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Forum Review Article

Gap Junctions in the Control of Vascular Function

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Abstract

Direct intercellular communication *via* gap junctions is critical in the control and coordination of vascular function. In the cardiovascular system, gap junctions are made up of one or more of four connexin proteins: Cx37, Cx40, Cx43, and Cx45. The expression of more than one gap-junction protein in the vasculature is not redundant. Rather, vascular connexins work in concert, first during the development of the cardiovascular system, and then in integrating smooth muscle and endothelial cell function, and in coordinating cell function along the length of the vessel wall. In addition, connexin-based channels have emerged as an important signaling pathway in the astrocyte-mediated neurovascular coupling. Direct electrical communication between endothelial cells and vascular smooth muscle cells *via* gap junctions is thought to play a relevant role in the control of vasomotor tone, providing the signaling pathway known as endothelium-derived hyperpolarizing factor (EDHF). Consistent with the importance of gap junctions in the regulation of vasomotor tone and arterial blood pressure, the expression of connexins is altered in diseases associated with vascular complications. In this review, we discuss the participation of connexin-based channels in the control of vascular function in physiologic and pathologic conditions, with a special emphasis on hypertension and diabetes. *Antioxid. Redox Signal*. 11, 251–266.

Introduction

Cell-to-cell communication is fundamental in vascular function, as blood vessels are complex, multicellular structures that must work as an unit, and thus, control of vasomotor tone depends on the fine synchronization of the smooth muscle and endothelial cell function along the length of the vessel wall. Synchronization and coordination is accomplished by an intricate system of radial and longitudinal cell-to-cell communication (10, 55, 170, 175). In addition, in the microcirculation, small arteries and arterioles form a complex network whose elements must work in concert to regulate blood-flow distribution and peripheral vascular resistance by precise, well-integrated changes in the luminal diameter of the vessels (55, 171, 173, 174, 177).

One mode of communication that plays a critical role in controlling the function of the vessel wall is the release of paracrine molecules such as nitric oxide (NO) and prostaglandins. This signaling pathway is widely recognized, and its role in vascular physiology has been extensively studied. A complementary mechanism of communication that has emerged as a key pathway to coordinate the vascular wall function is the direct cell-to-cell communication *via* gap junctions (Fig. 1). The expression of connexins has been reported to be altered in several animals models of pathologies associated with vascular complications (15), such as hypertension (56, 159) and diabetes (95, 215), which highlights the importance of the direct cell-to-cell interaction for vascular homeostasis.

Gap junctions are intercellular channels that directly connect the cytoplasm of adjacent cells, allowing the passage of current and small signaling molecules (molecular mass $<$1,000 Da), such as Ca^{2+} and IP_3 (49, 162) (Fig. 1). These intercellular channels comprise a protein family known as connexins (Cx), which are denoted according to their predicted molecular weight. Six connexins combine to make a connexon or hemichannel, and the association in the plasma membrane of two hemichannels provided by adjacent cells forms a functional gap-junction intercellular channel (49, 162) (Fig. 1). It is noteworthy that independent hemichannels may also remain unpaired and open to release paracrine signals such as ATP or NAD^+ (70, 163). Although it has been

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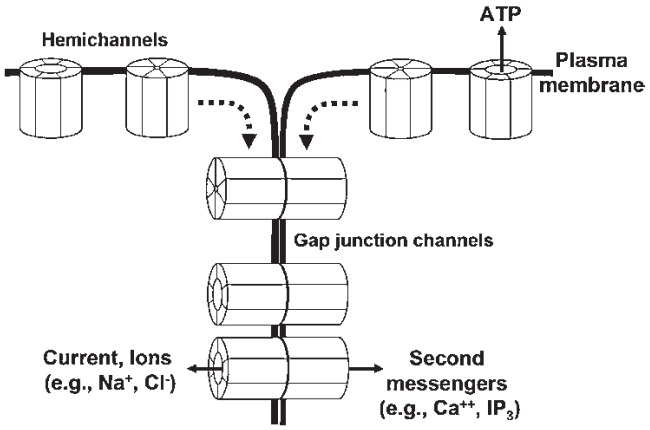

FIG. 1. Gap-junction channels in the plasma membrane. The family of proteins known as connexins constitutes the structural and functional unit of gap-junctional intercellular communication. Oligomerization of six connexin proteins forms a connexon or hemichannel (49, 162). In response to stimuli such as phosphorylation or Ca^{2+}, the associated six connexin subunits may coordinately change configuration to open the hemichannel and allow movement of paracrine signaling molecules such as ATP (70, 163). Hemichannels may diffuse laterally to the junctional membrane where they are in a position to dock with another hemichannel on the apposed membrane of the adjacent cell to complete a gap-junction channel (49, 162).

suggested that monocytes and macrophages expressed Cx37 hemichannels that regulate cell adhesion (207), this mode of connexin-based signaling has not yet been demonstrated to participate directly in the control of the vascular wall function (Fig. 1).

At least twenty connexin isoforms have been described in mammals, and one cell type often expresses more than one connexin (162). However, the expression of several connexins in one cell is not redundant. Gap junctions are not just simple channels that offer a low-resistance intercellular pathway for exchange of small solutes. Rather, the connexins mediate specific cell-to-cell signaling pathways, and the molecular selectivity as well as subcellular localization differs among connexin (55, 56, 129, 162, 203). Thus, although these proteins may have some overlap in function, they work in concert (55, 56, 79, 182, 183) and, in many cases, the function of one connexin cannot be replaced by other connexin isoform (79, 203, 206, 216). In addition, hemichannels can be composed of one (homomeric channels) or a mixture (heteromeric channels) of connexin proteins. Homomeric channels formed by different connexin isoforms typically differ in unitary conductance, permeability, and regulation and, as expected, the mixture of connexins in a heteromeric channel results in unique communication properties (28, 84, 131, 141), which provide an additional mechanism for fine regulation of gap junction–mediated signaling processes (12, 129, 204).

Connexin in the Vasculature

The vascular gap junctions are assembled from one or more of four connexin proteins: Cx37, Cx40, Cx43, and Cx45 (49, 55, 81, 178). The expression of connexins in the vessel wall is not uniform and seems to vary with vessel size, vascular territory, and species (85, 191, 192). In most cases, Cx45 is observed only in the smooth muscle cells (109, 122, 158). Although Cx37 is typically confined to endothelial cells (64, 178, 191), it has also been detected in the smooth muscle cells (158). In contrast, Cx40 and Cx43 may be expressed in both cell types (64, 126, 178, 191), but Cx40 is located predominantly in the endothelial cells (64, 191), and Cx43 is the most prominent gap-junction protein found in the smooth muscle cells (191). It should be noted, however, that in the mouse, Cx40 has been detected exclusively in the endothelium (36, 57, 91, 115, 183).

