There are several a priori reasons why tight cell-cell coupling between cells of the vascular wall might be essential. First, smooth muscle and endothelial cells are small compared with the size of the vessels that regulate blood flow and pressure. Regulation is accomplished by minute, precise changes in luminal diameter, during which the activities of many cells must be coordinated over distances of several millimeters and to parallel vessels in the vascular bed. Second, neural control of the vessels involves the release of transmitters from discrete neuronal varicosities, which can be widely separated along the vessel axis, and a system of longitudinal cell-cell communication in the vessel wall ensures coordinated responses to neurotransmitters. Third, smooth muscle and endothelium are in a sense both detectors and effectors in vascular function, and the activation of either cell type leads to coordinated responses in the other.

Diffusion of paracrine molecules through interstitial space (e.g., prostaglandin, endothelin, and nitric oxide) provides one well-established and critical pathway for cell-cell communication. The importance of this mode of cell-cell communication in vascular function has been widely studied and is well understood. Accumulating evidence demonstrates that gap junctions offer an additional means of coordinating vascular cell function by providing a pathway for movement of electric charge and small signaling molecules directly from one cell to another. Gap-junctional communication avoids the dilution and nonspecificity associated with diffusional transit through the interstitial space and provides a pathway to coordinate signaling processes in multiple cells.

Cell-Cell Interactions in the Vessel Wall: The Processes

Gap junctions appear to provide important pathways for communication, both along and across the vessel axis. Previous reviews have described the basic features of the longitudinal communication process (3, 45) as well as transverse signaling across the vessel wall (3). In this review we will briefly summarize our current understanding of those processes, but our major intent is to highlight gaps in our knowledge of the ways in which vascular cell-cell interactions are mediated by the gap junctions. In addition, we will begin to explore how junctions in the vascular wall might influence vascular physiology and pathophysiology.

FIGURE 1 summarizes two general classes of communication that are known to occur in the vessels. Longitudinal electrical conduction (red lines) coordinates membrane potential changes along a segment of vessel and produces more uniform cellular responses. Membrane potential changes modulate the opening of voltage-sensitive Ca²⁺ channels, and this in turn coordinates vasomotor tone over vessel segments several millimeters in length. Such coordination may be critical in minimizing flow heterogeneity in the microcirculation (46). Longitudinal movement of Ca²⁺ (blue lines) may occur as well, but to date this has only been demonstrated in the capillaries of the lung and primary cultures of porcine coronary artery endothelial cells (12, 60).

Longitudinal signaling is complemented by radial movement of current or signaling molecules such as Ca²⁺ or inositol trisphosphate (IP₃) between the two cell types via gap junctions located at the smooth muscle-endothelial cell interface [myoendothelial junctions (MEJ)] (1, 2, 17, 58). Whether Ca²⁺ or IP₃ is directly involved remains to be shown. FIGURE 1 highlights the tight coupling between the smooth muscle and endothelium at the MEJ. This coupling allows a rise in Ca²⁺ in one cell to trigger complementary or opposing signaling events in the other cell. For example, activation of the smooth muscle cell with an associated elevation in vasomotor tone and smooth muscle cell Ca²⁺ can drive Ca²⁺ through the myoendothelial gap junction into the endothelium (13). The rise in endothelial cell Ca²⁺ can activate Ca²⁺-sensitive processes that antagonize or minimize the magnitude of the smooth muscle contractile event. Recently, expression of voltage-sensitive Ca²⁺ channels has been
detected in the lung microvascular and adrenal medulla endothelium (54, 57), which offers a new opportunity for interactions between smooth muscle and endothelium. For example, when changes in smooth muscle membrane potential are coupled to the endothelium through the MEJ, gating of voltage-sensitive Ca\(^{2+}\) channels in the endothelial cells can modulate activity of both Ca\(^{2+}\)-sensitive K\(^+\) channels and endothelial nitric oxide synthase.

Endothelium-dependent smooth muscle hyperpolarization is a process commonly attributed to a freely diffusible, endothelium-derived hyperpolarizing factor (EDHF). In the present context, it should be noted that it has been hypothesized that EDHF is in reality simple transmission of an electrical signal from endothelium to smooth muscle via the MEJ (8). The change in endothelial cell membrane potential has been attributed to activation of the endothelial cell Ca\(^{2+}\)-activated K\(^+\) channels of small and intermediate conductance (6, 16, 37). Also, an endothelium-dependent cAMP accumulation in smooth muscle cells has been proposed to facilitate electrical coupling via MEJ (23).

