Vascular Gap Junctions in Hypertension

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The proteins that form the gap junctions (connexins) are widely expressed in organs that are central to the development of hypertension: endocrine organs, kidney, brain, heart, and vasculature (Figure 1). Surprisingly, there is little information on the modification of connexins in hypertension in any of these organs except the vasculature, the subject of our review, but it would be hoped that this lack of information might spur research on these organs.

Augmented vasomotor tone typically plays a key role in the development of hypertension, and tone depends on cell–cell communication established by paracrine molecules and, in addition, gap junctions. Paracrine-based linkages between cells of the vasculature are well known, and the possible roles of such linkages in the genesis of hypertension have been extensively explored. Much less is known of the roles of gap junctional linkages in the genesis of hypertension.

Structure and Regulation of Gap Junctions

The gap junctions are formed by joining 2 hexameric assemblies of connexin protein monomers, that is, hemichannels (1 in Figure 2A). In the vasculature, assays of message and protein show that the hemichannels can be assembled from combinations of 4 connexins, named according to their apparent molecular weight: Cx37, Cx40, Cx43, and Cx45. Two hemichannels are linked to form a gap junction, which can provide both homocellular and heterocellular signaling pathways at points of contact among vascular smooth muscle cells (VSMCs), pericytes, and endothelial cells (ECs). The connexin-mediated signaling between cells depends on ≥3 modalities that are highlighted schematically in Figure 2. The modalities are: (1) cytoplasmic continuity, (2) paracrine or autocrine signaling, and (3) intermolecular signaling. The most commonly recognized mode of signaling is via a water-filled pore, the gap junction, which is formed by the association of 2 hemichannels provided by adjacent cells. The gap junctions allow the passage of solutes (<1000 Da) and/or current between 2 cells. Gap junctional permeability can be regulated by small ions, such as Ca++, and H+, phosphorylation, and possibly NO.

Connexin isoforms manifest molecular charge, size, and solute selectivity and the translation of molecular selectivity into function can be inferred from the fact that deletion of one of the connexin isoforms can result in a unique phenotype that cannot be rescued by insertion or “knock in” of another connexin isoform (eg, see References 11–13). Hemichannels forming the gap junctions can be composed of mixtures of proteins that vary according to location within the cell. The mixtures of connexins may have a major impact on junctional permeability. Thus, understanding the molecular basis of connexin function and the potential involvement of connexins in hypertension will ultimately require knowledge of both the proteins present and the ways in which they are assembled. In the vasculature, these data are almost uniformly lacking, especially for the microvessels.

A second mode of connexin-mediated signaling involves independent hemichannels that have the ability to open and release ATP and/or NAD+ that can function as paracrine or autocrine signals (Figure 2A; for review see References 17 and 18). The regulation of hemichannel formation and permeability is a subject of intense debate (eg, see References 19 and 20), but it seems that the hemichannels can be induced or opened by elimination of extracellular calcium or inhibition of the electron transport chain. The hemichannels can be blocked by the peptide gap junctional inhibitors, but as yet there seems to have been no clear demonstration of the importance of this mechanism in vascular cells.

Provocative new evidence indicates that connexin proteins might also participate in cell–cell communication via direct, transcellular, protein–protein interactions (3 in Figure 2B). The C-terminal portion of the connexins is associated with a variety of cytoskeletal proteins and, in conjunction with N-cadherin, is linked directly to a number of cell-signaling cascades (Figure 2B). The importance of these interactions in vasomotor control remains to be explored.

Connexin Proteins and Gap Junctions in the Vasculature

Gap junctions between adjacent ECs are abundant and intimately associated with the tight junctional strands, thus forming a web of interconnected ECs. The purpose of these well-developed junctional complexes involving both the tight and gap junctions remains to be established, but early experiments disclosed that the association is changed in DOCA-salt hypertension. VSMCs, in contrast to ECs, are much less densely...
linked by gap junctions. In fact, at the electron microscopy level, the classical gap junctional plaques are often difficult to show, suggesting that the VSMCs may be linked by very small groups or even single gap junctions rather than by the classical plaques common in ECs.26