Consistent with the importance of gap junctions for the vascular homeostasis, it has been found that selective ablation of connexin genes results in severe vascular malformations. Cx45 knockout mice die at early embryonic stages (E9.5 to E10.5) and exhibit major defects in remodeling and organization of blood vessels. In addition, these animals fail to form a smooth muscle layer surrounding the major arteries (109). Deletion of Cx43 modifies the expression of many genes known to be involved in the differentiation and function of vascular cells, and cell-signaling pathways important for the regulation of vasculogenesis and angiogenesis (197), which produces several alterations in the pattern of coronary artery development, and the embryos die at birth of blockage of the right ventricular outflow tract (25, 154). Although Cx40-deficient embryos exhibit small septational defects, deletion of one allele of Cx43 increases the cardiac malformations of Cx40-knockout mice and leads to neonatal death. In contrast, haploinsufficiency of Cx40 did not affect the Cx43-knockout phenotype (105). Deletion of Cx37 is not lethal and has not been shown to produce any particular vascular phenotype. However, simultaneous ablation of Cx37 and Cx40 results in severe vascular abnormalities in skin, testis, intestine, stomach, and lung, and the animals do not survive past the first postnatal day (183), which highlights the role of the endothelial cell–gap junction communication in the development of the vasculature. Taken together, these data indicate that individual connexin isoforms are differentially involved in vascular development and emphasize the notion that although these gap–junction proteins may be coexpressed, they basically work in concert.

Connexins in Vascular Physiology

Vascular smooth muscle

Synchronization of vasomotor tone among the smooth muscle cells is critical for the function of blood vessels. The contractile state of the smooth muscle cells is determined by the cytoplasmic Ca^{2+} concentration and the Ca^{2+} sensitivity of the contractile apparatus. Consequently, the smooth muscle cell-membrane potential plays a central role in the tonic control of intracellular Ca^{2+} concentration by modulating the influx of Ca^{2+} *via* L-type, voltage-dependent Ca^{2+} channels. Gap junctions play a central role integrating the smooth muscle cell function by coordinating changes in both membrane potential and intracellular Ca^{2+} between adjacent smooth muscle cells (22–24).

In addition, gap-junctional communication of vascular smooth muscle cells seems to be involved in the development of the myogenic vasomotor tone in resistance arteries (42, 118). Interestingly, the participation of the gap junction in this process is not related to synchronization of Ca^{2+} sig-

naling, but rather to earlier signaling events such as coordination of the smooth muscle cell depolarization or, directly, the mechanosensitivity of the vascular smooth muscle. This hypothesis is supported by the fact that the gap-junction inhibitory peptide Gap27 or the gap-junction inhibitor 18α-glycyrrhetinic acid, in addition to blocking Ca^{2+} influx and vasoconstriction in mesenteric resistance arteries, also prevented pressure-induced smooth muscle cell depolarization (42). However, a nonspecific effect of the gap-junction blockers on ion channels cannot be discounted. In any case, the involvement of gap junctions in the myogenic response may be consistent with the finding that tensile stretch increased Cx43 expression as well as gap-junctional intercellular communication in vascular smooth muscle cells. Interestingly, this response was mediated by the formation of reactive oxygen species (29, 30, 190), which has been reported to contribute to the initiation of the myogenic constriction in mouse-tail arterioles (146).

In addition, Cx43 seems to be essential for coordination of cell proliferation and migration in the vasculature (55, 116, 151, 214), which was recently confirmed by the specific deletion of the Cx43 gene in the smooth muscle cell (124). In these animals, the injury to the carotid artery by vascular occlusion or wire injury resulted in an increase in the neointima and the adventitia formation (124), suggesting an accelerated growth of the smooth muscle cell with the Cx43 deletion, which was further confirmed by using cultured cells. In contrast to these findings, Chadjichristos *et al*. (19) showed that in heterozygous Cx43-knockout mice, the neointimal formation was reduced. However, in those animals, Cx43 was deleted from all cell types expressing Cx43, and the experiments included a high-fat diet, which may have influenced the result by either vascular adaptive responses to the diet or complex interactions between different cell types. Although the participation of Cx43 in neointimal formation demands further investigation, these data highlight the relevance of Cx43 in the feedback-control pathways necessary for vascular morphogenesis.

Endothelium

The endothelium plays a key role in the tonic control of blood pressure, and deletion of vascular connexin genes has disclosed that gap-junctional communication is essential in the coordination and integration of microvascular function by the endothelial cells in a very complex manner. Vascular endothelial cells–specific deletion of Cx43 (VEC $Cx43^{-/-}$) results in hypotension (123) and, in contrast, ablation of Cx40 produces a hypertension associated with an irregular vasomotion (36, 37) and a dysregulation of renin production (107, 195, 196). Although deletion of Cx37 does not appear to alter vascular function or blood pressure, polymorphisms of this connexin have been associated with myocardial infarction, coronary artery disease, and atherosclerosis (13, 86, 211, 212). In mice, Cx40 and Cx37 are expressed primarily in the endothelium, which emphasizes the importance of the endothelial cell–gap junction communication in the control of cardiovascular homeostasis and the idea that vascular connexins work in concert coordinating specific signaling processes.

Although the mechanistic bases of the hypotension observed in VEC $Cx43^{-/-}$ are still unknown, the plasma levels of angiotensin I and II as well as NO were elevated in these animals (123), suggesting that a dysregulation of NO production may have been responsible for the hypotension, and the renin–angiotensin system was activated as a compensatory mechanism. It is interesting to note that shear stress upregulates the expression of Cx43 in cultured endothelial cells (6, 38) and in the endothelium of rat cardiac valves (93), which is consistent with the idea that Cx43 is involved in the sensitivity to mechanical stimuli.

Smooth muscle–endothelium communication via gap junction

The smooth muscle cells and the endothelial cells can also be electrically and metabolically connected by gap junctions located at discrete points of contact between the two cell types at the myoendothelial junction (MEJ) (11, 46, 127, 166, 179, 209). This heterocellular communication seems to play a pivotal role in the Ca^{2+}-mediated responses induced by endothelium-dependent vasodilators, such as ACh (Fig. 2). These vasodilator responses are typically paralleled by hyperpolarization of the underlying smooth muscle cells (46, 71, 75). The relaxant pathway associated with smooth muscle hyperpolarization is thought to be independent on NO and prostacyclin production by the endothelial cells and has