The importance of the radial communication process appears to be heavily dependent on the type of vessel under consideration (3). In large vessels with thick media, MEJs are absent or much less dense than in the resistance vessels, and a signal arising in the endothelium and passing through the MEJ is quickly dissipated among a number of smooth muscle cells, with little resultant medial electrical response (3). In the smaller vessels in a circumstance in which each smooth muscle cell may contact multiple endothelial cells and vice versa (15), the coupling of the two cells can be much tighter. However, the extent of this coupling also appears to vary greatly with the particular microvessel examined (43).

**FIGURE 1.** Cell-cell signaling in the arteriolar wall

Gap junctions are thought to coordinate changes in membrane potential (\(\Delta E_m\); red lines), cell calcium (blue lines), and perhaps inositol trisphosphate (IP\(_3\); black lines). Coupling may be homocellular (solid lines) or heterocellular connecting endothelium and smooth muscle (dotted lines). Note that the smooth muscle and endothelium come into direct contact only at the perforations in the internal elastic lamina. Note that voltage-gated Ca\(^{2+}\) channels are shown in the smooth muscle and endothelium. Movement of both Ca\(^{2+}\) and IP\(_3\) between the two cell types is indicated; however, unequivocal evidence for one or the other remains to be established. ER, endoplasmic reticulum; NOS, nitric oxide synthase; T + L, T- and L-type Ca\(^{2+}\) channels; \(\alpha_1\) and \(\alpha_2\), adrenoreceptors; M, ACh muscarinic receptor; \(B_2\), bradykinin receptor.
Early in the study of longitudinal communication, basic electrical cable theory seemed to predict the patterns of the axially conducted electrical response, and it appeared that longitudinal conduction might simply be a passive spread of current along the vessel through gap junctions. More recently, striking divergences from cable theory have been noted. Both electrical stimulation and ACh can induce remarkable responses that spread over very long distances with little or no decay in magnitude (10, 18, 21). Absence of decay in the response indicates that an active and ultimately energy-dependent process must underlie the conduction, similar to that seen in a neuron. The molecular or structural basis for this conduction is not understood, and it remains an exciting area for future vascular research.

The pathway for the conducted responses may be along the endothelium, the smooth muscle, or both (35), and the pathway may vary with preparation and with the tissue (18). How a signal arising from application of an endothelial cell-dependent agonist such as ACh can move along the endothelium and return to the vascular smooth muscle to initiate avasomotor response is also controversial (14). The MEJ is most often invoked as the pathway for such heterocellular communication, but several other, more complex methods of signaling have been proposed and, as will be noted below, may be necessary.

Wall Morphology and Connexin Distribution

It is usually assumed that the gap junctions unite smooth muscle and endothelium and provide the pathway for the radial and longitudinal communication described above. However, there is surprisingly little direct evidence for this, and until recently the absence of specific gap junction blockers or cell-specific gap junction knockout mice has precluded unequivocal assessment of the roles of particular gap junctions in vascular function. The presence of vascular gap junctions has been ascertained in three ways: electron microscopy, dye movement between adjacent cells, and electrical coupling. It is important to note that these measurements need not yield the same information (34). Although it is often assumed that dye and electrical measurements are comparable, this clearly is not necessarily so, and the relative magnitude of electrical and dye coupling may be dependent on the type, as well as the density, of connexins composing the gap junction (19, 34).

Gap junctions were originally identified in transmission electron micrographs (EM) as pentalaminar structures at points of close membrane apposition (FIGURE 2B, INSET) (20). In endothelium, long sections of well-defined gap junctions are commonly observed, and in freeze-fracture EM, collections of individual gap-junctional particles often form large aggregates (plaques) of a few tenths of a micrometer in diameter (47). An association between the gap junctions and the tight-junctional strands is also striking and quite consistent (19, 47), leading to the belief that tight- or adherens-junctional organization is an integral part of proper gap junction formation between cells (35). The physiological significance of this association between tight and gap junctions remains to be established.

In contrast to endothelium, gap-junctional plaques in arterial smooth muscle have proved more difficult to visualize at the EM level, and often the junctional membranes are relatively widely separated (FIGURE 2C) even though the cells are dye and electrically coupled. This has led to the suggestion that in vascular smooth muscle, individual gap-junctional channels rather than gap-junctional plaques are responsible for cell-cell communication (4). The striking accumulation of cellular organelles at regions of close smooth muscle-smooth muscle contact (FIGURE 2C) raises the possibility that there exist other modes of transmission between adjacent smooth muscle cells. Recently it has been reported that hemichannels that are membrane-resident but unconnected gap-junctional hexamers can gate and briefly open, releasing, among other things, ATP (30). Such ATP release might act as a signal between two closely apposed but physically unconnected cells.

