Critical Review

Carbon Monoxide: A New Player in the Redox Regulation of Connexin Hemichannels

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Abstract

Carbon monoxide (CO) is a gaseous transmitter that is known to be involved in several physiological processes, but surprisingly it is also becoming a promising molecule to treat several pathologies including stroke and cancer. CO can cross the plasma membrane and activate guanylate cyclase, increasing the cGMP concentration and activating some kinases, including PKG. The other mechanism of action involves induction of protein carbonylation. CO is known to directly and indirectly modulate the function of ion channels at the plasma membrane, which in turn have important repercussions in the cellular behavior. One group of these channels is hemichannels, which are formed by proteins known as connexins (Cxs). Hemichannel allows not only the flow of ions through their pore but also the release of molecules such as ATP and glutamate. Therefore, their modulation not only impacts cellular function but also cellular communication, having the capability to affect tissular behavior. Here, we review the most recent results regarding the effect of CO on Cx hemichannels and their possible repercussions on pathologies. © 2015 IUBMB Life, 67(6):428–437, 2015

Keywords: *connexins; hemichannels; redox potential; gap junction channels; post-translational modification; gaseous transmitters*

Introduction

Connexins (Cxs) are a family of proteins that share a common plasma membrane topology: four transmembrane domains, two extracellular loops, one intracellular loop, and both the C- and N-termini located on the cytoplasmic side (Fig. 1A). At least 20 isoforms have been described in mammals (1), which are named according to their predicted molecular weight (*i.e.*, Cx46 is predicted to have a MW of 46 kDa). Cx isoforms exhibit considerable homology; however, the C-terminus is the most variable region, which in addition varies in length between isoforms. Thus, Cx23 presents a very short C-terminus when compared with Cx62, which has the longest one. Moreover, the C-terminus contains a number of regulatory sites, including consensus phosphorylation (2–5), oxidation (6–9), protein–protein interaction (10–12), and cleavage sites (13,14). Similar to the C-terminus of Cx channel, some post-translational modifications have been reported in the N-terminus and the intracellular loop (15). These modifications include ubiquitination (16), SUMOylation (17), acetylation (18), and hydroxylation (19). It is worth mentioning that the N-terminus is projected into the channel pore, which means that it forms part of the channel pore (only probed for Cx26; ref.

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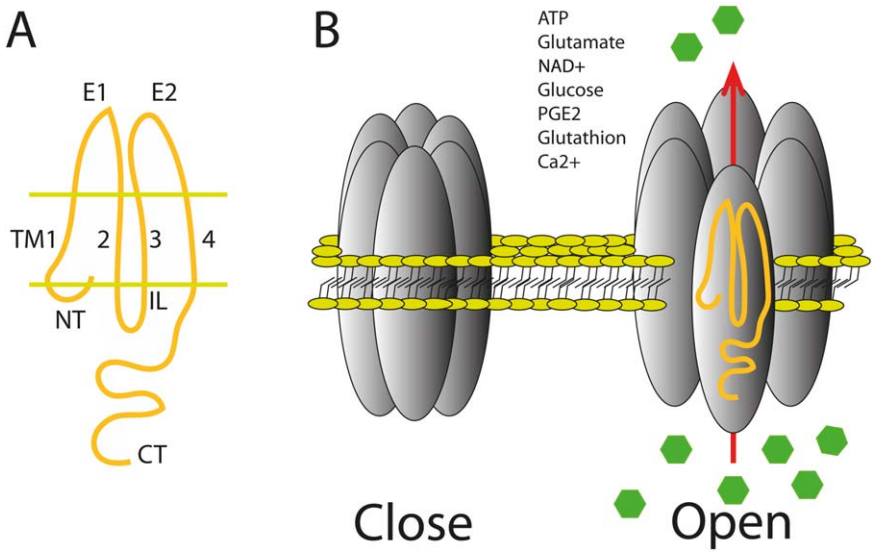

FIG 1

Connexins and hemichannels. A: Connexins (Cxs) are transmembrane proteins formed by two extracellular loops (E1 and E2), four transmembrane domains (TM1–4), and one intracellular loop (IL), and both the N- and C-terminals are located at the intracellular phase of the plasma membrane. The N-terminus can be located in the channel pore, forming a part of it. B: The oligomerization of six Cxs forms a hemichannel. Under normal conditions, hemichannels are mostly closed, but when open, they became a pathway for the interchange of molecules (green hexagons) between intracellular and extracellular space. The direction of the flow through the pore is driven by the electrochemical gradient of a given molecule. Thus, the release of molecules such as ATP, glutamate, NAD+, PGE2, and glutathione has been observed, and also the uptake of glucose and Ca^{2+}.

