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Connexin and pannexin mediated cell—cell communication

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Abstract

In this review, we briefly summarize what is known about the properties of the three families of gap junction proteins, connexins, innexins and pannexins, emphasizing their importance as intercellular channels that provide ionic and metabolic coupling and as non-junctional channels that can function as a paracrine signaling pathway. We discuss that two distinct groups of proteins form gap junctions in deuterostomes (connexins) and protostomes (innexins), and that channels formed of the deuterostome homologues of innexins (pannexins) differ from connexin channels in terms of important structural features and activation properties. These differences indicate that the two families of gap junction proteins serve distinct, complementary functions in deuterostomes. In several tissues, including the CNS, both connexins and pannexins are involved in intercellular communication, but have different roles. Connexins mainly contribute by forming the intercellular gap junction channels, which provide for junctional coupling and define the communication compartments in the CNS. We also provide new data supporting the concept that pannexins form the non-junctional channels that play paracrine roles by releasing ATP and, thus, modulating the range of the intercellular Ca^{2+}-wave transmission between astrocytes in culture.

Keywords

Hemichannels; gap junctions; Ca^{2+} waves; glia; astrocytes; purinergic receptors

The gap junction family of proteins: connexins, innexins and pannexins

Structurally, gap junctions of both vertebrates and invertebrates (see Epstein and Gilula, 1973) are recognized in thin-section electron microscopy as roughly parallel membrane interfaces separated by a 2—4 nm ‘gap’ and in freeze fracture as aggregates or plaques of hexameric particles located at the membranes of adjoining cells (Fig. 1A). In the animal kingdom, there are three families of gap junction proteins: the innexins (¾25 members in *C. elegans*) (reviewed in Phelan 2005;Yen and Saier, 2007) that are expressed only in protostomes (Nematoda, Mollusca, Plathyhelmintes, Arthropoda, Annelida) and the connexins (~20 members in humans) (reviewed in Willecke *et al.*, 2002), which are the deuterostome (Echinodermata, Urochordata, Cephalochordata and Vertebrata) gap junction proteins. Recently, a new group of gap junction proteins, pannexins, has been identified in deuterostomes. Pannexins (three members) share ~20% (significant) sequence homology with the innexins and display no homology with the connexins (Panchin *et al.*, 2000;Yen and Saier, 2007). Although it has been proposed that the term pannexin be expanded to include both deuterostome and protostome proteins (Baranova *et al.*, 2004), in this manuscript we use separate terms for clarity.

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Gap junctions contain intercellular channels that allow direct communication between the cytosolic compartments of adjacent cells (reviewed in Scemes and Spray, 2008). Each gap junction channel is formed by docking of two 'hemichannels' provided by the adjoined cells to form a bridge across the extracellular space. The hemichannels contributed by each cell are formed by six connexin/innexin/pannexin proteins and they are also called *connexons* if formed by connexin proteins, *innexons* if formed by innexins and *pannexons* if formed of pannexins (Fig. 1B). All of these proteins probably share the same predicted membrane topology; they are tetra-span proteins with the N- and C-terminal domains facing the cytoplasm of the cell (Fig. 1C). The two extracellular loops are the most conserved domains among connexins and are the sites that provide the strong interaction between the two hemichannels that enable the formation of an intercellular channel with virtually no leakage of either current or molecules to the extracellular environment. Gap-junction formation depends on the presence of conserved cysteine residues (Fig. 1C), three in each extracellular loop of connexins and two in each extracellular loop of innexins. Pannexins share the cysteine groups of innexins (two in each loop); however, glycosylation sites on the extracellular loop and the high level of glycosylation of pannexin proteins preclude an intimate interaction between pannexons (Boassa *et al.*, 2007; Penuela *et al.*, 2007). As a consequence, the formation of pannexin gap junction channels is impaired unless the glycosylation is removed (Boassa *et al.*, in press).

Thus, in contrast to innexins which provide both intercellular gap junction channels and hemichannels in non-chordates (Landesman *et al.*, 1999; Li *et al.*, 2003; Dykes *et al.*, 2004; Ducret *et al.*, 2005; Bao *et al.*, 2007), the roles of the other two members of the gap junction protein family seem, in most cases, not to overlap, with connexins primarily forming intercellular channels and pannexins composing the non-junctional pannexon channels (Dahl and Locovei, 2006). Exceptions to this might include the lens connexins Cx46 and Cx50, which readily form non-junctional channels when expressed in *Xenopus* oocytes (Srinivas *et al.*, 2005) and probably also in mammalian lens (Zampighi *et al.*, 1999). As noted below and reviewed elsewhere (Spray *et al.*, 2006), Cx43 is unlikely to perform this function except possibly under extreme pathological conditions.

Biophysical and pharmacological properties of gap junction proteins: how similar are they?

Gap junctions of both deuterostomes and protostomes are permeable to ions and other molecules of molecular weight <1 kDa (Schwarzmann *et al.*, 1981; Spray *et al.*, 2004), thereby allowing the exchange of signaling molecules between cells as well as of intracellularly injected dyes that are used to measure the extent of cell coupling in a cell population. Molecules that permeate gap junction channels include the second messengers Ca^{2+}, Ins(1,4,5)P_3, cAMP and metabolites (glucose, lactate, glutamate and ATP). Given that such broad range of molecules permeate these intercellular channels, it is not surprising that the function of gap junction channels (e.g. ionic dissipation, distribution of metabolites and transmission of Ca^{2+} waves) is diverse (reviewed in Scemes and Spray, 2008).