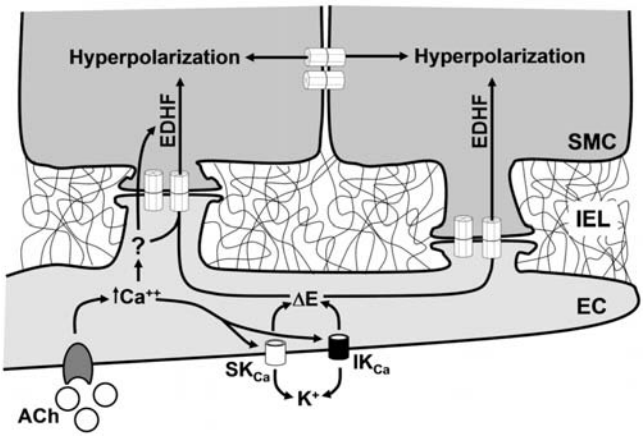

FIG. 2. Control of vasomotor tone by the endothelium-derived hyperpolarizing factor. Extensions derived from either the smooth muscle cells or endothelial cells may penetrate the internal elastic lamina (IEL) to make close contact with the other cell type. These points of contact, known as myoendothelial junctions (MEJs), provide the structural organization to achieve direct heterocellular coupling between the two cell types *via* gap junctions (40, 55, 166), and thus, one basis for endothelium-mediated smooth muscle hyperpolarization (often referred to as endothelium-derived hyperpolarizing factor, EDHF). Endothelium-dependent vasodilators such as acetylcholine (ACh) induce an increase in endothelial cell intracellular Ca^{2+} concentration, which, in turn, activates the Ca^{2+}-activated K^+ channels (K_{Ca}) of small (SK_{Ca}) and intermediate conductance (IK_{Ca}). The endothelial cell hyperpolarization is transmitted electrotonically to the underlying smooth muscle cells *via* gap junctions located at the MEJ, contributing to the endothelium-dependent vasodilation (18, 44, 51). The question mark highlights that the participation of a diffusible EDHF has not been definitely discarded.

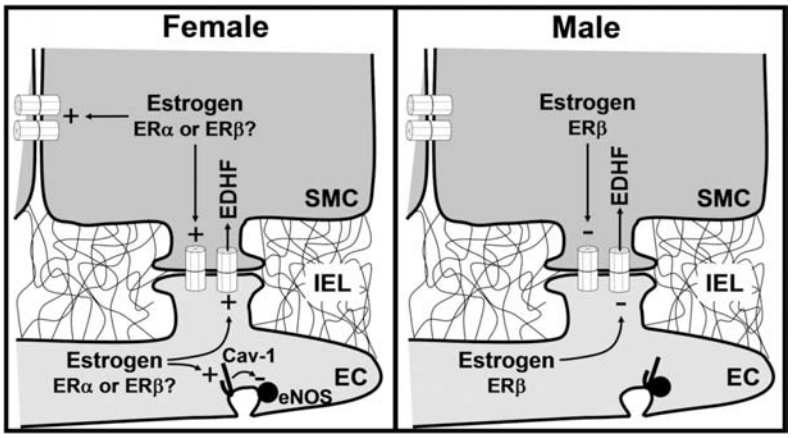

FIG. 3. Possible sex differences in the regulation of the endothelium-derived hyperpolarizing factor (EDHF). Nitric oxide (NO) and EDHF are the major endothelium-derived vasodilator signals in resistance vessels. The involvement of these two vasodilator signals differs between male and female animals. NO prevails over EDHF in males, and the contrary is observed in females (130, 169). This gender difference may be explained by the estrogen modulation of the myoendothelial gap junction–mediated smooth muscle hyperpolarization. In female animals, estrogen upregulates the expression of caveolin-1 (Cav-1) (128, 143), a structural protein of caveolae that, in turn, inhibits the activity of eNOS (52, 65, 100). In addition, this hormone enhances the expression of Cx43 and Cx40 (128, 143), two gap-junction proteins found at the myoendothelial junctions (35, 97). As a result, the NO-dependent vasodilation is reduced, and the gap junction–mediated EDHF signaling is increased. The involvement of the estrogen receptor α (ERα) or β (ERβ) remains to be determined. In contrast, in male animals, the activation of ERβ reduces the gap junction–dependent smooth muscle hyperpolarization (130).

been attributed to the release of an endothelium-derived hyperpolarizing factor (EDHF) (50, 193). The identity of EDHF remains controversial, and several EDHF candidates have been proposed, such as K^+ ions (43), epoxyeicosatrienoic acids (EETs) (4, 62), hydrogen peroxide (180), and C-type natriuretic peptide (CNP) (1, 20). However, the direct electrotonic transmission of a hyperpolarizing current from the endothelial cells to the smooth muscle cells *via* myoendothelial gap junctions (Fig. 2) has emerged as a most attractive hypothesis to explain the EDHF pathway (18, 40, 74). In this context, the increase in endothelial cell intracellular Ca^{2+} concentration activates the Ca^{2+}-activated K^+ channels (K_{Ca}) of small (SK_{Ca}) and intermediate conductance (IK_{Ca}), leading to the endothelium-dependent hyperpolarization of the smooth muscle cells *via* gap junctions located at the MEJ (18, 32, 44, 51, 148) (Fig. 2). Consistent with this hypothesis, the EDHF-dependent vasodilation is prevented by the connexin-mimetic peptides that are thought to specifically block the gap junction (21, 35, 104), as well as endothelial cell–selective loading of antibodies directed against the carboxyl-terminal region of Cx40 (134).

In addition to NO, shear stress has been reported to activate an EDHF-dependent vasodilator response (198), and the contribution of the EDHF-mediated responses seems to increase as the vessel size decreases (165, 181), which suggests that an EDHF pathway may be involved in the tonic control of peripheral vascular resistance. Consistent with this idea, intrarenal infusion of connexin-mimetic peptides homologous to the second extracellular loop of Cx43 (^{43}Gap 27) or Cx40 (^{40}Gap 27) not only decreased basal renal blood flow, but also increased mean arterial blood pressure of female rats, either in the presence or the absence of NO synthase (NOS) and cyclooxygenase (COX) blockade (35), suggesting that the connexin-mimetic peptides induced vasoconstriction by disrupting a tonic vasodilator signal.

Interestingly, in male animals, NO is the major endothelium-dependent vasodilator signal, but in female animals, EDHF prevails over NO or PGI_2 (169), and estrogen appears to be responsible for this gender difference (90, 128, 143). The endothelial isoform of NOS (eNOS) is found in a inhibitory association with caveolin-1, a structural protein of caveolae (52, 65, 100, 139, 140), and estrogen modulates the expression of this negative regulator of eNOS, and the EDHF-positive regulators, Cx43 and Cx40 (35, 97, 128, 143) (Fig. 3). Thus, in ovariectomized rats, the level of caveolin-1, Cx43, and Cx40 decreases in parallel with an increase in the NO-mediated vasodilation and a reduction in the EDHF-mediated vasodilation sensitive to the gap-junction blockers 18β and 18α-glycyrrhetinic acid (128, 143). As expected, all the changes are recovered after estrogen replacement (128, 143).