The smooth muscle and endothelium approach each other closely at perforations in the internal elastic lamina to form the highly restricted space and regions of cell-cell contact, the MEJ. A classic example of such a MEJ is shown in FIGURE 2D. Such structures are usually assumed to provide physical proximity for the gap junction communication pathway between the two cells. However, close examination of FIGURE 2D and other published EMs of the MEJ rarely disclose the characteristic pentalaminar structure of a gap junction. More commonly one sees either the widely spaced membranes shown in FIGURE 2D or simply areas of ill-defined electron opacity. It is noteworthy that the classic MEJs shown in FIGURE 2D show membrane separation on the order of 20 nm, which is a much greater distance than can be spanned by the molecules that make up the gap junction (e.g., compare FIGURE 2, A AND B with FIGURE 2D). We note that the wide space shown in FIGURE 2D might also be bridged by substances released by endothelial cell or smooth muscle hemichannels if present. Also in contrast to endothelial junctions, there is a lack of demonstrable tight or adherens junctions at the MEJ, which might stabilize or organize gap-junctional plaques. Nevertheless, as
mentioned for smooth muscle cells, it is possible that only individual gap junctions at the MEJ provide the pathway for endothelial-smooth muscle cell communication. Thus, although the functional evidence argues strongly for the presence of myoendothelial gap-junctional coupling, much work remains to be done to define the location and molecular structure of the pathway connecting the two cells.

Of the >20 known connexins, only Cx37, Cx40, Cx43, and Cx45 have been identified in the vasculature (19). Cx37, Cx40, and Cx43 commonly occur in the endothelium and may coexist within a given plaque (47). The presence of Cx40 is especially consistent in the endothelium and has been resolved by immunocytochemistry, Western blot, and electron microscopy in a variety of vessels and several species (47, 50). Cx37 is also found consistently in endothelium. However, endothelial cell expression of Cx43 is more controversial and appears to vary with vessel size, vascular region, and species (25, 32, 50).

Connexin expression in smooth muscle is not as well described as in endothelium. Cx43 and Cx40 are commonly observed in smooth muscle, and recently Cx45 has been reported as well (31). Cx37, which is usually thought to be an endothelial connexin, was also reported in smooth muscle (25).

The reasons for the divergent reports on connexin distribution remain unclear, but it must be emphasized that the uncertainty may reflect the limitations of the available methodology. Because of the mixture of cells in the vessels, especially the small arterioles, it is often difficult to obtain adequate amounts of a pure cell type for Western blots, and thus assessments of connexin distribution in vivo depend to a large extent on immunocytochemistry. Unfortunately, the ability of an antibody to accurately localize the connexins will depend on such factors as the access of the antibody to the desired epitope, the local environment of the connexin, its phosphorylation state, and cross-reactivity of antibodies among the connexin isoforms (47).

The availability of specific and selective gap junction inhibitors could greatly enhance our

**FIGURE 2. Cell-cell junctions in the vessel wall**

A: gap junction representation and dimensions as determined by electron crystallography. A functional gap junction is composed of two opposing hexamers made up of 6 connexin proteins. The hexamers are called connexons or hemichannels. Each connexin protein spans the membrane 4 times. Red lines show the boundaries for plasma membrane, extracellular gap, and cytoplasmic space. Adapted from Ref. 53, with permission. B: transmission electron micrograph of the capillary endothelium. j, Sites of tight and adherens junctions. Inset: pentalaminar gap junction at high magnification. Adapted from Ref. 48, with permission. C: electron micrograph showing the close apposition of membranes but apparent lack of gap junctions in smooth muscle of the coronary artery (d). Arrow shows junctional organelles. Adapted from Ref. 4, with permission. D: electron micrograph of an arteriolar myoendothelial junction (MEJ). Adapted from Ref. 40, with permission.
understanding of the role of connexins in vivo. However, an inhibitor that blocks either all gap junctions or specific connexins with a high degree of specificity in all model systems has yet to be developed (51). Traditional inhibitors such as halothane, octanol, and glycyrrhizic acid metabolites have serious nonspecific effects (5). Recently, gap-junctional blocking peptides have been used in isolated vessel preparations and in cell culture systems with some selectivity and specificity (5, 9). However, because of cost and metabolic clearance, these molecules may not be useful in an in vivo setting. Development of glycoconjugates for a specific connexon blockade has become an exciting prospect for studying connexin function in the future (33).