Innexin and pannexin channels share with connexin channels the property of permeation by moderately sized molecules (Landesman *et al.*, 1999; Li *et al.*, 2003; Dykes *et al.*, 2004; Ducret *et al.*, 2006; Bao *et al.*, 2007). For gap junctions formed by different connexins, however, there is evidence for differences in both charge- and size-selectivity (reviewed in Harris, 2007), although this has not been studied as thoroughly for the other types of gap junction proteins.

Unitary conductance of gap junction channels formed by connexins ranges considerably, from ~10 to >200 pS (reviewed in del Corsso *et al.*, 2006); conductances of hemichannels that correspond to these individual connexins are expected to be about twice as high as those of the cell—cell channel, which is consistent with their series alignment. Thus, Cx43 hemichannels,

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when over-expressed mammalian cells, have main-state conductance of ~220 pS (Contreras *et al.*, 2002) and Cx50 channels in oocytes are much larger, >500 pS (Srinivas *et al.*, 2005). Unitary conductances of protostome gap junction channels have been measured in only a few cases (Weingart and Bukauskas, 1993; Chanson *et al.*, 1994), but they appear to be in the same range as those of mammalian gap junctions, and hemichannel conductances are therefore also expected to be similar. Single pannexin1 (Panx1) channels in non-junctional membranes have conductances in the order of 500 pS (Bao *et al.*, 2004).

Gating of gap junction intercellular channels formed by connexins and innexins is voltage sensitive. For connexins the most prominent component of voltage dependence occurs as a consequence of the voltage gradient across the junctional membrane, regardless of the resting potentials of the individual cells; maximal junctional conductance occurs at zero transjunctional voltage and declines in response to hyperpolarization and depolarization of either coupled cell because of the increased probability of the channels residing in a sub-conductance state. The steepness of the bell-shaped voltage sensitivity of gap junction channels as well as the sub-state conductance varies depending on the composition of the gap junction channel (reviewed in del Corsso *et al.*, 2006; Gonzales *et al.*, 2007). Unlike connexin gap junction channels, however, innexin intercellular channels are sensitive to the actual membrane potential and less sensitive to the transjunctional potential, with depolarization closing the junctional channels (Loewenstein *et al.*, 1967; Verselis *et al.*, 1991; Chanson *et al.*, 1994). Nonjunctional gap junction channels (innexons, pannexons and some connexons, such as Cx46 and Cx50) are opened by membrane depolarization, although threshold for activation depends on the channel type (Ebihara and Steiner, 1983; Trexler *et al.*, 1996; Bruzzone *et al.*, 2005; Srinivas *et al.*, 2005; Bao *et al.*, 2007).

All gap junction proteins examined also show overlapping sensitivity to channel blockade by pharmacological agents, and the effectiveness of some of these agents varies depending on the protein type. The long chain alcohols heptanol and octanol, which inhibit junctional conductance in protostomes (Johnston *et al.*, 1980), are also effective blockers of connexin-based gap junctions (Spray *et al.*, 2002) and block dye uptake in response to $P2X_7R$ activation (Suadicani *et al.*, 2006), which presumably reflects pannexon closure (see below). Similarly, intracellular acidification blocks both innexin and connexin gap junctions, but the apparent p*Ka* and Hill coefficient vary depending on the type of channel-forming protein (Spray *et al.*, 2002). It has been proposed that differential sensitivity of connexons and pannexons to the glycyrrhetinic acid derivative carbenoxolone and to flufenamates (pannexons are ~5—10 times more sensitive to the former and ~10 times less sensitive to the latter) (Bruzzone *et al.*, 2005) might discriminate between these channels, although in this study Panx1 was only compared to a single type of gap junction hemichannel (Cx46). It is clear that more selective channel inhibitors are needed, and several laboratories are working on this.

One distinction that can be made is the sensitivity of gap junction channels and hemichannels of different types to intracellular and extracellular divalent ions. Whereas gap junction channels that are formed of both connexins and innexins are blocked by very high intracellular Ca^{2+} concentrations (Rose and Loewenstein, 1976; Spray *et al.*, 1982), nonjunctional pannexons and innexons are activated by micromolar concentration of this cation (Locovei *et al.*, 2006b; Bao *et al.*, 2007). With regard to extracellular Ca^{2+} concentration, connexon activity in oocytes increases greatly in the absence of Ca^{2+} (Srinivas *et al.*, 2005), whereas pannexon channels appear to be insensitive (Buzzone *et al.*, 2005; Locovei *et al.*, 2007).

That all three families of gap junction proteins share, to some extent, similar biophysical and pharmacological properties (which is expected for proteins with shared evolutionary history) imposes some constraints when interpreting results of pharmacological studies, particularly those that are related to the molecular identity of hemichannels.

