Published in final edited form as:
Trends Neurosci. 2003 November ; 26(11): 610–617. doi:10.1016/j.tins.2003.09.008.

New roles for astrocytes: Gap junction hemichannels have something to communicate

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Abstract

Gap junctions are clusters of aqueous channels that connect the cytoplasm of adjoining cells. Each cell contributes a hemichannel, or connexon, to each cell–cell channel. The cell–cell channels are permeable to relatively large molecules, and it was thought that opening of hemichannels to the extracellular space would kill cells through loss of metabolites, collapse of ionic gradients and influx of Ca^{2+}. Recent findings indicate that specific non-junctional hemichannels do open under both physiological and pathological conditions, and that opening is functional or deleterious depending on the situation. Most of these studies utilized cells in tissue culture that expressed a specific gap junction protein, connexin 43. Several such examples are reviewed here, with a particular focus on astrocytes.

Gap junctions mediate intercellular communication by providing ultrastructural cytoplasmic continuity, and they are integral to formation of the functional syncytium of astrocytes. Each of the joined cells contributes a hemichannel or connexon to each cell–cell channel (Figure 1; Box 1). Each hemichannel comprises a hexamer of connexins arranged around a central pore, and the cell–cell channels are gated by several stimuli, including transjunctional voltage, low pH and various pharmacological agents. Connexins are encoded by a gene family with at least 20 members in mammals [1].

Gap-junction pores are nominally 1.0–1.5 nm in diameter and are permeable to molecules of ~1 kDa [2]. Junctions formed from connexin 43 (Cx43), the predominant connexin in astrocytes, are permeable to Lucifer Yellow (443 Da, −2 charge) and propidium (420 Da, +2 charge). Other connexins appear to be more charge selective: Cx32 is more permeable to anions and Cx45 is more permeable to cations [2]. Single-channel conductance varies widely, from ~15 pS for Cx36 [3] and 110 pS for Cx43 [4] to ~375 pS for Cx37 [5]. The maximum size of permeant species also varies. Such diversity in selectivity and conductance are likely to account for the expansion of the connexin family.

Why shouldn't hemichannels be open?

Given the permeability and conductance of gap junctions, and the expectation that an open hemichannel would have twice the conductance and permeability of a cell–cell channel (in terms of ion or molecular flux, rather than selectivity), it was thought unlikely that hemichannels in the non-junctional membrane would open [6]. Direct evidence for this was provided by measurements during formation of the first channel between a newly apposed pair of cells. Each cell was held at a different potential, and then when the first channel

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opened, increased current was seen in the cell held at a more positive potential and decreased (more negative) current was seen in the other cell, but the sum of the currents clamping the two cells remained constant [7]. Thus, there was no change in the current flowing into the extracellular space, and the hemichannels were closed before opening to form a cell– cell channel. When expression of Cx46 in *Xenopus* oocytes was found to be fatal and to cause a large non-specific increase in membrane conductance in low Ca^{2+} conditions, it seemed to be a rule that proved the exception: opening of (non-junctional) hemichannels is bad for cells, and something unusual about Cx46 expressed in oocytes allows hemichannel opening unless relatively high Ca^{2+} concentrations are added to prevent it [8,9].

So are these really hemichannel currents?

The evidence for open hemichannels can be briefly summarized (and criticized). Macroscopically, the existence of hemichannels was initially inferred from a non-selective conductance that depended on exogenous expression of Cx46 in *Xenopus* oocytes. Now, there are many other examples in which cells expressing connexins endogenously or by transfection develop permeability or macroscopic currents consistent with expression of hemichannels [10–14]. Conversely, cells from connexin knockout animals do not show these properties, even though wild-type cells do [12,14]. (However, connexin transfection can induce changes in the expression of many other genes [15], and connexins have binding partners and might transport other channel molecules to the cell surface [16–19].) The permeability and/or currents are inhibited by gap-junction blockers [10–14] (although most of the blockers are of dubious specificity). The pathway is permeable to larger molecules than is the ‘normal’ surface membrane, as are gap-junction channels [10–14] (but there are $P2X_7$ receptors and mechanosensitive channels that let through larger molecules, including several of the gap-junction-permeant species [20,21]). The volume of this evidence is substantial, but it is still possible to doubt the existence of hemichannels.