Estrogen also enhances the EDHF-mediated vasodilation in response to flow (90), which suggests that the myoendothelial gap-junction–dependent signaling pathway may be more important in the control of blood pressure in female than in male animals. Recently, this idea was confirmed by using an eNOS/COX-1 double knockout. Deletion of eNOS and COX-1 did not alter the mean arterial blood pressure in female mice, whereas the double knockout resulted in hypertension in male mice (169). The endothelium-dependent relaxation was intact in resistance vessels of female mice and was mediated by the smooth muscle hyperpolarization (169), strongly indicating that EDHF plays a predominant role in the tonic control of blood pressure in female animals. These data suggest that EDHF rather than NO may underlie the higher resistance of premenopausal women to cardiovascular diseases such as hypertension.

Two estrogen receptor (ER) subtypes, ERα and ERβ, are expressed in vascular endothelial cells as well as smooth muscle cells of both female and male animals (135, 136, 217). It is thought that ERα mediates most of the vascular effects of estrogen, but the importance of ERβ is emerging, and ERβ-deficient mice develop hypertension as they age (217). In addition, recently Luksha *et al*. (130) proposed that ERβ contributes to the sex differences observed in the endothelium-dependent control of vasomotor tone by reducing the gap junction–mediated EDHF signaling in the male mouse (Fig. 3).

Conduction of vasomotor responses

Longitudinal conduction of vasomotor responses provides an essential means of coordinating changes in diameter and

flow distribution among vessels in a complex network. Vasomotor signals spread along the vessel length through gap junctions connecting cells of the vessel wall, and thereby, participate in the minute-to-minute coordination of vascular resistance by integrating function of proximal and distal vascular segments in the microcirculation (37, 55, 56). Although vasoconstrictor responses are thought to be conducted by the smooth muscle cells (8, 17, 202), the cellular pathway for conduction of vasodilator signals is more controversial and may be either exclusively the endothelium (47, 176) or both the smooth muscle and the endothelial cells (8, 17). The cellular pathway for conduction of vasomotor responses has been studied by selectively damaging a short segment of the endothelial cells or the smooth muscle cells with a light-dye (fluorescein-conjugated dextran) treatment. In feed arteries, selective damage of the endothelium completely blocked the ACh-induced conducted vasodilation (47, 176), but in arterioles, damage of either the endothelium or the smooth muscle did not affect the conduction of the response to ACh (8, 17), which led to the proposal that the cellular pathway for conducted vasodilations depends on the functional location of the vessel in the microvascular network (172). However, the cellular pathway of vasodilator signals may also depend on the stimulus that initiated the response, because, in contrast to ACh, selective damage of the endothelium blocked the vasodilation induced by bradykinin in arterioles (17, 202).

Direct measurements of membrane potential have shown that conducted vasomotor responses are associated with rapid propagation (milliseconds) of an electrical signal along the vessel length (47, 202, 208, 209). Because many observations have revealed an exponential decay of the conducted electrical signal, it was proposed that longitudinal spread of vasomotor responses reflects the passive, electrotonic conduction of changes in membrane potential *via* gap junctions connecting cells of the vessel wall (76, 150, 202). Therefore, the decay of the conducted vasomotor responses along the vessel length should be consistent with the length constant calculated from electrotonic potentials produced by current injection into the smooth muscle or endothelial cells of arterioles, which is between 0.9 and 1.6 mm (45, 87, 88).

Conduction of vasoconstrictor responses typically behaves as predicted by the electrotonic model. However, a simple electrotonic model often fails to predict conduction of vasodilator signals initiated by endothelium-dependent stimuli, such as ACh or bradykinin. These signals have been reported to propagate for many millimeters without showing noticeable decay in magnitude (36, 41, 47, 48) (Fig. 4). In addition, the electrical length constant of ACh-induced hyperpolarization has been shown to be longer than that measured for current injection (45), and the hyperpolarizing signal activated by ACh has been also reported to increase during the first 1,000 μm of longitudinal conduction (33). The lack of decay of these responses suggests that a regenerative, energy-dependent mechanism underlies the conduction process, similar to that described in neurons. Consistent with this idea, it was shown recently that electrical stimulation also activates a conducted, nondecremental endothelium-dependent vasodilation (Fig. 5) that is hypothesized to be mediated by a complex interplay between voltage-gated Na^+ channels (Na_v) and T-type, voltage-gated Ca^{2+} channels [T-Ca_v (54)], where Na_v underlies the conduction of the signal and T-Ca_v mediates the vasodilation (Fig. 6). Interestingly,

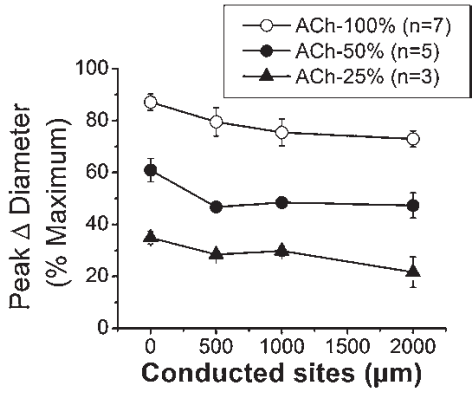

FIG. 4. Conduction of vasodilator responses induced by the stimulation of cremasteric arterioles with a pulse of ACh. The microcirculation of the cremaster muscle was prepared as described previously (54, 57), and arterioles were stimulated focally with the ejection of ACh (10 μM) by a pressure-pulse from a micropipette (inner diameter, 3–4 μm). The changes in diameter were measured first at the stimulation site (local), and then, at locations 500, 1,000, and 2,000 μm upstream in four separate stimuli. Variations in diameter were expressed as percentage of the maximal dilation possible (% maximum). The duration of the pulse (300–700 ms) and the ejection pressure (10–20 psi) were set to induce a local vasodilation of ~100%, ~50%, or ~25%. Maximal diameter was estimated during superfusion of 1 mM adenosine. Note that the magnitude of the response decays only from the local site to the 500-μm conducted site and does not show a further reduction thereafter.

deletion of Cx40 selectively eliminates the regenerative component of the conducted vasodilation induced by ACh, bradykinin (36), or electrical stimulation (57), leaving a decaying component consistent with the electrotonic model, which demonstrates that gap junctions are the pathways for the intercellular propagation of these vasodilator signals and suggests that Cx40 may be functionally associated with the endothelial Na_v (Fig. 6). Deletion of Cx37 did not affect conduction of vasodilator responses (Figueroa and Duling, unpublished observations), and replacement of Cx40 by Cx45 did not restore the nondecremental component of the conducted vasodilation activated by ACh or bradykinin (206), supporting the idea that individual connexins have different functions, but leaving much to be explained as to how such selectivity is conferred.