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Connexins and pannexins in the mammalian CNS

Connexin proteins are widely expressed encompassing all tissues except differentiated skeletal muscle, circulating erythrocytes and mature sperm cells. About half of the mammalian connexin genes are expressed in the nervous system and one third in the CNS, mainly in glial cells (Willecke *et al.*, 2002; Spray *et al.*, 2004). Each neural cell type expresses a different set of non-overlapping connexins. For example, astrocytes express mainly Cx43, but Cx30, Cx26, Cx45, Cx40 and Cx46 have also been reported (Dermietzel *et al.*, 1991; Naus *et al.*, 1997; Dermietzel *et al.*, 2000; Scemes *et al.*, 2000; Nagy and Rash, 2003), oligodendrocytes express Cx32, Cx29 and Cx47 (Altevogt *et al.*, 2002; Nagy *et al.*, 2003; Li *et al.*, 2004), Schwann cells express Cx32 and Cx29 (Altevogt *et al.*, 2002; Nagy *et al.*, 2003; Li *et al.*, 2007), and microglia express Cx36 (Dobrenis *et al.*, 2005). Cx36 is widely expressed by neurons in some regions of the brain and Cx45 is likely to be expressed by thalamic neurons (reviewed in Willecke *et al.*, 2002; Sohl *et al.*, 2004). In the retina, two additional neuronal connexins (Cx45 and Cx57) are also present as well as Cx36 (Kihara *et al.*, 2006; Shelley *et al.*, 2006).

Based on such differential distribution of connexin types among neural cell populations, it was suggested that gap junctions define communication compartments within the CNS, such that the astrocytic coupled compartment is separate from the oligodendrocyte and neuronal compartments (Spray and Dermietzel, 1996). However, because heterotypic channels might, in some cases, provide intercellular communication in heterologous systems, such as that which results from the pairing of astrocytic Cx30 or Cx26 with Cx32 from an oligodendrocyte (Barrio *et al.*, 1991; Rubin *et al.*, 1992; Yum *et al.*, 2007), communication between astrocytes and oligodendrocytes is possible. Although gap junctions between these two glial cell types have been demonstrated morphologically (Dermietzel, 1998; Massa and Mugnaini, 1985; Nagy and Rash, 2003; Altevogt and Paul, 2004), to date, there is no evidence for such a functional panglial syncytium either *in situ* or *in vitro*. Thus, it might be considered that connexin-based gap junction channels in the CNS determine the territories of communication within similar compartments. Moreover, it is interesting to note that a large portion of gap junctions present in glial cells is formed by ‘reflexive/autologous channels rather than intercellular channels. It has been proposed that the presence of autologous gap junctions at the Schmidt-Lanterman incisures provides a pathway between the successive inner and outer cytoplasmic membrane for radial diffusion of metabolites from the cell body to the innermost myelin lamellae of a Schwann cell (Bergoffen *et al.*, 1993; Scherer *et al.*, 1995; Balice-Gordon *et al.*, 1998). Although the function of such reflexive gap junctions in astrocytes has not been fully investigated, ~25% (10 000 gap junctions per cell) of the gap junction contacts are localized between the processes of a single cell *in situ* and ~1000 autocellular gap junctions are found in solitary astrocytes in culture (Rohlmann and Wolff, 1996; Wolff *et al.*, 1998).

Regarding expression of pannexin, Panx1, like Cx43, is expressed ubiquitously. In the CNS, Panx1 is reported to be expressed in neurons, astrocytes and oligodendrocytes (Vogt *et al.*, 2005; Zappala *et al.*, 2006; Huang *et al.*, 2007a). Panx2 seems to be restricted to neurons, and Panx3 is found mainly in epithelial cells (Bruzzone *et al.*, 2003). However, following ischemic insult and concomitantly with the appearance of astrogliosis, Panx2 is expressed transiently in astrocytes (Zappala *et al.*, 2007). Of the three pannexins, only Panx1 has been shown to form functional channels; however, the properties of Panx1 channels are modified by co-expression of Panx2, which indicates heteromeric assembly of these two proteins (Bruzzone *et al.*, 2003; Bruzzone *et al.*, 2005).

Since their discovery (Panchin *et al.*, 2000), the number of possible roles played by pannexins have increased. Early studies indicated that these proteins provide direct intercellular communication through gap junctions when expressed in *Xenopus* oocytes (Bruzzone *et al.*, 2003). However, presently there is no evidence that this ability is realized *in vivo*. On the

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contrary, several lines of evidence indicate that gap junction formation by pannexins is unlikely (Dahl and Locovei, 2006; Boassa *et al.*, 2007; Huang *et al.*, 2007b; Panuela *et al.*, 2007; see earlier section).

Panx1 has been shown to be activated by mechanical stretch, which indicates that it might function, in part, in the process of mechanotransduction (Bao *et al.*, 2004). More recently Panx1 has been proposed to provide a paracrine signaling pathway by forming membrane channels that are permeable to ATP (Locovei *et al.*, 2006a) and epoxyeicosatrienoic acid (Jiang *et al.*, 2007). Panx1 has also been proposed to be involved in immune responses by associating with $P2X_7$ receptor signaling cascade (inflammasome) involved in the activation of caspase-1 and the processing of interleukin 1β (IL-1β) from macrophages (Pelegrin and Surprenant, 2006; Kanneganti *et al.*, 2007). Moreover, Panx1 has been implicated in the leakage of Ca^{2+} from the endoplasmic reticulum (Vanden-Abeele, 2006), and has a role as a tumor suppressor (Lai *et al.*, 2007) and in the process of neuronal necrosis following CNS ischemia (Thompson *et al.*, 2006).