Data from single channels resolves the debate. Cells transfected with connexins exhibit channels that are about twice the conductance of the cell–cell channels, as predicted from simple series arrangement of the two hemichannels [22] (Figure 1; Table 1). The channels are absent in the parental cells. The altered gene expression and transport-related explanations of putative hemichannel currents are made much less likely, because the single hemichannel conductance is consistent with the cell–cell channel conductance. The channels exhibit a substate identical to that induced in cell–cell channels by transjunctional voltage, V_j. (Although, as will be discussed, the substate conductances in hemichannels and cell–cell channels are inconsistent; the deviation is similar for five different connexins.) The hemichannels have fastgating transitions between the fully open state and the substate, and slow-gating transitions between the closed state and either the fully open state or substate, as is observed with cell–cell channels [22]. The hemichannels exhibit pharmacology like that of the cell–cell channels. The differences between hemichannels and cell–cell channels will be discussed in a later section, but the inescapable conclusion from the electrophysiological data is that hemichannels can open (at least, in the opinion of an electrophysiologist).

Where are hemichannels formed, and are they precursors of cell–cell channels?

Early studies of connexin biogenesis and trafficking indicated that Cx43 monomers were synthesized in the endoplasmic reticulum (ER), assembled into hemichannels in a post-Golgi compartment and transported to the cell surface before they became incorporated into junctional plaques [23]. Recent data indicate that Cx32 oligomerizes in the ER before reaching the *trans*-Golgi [24]. (No connexins are known to be glycosylated, and there are no

consensus sequences for glycosylation in the extracellular domains of the connexins that have been examined in this regard; thus, one Golgi function is not required for connexin assembly [25,26].) Cx26 can be inserted into membranes post-translationally as well as co-translationally, and might never be found in the ER [27,28]. Brefeldin A, which disrupts the Golgi apparatus, prevents the trafficking of Cx32 and Cx43; however, Cx26 still reaches the cell surface.

Now, refined methods of labeling indicate that new channels are transported to the surface in an undirected manner and reach existing junctions by lateral diffusion, where they form new channels at their periphery [29,30]; channels being retired are removed from the interior of the junction [29]. This picture does not address the question of the initial formation of junctions [7]. Remarkably, gap junctions are internalized, piecemeal or in their entirety, with both junctional faces entering one of the cells. This mode of turnover requires the rupture and resealing of two membranes with no significant loss of cytoplasm. Moreover, a small amount of cytoplasm is transferred from one cell to the other (although this is membrane-bounded and presumably destroyed in lysosomes together with the junctions). The life cycle of hemichannels therefore involves a period when they are non-junctional and not opposed by another hemichannel.

What opens hemichannels?

Fibroblasts *in vitro* appear to have a few hemichannels open under basal conditions [31]. HeLa cells transfected with Cx43 show basal uptake of gap-junction-permeant molecules that is sensitive to pharmacological agents that block gap junctions [22]. However, calculations indicate that the observed uptake requires very little channel opening – a single channel per cell with a very low open probability would be sufficient. One can argue from these data that a cell can tolerate some hemichannel opening, but not very much. In several tissues, depolarization or polarization to positive potentials, particularly in low Ca^{2+} solutions, increases hemichannel opening measured by single-channel recording or tracer uptake. Low Ca^{2+} levels alone appear to cause hemichannel opening in astrocytes. One study indicates that significant opening can occur in astrocytes at low but not unreasonable external Ca^{2+} concentrations [32], whereas another study sees little effect at nominally zero Ca^{2+} [14]; the differences in these studies might arise from differences in culture conditions. Metabolic inhibition and ischemia can increase hemichannel opening. In cultured astrocytes, hemichannel opening induced by metabolic inhibition (measured by dye uptake) is reduced by nor-dihydroguaiaretic acid (NDGA), an inhibitor of lipoxygenase, suggesting control by a metabolite of arachidonic acid [14]. Although Cx43 is largely dephosphorylated by the time metabolic inhibition is inducing hemichannel opening, cell–cell coupling is not abolished, and dephosphorylation might not play a role in hemichannel opening. Protein kinase C (PKC)-mediated phosphorylation reduces hemichannel permeability to NAD^+ [33]. Cx43 hemichannels are opened by alendronate, a bisphosphonate that inhibits dexamethasoneinduced apoptosis and is used to promote survival of osteoblasts in the prevention of osteoporosis [12]. (Opening was detected by dye uptake, which was prevented by gap-junction blockers.)

Hemichannels in teleost and elasmobranch horizontal cells are opened by quinine and by depolarization in low-Ca^{2+} solution; dopamine-receptor and cAMP agonists reduce hemichannel and cell–cell channel conductance in these systems [34–38]. The connexins in these cases are paralogs of the (largely) neuron-specific mammalian connexin Cx36. Hemichannels in horizontal cells of carp are implicated in an electrical feedback mechanism that acts on release of glutamate from the receptor cell [39]. The putative connexin is a paralog of mammalian Cx26, which is not known to form functional hemichannels and is

not expressed in mammalian horizontal cells [40]. If the same mechanism is present in mammalian retina, it is likely to involve a different connexin.