20). Hence, the N-terminal is a core element in the permeability and voltage dependency of Cx base channels (21). Therefore, any post-translational modification to this segment is supposed to have profound repercussions on channel permeability, conductance, and open probability.

Almost all Cxs (except for Cx23; ref. 22) have six conserved extracellular cysteines (Cys), which have been proposed to form intramolecular disulfide bonds that are essential for hemichannel docking and the formation of gap junction channels (GJCs; ref. 23). However, it has been recently suggested that, at least in Cx46, some of these extracellular Cys can be in the form of —SH, being part of the hemichannel redox sensor (24). Finally, it is important to note that in mammals, almost all cell types express one or more Cxs and that there are major differences between tissue expressions of Cx isoforms. For example, Cx43 is the most ubiquitously expressed (25–27), whereas Cx46 has been described in the lens (28) and lungs (29). The wide expression of Cxs suggests that they are important in several physiological processes and, because of their unique properties, they can support cellular processes that cannot be replaced by any other Cx type (30).

GAP Junction Channels and Hemichannels

Gap Junction Channels

Gap junction channels are formed by the docking of two hemichannels at the so-called junctional membrane of adjacent cells. GJCs mediate passive fluxes of ions and other solutes between adjacent cells both *in vivo* and *in vitro*. It is well accepted that channels formed by Cxs have pores that allow the passage of molecules up to 1.2 kDa because of their large diameters (about 14 Å; ref. 20). However, experimental data strongly suggest that GJCs filter molecules not only based on their size but also their charge and shape, involving specific interactions within the pore wall (31). Thus, neighboring cells can share molecules such as ATP, ADP, glucose, glutathione, glutamate, and second messengers such as cAMP, IP_3, and Ca^{2+} (32–37). Nevertheless, the particular solute selectivity of GJCs will depend on the Cx isoforms expressed in a given tissue. This is so because Cxs can form GJCs (and also hemichannels) formed by more than one type of Cx (heterotypic/heteromeric; for more details see ref. 38). Unfortunately, permeability and general characteristics of the so-called heterotypic/heteromeric GJCs have been less explored because of technical difficulties. However, it seems very relevant to overcome this lack of knowledge because *in vivo* heterotypic/heteromeric GJC-mediated communication could be present at a higher proportion than that of GJC formed by only homomeric/homotypic channels.

The mechanisms for GJC opening and closing (gating) have been studied in detail and have been recently reviewed by Rackauskas et al. (27). Briefly, GJCs are controlled by "transjunctional voltages" which are voltages between the membranes of two adjacent cells. Thus, two voltage-gating mechanisms have been described: the "fast gating" which is located at the cytoplasmic side of the channels, and the "loop gating" located at the extracellular face (39). It has been

demonstrated in hemichannels that loop-gating voltage dependence is strongly regulated by extracellular divalent cations (40) and involves the movement of a region composed of a segment of transmembrane 1 (M1) and the first extracellular loop (E1), narrowing the pore lining (41). Until now, it is unknown if these molecular movements are also present in the GJCs. Another control mechanism for GJCs is through phosphorylation. GJCs present multiple sites for phosphorylation with several kinases, including PKC, PKA, PKG, MAPk p38, and Src (5,42). Phosphorylation of one or more sites (including serine, threonine, or tyrosine residues) is known to affect unitary conductance (43,44), GJC turnover (5), GJC assembly (45), and large-solute permeability (46,47). Thus, depending on the cell status, Cx phosphorylation can modulate GJC properties, which in turn will affect cellular and tissue functions. Additionally, other molecular mechanisms have been studied, such as intracellular acidification (48) and intracellular free Ca^{2+} (48–50). The effect of Ca^{2+} seems to be mainly mediated by a calmodulin-dependent mechanism, in which direct interaction of calmodulin with both the N- and C-termini decreases GJC activity (49,51). However, whether calmodulin affects or not the GJC open probability or permeability is still unknown (51).