Heterocellular gap junctional contact is also common in the vasculature; ECs, VSMCs, renal juxtaglomerular (JG) cells, and pericytes can all be joined by gap junctions.27–32 The point of contact between VSMCs and ECs is called the myoendothelial junction (MEJ), and this tight link is a likely locus for the involvement of the connexins in the pathology of hypertension.33

The relative expression levels of the connexins have been shown to differ with vessel size/location and species. The typical observation is that Cx37, Cx40, and Cx43 are found in the ECs, whereas Cx43 is common in VSMCs, with occasional reports of Cx37 and Cx45 being present in this cell type as well (eg, see References 34–37). There has been no systematic investigation of the distribution of the connexins in microvessels, although Cx43 has been reported to be present in the ECs of larger arterioles in the mouse but absent in the smallest arterial vessels.38 Cx43 is absent from the ECs of rat coronary arteries but present throughout arteries of a similar size in the mesenteric circulation.34,39 Cx37 is not found in bovine and guinea pig aorta, but it is abundantly expressed in rat and mouse aorta.34,40 Remarkably, the literature offers no examination of the presence or absence of connexins in capillaries, veins, or venules; the latter is a major oversight in view of the importance for capillaries in vascular signaling41–43 and of venous vasomotor activity in cardiac filling.

The factors that determine connexin expression in the different vascular segments are largely unknown, although it has been proposed that species differences in expression patterns of vascular connexins can be related to or associated with variations in cardiac function.44 In addition, physical factors, such as the intravascular flow pattern, may have profound effects on connexin expression, especially in the case of Cx43.45–47

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The literature on connexins in the vasculature is confusing and sometimes contradictory, and changes in hypertension seem to depend on experimental model, species, and method of measurement.

Renal Hypertension

In general, the expression of Cx40 and Cx43 increase in renal hypertension. In the rat DOCA-salt, and the 2-kidney, 1-clip Goldblatt (2K1C) models of hypertension, both mRNA and protein for Cx43 are increased in a vessel and tissue-specific manner.48–50 Cx40 also colocalizes with the renal renin-secreting cells, and in the 2K1C model of hypertension, JG cell Cx43 increased only in the unclipped kidney, whereas Cx40 increased in both kidneys,52 suggesting that Cx43 may be responding to the rise in intravascular pressure, whereas Cx40 may be responding to a more generalized stimulus, perhaps circulating angiotensin II (Ang II). Cx43 seems to be sensitive to hemodynamic change, rather than renin, because Cx43 is elevated in the VSMC of the aortae in both high-salt and 2K1C models of hypertension, models that manifest opposite changes in plasma renin.48 In a novel approach to studying the connexins in hypertension, it has been shown that, in mice in which the Cx43 gene is replaced by the Cx32 gene, plasma renin levels are reduced, and 2K1C animals do not become hypertensive. This experiment highlights the importance of Cx43 in the regulation of renin secretion and supports the idea that the individual connexins may have different functions (ie, Cx32 instead of Cx43).13

Hypertension Produced by a Reduction of NO

Hypertension induced by inhibition of NO synthase is associated with a decrease in Cx43 instead of the increase observed in a renovascular model.50–52 Cx37 was also reported to be reduced without any change in Cx40.52 Yeh et al52 found that hypertension-associated expression of ECs Cx43 and Cx37 was largely reversed in N'-nitro-L-arginine methyl ester–induced hypertension by treatment with carvedilol, an adrenergic blocker. Western blot analysis indicated that Cx37 was more phosphorylated in the aortae of the 2K1C rats mentioned above than in the animals receiving N'-nitro-L-arginine methyl ester, indicating that there may be a different regulatory process for aortic Cx43 in the 2 models of hypertension53 and emphasizing the potential importance of phosphorylation in the regulation of connexin function.