A somewhat controversial gating mechanism is mechanical stimulation [13,41]: increased connexin expression and low Ca^{2+} solution increase sensitivity but the specificity of this is not well established.

Are cell–cell channels just two hemichannels in series?

For connexins that form functional hemichannels, one can ask how the hemichannel conductance relates to the cell–cell channel conductance. In the five connexins for which such data are available, the open hemichannel conductance is close to twice the open cell–cell channel conductance, as would follow from simple series connection [4,22,42–47] (Table 1, Figure 1). The same conclusion has been reached for several connexins that form heterotypic junctions: the heterotypic channel conductance was the series sum of the inferred hemichannel conductances (twice the single channel conductance of the homotypic junctions [47–49]). Moreover, in Cx43–Cx45 junctions, the single channel conductance was as predicted, but the sensitivity of the two hemichannels to V_j was also altered, consistent with a greater or lesser fraction of V_j falling across the lower-conductance and higher-conductance hemichannels, respectively [47].

It is typical of gap-junction channels to have a main or fully open state and a substate with a conductance of 10–30% of the main state; hemichannels exhibit similar main states and substates. In cell–cell channels, transitions between these states are induced by application of different magnitudes of V_j. Transitions between the fully open state and substate are rapid (<1 ms) and unresolved. However, the rate constants tend to be long (tenths of seconds) compared with those of excitable membrane channels. Voltage sensitivities depend greatly on the connexin, and the most sensitive are comparable in this parameter with the voltage-sensitive channels of excitable membranes [2]. The substates of cell–cell channels and hemichannels generally differ consistently from the prediction of simple series arrangement in cell–cell channels (Table 1). When the substate conductance of the cell–cell channel is calculated as the series sum of the open-state and substate conductances of the hemichannel, the result is too large by a factor of about two, although one published value for Cx46 does match the prediction [44]. Note that one side of a cell–cell channel is closed to the substate by one polarity of V_j, which tends to open the V_j gate on the other side; however, closing of both V_j gates at the same time must be a very rare event. (Calculating in the opposite direction, from the substate conductance of the cell– cell channel, which is a hemichannel in the substate in series with a hemichannel in the open state, generally produces a hemichannel substate conductance that is too small by a factor of about two.)

How can we explain these data? Not without handwaving. Most simply, the extracellular region of the hemichannel when it is at the substate conductance could have a very different configuration to that of the same region in the cell–cell channel. V_j or fast gating to the substate in cell–cell channels is thought to involve the cytoplasmic end of the channel, and charges implicated in the voltage sensor are located there; however, other charged residues affecting gating are found at the beginning of the first extracellular loop [45,50]. Although not yet adequately characterized, there are differences in singlechannel rectification in hemichannels. An open hemichannel could well exhibit asymmetry in fixed charges that causes rectification, whereas the asymmetry is neutralized in the cell–cell channel [45,51].

There are, at most, only small differences in permeability between hemichannels and cell–cell channels. Part of the evidence for opening of hemichannels is permeation of the surface membrane by relatively large molecules to which gap junctions, but not the surface membranes, are permeable. In quantifying permeability, one needs to know the number of

open channels, and (for cell–cell channels) junctional conductance, which is essentially a measure of K^+ permeability and allows comparison between cells expressing the same connexin. As a first approximation, permeability normalized to conductance should be the same for cell–cell channels and hemichannels – the hemichannel has twice the conductance and should have twice the permeability (in so far as series hemichannels make up cell–cell channels without change in properties).

Hemichannel open probability is small

Although there is no direct measure of the number of hemichannels inserted in the membrane using connexins labeled with enhanced green-fluorescent protein (eGFP) [22], reaction with antibodies to the extracellular loop indicates substantial and readily visualized accessibility of extracellular epitopes of Cx43 hemichannels in astrocytes [52]. Thus, the observations that only a few hemichannels open in Cx43-expressing cells indicate that open probability, P_o, is very low. This conclusion holds, even in comparison with the perhaps surprisingly small fraction of gap-junction channels that are open: ~0.1 for Cx43 gap junctions [53]. Moreover, opening of cell–cell channels is not observed in plaques containing up to several hundred channels (i.e. $P_o = 0$). This result suggests that cooperative interactions are necessary for cell–cell channel opening. A requirement for cooperativity could also explain the low open probability of hemichannels: a threshold concentration of hemichannels might be necessary for opening, which would be reached only infrequently in the random diffusion of hemichannels in the surface membrane [54]. The single-channel recordings suggest that when a hemichannel is opening, it is not that different from cell–cell channels in terms of open time and stability in the membrane.