Hemichannels

Those are composed of six Cxs monomers (Fig. 1B). They are assembled in the endoplasmic reticulum, Golgi apparatus, or post-Golgi vesicles and then transported to plasma membrane (52,53). The presence of undocked hemichannels at the plasma membrane has been demonstrated in several cell types, using different techniques including cryomicroscopy (54), biochemical (6,55), electrophysiological (56–58), optical (59), and functional approaches (*i.e.*, dye uptake or ATP release; ref. 60). Because of the strong evidence, the presence of hemichannels at the plasma membrane is not a matter of controversy nowadays. However, their role in physiological or pathophysiological processes is under thorough study.

Because hemichannels are permeable to molecules up to 1.2 kDa, it was believed for several years that they had to be closed to prevent cell damage and death. Accordingly, if hemichannels were to remain open, molecules such as ATP, amino acids, and cofactors would be lost, and Ca^{2+} would enter the cells, thus having deleterious effects. However, recent studies show that, in some circumstances, hemichannels can open under physiological conditions without affecting cell viability. Hemichannels partially exert their action by allowing the release of signaling molecules such as ATP (61,62), cyclic ADP ribose (63,64), prostaglandin E2 (PGE_2; ref. 65), glutamate, and aspartate (66). Although some of these studies were carried out under nonphysiological conditions (*i.e.*, in the absence of Ca^{2+} and Mg^{2+}), it is clear that hemichannels may open in solutions with physiological concentrations of Ca^{2+} and Mg^{2+}, as observed in the generation and spreading of calcium waves in several cell types (67–70). In addition, hemichannels appear to be involved in Ca^{2+} permeation across the plasma membrane (71,72), osteoblast viability induced by biphosphonates (73), cell proliferation (74), cell migration (75), light processing by the retina (76,77), mechanotransduction (78), glucose uptake (79), and synaptic plasticity (80). Based on the above findings, the activity of hemichannels has profound consequences on cellular function. Therefore, cells have several mechanisms for hemichannel activity control, which include phosphorylations (81), changes in plasma membrane potential (82–85), alterations in extracellular Ca^{2+} concentration (86,87), and unsaturated fatty acids (88,89). In conclusion, under physiological conditions, Cx hemichannels may be activated by specific signals, and their opening results in the release of paracrine–autocrine molecules and/or the modulation of other important cell functions (*i.e.*, spread of calcium waves).

On the other hand, massive and/or prolonged hemichannel opening has been proposed to induce or accelerate cell death under certain pathological conditions. These include Charcot-Marie-Tooth disease (90), metabolic alterations, such as ischemia (6,60,91,92), oculodentodigital dysplasia (93), hidrotic ectodermal dysplasia (94), other skin diseases (95), inflammatory processes (79,96,97), cadmium-induced oxidative cellular stress (98), and deafness (99,100). Although the exact mechanism by which hemichannels induce cell death is unknown, it is highly probable that it may be due to a massive loss of important metabolites such as ATP, amino acids, and reduced glutathione (101), loss of transmembrane ion gradients, membrane potential, and the massive entry of Ca^{2+} (71,72). In summary, a considerable body of evidence supports the idea that controlled hemichannel opening allows physiological autocrine/paracrine cell signaling; however, in contrast, massive and/or uncontrolled hemichannel opening induces or accelerates cell death (102). This is why it is so important to study the molecular mechanisms that keep hemichannels closed under normal conditions or induce their massive opening under pathological conditions. Future studies will allow the generation of new tools (*i.e.*, small molecules and iRNA) for the treatment of those diseases in which hemichannels have an important role.