Distinct roles of connexins and pannexins: evidence from intercellular Ca^{2+} wave spread between astrocytes

Two distinct pathways for intercellular Ca^{2+} wave (ICW) transmission in cells have been identified; one involves the diffusion of Ca^{2+}-mobilizing second messengers (i.e. Ca^{2+}, IP_3 and cADP-ribose) from the cytosol of one cell to that of another through gap junction intercellular channels, and the other, extracellular, pathway involves the diffusion of molecules released from one cell acting on cell surface membrane receptors of its neighbors (Fig. 2) (reviewed in Scemes and Giaume, 2006). In several cell types, the paracrine/autocrine pathway involves the release of ATP through vesicles and/or ion channels activating ATP-sensitive purinergic receptors (P2Rs), which comprise metabotropic P2Y and ionotropic P2X receptors.

Evidence for the participation of an extracellular pathway in the spread of ICWs in astrocytes, which was described initially for non-coupled mast cells (Osipchuk and Cahalan, 1992), was provided by Enkvist and McCarthy (Enkvist and McCarthy, 1992), showing that Ca^{2+} waves cross cell-free areas in confluent cultures of cerebral astrocytes. Later, Hassinger *et al.* (Hassinger *et al.*, 1996) showed that electrically-induced Ca^{2+} waves in cultured astrocytes could cross cell-free areas of up to 120 μm, and travel with similar velocity between confluent cells and through the acellular lanes. Moreover, the extent and direction of waves traveling in confluent cultures were shown to be affected by superfusion (Hassinger *et al.*, 1996). Subsequently, it was demonstrated that ATP was the extracellular molecule released by stimulated astrocytes and that ATP also mediates the transmission of Ca^{2+} waves (Guthrie *et al.*, 1999).

During this time studies from our group and others have provided evidence that the properties (velocity, amplitude and extent of transmission) of ICWs in astrocytes depended upon the subtype of P2R expressed and on gap junctional communication (Blomstrand *et al.*, 1999; Gallagher and Salter, 2003; Suadicani *et al.*, 2004). Moreover, our studies indicate that Cx43 gap junction channels coordinate ICWs mediated by $P2Y_1R$ activation, which, in the absence of coupling, display a 'saltatory' behavior (Suadicani *et al.*, 2004). Such coordination of ICW transmission by gap junction channels might result from the increase in the effective volume of the intracellular compartment, which might lead to the dissipation of the gradients of P2YR-generated second messengers to below threshold levels, thereby terminating ICW spread (Giaume and Venance, 1998; Suadicani *et al.*, 2004). By contrast, gap junction channels might also recruit non-responsive cells into a network of cells expressing more effective P2 receptor subtypes (Venance *et al.*, 1998; Suadicani *et al.*, 2004), thus, expanding the ICW transmission range.

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Certainly, the ability of astrocytes to convey Ca^{2+} signals also depends on the machinery involved in the release process of active substances. In this regard, distinct mechanisms of 'gliotransmitter' release from astrocytes have been proposed including regulated exocytosis and diffusion through ion channels (reviewed in Parpura *et al.*, 2004; Duan and Neary, 2006; Kimelberg *et al.*, 2006; Montana *et al.*, 2006; Oliet and Mothet, 2006; Spray *et al.*, 2006). Among the ion channels that contribute to the paracrine pathway, unpaired connexon channels and the pore-forming $P2X_7R$ have been proposed as sites of ATP release from astrocytes. Previous studies have indicated that Cx43 hemichannels provide sites of ATP release, amplifying the extent of Ca^{2+} signal transmission between cultured astrocytes exposed to low divalent cation solution (Cotrina *et al.*, 1998; Stout *et al.*, 2002; Stout and Charles, 2003). This conclusion is based mainly on pharmacological studies indicating that gap junction-channel blockers prevent ICW amplification. However, as noted above, gap junction blockers also inhibit Panx1, and direct evidence against the participation of Cx43 hemichannels as sites of ATP release from astrocytes is provided by our study showing that ICW amplification is present in Cx43-null and absent in $P2X_7R$-null astrocytes (Suadicani *et al.*, 2006), which indicates that the pore-forming $P2X_7R$ is the most likely candidate to mediate ATP release (Fig. 3).

All the gap junction channel blockers evaluated by Suadicani *et al.* prevent $P2X_7R$-mediated influx of the large fluorescent molecule YoPro-1 (Suadicani *et al.*, 2006). Following these results, which show that the pore-forming $P2X_7R$ has a similar pharmacological profile to that reported for gap junction channels (Suadicani *et al.*, 2006), we investigated whether the gap junction protein Panx1 is the pore recruited by activation of the $P2X_7R$ that is sensitive to gap junction-channel blockers. Indeed, we and others (Pelegrin and Surprenant, 2006; Locovei *et al.*, 2007) found that Panx1 is part of the $P2X_7R$ complex that mediates dye uptake, and that Panx1 mediates the release of ATP from erythrocytes (Locovei *et al.*, 2006a) and from taste bud cells (Huang *et al.*, 2007b).