An extracellular signal released through hemichannels

Cyclic ADP-ribose (cADPR) is a signaling molecule that causes release of Ca^{2+} from intracellular stores. Surprisingly, it is synthesized by the ectoenzyme CD38, an integral membrane protein with an extracellular active site that cyclizes NAD^+ to form cADPR (Figure 2). cADPR, which does not permeate Cx43 hemichannels [31,33], reaches its site of action on ryanodine receptors by both active and passive transport across the surface membrane [55]. It can also have a paracrine action, which might be why an ectoenzyme is used. The precursor NAD^+ is released from the cytoplasm into the medium through Cx43 hemichannels, as indicated by: (i) dependence on level of Cx43 expression in transfected or antisensetreated cells; (ii) sensitivity to gap-junction blockers; and (iii) influx into and efflux from liposomes incorporating isolated Cx43. cADPR is also synthesized in intracellular vesicles transporting CD38 to or from the surface; NAD^+ enters the vesicles through Cx43 hemichannels and cADPR moves to the cytoplasm by action of the same nucleotide transporters that operate in the surface membrane [33,55]. This pathway of synthesis would prevent loss of NAD^+ and cADPR by diffusion away from an ectoenzyme, but also prevent paracrine action. A negative-feedback loop exists in which rises in intracellular Ca^{2+} concentration activate PKC, which phosphorylates Cx43, thereby reducing permeability of or blocking hemichannels. A proposed mechanism is that access to substrate for vesicular or extracellular CD38 is regulated negatively by intracellular Ca^{2+}, thus preventing excessive loss of NAD^+, rise of cADPR, emptying of intracellular Ca^{2+} stores and possible cytotoxicity.

CD38 is expressed by astrocytes, which in culture respond to extracellular cADPR with elevations in Ca^{2+} levels [56]. Rises in intracellular Ca^{2+} concentration in astrocytes can cause glutamate release and rises in Ca^{2+} levels in nearby neurons. The rises in neurons are not completely blocked by glutamate-receptor antagonists, indicating the presence of other

mediators, which could include cADPR. This pathway of glial–neuronal signaling might be important in the normal operation of the nervous system [57].

What about Ca^{2+} waves?

Rises in intracellular Ca^{2+} concentration that spread in an epithelium [58] or a monolayer of cells in culture are termed Ca^{2+} waves. In general, the waves proceed in all directions from a stimulated cell and are limited in extent. It is arguable that they spread passively without regeneration (although, as will be discussed, this point is controversial and probably not always true). Ca^{2+} waves are observed in many cell types, including in cultured astrocytes [59–60], in white matter slices, where mediation is glial [61], and in gray matter, where neurons are involved and mediation might be more complex [62–64].

In the initial descriptions of Ca^{2+} waves, gap junctions were implicated in spread of inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] generated in a stimulated cell to neighboring cells, where it caused release of Ca^{2+} from intracellular stores [58] (Figure 3a). (Detection of Ins(1,4,5)P_3 would be non-linear because of Ca^{2+}-evoked Ca^{2+} release.) This explanation of Ca^{2+} waves was challenged by observations that Ca^{2+} waves could cross cell-free lanes and thus were mediated by an extracellular signal [60,65] (Figure 3b). Unfortunately, gap-junction blockers are ambiguous in distinguishing the two pathways, as hemichannels might be the source of extracellular signals (of which ATP is certainly one), and coupling between cells might contribute to the extracellular signal by allowing diffusion of the signal from neighboring cells into the releasing cell. After doubts were raised about gap-junction mediation, the situation was clarified by experiments showing that both mechanisms of wave propagation could be seen in the same cells, in this case HeLa cells transfected (or not) with eGFP-labeled connexins [66]. Where connexin hemichannels mediate ATP release, dependence of waves on connexin expression holds for both mechanisms. Waves mediated by an extracellular signal could be blocked by apyrase (which hydrolyzes ATP) and purine-receptor antagonists (e.g. suramin), and altered in extent by perfusion of medium across a monolayer [65] (Figure 3). Waves mediated by an intracellular signal required connexin expression and were not blocked by extracellular apyrase or purine-receptor antagonists. Moreover, the somewhat problematic initiation by mechanical stimulation was replaced by photorelease of Ins(1,4,5)P_3, which is repeatable and non-damaging [66]. Rises in Ca^{2+} levels were seen to spread through a cell and then to initiate rises in an adjoining cell at an eGFP-tagged gap junction localized by its fluorescence. During extracellularly mediated waves, Ca^{2+} levels first rose at the ER owing to the action of an intracellular second messenger activated by extracellular ATP. To make the parallel system a little more complicated, intracellular Ins(1,4,5)P_3 can induce release of ATP by a mechanism inhibited by a bath-applied peptide with the sequence of an extracellular region of Cx43, an agent likely to be a highly specific blocker of hemichannels [67]. These data suggest that ATP can induce ATP release, which would confer regenerative properties on Ca^{2+} waves. The extent of Ca^{2+} propagation in slices of corpus callosum is also indicative of regenerative propagation [61]. The possibility of regenerative release of ATP with the prospect of indefinitely propagating waves was raised early in the investigation of the extracellular pathway [68]. However, it was contraindicated by Arcuino *et al.* [60], who saw no difference in extent between a finite wave propagating across a cell-free area and one propagating across confluent cells. Thus, the presence of active propagation depends on cell type and specific conditions.