Redox Signaling Controls Hemichannel Properties

As mentioned above, there are several molecular mechanisms that operate to maintain hemichannel activity as low as possible. Thus, under physiological conditions (*i.e.*, 1.2 mM extracellular Ca^{2+}, negative membrane potential, and phosphorylation), Cx hemichannels are expected to present a very low open probability (57,103). Recently, a new hemichannel control mechanism has been found, which operates through changes of redox potential (Fig. 2; ref. 8). One of the first observations of hemichannels being controlled by changes in redox potential was done in primary cultured astrocytes, where Cx43 hemichannels opened when astrocytes were exposed to cytotoxic hypoxia for 75 min (60). Hemichannel opening was prevented by the addition of a free radical

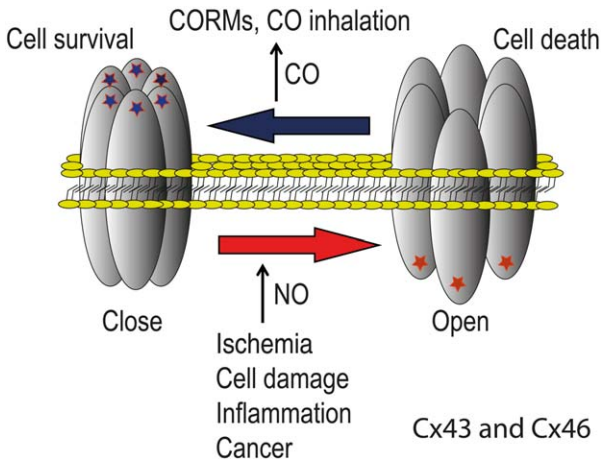

FIG 2

*Cx hemichannels are sensitive to redox signaling. Under normal conditions, Cx hemichannels are mostly closed. When a pathological condition appears (*i.e.*, ischemia, cell damage, inflammation, and cancer), the levels of nitric oxide (NO) are usually elevated. This NO increase could modify the activity of Cx43 and Cx46 hemichannels, inducing the opening of them. It has been suggested that Cx43 and Cx46 have intracellular cysteines that can be modified by NO inducing their S-nitrosylation (red stars). The massive and/or prolonged hemichannel opening have been associated with cell death. If the concentration of carbon monoxide (CO) is increased [*i.e.*, due to CO inhalation or administration of CO-releasing molecules (CORMs)], hemichannel activity can be decreased, preventing cell death. It has been suggested that the CO sensor, at least in Cx46, could be located in the extracellular space of the protein and that the CO could induce the carbonylation of Cx46 (blue stars).*

scavenger, Trolox, to the extracellular media (60). This strongly suggests that hemichannels are somehow sensitive to free radicals. Then, in the same model, it was shown that hemichannels from metabolically inhibited astrocytes become closed in response to dithiothreitol (DTT) and reduced glutathione (6), which indicates that oxidation of free Cys mediates Cx43 hemichannel opening. Additionally, it was observed that a nitric oxide donor (GSNO) induced the opening of Cx43 hemichannels and that both GSNO and metabolic inhibition induced the S-nitrosylation of Cx43 hemichannels (6). This was the first indication that Cxs are modified post-translationally by NO, suggesting that Cx hemichannels are directly sensitive to redox changes. However, whether GJC can also be directly affected by NO in this model remains unanswered. In a physiological model, however, it was demonstrated that Cx43 is S-nitrosylated in Cys271. This modification induces changes in IP_3 permeability between endothelial and smooth muscle cells (9), which strongly suggests that Cx43 GJC can also be modified (at least their permeability) by NO. We want to point out that despite the sensitivity of Cx43 hemichannels to redox changes, the net effect will depend on cellular status (58). This hypothesis is supported by the fact that DTT induces the opening of Cx43 hemichannels when cells are healthy, but induces hemichannel closing when the cells are metabolically inhibited (58). Thus, perhaps there is a cross-talk between the redox and phosphorylation status, and this relationship would lead to the final effect.