These new findings, which indicate a partnership between the purinergic receptor and the non-connexin gap junction protein, greatly favor the hypothesis that Panx1 participates in the amplification of ICWs in astrocytes. Indeed, as shown in Fig. 4A, knockdown of Panx1 expression in astrocytes using siRNA prevents the expansion of ICW transmission in low divalent cation solutions. The efficiency of Panx1 downregulation is evidenced by the fact that all three mouse Panx1 siRNA sequences used, but not the control siRNA, significantly reduced Panx1 expression in astrocytes in culture (Fig. 4B) (Locovei *et al.*, 2007). That Panx1 channels contribute to the diffusion of large molecules is indicated by the significant reduction in the uptake of YoPro-1 observed in Panx1-knockdown astrocytes compared to control cells when exposed to a P_2X_7R agonist (Fig. 4C). Moreover, evidence that Panx1 channels provide sites for ATP release is illustrated in Fig. 4D because the amount of ATP present in the solution bathing P_2X_7R-expressing astrocytoma cells is decreased significantly by siRNA-mediated knockdown of Panx1.

Thus, these new results (Fig. 4) together with our previous reports (Suadicani *et al.*, 2006;Locovei *et al.*, 2007) strongly support the idea that the $P2X_7R$-Panx1 complex participates in the amplification of ICWs and that Panx1 provides sites of ATP release from astrocytes.

CONCLUSIONS

In mammals the two gap junction families of proteins, connexins and pannexins, are likely to have non-overlapping roles, one providing gap junctional communication and the other a paracrine signaling pathway. There is no question that connexins are the molecules of which gap junction intercellular channels are composed in deuterostomes. However, whether these proteins form the 'hemichannels' has been a matter of intense debate. As mentioned above,

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and emphasized by Spray *et al.* (Spray *et al.*, 2006), the criteria used to identify the contribution of connexin hemichannels in diverse physiological and pathological processes has been mainly circumstantial, based on measurements of transmembrane flux of large fluorescent molecules and the use of pharmacological agents that block gap junction channels. As pointed out in this review, these are properties shared by all members of the gap junction family of proteins, including the protostome innexins and the deuterostome pannexins.

Because pannexins were first to be shown to provide intercellular channels (Bruzzone *et al.*, 2003) it made sense, from an evolutionary standpoint, to propose that pannexins and connexins had an overlapping role in forming of gap junction channels, because of the use of pannexin homologues for this purpose in protostomes. However, it is becoming apparent that pannexins are non-junctional channels and are, thus, more likely to provide the pathway for the paracrine signaling attributed to connexin ‘hemichannels’.

The pharmacological profile and permeability of pannexons overlap to a great extent with those described for gap junctions, which is likely to confound interpretation regarding the molecular identity of, so-called, hemichannels. There are, however, reported differences in activation and inactivation between connexin hemichannels and pannexon channels that warrant further exploration. One of these is the sensitivity to extracellular divalent cations, which, at low concentrations, opens some connexin hemichannels but has no effect on the voltage dependence of Panx1 channels. However, considering that the, so-called, P2Z receptor, which is permeable to dye molecules of ~800 Da (Steinberg *et al.*, 1987), is now believed to be composed of Panx1 in a complex with the purinergic P_2X_7 receptor (Pelegrin and Surprenant, 2006; Locovei *et al.*, 2007), and that the affinity of P_2X_7R for its agonists (e.g. ATP) is markedly increased by lowering the extracellular concentration of divalent cations (Virginio *et al.*, 1997), opening of Panx1 channels can occur under this condition and, thus, mimic the reported sensitivity of connexin hemichannels to low concentrations of divalent cations.

Recent studies have indicated that ATP release from cells can occur through Panx1 (Locovei *et al.*, 2006a; Huang *et al.*, 2007b). Although our results (see Fig. 4) also indicate that pannexons can be sites of ‘gliotransmitter’ release from astrocytes when exposed to a low divalent cation solution, further studies are necessary to reveal their contribution to cell—cell communication under physiological conditions. However, because Panx1 can be activated by elevation of intracellular Ca^{2+} concentration (Locovei *et al.*, 2006a) (Fig. 4), the function of these channels can, to some extent, overlap with the machinery for exocytotic release of ‘gliotransmitters’.

In summary, in this review we present and discuss evidence that both types of mammalian gap junction proteins, connexins and pannexins, mediate cell communication in several tissues, including the CNS. However, pannexins but not connexins have a paracrine role, releasing ATP, whereas connexins but not pannexins form gap junction channels that coordinate the activity of groups of cells and the direct transmission of Ca^{2+} signals between astrocytes. In a broader sense, it is plausible to consider that the two types of mammalian gap junction proteins have distinct roles; with connexin gap junction channels defining communication compartments within the CNS, whereas pannexons provide amplification of signaling both within and between compartments.