Connexin expression and ATP-mediated Ca^{2+} wave propagation have been correlated in a transformed astrocyte line [13,59]. However, connexin transfection markedly increased basal conductance of isolated cells to a degree that would require opening of many hemichannels (~50); yet the cells were healthy and not depolarized. A subsequent study

showed ATP release (visualized by luciferase-mediated light emission) from single cells within a confluent monolayer in zero-Ca^{2+} solution [60]. ATP release was prevented by gap-junction blockers and the release 'channel' was permeable to molecules that would permeate gap junctions. However, the current when the release channel opened was not appropriate for hemichannels; it was too big for one hemichannel and did not appear to consist of multiple openings of Cx43 hemichannels. The data might result from cooperative opening of several hemichannels at the same time and from the rather unpredictable channel behavior sometimes observed with hemichannels under conditions of slow gating [22].

Hemichannels in the CNS

Astrocytes contain millimolar levels of free glutamate in the cytoplasm; they also express hemichannels. Under some culture conditions, low extracellular Ca^{2+} levels cause release of glutamate from astrocytes, evidently by opening of hemichannels (as evidenced by sensitivity to gap-junction blockers and dye uptake [32]). What is striking about these observations is that significant release was observed at Ca^{2+} levels that might well occur under conditions of ischemia, seizures or spreading depression. It will be of interest to evaluate Cx43-deficient astrocytes for glutamate release, but the other obvious suspects, reverse operation of the uptake system and $P2X_7$ receptors, have been excluded pharmacologically. A low-Ca^{2+} solution was shown to induce release of glutamate from optic nerve (which contains astrocytes and axons but no neuronal cell bodies or synaptic terminals). Extending these observations to gray matter is likely to be difficult given all the other possible sources of glutamate.

In astrocytes in culture, lanthanum ions (La^{3+}) applied extracellularly block hemichannels but not gap junctions [14]. Metabolic inhibition causes opening of hemichannels, and La^{3+} application delays cell death caused by metabolic inhibition; thus, hemichannel opening is deleterious under these conditions. Gap-junctional communication between healthy and unhealthy cells can increase survival of unhealthy cells or death of healthy cells depending on the relative numbers of cells and degree of coupling between the two groups. When the balance of unhealthy to healthy cells is unfavorable for cell survival, gap-junction blockers reduce cell death [69]; where gap junctions are bad, hemichannels are likely to only make things worse.

Concluding remarks

There can be little doubt that opening of gap-junction hemichannels is part of the cell repertoire. Hemichannels do provide a 'leaky' pathway, and quantification of permeation and electrical conductance needs to be extended. The predominant connexin in astrocytes is Cx43, but other connexins are expressed in astrocytes and neurons. Where hemichannels are carrying out physiological signaling, their value is clear. As part of a signaling pathway, they fail to discriminate between transmitter and 'postsynaptic' target when compared with the classical chemical synapses. When hemichannels open under conditions of metabolic inhibition or low Ca^{2+} levels, there is less reason to think that the response is a useful one, although limited cell death is not always bad for the organism. Untangling the many different forms of chemical communication in the CNS to identify those mediated by hemichannels will not be simple (Questions for Future Research); additional pharmacological and genetic tools will probably be required.

Acknowledgments

Our research is partially funded by grants from the National Institute for Health (NS45837 to M.V.L.B. and NS36706 to F.F.B.), from the F.M. Kirby Foundation Program in Neuroprotection and Repair, and from Fondo

Nacional para el Desarrollo de Ciencia y Tecnología (FONDECYT 1030945 to J.C.S.). M.V.L.B. is the Sylvie and Robert S. Olnick Professor of Neuroscience.

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Box 1. Gap junctions – an overview

- Gap junctions provide high-conductance, high-permeability pathways between cells, and mediate electrical coupling and exchange of small molecules.
- Gap-junction channels comprise two hemichannels or connexons, one contributed by each of the participating cells.
- A hemichannel is generally closed before docking with another hemichannel; both hemichannels then open in a ligand-gating reaction where the ligand and gate are the same molecules.
- Recent data indicate that unapposed, non-junctional hemichannels can open under some conditions.

