cx30 in astrocytes, no data are still available regarding the effect of free radicals on Cx30 hemichannels or gap junction channels. However, Cx30-null mice are known to exhibit an increased concentration of free radicals in the inner ear compared to wild type animals (Chang et al., 2008), suggesting that Cx30 expression may affect the total amount of free radicals in certain cells. Future experiments could elucidate whether free radicals can regulate Cx30, and if its expression may modulate free radical levels in other cell types of the CNS.

Cx32. Although Cx32 is mainly expressed in oligodendrocytes, other studies have described its presence in some neurons (Scherer et al., 1995; Snipes and Suter, 1995). To our knowledge, there are no data available regarding the effect of ROS on Cx32 in oligodendrocytes or any other CNS cell type. Additionally, it remains unknown whether Cx32 is modified by ROS in heterologous systems. Therefore, further studies are needed to determine the real effects of free radicals on Cx32, as well as on cells that normally express Cx32, such as oligodendrocytes, or in exogenous expression systems (e.g., HeLa cells).

Cx36. Cx36 is the mainly connexin expressed in neurons of the CNS (Condorelli et al., 1998). In spite of the lack of studies

Table 3
Regulation of gap junction channels and hemichannels by ROS/RNS in brain cells.

Agent	Cx/Panx type	Effect on GJCs	Effect on HCs	Used technique	Cell type	References
Arachidonic acid	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Martínez and Sáez, 1999
	Cx43	n/a	↑	ATP release	C6 glioma – Cx43	De Vuyst et al., 2009
	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Giaume et al., 1991
Dithiothreitol	Cx43	n/a	↑↓	Dye uptake	HeLa – Cx43	Retamal et al., 2007b
	Cx43	N.E.	↓	Dye uptake	Cortical astrocytes	Retamal et al., 2007a
	Cx43	N.E.	↓	Dye uptake	Cortical astrocytes	Orellana et al., 2010
	Cx43	n/a	↓	Dye uptake	Cortical astrocytes	Retamal et al., 2006
H_2O_2	Cx43	↑	n/a	SLDT	Striatal astrocytes	Rouach et al., 2004
Nitric oxide	Cx36 ?	↑	n/a	Dye coupling	Neostriatal neurons	O'Donnell and Grace, 1997
	Panx1?	n/a	↑	Dye leakage	Hippocampal neurons	Zhang et al., 2008
	Cx43	↓	n/a	SLDT	Cortical astrocytes	Bolanos and Medina, 1996
	Cx43	↓	n/a	Dye coupling	Cortical astrocytes	Ball et al., 2011
	Cx43	n/a	↑	ATP release	C6 glioma – Cx43	De Vuyst et al., 2009
(GSNO)	Cx43	n/a	↑	Dye uptake	Cortical astrocytes	Retamal et al., 2006
O_2^-	Cx43	↓	n/a	SLDT	Cortical astrocytes	Bolanos and Medina, 1996
TCEP	Panx1	n/a	↓	Whole-cell patch-clamp	Xenopus Oocytes	Bunse et al., 2009
Trolox	Cx43	n/a	↓	Dye uptake	Cortical astrocytes	Contreras et al., 2002

Cx, connexin; Panx, pannexin; GJCs, gap junction channels; HCs, hemichannels; NE, no effects; n/a, data not available or unknown; ? = Data suggesting a direct effect on. SLDT, scrape-loading and dye transfer; FRAP, fluorescence recovery after photobleaching.

supporting any direct effect of ROS on hemichannels and/or gap junction channels formed by Cx36, there are some pieces of evidence suggesting that at least gap junction channels formed by this connexin are modulated by ROS. For instance, activation of cortical afferences increases GJIC among rat neostriatal neurons recorded *in vitro* (O'Donnell and Grace, 1997). This phenomenon is mimicked by nitric oxide (NO) donors and prevented by NO synthase (NOS) inhibitors, suggesting that GJIC between striatal neurons could be upregulated by NO. Note that neurons in the striatum actually do express Cx36 (Belluardo et al., 2000; Cummings et al., 2008) and thus, Cx36 gap junctions might be directly affected by NO.