Spontaneously Hypertensive Rat

Observation on changes in connexins in spontaneously hypertensive rats (SHRs) are mixed. Cx40 and Cx37 were found to be reduced in ECs and VSMCs,39,53–55 and both increases and decreases in Cx43 have been reported.30,55 In ECs isolated from mesenteric arteries of SHRs, Goto et al56 found no change in either Cx40 or Cx43 but a decrease in Cx37. In the SHRs, Cx45 was increased in cerebral VSMCs.57 In mesenteric arteries from stroke-prone SHRs, Cx43 mRNA was similar to that observed in control Wistar–Kyoto rats, and no clear correlation between gap junctions and hypertension could be established.58

Normalization of blood pressure in the SHR using an angiotensin-converting enzyme inhibitor restores connexin expression to normal in the endothelium but not in the media.55 In addition, treatment with candesartan but not with a combination of hydralazine and hydrochlorothiazide restored the expression of Cx37 and Cx40 in the SHR in parallel with the normalization of blood pressure.59

Gene Modifications

Perhaps the clearest indication to date of a causal link between connexins and hypertension is the discovery that deletion of the Cx40 gene results in a marked, sustained hypertension.59 This deletion was associated with segmental constrictions and irregular vasomotion in small arterioles, suggesting a direct link among connexins, vasomotor tone, and blood pressure. The measurements of renin levels in these animals would be extremely interesting. Additional evidence for a central role for the connexins in the regulation of blood pressure derives from the fact that conditional deletion of Cx43 in the ECs produces hypotension, but it is a very complex response in which both Ang II and NO are increased.60

Recently, genetic screens for vascular connexins have provided additional correlative evidence for the importance of the connexins in vascular disease and hypertension in humans. Directly related to the present concern, a polymorphism in human Cx40 gene promoter is associated with increased risk of hypertension in humans,61 especially in men.62 Also, a polymorphism in the human Cx37 gene has been found to be strongly correlated with a risk for myocardial infarct.63,64

Methodologic Issues

Connexin protein turnover is very rapid, and removal of the connexins from the membranes is at least as important as their synthesis, thus making dissociation between mRNA and protein levels likely, and precluding a meaningful conclusion based on mRNA measurement alone.1,65 Defining a role for the connexins in hypertension will, thus, require assessment of both connexin protein and message levels in VSMCs and ECs, and such measurements must be made on the resistance vessels. Analysis of mRNA or protein in the intact resistance vessels is likely to be of little use because of the diversity of message and protein expression in the 2 cell types and the fact that selective isolation of either smooth muscle or endothelium from arterioles is difficult.66 Thus, we are almost entirely dependent on immunocytochemistry for analysis of connexins in resistance vessels. Unfortunately, immunocytochemistry is a technique that is markedly influenced by factors such as fixation,67 antibodies used,66,67,68 use of detergents,69 and phosphorylation state of the epitope probed by the antibody.70 These issues pose a major challenge for research in this field.

Connexin isoforms may also be coregulated, thus linking experimental modification of one connexin to an alteration of another. In EC, knockout of Cx40 has been reported both to increase and decrease Cx37,29,71 and in view of the profound effect of deletion of Cx40 on blood pressure, the associated changes in Cx37 must be re-evaluated.

Coordinated regulation of the connexins has even been reported across cell types, that is, alteration in EC connexin has been associated with an alteration in VSMC con-
nexin. We speculate that assembly and/or docking of hemichannels composed of multiple connexin isoforms is linked to the composition of the hemichannels on the opposing cell, but the feedback signals linking expression of connexin isoforms in the 2 cells remain to be determined. The importance of coregulation to the understanding of a role for these proteins in hypertension was emphasized by Rummery and Hill, who noted that "... determination of a role for specific connexins in the control of blood pressure must await the development of animals in which connexin expression can be modulated in a more complex temporal and tissue-specific manner." Thus, much remains to be done in elucidating the role of connexins in hypertension, and an excellent starting point is to review what is known of the role played by gap junctions in VSMC and EC function in the vasculature.

Mechanisms: Smooth Muscle Cell–Smooth Muscle Cell Communication

The vascular origin of many forms of hypertension depends on the modification of vasomotor tone. In VSMCs, gap junctions play an important role in the synchronization of changes in cytoplasmic Ca\(^{2+}\) in adjacent cells and in the overall development of tone. In addition, the gap junctions in the VSMC may provide a preferred pathway for the conduction of vasoconstrictor signals along the length of the microvessels. In the DOCA-salt hypertension model, there is an enhanced expression of Cx43, which is correlated with greater sensitivity of the VSMC to stimulation and enhanced tendency of the vessel tone to oscillate.

