Published in final edited form as:

Microcirculation. 2009 May ; 16(4): 307–322. doi:10.1080/10739680902744404.

The myoendothelial junction: breaking through the matrix?

Katherine Heberlein^{1,2}, Adam Straub^{1,2}, and Brant E Isakson^{1,2,*}

¹Department of Molecular Physiology and Biological Physics, Charlottesville VA 22908 USA

²Robert M. Berne Cardiovascular Research Center, PO Box 801394, Charlottesville VA 22908 USA

Abstract

Within the vasculature, specialized cellular extensions from endothelium (and sometimes smooth muscle) protrude through the extracellular matrix where they interact with the opposing cell type. These structures, termed myoendothelial junctions, have been cited as a possible key element in the control of several vascular physiologies and pathologies. This review will discuss observations that have led to a focus on the myoendothelial junction as a cellular integration point in the vasculature for both homeostatic and pathological conditions and as a possible independent signaling entity. We will also highlight the need for novel approaches to studying the myoendothelial junction in order to comprehend the cellular biology associated with this structure.

Keywords

myoendothelial junction; microdomain; gap junction; heterocellular signaling; endothelium; smooth muscle

Introduction

The myoendothelial junction (MEJ) is commonly described as the structural location at which an endothelial cell (EC) or vascular smooth muscle cell (VSMC) extension protrudes through the internal elastic lamina (IEL), resulting in plasma membrane juxtaposition with the opposite cell type. The MEJ is an interesting structure in vascular physiology as it has been invoked to explain numerous pharmacological experiments detailing how stimulation of either EC or VSMC can have an effect on the unstimulated cell type, but almost nothing is known about what, if any role, the MEJ may have in these pharmacological events. This review will present some of the more descriptive data on the MEJ structure and how these may suggest a physiological significance for the structure.

The MEJ was first described in 1957 in a transmission electron microscopy (TEM) study of small arteries in the dog heart (67). A physiological function was later hypothesized for the MEJ as a structure that may serve as a conduit for the transfer of solutes between blood and the vessel (22). In 1967, Rhodin produced some of the most resolvable TEM images of the MEJ from rabbit kidney arterioles, revealing that the cytoplasmic density of EC extensions was many times greater than the nerve endings he observed innervating the VSMC (70). Based on the thick cytoplasmic density of the MEJs, it was hypothesized that these structures were possible localizations of specific cellular components, and that MEJs were “conductive devices for humoral transmitter substances” (70). These original hypotheses about the MEJ were based on anatomical observation without any direct tests of the structure’s function. Over forty years later, significant progress has been made in understanding the MEJ, however, testing for the

*to whom correspondence should be addressed, T: 434-924-2093, F: 434-924-6899, bei6n@virginia.edu.

physiological function of the structure is arduous and it remains difficult to place the MEJ in context of normal vascular physiology and/or pathologies. Further complicating this problem is the debate on the role of gap junctions at the MEJ and the exact nature of endothelium derived hyperpolarizing factor (EDHF). This review will attempt to place the current knowledge about the MEJ into the context of its possible physiological functions.

Ultrastructure of the Myoendothelial Junction

The MEJ is described as a cellular extension protruding through the IEL and is approximately 0.5 μm in width by 0.5 μm in depth (although this is highly variable depending on the IEL thickness), as shown by TEM (70;78;91). Generally, the frequency of MEJs increases with decreasing vessel size. The exact number of MEJs per cell is variable depending on the type (e.g., vein vs. artery) and diameter (e.g., arteriole vs. artery) of the vessel being studied, however, due to the arrangement of the EC and VSMC in the vasculature (31), a first-order arteriole could have approximately seven MEJ structures per EC and three MEJ structures per VSMC (78;91). Although MEJs are usually observed in smaller resistance arteries and arterioles, they have also been identified in the veins and aorta during development (65) as well as in veins and larger vessels in the pulmonary circulation (89;93). There is currently no explanation regarding the diverse distribution of the MEJs in veins or the pulmonary circulation.