Cx43 is not the only Cx sensitive to redox changes. Cx46 hemichannels expressed in *Xenopus laevis* oocytes are also sensitive to nitric oxide (7). In this case, NO induces changes in the kinetics of hemichannel opening and closing, the appearance of a current inactivation at voltages above +40 mV and changes in permeability to large molecules (*i.e.*, ethidium (Etd), MW = 394.3). Contrary to Cx43 hemichannels, Cx46 hemichannel oxidation is not correlated with any obvious changes in hemichannel open probability. Moreover, NO induced slight modifications in hemichannels formed by a Cx46 (Cx46C3A) without intracellular Cys (7). The data above suggest that Cx46 intracellular Cys are responsible for sense changes in NO production. Recently, Cx32, Cx37, and Cx40 have also been proposed to be sensitive to NO, and more interestingly, hemichannels formed by these Cxs are permeable to this gaseous transmitter, hence facilitating crossing through the plasma membrane (104).

Carbon Monoxide and Gaseous Transmitters

There are at least four gaseous transmitters, such as nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S; ref. *105*), and sulfur dioxide (SO_2; ref. 106). Traditionally, CO was cataloged as a toxic molecule because it binds with high affinity to hemoglobin (forming carboxyhemoglobin), decreasing the amount of O_2 that this protein can carry and delivering it to the tissues (107,108). In addition, it also has the ability to bind both to cytochrome oxidase and cytochrome P450, inhibiting cellular respiration (109,110). However, nowadays, there is a growing body of evidence indicating that CO is a physiological molecule involved in a plethora of cellular processes (110). Under physiological conditions, CO is produced by heme oxygenase (HO) enzymes, which catalyze the decomposition of heme groups (111). The physiological importance of CO is supported by the knowledge that HO-I knockout mice die after birth and cell cultures from these animals present high concentration of free radicals (111). Under physiological conditions, CO can act through two possible cellular pathways. First, it can be through the activation of guanylate cyclase, which increases the cGMP levels and the activation of PKG, and second by direct carbonylation of amino acids, such as proline, threonine, lysine, and arginine (112). For many years, protein carbonylation was synonymous to proteins degradation (113). However, recent evidence suggests that there is a natural occurring process of decarbonylation (114). This mechanism involved an unknown thiol-dependent enzymatic process, in which the enzymes thioredoxin (Trx) and glutaredoxin (Grx1) seem to be involved (114) Therefore, the effect of CO on

protein activity can be reversed and controlled by the redox status of a cell. Nevertheless, the exact molecular mechanism of decarbonylation is still unknown.

Carbon Monoxide Modulates Ion Channels

In 1992, it was reported that continuous exposure to high levels of CO induces degeneration of hippocampal CA1 pyramidal cells in a NMDA-dependent process (115). This suggests that CO may partially induce neuronal cell death through a modulation of ion channels activity. Since then, several reports strongly supported the notion that CO acts as an ion channel modulator under physiological as well as in pathological conditions. Thus, it has been reported that CO directly increases the open probability of calcium-activated K (KCa) channels in vascular smooth muscle cells (116) and human umbilical vein endothelial cells (117). The molecular mechanism of this phenomenon is not well understood, but there are several propositions, including enhancement of Ca^{2+} sensitivity (116); dependency on the expression of the KCa-α but not on β-subunits (118); modulation by NO (119), which in the case of KCNMA1 channel would depend on the S9-S10 C-terminal segments (120); and apparently of an aspartic acid 367 as well as two histidine 365 and 394 residues located in the cytoplasmic RCK1 domain (121). Finally, C911 has also been proposed to be located in the vicinity of the "calcium bowl" of the KCa-α subunit and is important for CO activation. It was also suggested that these Cys groups coordinate CO in a manner similar to the transition metal-dependent coordination (122,123). Other potassium channels have also been demonstrated to be affected by CO. CO activates a 70-pS K^+ channel in the thick ascending limb (124) and inhibits Kv2.1 expressed in HEK293 based on a mechanism dependent on mitochondrial ROS production and PKG activation (125), whereas it has a biphasic effect on TREK-1 channels when expressed in HEK293 (126).