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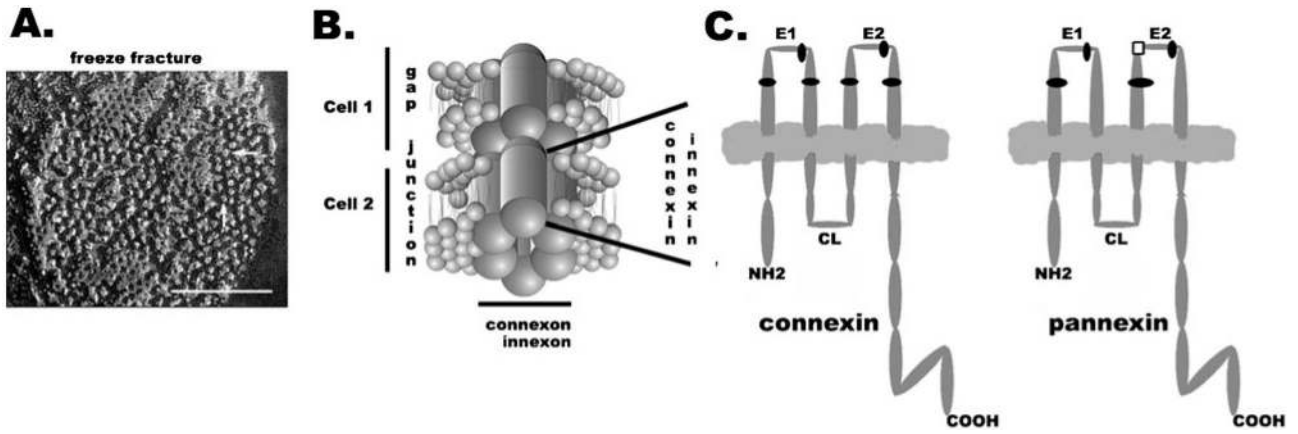

Fig. 1. Gap junction structure and protein membrane topology

(A) P-face of a gap junction plaque between two astrocytes obtained by freeze fracture showing the connexin particles (arrow). Scale bar, 100 nm. Modified from Duffy *et al.* (2000; copyright 2000 by the Society for Neuroscience). (B) Schematic drawing of a gap junction channel formed by the docking of two hexameric structures called 'hemichannels' (either connexons or innexons) provided by two adjoining cells. Each hemichannel is formed by six subunits, the connexins or innexins. (C) Membrane topologies of connexin and pannexin proteins, which are tetra-span proteins with the N- and C-termini, and a cytoplasmic loop (CL) in the cell. The two extracellular loops (E1 and E2) of connexins have three cysteine domains (black ellipses) in each loop that provide the strong interaction with the apposing extracellular loops of connexins in the adjoining cells. By contrast, pannexins have only two cysteines in each extracellular loop and a glycosylation site (white square) within E2 that prevents strong interaction between two pannexons. Adapted from Scemes and Spray (2008).

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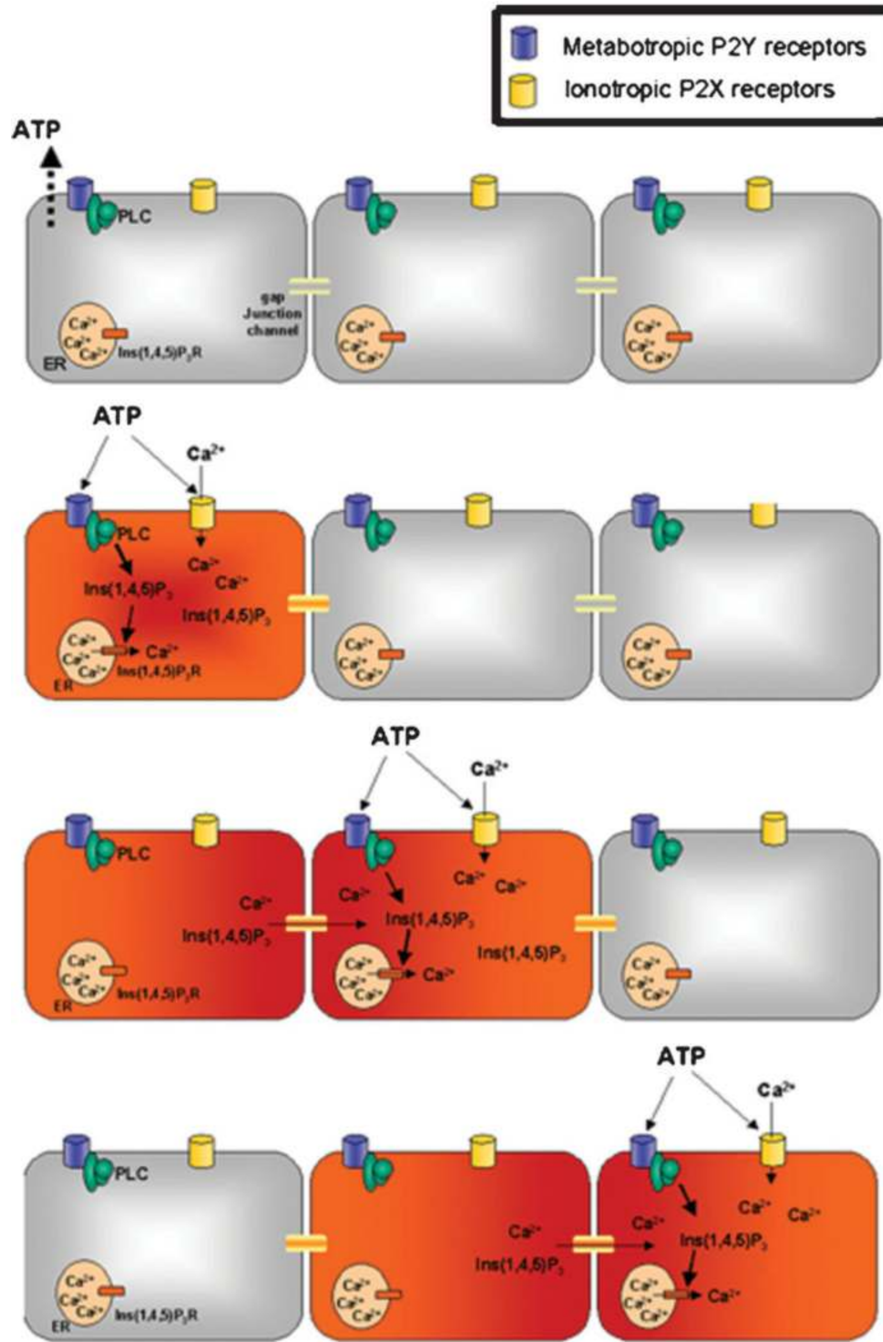