Mechanisms: EC–EC Communication

In mice, Cx40 and Cx37 are strongly expressed in the EC, and ablation of Cx40 produces marked hypertension. Deletion of Cx37, a major EC connexin in the mouse, does not alter blood pressure (X.F. Figueroa, unpublished observations, 2005). The lack of effect of Cx37 deletion on blood pressure is surprising in view of its abundance in the ECs and the fact that polymorphism of this connexin in humans has been associated with myocardial infarction, coronary artery disease, and atherosclerosis. However, it must be remembered that this is an observation made on mice in which a lifelong, global deletion has existed and in which many compensatory mechanisms may have occurred.

Mechanisms: Smooth Muscle–EC Communication

VSMCs or pericytes can share a common membrane potential with ECs, and small solutes can move between VSMCs and ECs or between ECs and pericytes through the heterocellular junction. Recently, the potential physiological relevance of gap junctional coupling at the MEJ was highlighted by the finding that inositol triphosphate can move from VSMCs to ECs and by the observations that gap junction inhibitors and connexin antibodies will block endothelium-derived hyperpolarizing factor (EDHF). The small size of the MEJ (≈0.5 μm) and its location within the internal elastic lamina (IEL), have made the investigation of the heterocellular gap junctions formed there quite difficult.

Electron microscopy provides the most direct approach, sometimes disclosing the presence of the classical pentameric organization of gap junctions and the connexin proteins. However, measurements of functional MEJ coupling between the 2 cells, assessed by dye movement and electrical continuity, have yielded variable results, which may depend on the vessel size or branching order.

The conditional deletion of EC Cx43 has been reported to result in hypertension, but this is controversial and demands further observation. In any case, the blood pressure change that was reported must have been multifactorial, because it includes increased plasma levels of both Ang II and NO. The specific signaling pathways that lead to these changes remain to be established, but a plausible hypothesis is that the EC Cx43 deletion increases EC Ca\(^{2+}\) in some way, thus leading to formation of NO, and that the rise in Ang II is compensatory for the associated tendency toward hypertension. An alternative hypothesis is that Cx43 is a key component of the junction linking renal JG cells and endothelium and that its deletion modifies Ca\(^{2+}\) concentration in the JG cells and, thus, their synthesis of renin. This is consistent with the recent observation that replacement of Cx43 by Cx32 in the mouse reduced the plasma renin levels by half, abolished renin salt sensitivity, and eliminated hypertension in the 2K1C model.

EDHF, the MEJ, and Hypertension

Vasodilator responses induced by stimulation of EC are typically accompanied by hyperpolarization of the overlying VSMC, purportedly because of the release of an EDHF. Alteration of EDHF signaling has been linked to hypertension in a variety of investigations. The mechanistic basis for EDHF signaling remains controversial, but gap junctions found in the MEJ provide a way to directly couple hyperpolarization of the EC to the VSMC. Connexin-mimetic peptides that selectively block gap junctions, as well as EC-selective loading of antibodies directed against the carboxyl-terminal region of Cx43, blocked EDHF-dependent vasodilation. Both observations are consistent with the idea that gap junctions play a pivotal role in EDHF coupling. In addition, the contributions of the EDHF-mediated responses are thought to increase as the vessel size decreases, demonstrating a pattern similar to that followed by the MEJ.

Consistent with the participation of MEJ in the control of vasomotor tone, EDHF-mediated vasodilation seems to be altered in hypertension. In 2K1C and high-salt diet-induced hypertension, the vasodilation induced by acetylcholine (ACh) was not changed, but in these animals, the response was mainly mediated by an EDHF-dependent pathway instead of NO. This change might be expected, because the expression of Cx43 is increased in the 2K1C model, and this connexin is essential in the EC–VSMC coupling. In contrast to the findings in the 2K1C animal, ACh-mediated vasodilation and the gap junction–dependent smooth muscle hyperpolarization were reduced in the SHR. This finding may have been the result of structural changes in the media, because the heterocellular coupling was enhanced in these animals. Taken together, these data suggest that a gap...
junction–mediated EDHF signaling is an important compensatory vasodilator mechanism during hypertension and one that demands further investigation.