The electrotonic and regenerative components of the conducted vasodilation activated by an endothelium-dependent vasodilator such as ACh are highlighted schematically in a hypothetical model shown in Fig. 6. The opening of inward-rectifier K^+ channels (K_{ir}) induced by the smooth muscle hyperpolarization may be an alternative hypothesis to explain the extended conduction of vasodilator responses. An intrinsic biophysical property of K_{ir} channels is that they increase their activity on cell hyperpolarization, and it has been proposed that the activation of these K^+ channels in the smooth muscle cells amplifies the hyperpolarizing current initiated by ACh, thereby facilitating the conduction of this signal (98). However, as mentioned earlier, current-induced hyperpolarization decays faster than the response induced by ACh (45), which argues against the participation of K_{ir}

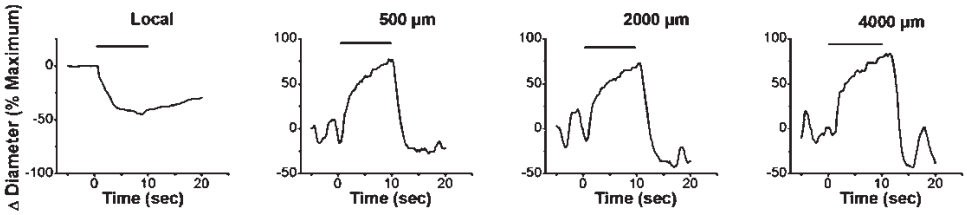

FIG. 5. Representative tracings of the conduction of the vasomotor responses induced by focal electrical stimulation of cremasteric arterioles. The microcirculation of the cremaster muscle was prepared as described previously (54, 57). An Ag/AgCl reference electrode immersed in the superfusate was positioned symmetrically around the cremaster, and the arteriole was stimulated with a depolarizing train of pulses (30 Hz, 2 ms, 30 V) for 10 s by using a beveled micropipette (inner diameter, 3–4 μm) filled with 1 *M* NaCl. The stimulating pipette was inserted under the cremasteric mesothelium and positioned directly above the arteriole at a distance selected to evoke a local constriction of ~50% (54, 57). In separate stimuli, the changes in diameter were observed at the stimulation site (local), and at locations 500, 2,000, and 4,000 μm upstream. Variations in diameter were expressed as percentage of the maximal constriction or dilation possible (% maximum). Maximal diameter was estimated during superfusion of 1 m*M* adenosine. Focal electrical stimulation of the arteriole evoked a vasoconstriction that was restricted to a short vessel segment (~70–100 μm) at the stimulation site and, in addition, activated a rapid conducted vasodilation that spread along the length of the entire vessel without decay. Horizontal bars indicate the stimulation period.

alone in the nondecremental component of the conducted vasodilation and suggests that further investigation is needed to elucidate the mechanisms involved in the conduction of vasomotor responses.

Theoretic analysis of vascular adaptation to hemodynamic and metabolic stimuli indicate that conduction of vasodilator signals may also play a role in the long-term control of peripheral resistance by contributing to the maintenance of the structural homeostasis of the microvascular network (152). The vascular rarefaction observed during the development of hypertension may be an example of this. The phenomenon of rarefaction is manifested as a reduction in the number or density of microvessels, which occurs in two phases: a functional and an anatomic rarefaction (120). The functional rarefaction involves a reduction in the perfusion of microvessels that appears to reach a nadir at which they may then be disassembled, leading to the structural or anatomic rarefaction. Interestingly, mathematical simulations suggest that conduction of vasomotor responses induced by metabolic stimuli may be essential to preclude the disassembly of microvessels observed in the functional rarefaction (152, 153). Therefore, the functional longitudinal communication from capillaries up to arterioles *via* gap junctions (9, 185) provides pathways for both short-term modulation of diameter and for long-term regulation of the microvascular network architecture in physiologic and pathologic conditions.

Neurovascular coupling

Conduction of vasomotor signals is also involved in the control of cerebral microcirculation by neuronal activity, referred to as neurovascular coupling (3, 83, 121). In this case, however, vasomotor signals seem to be conducted by astrocytes, as opposed to smooth muscle or endothelium (2, 106, 137, 142, 189, 218). Tight spatial and temporal coupling between neuronal activity and blood flow is essential for the brain function (2, 83, 121, 161), and astrocytes are found in a strategic location between neurons and the microvasculature, with the astrocytic endfeet ensheathing the vessels (Fig. 7). This spatial organization places the astrocytes in a key position to orchestrate the neurovascular coupling, and an increasing body of evidence shows that the astrocyte transduces and conducts neuron-generated vasomotor signals to

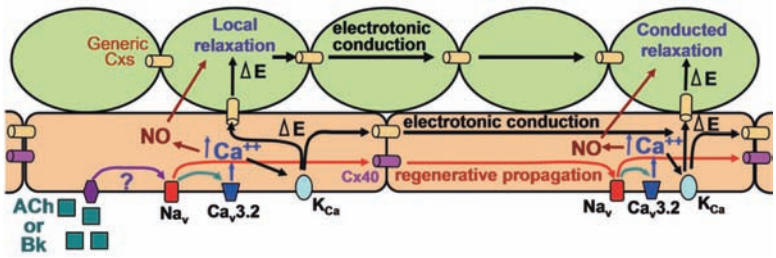

FIG. 6. Hypothetical model of conducted vasodilator responses activated by endothelium-dependent vasodilators [based on data from (36, 54, 57, 206)]. Stimulation of the endothelial cells with ACh or bradykinin triggers a regenerative, conducted vasodilator signal that is mediated by the activation of voltage-dependent Na^+ channels (Na_v) and rapidly propagated along the endothelium selectively *via* Cx40 gap junctions (*red lines*). The Na_v-mediated conducted electrical signal is transduced into vasodilation by activation of the T-type, voltage-dependent Ca^{2+} channels $Ca_v3.2$ and the subsequent initiation of the production of Ca^{2+}-sensitive vasodilator signals such as NO and Ca^{2+}-activated K^+ channel (K_{Ca})-mediated smooth muscle hyperpolarization (*black lines*). The K_{Ca}-dependent hyperpolarization may be conducted electrotonically along the longitudinal axis of the vessel by either the smooth muscle cells or the endothelial cells *via* Cx40 or other connexins present in the vascular wall, which, in this schematic, we designated "generic" Cxs. This hypothetical model is compatible with the evidence showing that deletion of Cx40 completely eliminates the regenerative component of the conducted vasodilator response (36, 57, 206). The decremental conducted vasodilation that remains in the absence of Cx40 may correspond to the electrotonic conduction of the K_{Ca}-dependent hyperpolarizing signal. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.lieberonline.com/ars)