Questions for Future Research

- What stimuli cause hemichannel opening?
- Why is the open probability of hemichannels so low?
- Do open hemichannels account for movement across non-junctional surface membranes of gap-junction-permeant species?
- Do all connexins form hemichannels that can open?
- How important is hemichannel opening in cell physiology?

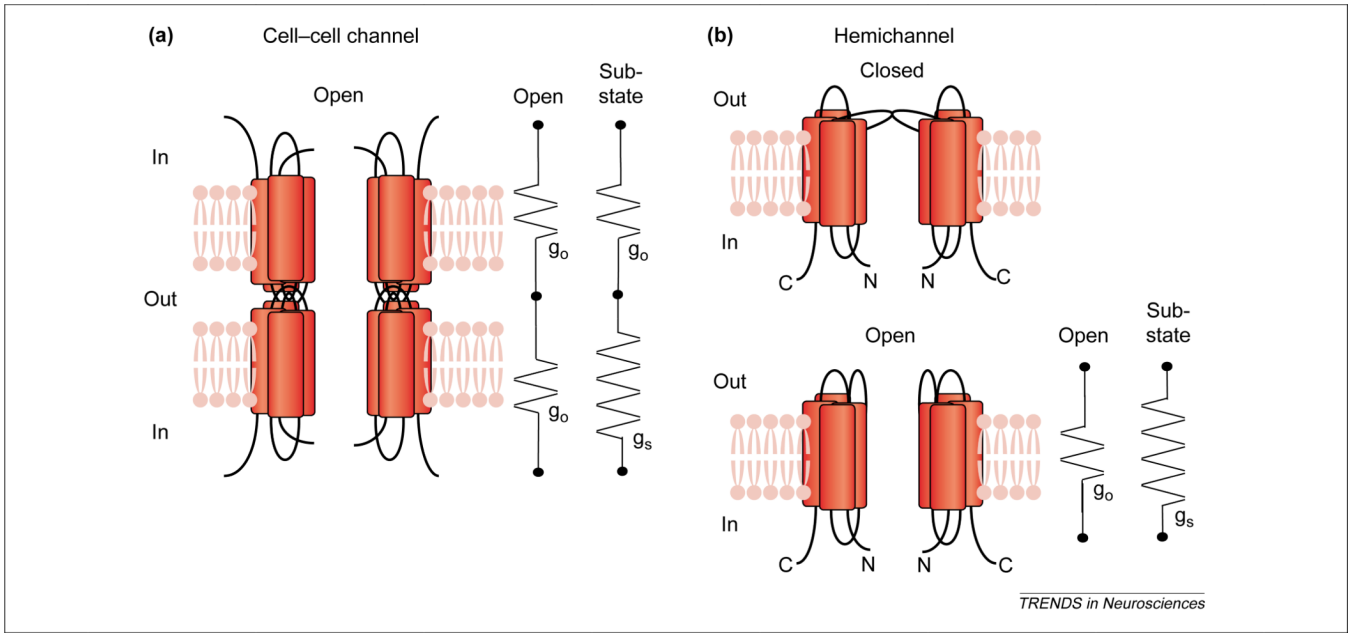

Figure 1.

Diagrams and equivalent circuits of cell–cell channels and hemichannels. (a) In the cell–cell channel, the conductances of the two hemichannels are in series; the circuits show both gates open at the fully open or main-state conductance, g_o (left) and one gate open at g_o and the other closed to the substate conductance, g_s (right). (b) For the hemichannel, a single element represents each state: an open-state conductance (g_o) and a substate conductance (g_s). Table 1 shows that the cell–cell open-state conductance is about half that of the hemichannel open state, consistent with series arrangement in the cell–cell channel. By contrast, the substate conductance of the hemichannel is larger than predicted from the cell–cell channel (and the substate conductance of the cell–cell channel is smaller than predicted from the hemichannel, except in Ref. [44]).