Recently, the participation of EDHF in the control of blood pressure was confirmed in intact animals. Intrarenal infusion of connexin-mimetic peptides homologous to the second extracellular loop of Cx43 (Gap 2737,43) or Cx40 (Gap 2740) not only inhibited the EDHF-mediated response to ACh but also decreased basal renal blood flow and increased mean arterial blood pressure of female rats both in the presence and absence of NO synthase and cyclooxygenase blockade. This strongly supports the idea that gap junction-dependent EDHF may be involved in the control of basal vascular tone. In addition, these findings suggest that gap junctional connexin blockade modifies renin secretion, perhaps by interrupting the connection between the JG cell and EC, but the signaling involved in such an interaction remains to be established.

A Role for Conducted Responses in the Pathogenesis of Hypertension
Vasomotor responses spread along the vessel length through gap junctions and play an important role in the moment-to-moment coordination of vascular resistance by longitudinal integration of segments of the microcirculation. Thus, conduction of vasomotor responses represents an attractive mechanism by which vascular gap junctions could participate in the control of arterial blood pressure. Consistent with this idea, conducted vasodilatation is impaired in spontaneously hypertensive hamsters, SHRs, and Cx40 knockout animals.

It is worth noting that theoretical considerations suggest that gap junctional modulation of conduction of vasomotor signals may also play a role in the long-term control of peripheral resistance. This is based on the idea that the development of hypertension is typically associated with vascular rarefaction, a reduction in the number or density of microvessels. This process may occur in 2 phases, first a functional and subsequently an anatomic rarefaction. Mathematical simulations show that conduction of vasomotor responses induced by metabolic stimuli may play a role in the remodeling, and the fact that there is EC communication along the vascular axis from capillaries up to arterioles provides a mechanism by which the capillaries might initiate a centrifugal regulation of structural adaptations, as well as moment-to-moment regulation of arterial diameter.

Perspectives
The foregoing observations and reports make it clear that the connexins can play a multifaceted role in the establishment of vasomotor tone, and they establish a strong association between hypertension and changes in connexin expression. Moreover, treatment of hypertension can reverse the changes in connexin expression, although not invariably. Our knowledge of the role of connexins in cell–cell communication in general and in the control of vasomotor tone in particular indicates that alterations in connexin expression could provide the substrate for modification of vasomotor tone and, thus, the genesis of hypertension. However, the current literature does not allow one to include or exclude the idea that the connexins are causative elements in the pathogenesis of hypertension. Methodologic limitations, especially those related to immunocytochemistry, have not yet allowed us to be certain of the presence or absence of a particular connexin in the resistance vessels. Given the suggestion that the connexins, especially Cx43, may act as sensors of mechanical stress, it is essential to hold the thought that the changes in expression of vascular connexins in hypertension may be secondary to alterations in pressure and/or flow, rather than causative. This is particularly true in view of the divergent results obtained with different experimental models of hypertension and in different species.

The fact that there is coregulation of the connexins makes it imperative that multiple connexins be analyzed in hypertensive models to insure that a change in one connexin is not incidental to a secondary change in a different connexin. These analytical and regulatory issues may explain the different outcomes of experimental treatments, and it is important to repeat key experiments with these complex interactions in mind. A major area of investigation that remains virtually untouched is the possible role of posttranslational modification of connexin in the regulation of cell–cell communication in hypertension, because such modifications might explain experimental variability, as well as the responses to and the participation of connexins in hypertension.

Both heterocellular and homocellular communication in the vessel wall could be modified in ways that might lead to hypertension. An effort to understand the gap junction composition, especially at the MEJ, should be very helpful in correlating changes in connexin expression with changes in function. In addition, it is critical to recognize that control of gap junctional permeability by phosphorylation may be important in regulating vascular tone, and the tools for addressing this possibility are just becoming available. It can be anticipated that the use of connexin-specific inhibitors (eg, connexin-mimetic peptides) in combination with the large selection of vascular-specific connexin knockout animals now available should begin to clarify the function of gap junction–mediated communication in the vessel wall and ultimately the participation of the gap junctions in the pathogenesis of hypertension.

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