At the MEJ, the plasma membranes of EC and VSMC come into close apposition (Fig 1), producing three distinct “types” of MEJs as observed in the literature. In order of prevalence, these include: 1) EC extensions through the IEL that make contact with the VSMC, 2) VSMC extensions through the IEL that make contact with the EC, or 3) both EC and VSMC extensions into the IEL (65). While extensions from the VSMC to the EC layer are seen, EC extensions are typically the most prominent regardless of vessel type or location (65), indicating that possible signals from the VSMC may induce morphological changes in the EC. There are also differences between vessels regarding the way cellular extensions form, being either flat appositions of EC and VSMC or club shaped extensions. It is not clear why these two shapes have developed as MEJ structures, although explanations for the observed variance in shape may concern the potential function for each MEJ shape. It is possible the club-shaped protrusions could provide an increased surface area, potentially facilitating the transfer of signal (electrical or chemical) between cell layers, however this has not been studied.

Ultrastructure of the myoendothelial junction: *gap junctions*

Gap junctions are dodecameric channels composed of two hexameric hemichannels, with each adjacent cell's plasma membrane contributing one hemichannel to the junction. These highly regulated channels allow direct cytoplasmic transfer of chemical and electrical signals between cells (for review see, (5)). In 1973, Johnson et al demonstrated for the first time that cellular extensions >10 μm from two different cells could form heterocellular gap junctions, as revealed by freeze-fracture TEM images, detailing the typical gap junction hexagonal array in the protoplasmic face (P-face) and the extracellular face (E-face) (45). In 2000, Sandow et al performed exquisite 3D reconstructions of the MEJ from sequential TEM sections which confirmed that very small gap junction plaques could exist between EC and VSMC (78). However, there are also reports that demonstrate close apposition of EC and VSMC at the MEJ, but no observable pentalaminar structure resembling gap junction plaques (12;30). These observations bring to the forefront the difficulty in studying not only gap junctions at the MEJ, but the structure as well. Additionally, fixation issues associated with TEM may pull MEJs apart (for example, MEJs with flat appositions as seen in arterioles (91)) and it is also reported that the pentalaminar structure typically associated with gap junction plaques are found in very

small clusters at the MEJ (78), making it unlikely that gap junction plaques could always be satisfactorily resolved.

Dye transfer experiments designed to test permeability of open gap junctions in hamster cheek pouch arterioles and rat mesenteric arteries demonstrated the potential presence of heterocellular gap junctions, showing the transfer of dye between the EC and VSMC (56;63). A more physiologically compelling argument compared the membrane potential of opposing EC and VSMC which were shown to be similar (19), suggesting functional coupling of the two cell types, thereby confirming earlier works by Xia, et al (102;103). However, evidence has also suggested that gap junction coupling at the MEJ may be limited or non-existent (6; 82;84). For example, in the mouse cremaster vascular bed, the conducted responses in EC and VSMC were independent of each other (6) and separate experiments in the same vessel type show that not only were the membrane potentials of the EC and VSMC different, dye could not pass between cell types, which directly opposes observations made in previous work (84).

The ability to identify functional gap junctions at the MEJ is further complicated by a lack of specific gap junction inhibitors (71). Typical gap junction inhibitors such as heptanol or octanol contract plasma membranes, subsequently acting on a large number of other plasma membrane channels. Carbonoxolone and its derivatives (e.g., 18 α -glycyrrhetic acid) were considered to be relatively specific gap junction inhibitors, however they have recently been shown to induce an up regulation of gap junction channels (73). There has also been an on-going interest in peptides derived against the extracellular loops of the proteins that compose gap junctions, connexins, which in principle could specifically and reversibly inhibit gap junction channels (20). These peptides work well in in vitro preparations (e.g., (62)), and have been shown to be useful for many in vivo preparations as well (e.g., (11)). However, within in vivo preparations, concentrations of the peptides are reported to be as high as 600 μ M and there are additional reports that they are not efficacious in rat mesentery (63). Furthermore, whether the gap junction inhibitors are acting on homocellular or heterocellular communication complicates the interpretation of pharmacological closure of gap junctions to an even greater extent.