Cx43. Under control conditions, Cx43 is mainly expressed in astrocytes. However, under pathological conditions, Cx43 is upregulated in microglia (Eugenín et al., 2001). In both astrocytes and microglia, Cx43 can form functional hemichannels and gap junction channels (Garg et al., 2005; Orellana et al., 2011c; Retamal et al., 2007a), which play a fundamental role in physiological and pathological processes. For example; Cx43 is involved in glia–glia and glia–neuron paracrine/autocrine communication (Sáez et al., 2003b; Stehberg et al., 2012) and participate in the inflammatory responses of glial cells (Bennett et al., 2012). To our knowledge, the first report suggesting that astroglial Cx43 hemichannels could be modulated by ROS was published by Contreras and colleagues (Contreras et al., 2002). They showed that the cell membrane rat cortical astrocytes expressing Cx43 became permeable to fluorescent dyes (ethidium and LY) after 75 min of metabolic inhibition. This increase in cell membrane permeability was suppressed by hemichannel blockers (octanol and AGA), supporting the idea that astroglial hemichannels are opened in response to ischemia-like conditions. This response was paralleled by a reduction in GJIC but the molecular mechanism involved and functional consequences were not elucidated (Contreras et al., 2002). Interestingly, the increase in dye uptake was prevented by Trolox, which is a free radical scavenger, suggesting that redox potential could induce opening of Cx43 hemichannels. A few years later, it was demonstrated that astrocytes treated with an NO donor (GSNO) exhibit increase opening of Cx43 hemichannels and their cell membrane become permeable to small molecules (Retamal et al., 2006) (Table 3). Additionally, DTT, being a –SH group reducing agent, inhibited completely the increase in membrane permeability induced by metabolic inhibition or GSNO, implying that cystine oxidation could occur in Cx43 under this condition. This idea was confirmed by a biotin-switch assay, which showed that both GSNO and metabolic inhibition induced Cx43 S-nitrosylation. The cystine involved in these processes should be intracellular because extracellular reduced glutathione (GSH), which is membrane-impermeant, did not reverse the opening of Cx43 hemichannels induced by metabolic inhibition, whereas GSH ethyl ester, which is membrane permeant, completely reverse the response (Retamal et al., 2006). Cys 271 may participate in the increase Cx43 hemichannel activity induced by metabolic inhibition or GSNO, because it has been demonstrated to be S-nitrosylated in endothelial cells (Straub et al., 2011).

Later on, it was showed that DTT induces Cx43 hemichannel opening in HeLa cells transfectants (Retamal et al., 2007b) (Table 3). Then, the following question was raised: How is it possible that DTT, being a reducing agent, and NO, an oxidant agent, can both induce Cx43 hemichannel opening? This question was answered through metabolic inhibition experiments performed in primary astrocyte cultures. When cells were stimulated with DTT during short time periods (0–20 min) of metabolic inhibition (when the abundance of oxidant agents is limited), Cx43 hemichannel opening increased. However, when DTT was added after 40 min of metabolic inhibition (when the abundance of oxidant agents overcomes the concentration of endogenous reducing compounds), Cx43 hemichannel opening decreased (Retamal et al., 2007b). Moreover, in astrocytes under short period of metabolic inhibition Cx43 hemichannels are mainly phosphorylated (Retamal et al., 2006), but at longer times of metabolic inhibition (more than 30 min) the ATP concentration is low (Contreras et al., 2002) and Cx43 hemichannels are dephosphorylated (Retamal et al., 2006). Therefore, it is possible that the functional consequences on Cx43 hemichannels might be the result of interplay between redox status and phosphorylation state of Cx43. However, more experiments are needed to solve this hypothesis. In another approach, similar to that observed in cerebral ischemia, rat or mouse cultured astrocytes were exposed to hypoxia (in normal or high glucose) followed by reoxygenation. Cx43 hemichannel opening was observed, but accompanied by a reduction in GJIC (Orellana et al., 2010). Consequently, it was suggested that ROS/RNS (i.e., NO) could affect Cx43 hemichannel gating in ischemia-like conditions.

Other conditions known to produce free radicals are inflammatory processes. For example, primary cultures of astrocytes exposed to LPS-activated microglia cells or pro-inflammatory cytokines (TNF-α plus IL-1β) became permeable to large fluorescent molecules due to Cx43 hemichannel opening (Retamal et al., 2007a). This cell permeabilization is completely inhibited by DTT and L-NAME (a broad range NOS inhibitor), in agreement with the modulation of Cx43 hemichannels by NO. Additionally, GJIC decreases under the above conditions, and this is prevented by a p38 MAPK inhibitor, but not DTT or L-NAME, suggesting that Cx43 gap junction channels are not affected by NO or that they are much less sensitive than Cx43 hemichannels to changes in redox potential under these conditions. However, Cx43 gap junction channels seem to be affected by redox signaling in other experimental models, one of which involved rat astrocyte cultures in normal glucose (5.5 mM) or high glucose (25 mM) for two weeks. Under these conditions, dye coupling between astrocytes grown in high glucose was much lower compared to that of astrocytes maintained under control conditions (Orellana et al., 2010). Moreover, control astrocytes exposed to NO donors exhibited prominent inhibition of GJIC (Orellana et al., 2010). Additionally, the uncoupling effect induced by high glucose was restored by DTT (Ball et al., 2011). Interestingly, the NO donor S-nitroso-N-acetylpenicillamine and $O_2^{\cdot-}$ decreased gap junction channel activity in astrocytes (Bolanos and Medina, 1996). The authors proposed that the peroxynitrite anion formed by the reaction between NO and $O_2^{\cdot-}$ could be responsible for gap junction inhibition. Similarly, rat cortical astrocytes stimulated with arachidonic acid showed decreased dye coupling (Giaume et al., 1991), which was prevented by cyclooxygenase and lipoxygenase inhibitors, or by melatonin (a free radical scavenger), but not by L-NAME (Martínez and Sáez, 1999). Furthermore, gap junction channels in astrocytes seem to be modulated by different kinds of ROS. Confirming this notion, H_2O_2 has been shown to increase GJIC in cultured astrocytes, and this response was reverted by reducing agents (Rouach et al., 2004). Therefore, although free radicals like NO, decrease GJIC, others such as H_2O_2 or one of their sub-products (i.e., oxidized gluthathione; GSSG) may have the opposite effect. Additional research is necessary to clarify the effect of different ROS on Cx43 hemichannels and gap junction channels.