Although there is much more data about the molecular mechanism underlying the action of CO action on K^+ channels when compared with other ion channels, the effects of CO are not limited to K^+ channels. The list of other ion channels affected by CO include the following: 1) the amiloride-sensitive channel (127); 2) Nav1.5 channels in which the inhibition observed presents a DTT- and NO-dependent pathway (128); 3) Cav3.2 T-type Ca^{2+} channels, inhibited by an extracellular Trx-dependent mechanism (129); and 4) L-type Ca^{2+} channel inhibition mediated by free radicals produced by the mitochondria (130) and enhancement of ATP-dependent currents in HEK293 cells expressing P2X2 receptors (131).

Carbon Monoxide Modulates Cx Hemichannels

Recently, CO has been presented as a new hemichannel modulator (Fig. 2; ref. 24). León-Paravic et al. showed that a CO donor (CORM-2) induced a dose-response inhibition in Cx46 hemichannels expressed in *Xenopus laevis* oocytes. This inhibition displayed an IC_{50} around 3.4 μM, making Cx46 hemichannels an excellent CO sensor under physiological (>1 μM) and pathological (>10 μM) conditions (132). The CORM-2 effect was fully prevented by the addition of hemoglobin (a CO scavenger) to the bath solution, indicating that the effect of CORM-2 was mediated by the release of CO. As expected, CORM-2 induced the carbonylation of purified Cx46, and this post-translational modification, in turn, induces important rearrangements in protein structure *in vitro*. Interestingly, the *I/V* analyses indicate that the inhibitory effect of CO on Cx46 hemichannels did not involve important changes in the number of gating charges of channel activation and the voltage at which the half of maximal current is reached, suggesting that CO does not modify neither of these hemichannel biophysical parameters. Here, it was also possible to observe that CO does not modify the kinetics of hemichannel closing and opening. These data indicate that CO decreases the number of hemichannels able to open. One possibility is that CO induces hemichannel internalization, which could induce a decrease in hemichannel current. Data suggest that this is not the case as the inhibitory effect of CO was rapidly (less than 1 min) and fully recovered by reducing agents (DTT, β-mercaptoethanol, and reduced glutathione). In addition, no changes were observed in Cx46-GFP distribution in HeLa cells (unpublished data). The fact that reduced glutathione (which cannot permeate the plasma membrane) was able to recover Cx46 hemichannel currents indicates that some extracellular Cys are involved in these processes. This hypothesis was complemented with the fact that hemichannels formed by a Cx46 without intracellular Cys (Cx46C3A) were inhibited in the same proportion as hemichannels formed by the Cx46 wild type. However, hemichannels formed by a Cx46 without extracellular Cys were much less sensitive to CORM-2 when compared with Cx46 wild-type hemichannels. The recovery of hemichannel current by reducing agents supports the hypothesis that there is a redox-sensitive mechanism of decarbonylation (114). However, in the case of Cx46, the Trx component was not important. The study of the molecular mechanism of Cx46 decarbonylation in *Xenopus laevis* oocytes is still pending, as well as studies to find out whether this mechanism is also present in mammalian cells. Hemichannels formed by Cx43 or Cx46 expressed in HeLa cells were inhibited by CORM-2; however, the recovery by reducing agents was not studied. Interestingly, hemichannel activity progressively increased in HeLa cells at concentrations above 10 μM of CORM-2, indicating that the effect of CO on hemichannels formed by Cxs in mammalian cells may be biphasic and could have an inhibitory effect under physiological conditions (concentration close to 1 μM) and induce an activation at higher levels (concentrations higher than 10 μM); however, this hypothesis must be tested. Preliminary results in our laboratory show that CO modifies Ca^{2+} sensitivity of Cx46 hemichannels similarly to what has been observed in KCa channels (116). This evidence is

interesting because extracellular Ca^{2+} modulates the voltage dependence of loop gating (39,40). Therefore, CO could be somehow modulating this so-called slow gating or loop gating (41) through modifications in the mobility of some segments of the extracellular Cxs loops. In summary, CO inhibits Cx hemichannels in *Xenopus laevis* oocytes in a wide range of concentrations (1–100 μM); however, the effect in mammalian cells is more complex, because it presents a dual response depending on CO concentration. The CO effect can be reverted by a reducing agent–dependent process; however, the molecular mechanism is still unknown. The CO effect seems to influence the loop gating of hemichannels; however, much more evidence is needed to test this hypothesis. Finally, experiments to verify if the inhibitory effect observed in HeLa cells is reverted and if CO affects GJC expressed in *Xenopus oocytes* and HeLa cells are needed.