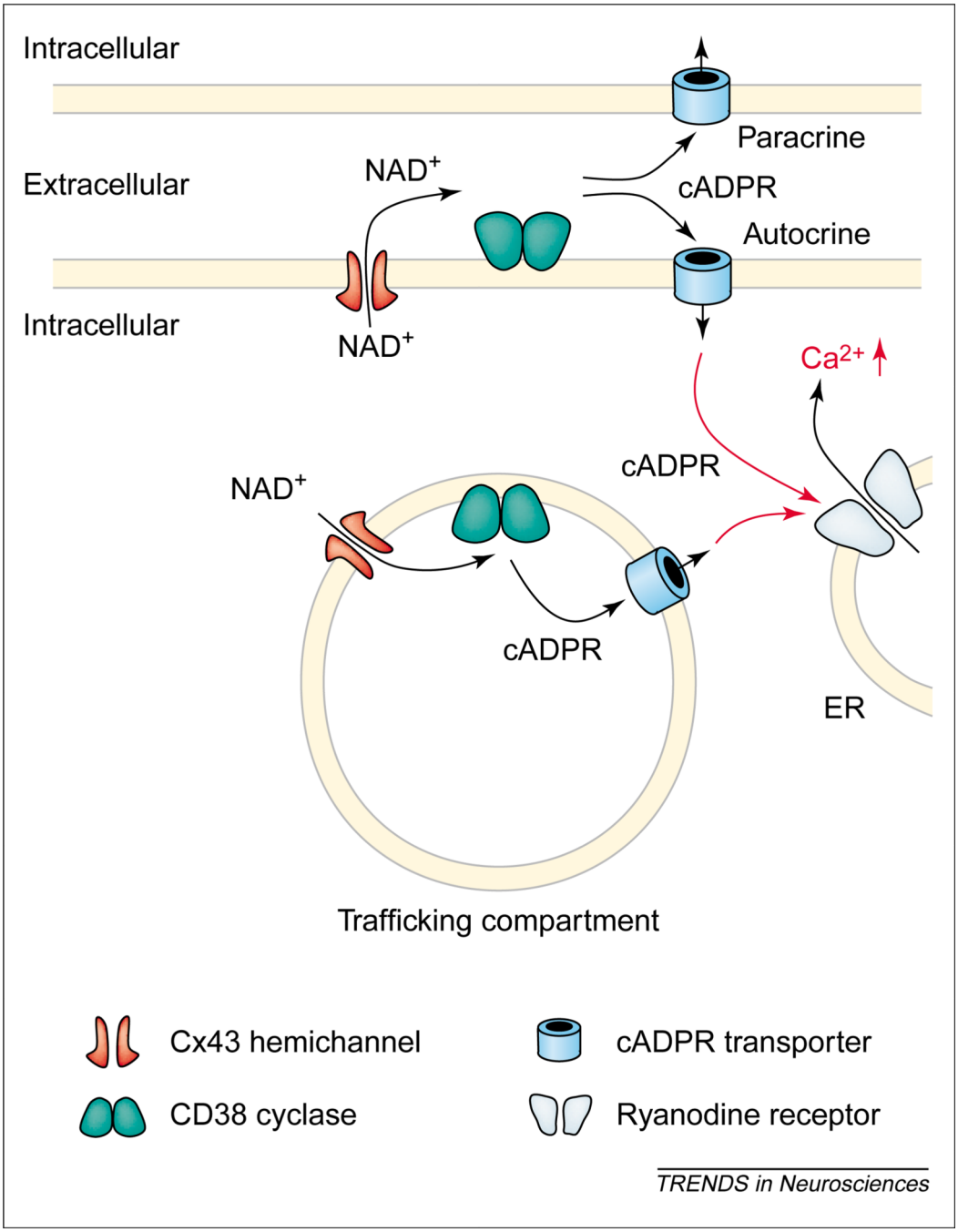

Figure 2.

Connexin 43 (Cx43) hemichannels mediate cyclic-ADP-ribose (cADPR) signaling by allowing outward passage of NAD^+. Cells, including astrocytes, express CD38, an ectoenzyme that cyclizes NAD^+ to form cADPR. cADPR then has to cross the surface membrane to reach ryanodine receptors on the endoplasmic reticulum (ER) to trigger release of Ca^{2+} into the cytoplasm; this action can be autocrine or paracrine. A pathway using intracellular vesicles can largely restrict action to the single cell.