**Regulation of Gap Junctions and Modulation of Function**

Isolated cell studies have demonstrated that the gap junctions can be regulated, although the functional consequence of regulation of connexins in intact vascular tissue remains virtually unexplored. All of the vascular connexins have phosphorylation sites on the COOH terminus, and both protein kinase A and protein kinase C can affect connexin phosphorylation. Accordingly, increases in intracellular Ca\(^{2+}\) concentration and cAMP may alter connexin protein phosphorylation and function (11, 19).

In addition to regulating connexin gating, phosphorylation may modulate assembly of connexons into plaques as well as modulate residence time at the junction (19, 42). The density of functional connexins at the membrane can be influenced by modulation of transport along cytoskeletal elements such as microtubules. Once at the plasma membrane, a gap junction's residence time is likely to be short, because they are recycled every 2–5 h (19). Junctions may be endocytosed into lysosomes and proteasomes, or the entire plaque may be autophagocytosed as part of the cycling process. Growth factors, cAMP, specific G proteins, and extracellular matrix proteins have all been shown to regulate individual gap junction internalization or plaque autophagy (19). It is remarkable that, although the literature is abundant on the regulation of the connexins, there is virtually nothing known of how these regulatory processes influence vascular function in vivo.

The function of the gap junctions may also be regulated by associations and interactions with a variety of other proteins. The association between the tight and gap junctions was mentioned above. Interactions among the connexins themselves may also play a role in expression, because it has been reported that modification of the expression of one connexin can alter the expression of another (32, 50). More recently, other specific protein-protein interactions have been defined, and there is accumulating evidence that the gap-junctional proteins can be targeted to selected membrane microdomains such as lipid rafts and caveolae (36, 44). Such targeting places the gap junctions in special proximity to other membrane proteins and might have a profound effect on the function of the gap junctions. For example Cx43 interacts with the second PDZ domain of the zonula occludens-1 (ZO-1) protein (22), an integral tight junction protein that mediates anchoring to the cytoskeleton. Apparently, ZO-1 not only tethers Cx43 at the tight junction complex but also participates in the transport of connexons from intracellular compartments to the cell surface (52, 56). Cx43 can interact directly with two distinct domains of caveolin-1 (44), a typical structural protein of caveolae. Likely molecular associations in the endothelium are illustrated in FIGURE 3.

Such selective localization of the connexins might serve multiple purposes. The tight junctions might simply provide physical support for gap junctions. Positioning of the gap junctions at tight junctions could also indirectly modulate channel activity by establishing and maintaining a special extracellular microenvironment in the space formed by apposed microdomains created by the junctional proteins. Such a system has been proposed to play a key role in cardiac muscle (e.g., see Ref. 27), and early findings are that the components of the junction may be similar in the vessel wall. A high degree of spatial organization could also place the connexins in close proximity to other highly relevant signaling molecules, such as kinases, phosphatases, nitric oxide synthase, calmodulin, plasma membrane ion pumps, adenylate cyclase, and ion channels, which might facilitate fine regulation of the connexons and coordinate the apposed signaling microdomains of adjacent cells. Although speculative at this time, FIGURE 3 highlights the interactive elements that are likely to be involved in intercellular signaling and emphasizes the fact that important gap-junctionally mediated signaling events may be confined to very localized regions of the endothelial cell as well as that new methods may be required to uncover such signaling processes.

**Physiological Expressions of Vascular Gap-Junctional Communication**

It is becoming evident that gap-junctional communication may be closely linked to overall vascular physiology or pathophysiology. Cx40 is involved in the control of vasomotor tone, as
hypertension associated with deletion of Cx40, endothelial cell-specific deletion of Cx43 causes hypotension. The origin of the hypotension is not at all clear, however, because the animals with this deletion also show elevation in plasma levels of both angiotensin II and nitric oxide (32). Selective deletion of vascular gap junction genes has revealed an important role of these intercellular channels in cardiovascular development as well. Cx45-deficient embryos show apparently normal initiation of vasculogenesis, but they then manifest major defects in remodeling and organization of blood vessels and failure to form a smooth muscle layer surrounding the major arteries (26). Cx43 knockout embryos die at birth as a consequence of blockage of the right ventricular outflow tract from the heart and manifest a variety of alterations in the pattern of coronary artery development (39). Cx37 deletion is not lethal, and its deletion has yet to be shown to produce any vascular phenotype or blood vessel-developmental defects, although the expression of this connexin is induced in vascular smooth muscle during coronary arteriogenesis (7). Cx37 is not inert, however, since simultaneous ablation of Cx37 and Cx40 results in severe vascular abnormalities and, ultimately, lethality (49). This emphasizes the concept that connexins may either work in concert or may be redundantly expressed and shows that competent endothelial coupling is essential for the normal development of the vasculature.