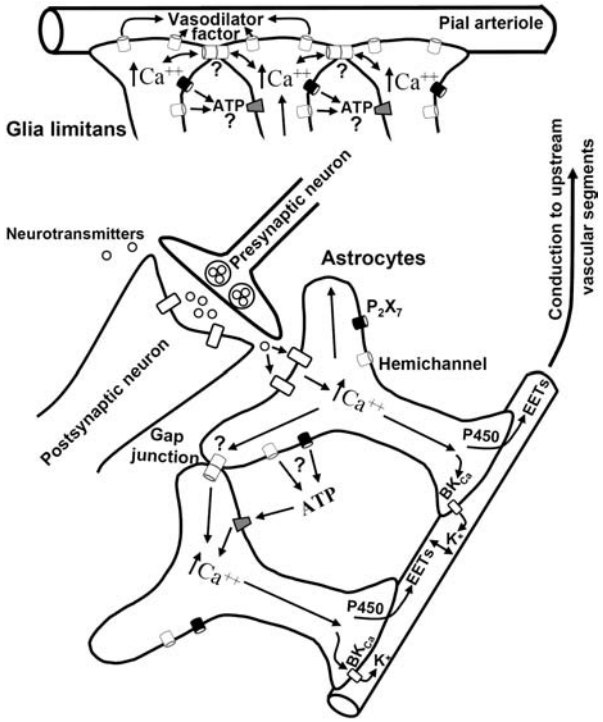

FIG. 7. Connexin-based channels likely to be involved in neurovascular coupling. Neurotransmitters released on an increase in neuronal activity may exit the synaptic cleft and activate receptors on astrocytes (2, 218). These receptors cause an increase in astrocyte intracellular Ca^{2+} concentration that leads to the activation of large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) and/or cytochrome P450 epoxygenase (P450) at the astrocytic endfeet. The efflux of K^+ through BK_{Ca} and/or production of epoxyeicosatrienoic acids (EETs) by P450 results in vasodilation of the parenchymal arterioles (2, 58, 59, 82, 187). The astrocyte-mediated vasodilator signal may be coordinated by the propagation of an interastrocyte Ca^{2+} signal *via* ATP release or directly by gap-junction communication. ATP may be released by either P_2X_7 receptors or unpaired hemichannels (70, 188). The potential role of interastrocyte Ca^{2+} waves in the coordination of the neurovascular coupling remains to be clearly defined. Local vasodilation of parenchymal cerebral arterioles must be communicated to upstream vascular segments to produce a functional increase of blood supply, and astrocytes may also communicate the vasodilator response to upstream vessels, such as the pial arterioles. These arterioles overlie a thick layer of astrocytic processes, called the glia limitans. The vasodilator signal triggered by neuronal activity reaches the glia limitans and induces a Cx43-based channel-dependent vasodilation (210). The mechanism by which Cx43 controls the astrocyte-mediated vasodilation has not been established, but coordination of the response between astrocytes, *via* gap-junction communication, or ATP release by hemichannels, is an interesting hypothesis that must be explored. An alternative hypothesis may be that a vasodilator factor is released at the endfeet *via* Cx43-based hemichannels.

the local microvasculature (2, 138, 142, 189, 218). As a result, astrocytes couple neuronal activation to vasodilation of local parenchymal arterioles (Fig. 7), which, in turn, leads to an increase in blood-borne oxygen and glucose supply to satisfy the enhanced metabolic demand rapidly (2, 83, 121, 161).

Calcium seems to be the intracellular vasomotor signal of the astrocyte-mediated neurovascular coupling. The increase in neuronal activity results in astrocytic calcium signaling that propagates through the astrocytic processes into the endfeet (2, 58, 142, 187, 218). The increase in cytosolic calcium concentration in the endfeet ultimately causes the release of vasoactive factors and arteriolar dilation (2, 58, 59, 187, 218) (Fig. 7). Interestingly, astrocytes express gap junctions (132, 133, 155, 162), and a calcium signal may propagate between neighboring astrocytes in a wavelike manner (27, 144, 145), coordinating the neurovascular coupling in the local cerebral microcirculation (2, 58, 142, 187, 218). However, the possible participation of gap junctions in the coordination of the astrocyte-mediated vasomotor signals remains to be established.

As described in the peripheral microcirculation (171, 177), local vasodilation of cerebral arterioles must be communicated to upstream vascular segments to produce a functional increase of blood-flow supply and to match the local metabolic demand (31, 92). Although vasomotor responses have been observed to be conducted in cerebral arterioles (39, 89), recently Xu *et al*. (210) demonstrated *in vivo* that astrocytes also play a central role in integrating the function of local arterioles with upstream cerebral vessels involved in the neurovascular coupling. Pial arterioles are important upstream vessels of the parenchymal cerebral arterioles. It is important to note that pial arterioles overlie a thick layer of astrocytic processes, known as the glia limitans, which isolate these arterioles from the neurons that are located right below (Fig. 7). Vasodilation of pial arterioles associated with neuronal activation was blocked by the selective elimination of astrocytes through treatment with L-α-aminoadipic acid, and, interestingly, this response was also sensitive to the inhibition of Cx43-based channels with the specific connexin-mimetic peptide gap-27 (161, 210). The selectivity of the Cx43 inhibition was confirmed by using two connexin-blocking peptides: gap-27, which targets Cx43/37-based channels, and gap-26, which is a Cx40/37-selective peptide (210). Taken together, these data suggest that Cx43 is essential in the astrocytic signaling that mediates the neurovascular coupling. In astrocytes, Cx43 may be found forming unpaired hemichannels or gap-junction intercellular channels (155, 162, 186). Thus, astrocytic, Cx43-based channels could be involved in the coordination of calcium waves between astrocytes or in the release of vasoactive factors (Fig. 7), which is an interesting scientific challenge that requires further investigations.

Vascular Connexins in Pathology

Hypertension

Consistent with the participation of gap junctions in the control of vasomotor tone, the expression of vascular connexins is altered in hypertension (56). However, changes in several parameters associated with this pathology, such as mechanical load (30), bioavailability of NO (80, 101, 156, 213), shear stress (93, 99, 117), or angiotensin II (78, 103), may modulate the expression of connexins. In addition, gap junctions are involved in different and sometimes antagonistic (vasodilation *vs*. vasoconstriction) functions in the control of vascular function, and changes in connexin expression vary depending on the experimental model of hypertension or

vascular territory studied (72, 77, 78, 80, 103, 157, 160, 199, 213). Therefore, a simple analysis of connexin expression does not allow one to determine whether connexins play a role in the genesis of hypertension or whether the changes observed are a consequence of the development of the pathology. Also, it is important to note that the heterocellular coupling between the smooth muscle and endothelial cells has been shown to be enhanced in spontaneously hypertensive rats (SHRs). However, in these animals, the vasodilation induced by ACh and the gap junction–mediated smooth muscle hyperpolarization were reduced, which was assumed to be the result of structural changes in the media, and that the greater heterocellular coupling was a compensatory mechanism to maintain the EDHF signaling in this model of hypertension (164). A recent review describes in detail the alterations of gap junctions in hypertension (56), and therefore, in this section of the review, we focus on the genetic manipulations of connexin expression that result in an altered control of arterial blood pressure.