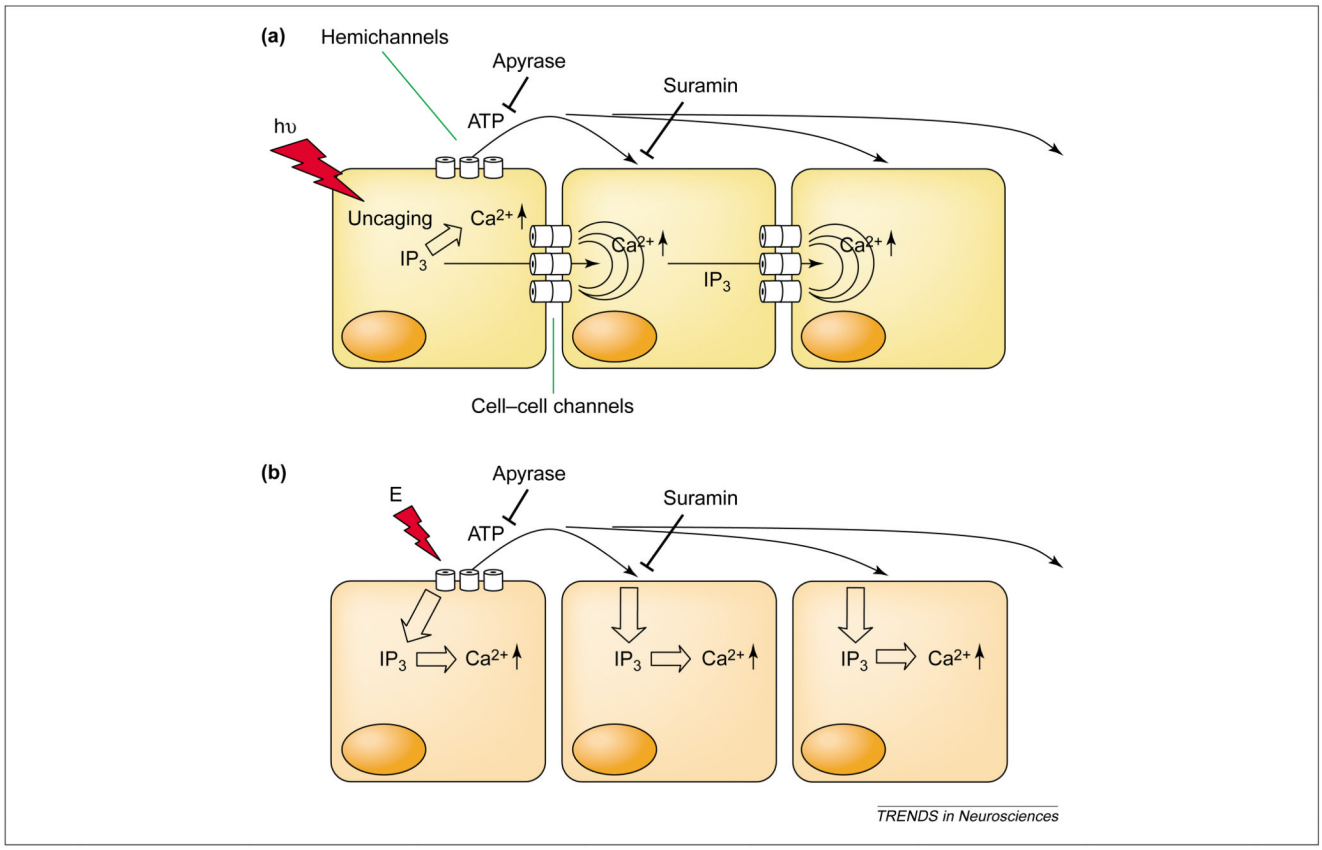

Figure 3.

Two models for conduction of Ca^{2+} waves. (a) Ca^{2+} waves are mediated by diffusion of cytoplasmic inositol (1,4,5)-trisphosphate (IP_3) through gap junctions between cells. Evidence for this mechanism includes: (i) the waves are dependent on gap junctions, and Ca^{2+} increases in a downstream cell begin at junctions; (ii) the waves are not blocked by extracellular apyrase, an ATPase; (iii) the waves are not blocked by purine-receptor antagonists such as suramin; and (iv) the waves do not jump a gap between cells. The red lightning bolt represents a photo-uncaging stimulus (hν). (b) Ca^{2+} waves are mediated by ATP released through hemichannels. Evidence for this mechanism includes: (i) the waves require connexin expression; (ii) gap junction and hemichannel blockers prevent the waves; (iii) ATP is released by the initiator cell, and the Ca^{2+} wave extends as far as the ATP diffuses; (iv) the waves are blocked by extracellular apyrase; (v) the waves are blocked by suramin; and (vi) the waves jump cell-free gaps and are deflected by flow of medium. The red lightning bolt represents an electrical stimulus (E) that causes release of ATP by unclear and perhaps nonspecific mechanisms.

Table 1

Comparison of main and substate conductances of cell–cell channels and hemichannels (pS)[a]

Connexin	Cell–cell: open	Substate observed (calculated)	Refs	Hemichannel: open	Substate observed (calculated)	Refs
Cx30	146	25 (41)	[43]	283	48 (30)	[44]
Cx43	115	30 (56)	[4]	220	75 (40)	[22]
Cx45	30	4 (12)	[47]	62	15 (4.6)	[46]
Cx46	150	28 (75)	[45]	300	100 (33)	[45]
		(32)		250	37 (34)	[44]
Cx50	203	33 (60)	[44]	357	77 (39)	[44]

[a]Substate conductances usually do not follow from simple series arrangement of hemichannels in cell–cell channels (Figure 1) (access resistance is neglected).