Glial cells open their Cx43 hemichannels in response to rises in intracellular calcium ion concentration ($[Ca^{2+}]_i$) (De Vuyst et al., 2009). The possible involvement of several pathways was tested and concluded that increases in $[Ca^{2+}]_i$ induced Cx43 hemichannel opening in an indirect way, which was mediated in part by NO. Up to now, most data indicate that the pattern of connexin-based channel modulation by ROS/RNS may depend on the metabolic status of a given cell. Clearly, future studies could resolve the relationship between phosphorylation and redox signaling as Cx43 hemichannel gating signaling.

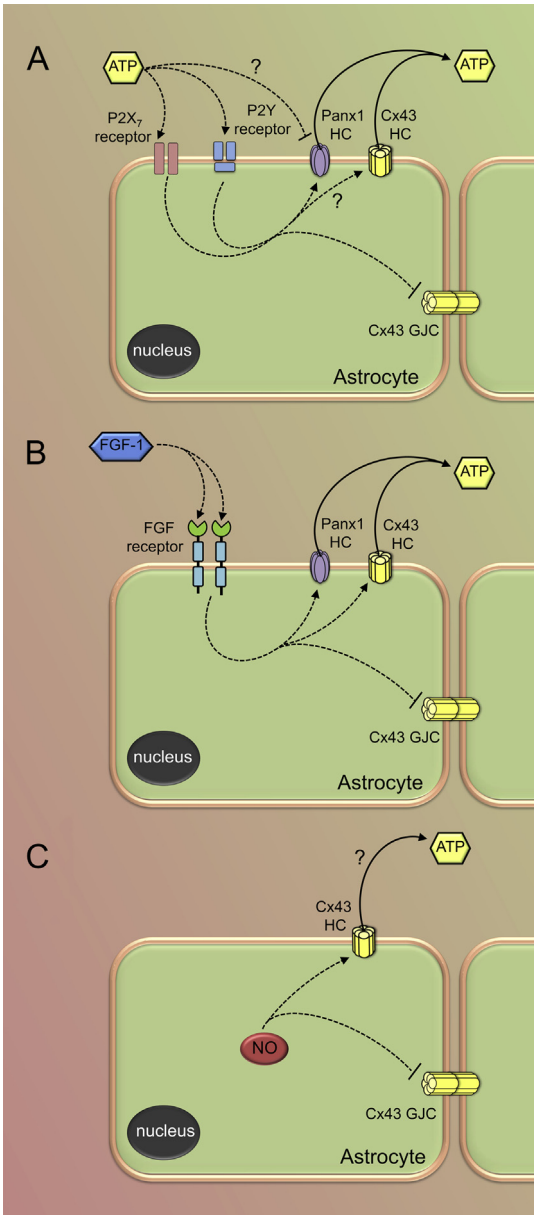

Fig. 2. Hemichannels and gap junction channels are oppositely regulated by diverse signaling molecules in astrocytes. (A) ATP released from vesicles and/or plasma membrane channels diffuses through the extracellular space and activates membrane purinergic (P2) receptors in astrocytes. Stimulation of metabotropic P2Y receptors leads to activation of phospholipase C (PLC) and formation of IP_3. Whereas activation of ionotropic P2X receptors leads to Ca^{2+} influx, the increase in free $[Ca^{2+}]_i$ induced by IP_3 and P2X receptor openings induces activation of Panx1 hemichannels and increase in total Cx43 hemichannels at the cell surface. The release of ATP via astroglial hemichannels could extend Ca^{2+} waves to neighboring cells. At the same time, diacylglycerol activate phospholipase C, leading to the formation of arachidonic acid (AA) upon the action of diacylglycerol lipase. Finally, AA, through an unknown mechanism, could induce the closure of astroglial gap junction channels. (B) FGF-1 binds to its (dimeric) receptor in spinal astrocytes, causing a slight rise in $[Ca^{2+}]_i$, and further release of ATP from vesicles (not shown). Autocrine action of ATP via $P2X_7$ receptors strongly increase the $[Ca^{2+}]_i$, leading to the early opening of Panx1 hemichannels and the later activation of Cx43 hemichannels. Alternatively, FGF-1 by an unknown mechanism could directly induce the activation of Panx1 and Cx43 hemichannels and the closure of gap junction channels. (C) Under pro-inflammatory conditions, a series of intracellular signal transductions induces iNOS activation and the production of NO in astrocytes. High levels of NO could induce cysteine S-nitrosylation of Cx43 hemichannels, enhancing their activity. Simultaneously, NO could reduce the functional activity of gap junction channels by a mechanism linked to peroxynitrite anion upon reaction between NO and $O_2^{\cdot-}$. HC, hemichannel; GJC, gap junction channel.