Fig. 2. Components of astrocyte Ca^{2+} waves

Schematic representation of the steps involved in the transmission of intercellular Ca^{2+} waves. ATP released from vesicles and/or ion channels diffuses through the extracellular space and activates membrane purinergic (P2) receptors. Stimulation of metabotropic P2Y receptors leads to activation of phospholipase C (PLC) and formation of Ins(1,4,5)P_3, and activation of ionotropic P_2X receptors leads to the influx of Ca^{2+}. Ins(1,4,5)P_3 and Ca^{2+} promote the release of Ca^{2+} stored in the endoplasmic reticulum (ER), and thereby increase intracellular Ca^{2+} levels in the stimulated cell. Diffusion of these two, intracellular, Ca^{2+}-mobilizing second messengers through gap junction channels, together with the activation of P2 receptors on a nearby cell,

contribute to the Ca^{2+} rise in the nearby cell. This process continues until the concentrations of ATP, Ins(1,4,5)P_3 and Ca^{2+} are not sufficient to trigger intracellular Ca^{2+} rises.

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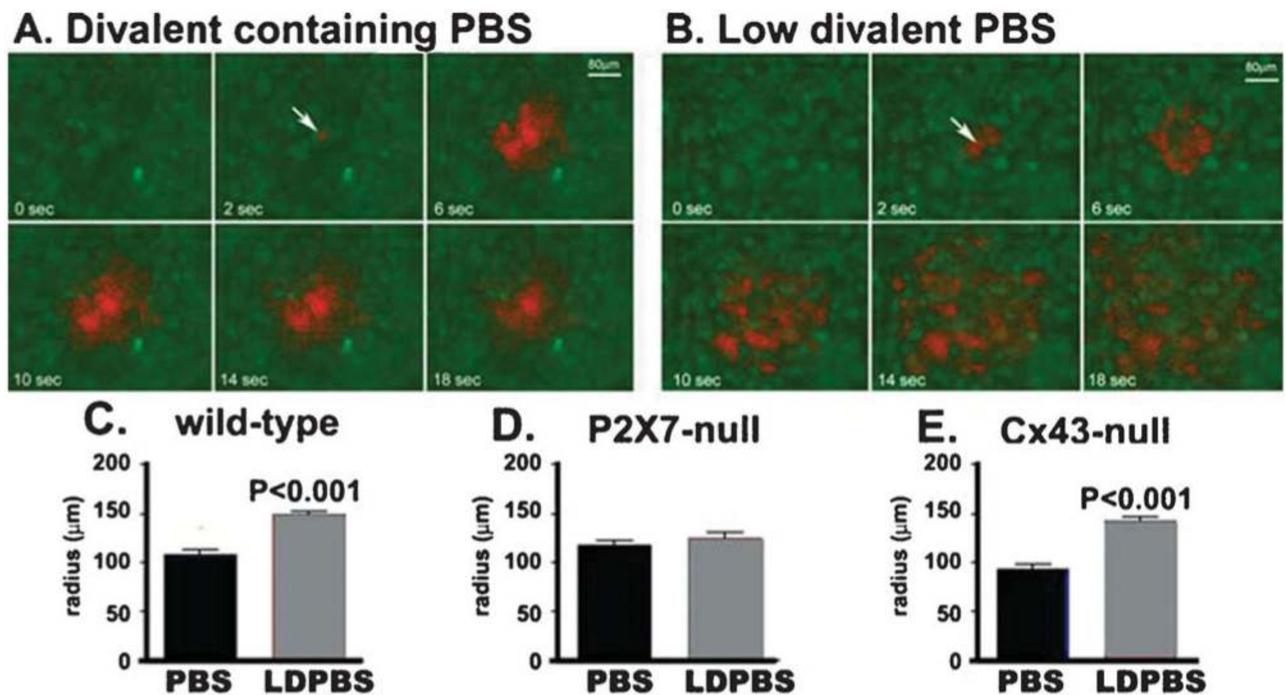