Probably the clearest participation of gap junctions in hypertension is through the control of renin secretion. Cx37, -40, and -43 are expressed in the kidney and link the cells of the juxtaglomerular apparatus (JGA) (5, 114, 215). These three gap-junction proteins are expressed in the endothelial cells of afferent arterioles. In addition, Cx37 and Cx40 are found in renin-secreting cells and mesangial cells (5, 114, 215). Interestingly, endothelial cell communication *via* Cx43 appears to be a key in the control of renin secretion, because replacement of Cx43 by Cx32 (Cx43KI32) disrupted the regulation of renin synthesis and secretion (79). In Cx43KI32 mice, expression of renin was reduced, and the levels of this hormone were not downregulated by a high-salt diet or increased by clipping a renal artery in the two-kidney, one-clip (2K1C) model of renin-dependent renovascular hypertension (79).

In contrast to the inhibition of renin secretion observed in Cx43KI32 mice, deletion of Cx40 resulted in increased renin production and plasma renin concentration (Fig. 8), leading to the proposal that this connexin plays an essential role in the tonic inhibition of the renin system in the kidney (107, 196). Consistent with this idea, intrarenal pressure and angiotensin II failed to attenuate renin secretion in Cx40-deficient mice (196). Although Cx40 is the dominant gap-junction protein in renin-secreting cells, the dysregulation of the renin system observed in Cx40-knockout mice may depend, not on direct cell-to-cell signaling *via* Cx40 gap junctions, but on structural changes in the JGA architecture, as shown by Kurtz *et al*. (114). Ablation of Cx40 seems to affect the JGA development and, in the absence of Cx40, renin-expressing cells were not present in the terminal part of the afferent arteriole wall; instead, renin was found in cells of the extraglomerular mesangium and periglomerular interstitium (114). Taken together, these studies highlight the importance of gap-junction communication in the control of JGA development and renin secretion. It is important to remember that Cx37 is also expressed in the JGA, and although deletion of Cx37 does not affect arterial blood pressure, the participation of this connexin in JGA development and control of the renin system remains to be determined.

Although it may be controversial (107), the increase in renin secretion does not fully account for the hypertension observed in Cx40-knockout mice because blockade of the angiotensin II receptor AT1 with candesartan (37) or the angiotensin I–converting enzyme (ACE) with enalapril (196) reduced, but did not revert the blood pressure of these animals to the normal values (Fig. 8). As mentioned earlier, deletion of Cx40 is also associated with an irregular vasomotion of microvessels and an impaired conduction of vasodilator signals (37). Therefore, the renin/angiotensin II–independent component of the hypertension triggered by the absence of Cx40 probably is caused by an interruption of the control and coordination of the cells of the vessel wall (Fig. 8). In support of the importance of coordination and integration of vasomotor tone in the control of arterial blood pressure (37, 55, 56) and structural adaptation of the microvascular network (152, 153), conduction of vasodilator responses has been found to be impaired in spontaneously hypertensive hamsters (110, 111) and SHRs (72).

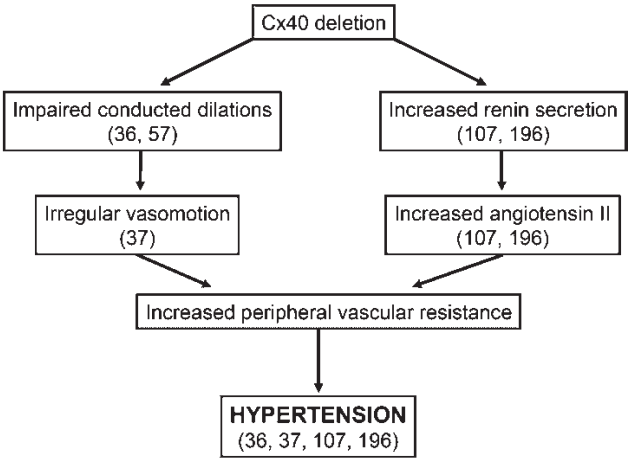

FIG. 8. Mechanisms of the control of arterial blood pressure by Cx40. Cx40 seems to play a central role in the control of arterial blood pressure by the parallel coordination of the vasomotor tone of resistance vessels and by renin secretion in the juxtaglomerular apparatus (JGA). Deletion of Cx40 affects the endothelial cell–dependent longitudinal synchronization of the vessel wall function, which results in an impaired conduction of vasodilator responses (36, 57), an irregular vasomotion and segmental constrictions (37). At the same time, the absence of Cx40 in the renin-secreting cells at the JGA disrupts the tonic inhibition of renin system, leading to an increase in renin secretion with the consequent increase in angiotensin II levels (107, 196). The dysregulation in the longitudinal communication of microvessels and renin secretion converges to increase the peripheral vascular resistance and produce the hypertension observed in Cx40-knockout animals (36, 37, 107, 196).

The potential importance of endothelial cell Cx40 in the development of hypertension has also been noted in SHRs (103, 160). In the caudal artery of these animals, Rummery *et al*. (160) found decreased endothelial cell size and a reduction in the density of endothelial gap-junction plaques containing Cx40. Again, the heterogeneity in connexin participation arises because, in these experiments, no changes were found in the expression of Cx37 or Cx43.

Consistent with the key role played by Cx40 in the control of renin secretion and coordination of vasomotor tone, two closely related polymorphisms (−44A and +71G) within

the regulatory region of the human Cx40 gene have been associated with increased risk of hypertension in men (61, 73). As expected, these polymorphisms seem to alter the activity of the Cx40 promoter, because *in vitro* reporter assays revealed that the Cx40 haplotype −44A/+71G reduced the expression of luciferase by 50%, and the −44A polymorphism negatively affects the promoter regulation by the transcription factors Sp1 and GATA4 (60, 61, 73).

Diabetes

As observed in hypertension, control and coordination of vasomotor tone play a critical role during the development of diabetes, and vascular complications are the leading causes of mortality in this pathology. Diabetes and hyperglycemia may lead to vascular dysfunction by several mechanisms, such as nonenzymatic glycosylation (16, 194), oxidative stress (69), polyol-myoinositol alteration (63), and activation of diacylglycerol (DAG)-protein kinase C (PKC) pathways (119, 205).

A large body of evidence shows that PKC-dependent phosphorylation of Cx43 affects the gap-junction intercellular communication (125, 162), and, in the vasculature, the direct activation of PKC with phorbol myristate acetate (PMA) reduces the Cx43-mediated dye coupling in primary cultures of human tonsil high endothelial cells (53). In accordance with these findings, high glucose concentrations inhibited gap-junction dye transfer through the activation of a PKC-dependent signaling pathway in cultured bovine aortic endothelial cells (94, 95). In addition, exposure to a high-glucose medium reduced gap-junction activity in rat microvascular endothelial cells (167), and the reduction in dye coupling observed in these cells was correlated with a decrease in Cx43 mRNA and protein levels (Fig. 9), whereas the expression of Cx37 and Cx40 was not affected (167). Together, these data suggest that inhibition of gap junction–endothelial cell communication by an increase in glucose concentration may be involved in the endothelial cell dysfunction typically observed in diabetes (Fig. 9).