Panx1. Panx1 is expressed in the CNS (Bruzzone et al., 2003) specifically in neurons (Bruzzone et al., 2003; Vogt et al., 2005) and in glial cells (Iglesias et al., 2009; Thompson and Macvicar, 2008). It is important to highlight that ischemia-like conditions open Panx1 hemichannels in neurons, which is an effect strongly inhibited by DTT and L-NAME, indicating that NO is involved in neuronal Panx1 hemichannel opening (Zhang et al., 2008). Accordingly, Panx1 hemichannels are inhibited by reducing agents under control conditions (Bunse et al., 2009). These data suggest that, under physiological conditions, when GSH levels are high, Panx1 hemichannels are preferentially in the closed state. However, in pathological conditions such as ischemia, where free radicals are elevated, Panx1 hemichannels are preferentially open. Recently, was shown that intracellular mutation of Cys346 induces the formation of "leaky" Panx1 hemichannels (Bunse et al., 2010), pinpointing Cys as a probable sensor of redox potential in neurons. Importantly, it was demonstrated that S-nitrosylation of Panx1 inhibits plasma membrane currents and ATP release in HEK 293T cells expressing wild type Panx1 and mouse aortic endothelial cells (Lohman et al., 2012). Using single cysteine-to-alanine substitutions in Panx1, Lohman and colleagues found that mutation of both Cys-40 and Cys-346 prevents Panx1 S-nitrosylation by GSNO as well as the GSNO-mediated inhibition of Panx1 current and ATP release. Opposite effects of NO on Panx1 hemichannel activity in neurons (Zhang et al., 2008;) and aortic endothelial cells (Lohman et al., 2012), could be explaining by different constitutive mechanisms of gating regulation of Panx1 hemichannels found in these cell types.

5. Conclusions

Intercellular communication plays a crucial role in brain processing of external and internal stimuli. Under physiological conditions, the release of paracrine and autocrine molecules may regulate GJIC, allowing the appropriate diffusion of energy metabolites and dissipation of toxic substances required to sustain neuronal activity and survival. At the same time, intracellular signaling pathways activated by extracellular molecules could modulate hemichannel opening, allowing the release of paracrine substances necessary to self-perpetuate long distance communication through calcium waves. Interestingly, in some circumstances the molecules may increase both hemichannel and gap junctional opening, but in other cases they could regulate the activity of these channels in an opposite direction. The former alternative has broadly been demonstrated to occur in astrocytes when glutamate is applied (Table 2). In contrast, gap junction channels and hemichannels in astrocytes are oppositely regulated by ATP, FGF-1 and NO (Fig. 2 and Tables 1–3). The final outcome of connexin and pannexin-based communication will depend on the brain zone, cell type, cell stage, and the connexin and pannexin subtype expressed. For example, dopamine or related dopamine receptor agonists could increase GJIC in striatal and hippocampal neurons, but could decrease it in cortical neurons and amacrine cells (Table 2). Understanding how paracrine signaling modulates connexin and pannexin-based channels might contribute to the knowledge of how cellular interaction can affect brain processing under physiological conditions. With regard to this, GJIC via connexins is crucial in several brain processes, including neuronal energy supply, electrical and chemical synapses, calcium waves, spatial buffering of K^+ and glutamate, maintenance of myelin and blood–brain barrier integrity, among others. In the same way, connexin and pannexin hemichannels accomplish essential roles in the CNS, such as brain glucosensing, cellular migration, fear memory consolidation and ischemic tolerance. Taking in account the above-mentioned, dysregulation and failure in these functions in which

connexin and pannexin-based channels are involved, could contribute to the development of several CNS pathologies. Among these pathologies, stroke, meningitis, Alzheimer's disease, epilepsy, demyelinating diseases and brain trauma are included. Therefore, connexins as well as pannexins might represent potential and alternative targets for therapeutic intervention in brain diseases.

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