Connexins may also play a pivotal role in vascular disease. Kwak et al. (29) showed that in LDL receptor (LDLR) knockout mice, which have a pre-

FIGURE 3. Association of gap junctions with other membrane proteins in specialized membrane regions of the endothelium

Each of the proteins shown has been found in lipid rafts or caveolae, a location where connexins are also observed. The light blue area in the center represents what would be a highly localized signaling region and which interacts with cellular Ca^2+ stores. In the myocardi- um, such associations create highly localized environ- ments, and there is reason to believe that the association plays a key role in gap-junctional transmission (27). eNOS, endothelial NOS; VE-cadherin, vascular endothelial cadherin; Ca_v, voltage-dependent Ca^2+ channel; K_Ca, calcium-activated potassium channel; ZO-1, zonula occludens-1.
disposition to atherosclerosis, Cx43 was expressed in aortic endothelium at the leading edge of the atherosclerotic plaque. Moreover, in mice heterozygous for deletion of the Cx43 gene and homozygous for deletion of the LDLR gene, there was a significant reduction in the progression of atherosclerotic plaques (29). Thus upregulation of Cx43 in mouse aorta may enhance the formation of atherosclerotic plaques, implicating a role for connexins in vasculature pathology.

Consistent with the participation of gap junctions in vascular development and control of cell proliferation and migration is the observation that connexin expression is regulated in regenerating blood vessels in a highly specific manner. In smooth muscle cells, after mechanical injury, Cx43 expression was upregulated in the neointima of rabbit carotid artery, whereas no changes in the expression of this connexin were observed in rabbit iliac artery (38, 59). Interestingly, in cultured endothelial cells, wound-induced migration elicited an increase in Cx43 expression, a downregulation of Cx37 expression, and no change in Cx40. In cells transfected with dominant negative connexin inhibitors, the rate of wound repair was markedly reduced (28). These findings led to the hypothesis that stimulatory signals received by cells at the migration front of the developing plaque may be passed via Cx43 gap junctions to endothelial cells outside of the wound and that the inhibitory signals originating from endothelial cells in a confluent monolayer may not reach cells at the wound edge because of a lack of Cx37. Thus vascular connexins seem to be essential to the coordination of cell proliferation and migration during the wound-repair process and angiogenesis, which highlights the critical balance between pathological and/or homeostatic function that connexins may serve.

Summary

Connexins appear to play a central role in coordination of cellular function in the vasculature. Roles for the connexins in conduction and in cell-cell solute exchange are well established, and an extensive literature exists on the localization, regulation, and placement of the connexins in cell systems. However, much remains uncertain regarding both the morphology and the function of the connexins in intact vessels and in vivo. We know little about the specific functions of any of the vascular connexins. There is a divergence of opinion regarding the connexin isoform distribution within the vasculature, with little understanding of the rules governing expression. Although the literature is rife with references to the presence of gap junctions in MEJs and in uniting vascular smooth muscle cells, gap junctions are rarely seen in published EMs, an omission that must be remedied if the connexin isoforms that define the MEJ are to be established. The emerging structural information, our growing understanding of the regulation of the connexins, the discovery of new and specific connexin blockers, all combined with the increasing availability of genetically engineered mice offers us a wealth of new tools to investigate this important area.

We thank Dr. Jean-Louis Beny for critical review of this manuscript. We also thank Dr. Patricia E. Martin for stimulating conversations regarding hemichannels at the MEJ. David N. Damon and Kathleen H. Day were key participants in the discussions and the experiments that led to this manuscript.

In the interest of brevity, we have severely limited the number of citations in this review and relied heavily on reviews to place our comments in context. Accordingly, our references may fail to recognize precedence or key contributions, and for this we apologize. We wish to emphasize that all of the elements of Figure 1 and much of the text are based on the hard work of many individuals and groups of investigators, and although specific citations are not given, a literature search on a particular topic will yield many of the references consulted in writing this paper.