Glucose and diabetes may also alter vascular function by affecting gap-junction activity in vascular smooth muscle cells (102). In this regard, Kuroki *et al*. (95, 113) showed that high glucose levels reduce gap-junction intercellular communication of cultured aortic smooth muscle cells through the activation of a PKC-dependent pathway and the subsequent Cx43 phosphorylation (Fig. 9). In view of the importance of Cx43 gap junctions in smooth muscle cell proliferation and migration, as well as in vascular morphogenesis (55, 112, 124, 151, 214), the effect of high glucose on Cx43 gap-junction activity may contribute to the development of the diabetes-associated angiopathy. In addition, the permeability properties of the Cx43-derived gap-junction channel were reported to be altered in corporeal vascular smooth muscle cells isolated from streptozotocin-induced diabetic rats (14) (Fig. 9). As Cx43 appears to play a central role in the development of myogenic vasoconstriction, the alteration in the selectivity filter observed in the gap-junction communication of corporeal vascular myocytes may be involved in the diabetes-related erectile dysfunction.

The retina is a particularly sensitive tissue to be damaged by diabetes. Chronic hyperglycemia in diabetes is associated with the development and progression of pathologic changes in the retinal vasculature, and diabetic retinopathy is the leading cause of blindness in the working population (26). Retinal microvessels are well coupled *via* gap-junction channels, and, as may be expected, soon after the onset of streptozotocin-induced diabetes, the intercellular communication of the retinal microvascular network is disrupted (149). Therefore, the breakdown in the cell-to-cell organization *via* gap junctions may contribute to the early vascular dysfunction typically observed in diabetes (Fig. 9).

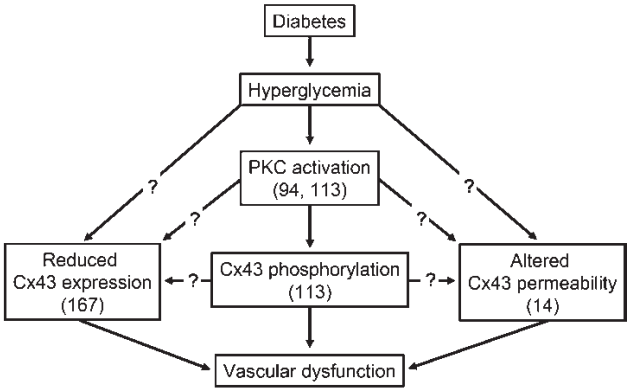

FIG. 9. Diabetes affects the Cx43-mediated communication in the vascular wall. The hyperglycemia associated with diabetes leads to a reduction in the gap-junction intercellular communication in the endothelial cells and smooth muscle cells through the activation of PKC (94, 113). The reduction in gap-junction communication induced by hyperglycemia is associated with a decrease in Cx43 expression (167), Cx43 phosphorylation (113), and changes in the permeability properties of the Cx43-based channels (14). Although the phosphorylation of Cx43 is mediated by PKC, the participation of this protein kinase in the changes of Cx43 expression and permeability remains to be established. Cx43 is an important gap-junction protein in the vasculature, and the disruption of Cx43-mediated gap-junction communication induced by hyperglycemia may contribute to the vascular dysfunction typically observed in diabetes.

Concluding Remarks

Gap junctions play a multifaceted role in the vasculature that is essential in the control of gene expression, vascular development, and vascular function. However, the function of gap junctions in the vasculature does not depend only on the molecular selectivity or permeability of the different vascular connexin isoforms. It is now evident that connexins work in concert, and thus, the same connexin isoform may have distinct and sometimes antagonistic functions, depending on organization of the connexins and the cell type of the vessel wall in which they are expressed (*e.g.*, the endothelial cells or the smooth muscle cells). In addition, the function of gap junctions may be influenced by the subcellular localization of the connexin-based channels. Gap junctions may be targeted to signaling microdomains such as tight junction, lipid raft, and caveolae (55, 147, 168). In accordance with that localization, connexins have been reported to be associated with cytoskeletal proteins and caveolin-1 (7, 56, 66–68, 147, 168). Such targeting positions the connexins in a strategic spatial relation with a variety of cell-

signaling cascades in which they may participate in cell–cell signaling both by direct protein-to-protein interactions and by coordination of signaling pathways between two neighboring cells (34, 55, 147, 200, 201). Therefore, simple analysis of the level of connexin expression is not sufficient to understand fully the participation of the gap junction in cardiovascular function in normal and pathologic conditions. In addition, the cell-type distribution and, if it is possible, the subcellular localization of the specific connexin isoforms must be determined.

The apparent co-regulation of the connexin expression is another potentially important point in the study of vascular gap junctions. That is, deletion of one connexin isoform may affect the expression of another isoform (96, 108, 123, 184). Interestingly, this interaction may traverse the cell boundaries, and connexins present in one cell of the vascular wall may be linked to the expression of connexins in the functional associated cell type, as observed by Liao *et al*. (123), who reported a reduction in the Cx43 levels in the smooth muscle cells of VEC $Cx43^{-/-}$ mice. Consequently, changes in one connexin may be secondary to the alteration of a different connexin, which leads to a demand for parallel analysis of multiple connexin isoforms, especially during the study of gap junctions in chronic vascular diseases.

Although the participation of the gap junction in vascular function seems to be very complex, the development of connexin-knockout animals has been a great contribution to our understanding of how these proteins work in the vasculature. In addition, connexin-mimetic peptides have demonstrated to be an effective tool to dissect the participation of gap junctions in vascular function. However, the potential manipulation of connexin function and/or expression in therapeutic interventions must await the expected future development of selective pharmacologic tools that allow targeting gap junctions in a connexin-isoform and cell type–specific manner.

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Abbreviations

ACE, angiotensin I–converting enzyme; Ach, acetylcholine; ATP, adenosine 5′-triphosphate; COX, cyclooxygenase; Cx, connexin; Cx43KI32, replacement of Cx43 by Cx32; DAG, diacylglycerol; EDHF, endothelium-derived hyperpolarizing factor; eNOS, endothelial nitric oxide synthase; ER, estrogen receptor; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; IK_{Ca}, Ca^{2+}-activated K^+ channels of intermediate conductance; IP_3, inositol 1, 4, 5-triphosphate; JGA, juxtaglomerular apparatus; K_{Ca}, Ca^{2+}-activated K^+ channels; 2K1C, two-kidney, one-clip model of hypertension; MEJ, myoendothelial junction; NAD^+, nicotinamide adenine dinucleotide; Na_v, voltage-gated Na^+ channel; NO, nitric oxide; NOS, nitric oxide synthase; PGI_2, prostacyclin; PKC, protein kinase C; PMA, phorbol 12- myristate 13-acetate; SHR, spontaneously hypertensive rat; SK_{Ca}, Ca^{2+}-activated K^+ channels of small conductance; T-Ca_v, T-type, voltage-gated Ca^{2+} channels; VEC $Cx43^{-/-}$, vascular endothelial cell–specific deletion of Cx43.

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