Possible Interplay Between CO and NO in Cx Hemichannel Activity

CO and NO are two gaseous transmitters that activate similar intracellular pathways. Thus, it is well known that both CO and NO stimulate soluble guanylyl cyclase to produce cGMP, which in turn activates PKG (133). Therefore, it is possible to suggest that these two gases can at some point interact and modulate their cellular effect. Accordingly, it has been shown that both CO and NO act as a safety mechanism in renal afferent arteriolar vasoconstriction regulation. Thus, in the presence of NO, CO does not induce evident changes in arterial diameter, but when NO production is inhibited, CO is able to induce vasodilatation (133). In this case, CO and NO are performing similar effects in renal arteries. However, this is not always the case. For example, when iNOS is activated, an increase of NO occurs, which in turn increases cell expression of HO-1 with the consequent production of CO (134). An increase of CO will have a negative effect on iNOS activity, thus decreasing the levels of NO (134). In general terms, an increase of CO concentration has been associated to protective cellular effects (135,136), whereas increases in NO concentration have been associated to deleterious effects (137). According to this, the addition of NO donors to astrocytes in culture induces a massive Cx43 hemichannel opening (6); however, when a CO donor is added to HeLa cells, Cx43 hemichannel became closed (24). However, it is unknown whether astrocytes exposed to CO donors also close their Cx43 hemichannels, and whether the effect of NO and CO over Cx43 hemichannels is synergic or antagonic is also unknown. The final effect of these two gases *in vivo* could also be affected by the intracellular distribution of enzymes that produce NO and CO, thus it is known that HO-1 isoform is located mainly in the endoplasmic reticulum (138) and nucleus (139), whereas HO-2 is mainly located in the endosomes (140). On the other hand, the endothelial nitric oxide synthase (eNOS) is mainly located at the plasma membrane and Golgi apparatus (141), the neuronal type (nNOS) is mainly in the cytoplasm (142), and the inducible form is located mainly in the cytoplasm (143). The differences in localization of enzymes that produce NO and CO will certainly have a differential impact in Cx regulation.

Another type of interaction between NO and CO could occur at the molecular level. NO interacts with Cys groups inducing protein S-nitrosylation (144), whereas CO can induce secondary carbonylation in Cys as well (145). Thus, both can compete for Cys groups and exert their modulation in a competitive way. Obviously, this competition for Cys groups will be affected by the concentration and localization of HO and NOS enzymes. Coimmunoprecipitation and high-resolution confocal studies are needed to understand the interactions of these enzymes and Cx hemichannels and thus understand the overall effect of these two gaseous transmitters in the intercellular communication based on Cxs.

Future Directions

It is known that CO is neuroprotective in cerebral ischemia (146,147); however, the molecular mechanisms are not well understood. Hemichannels are massively open in ischemia/metabolic inhibition conditions as observed in astrocytes (6,60) and neurons (148), and this in turn affects neuronal viability (149). We propose that CO could induce Cx36 and/or Cx43 hemichannel closing and, thus, prevent cell death. Additionally, pannexin channels (Panx), which also form channels at the plasma membrane with similar characteristic as Cx hemichannels (150), are involved in neuronal death in ischemia episodes (151,152). It would be interesting to study if Panx are also affected by CO. Another example of the use of CO as treatment for a pathological condition would be for cancer (153,154). Recently, it has been proposed that Cx hemichannels have a role in cancer progression (155), where these channels could increase the P2X/Y signaling that in turn would affect the intracellular Ca^{2+} concentration (155), which is a powerful signaling in cancer cells (156). Therefore, it is plausible to speculate that CO may affect hemichannels in cancer cells and thus modulate intracellular calcium levels.

There are many other human pathologies where CO is used for their treatment (157), and in which hemichannels could be involved. Thus, the study on the effect of CO on Cx-based hemichannels, GJCs, and Panx will help to understand the underlying molecular mechanism of action involving CO in pathological as well as in physiological conditions.

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