Fig. 3. P2X$_7$ receptor-mediated amplification of intercellular Ca^{2+} waves (ICWs)

(A,B) Sequential fluorescence images of cultured astrocytes loaded with Fura-2-AM and bathed in PBS containing divalent cations (A) and low divalent cations (B). The Ca^{2+} signal initiated by focal mechanical stimulation (white arrows) of single astrocytes is transmitted to neighboring cells. The distance traveled by the ICW increases when cells are bathed in low divalent cation solution (compare A and B). (C) The radius of the ICW spread between astrocytes increases significantly when the cells are bathed in low divalent cation PBS (LDPBS: zero Ca^{2+},1 mM MgCl$_2$, 1 mM EDTA). (D,E) ICW amplification is absent in P$_2$X$_7$R-null (D) and present in C$_{x43}$-null (E) astrocytes exposed to LDC-PBS. Data are mean ± SEM. Adapted from Suadicani *et al.* (2006; copyright 2006 by the Society for Neuroscience).

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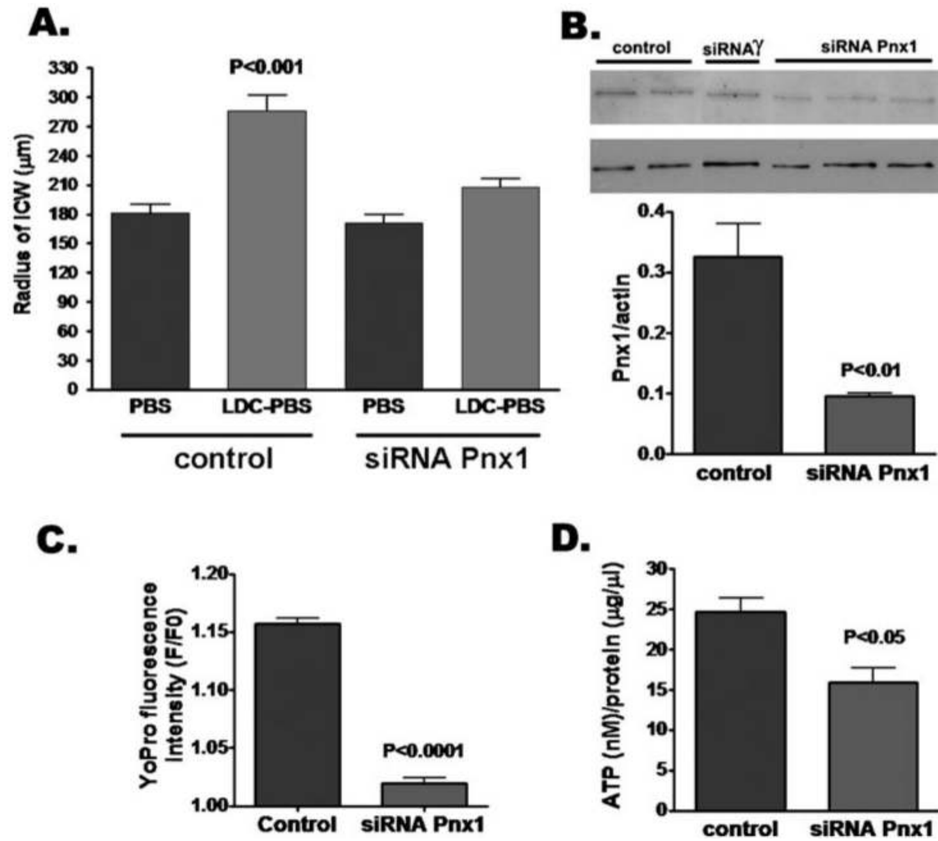

Fig. 4. Panx1-mediated amplification of intercellular calcium wave (ICWs)

(A) ICW radii obtained from untreated (control) and pannexin1 (Panx1)-siRNA treated astrocyte cultures bathed in PBS containing divalent cations, and in low divalent cation (LDC)-PBS. Note that ICW amplification occurs in untreated (control) astrocytes following exposure to LDC-PBS but not in Panx1-siRNA treated cultures. At least four independent experiments were performed. *P* values were obtained from ANOVA analysis of variance followed by Newman Keuls' test. (B) Panx1 expression in untreated astrocytes (control) and astrocytes treated with control siRNAγ and Panx1 siRNA shows the significant reduction in Panx1 in cells treated for 48 hours with mouse Panx1 siRNA. *P* value obtained by unpaired *t*-test. (C) Yo-Pro uptake following BzATP (300 μM) stimulation of control and Panx1 siRNA-treated astrocytes. *P* valueobtained using unpaired *t*-test. (D) ATP release from mechanically stimulated P_2X_7R-1321N_1 astrocytoma cells untreated (control) and treated with Panx1 siRNA. *P* value obtained by unpaired *t*-test. The mouse and human Panx1 siRNA sequences used in the present study have been reported previously (Locovei *et al.*, 2007). All data are mean ± SEM.