While it appears gap junctions may be localized to the MEJ, whether they are present in every vascular bed and perhaps even more importantly if they are open or closed, remains unknown. The proteins that compose gap junctions, connexins (Cx), impart selectivity of the gap junctions to various solutes and therefore may be important for fine regulation of physiological function (e.g., (18)). It is for this reason that identifying particular connexins at the MEJ is important and antibodies are commonly used to identify the different isoforms. Antibodies have been made against the different tertiary structures and phosphorylation states of Cx43 (90), highlighting the multiple post-translational modifications these proteins can undergo, making the connexins, particularly Cx43, sometimes difficult to identify. However, it should be noted that connexin antibodies can be highly cross-reactive and subject to fixation artifacts, making their efficacy in identifying connexins variable (23;39;83)

Four connexins (Cx37, Cx40, Cx43, and Cx45) have been identified in the vasculature, depending on the species and vascular bed (e.g., (30;38;58;79;94)) and all but Cx45 have been identified at the MEJ (32;38;80). Evidence for functional gap junctions with particular connexin isoforms at the MEJ was shown in studies from rat mesentery, in which Cx40 antibodies were loaded into EC of rat mesenteric arteries and found to block carboxyfluorescein dye transfer from EC to VSMC, as well as EDHF (63). Using immunolabeling on TEM sections, other studies have found Cx37 and Cx40 localized at the MEJ in rat mesentery, and rat cerebral vessels (32;80) while Cx37, Cx40 and Cx43 were found in mouse mesentery (74). Recently, semi-quantitative analysis of antibody binding on actin-bridges between EC and VSMC in mouse cremaster arterioles identified Cx40 and Cx43 as

the most commonly expressed connexins on the actin bridges, with Cx37 being the most variable, and Cx45 being the most difficult to detect (38). One possible explanation for the differential localization of connexins at the MEJ may lie in their ability to be differentially regulated by particular second messengers for opening and closing at the MEJ. For example, both Cx37 and Cx43 gating has been shown to be regulated by nitric oxide (NO; (46;105)), which may have important implications for control of vessel tone. In addition, an abundance of work on connexin phosphorylation has demonstrated that multiple serine and/or tyrosine residues, typically on the carboxy tail of the protein, may be key regulation sites for the opening and closing of gap junction channels (for review, see (51)) and all of the connexins currently identified at the MEJ have phosphorylation sites (21). Recent evidence has indicated that Cx43 is phosphorylated at the serine 368 residue at the MEJ in mouse cremasters in vivo (38). Phosphorylation at this particular residue is associated with protein kinase C, and has been shown to initiate a more closed confirmation state of the gap junction channel (52). However, more research into possible effect of connexin phosphorylation on heterocellular communication is required to understand what this observation means.

Because differential expression of connexin isoforms at the MEJ are seen in vivo and in vitro (e.g., (32;38;80)), this may indicate that the connexins at the MEJ are selectively organized into gap junctions to regulate which solutes may move between the two cell types. This concept has been difficult to test in vivo because it has been shown that knockout of Cx40 can cause up-regulation, down-regulation, or re-distribution of other connexins (39;49;86) and conditional knockouts of Cx43 can have a dramatic effect on a large number of other genes (36). Therefore, experiments from these animals become difficult to interpret. When connexins at the MEJ were individually altered in an in vitro model of cremaster arterioles, the movement of inositol 1,4,5-trisphosphate (IP₃) and/or Ca²⁺ between EC and VSMC were not altered (37). The data suggests that particular connexin isoforms at the MEJ may not be as important for specific solute movement through the channels, but may be more important for their regulated opening and closing (e.g., via phosphorylation) in response to particular stimuli. However, this concept has yet to be tested in vivo.

Ultrastructure of the myoendothelial junction: *Additional components*

A persistent question regarding the ultrastructure of the MEJ is why there are no identifiable adherens or tight junctions. There is a close association between tight junctions or adherens junctions and gap junctions (85;97), however, while there are reports, both in vivo and in vitro of N-cadherin being present between EC and VSMC (72), classical TEM evidence of adherens or tight junctions has yet to be identified. This issue needs to be resolved because not only can tight/adherens junctions provide another mechanism for cellular signaling (e.g., (66)), but a lack of these junctions would indicate the first identified instance of gap junctions providing the only structural linkage between two cell types.

Ultrastructural evidence from TEM has revealed clues as to a possible physiological function of the MEJ, however some controversy does exist, in particular regarding the exact nature, role, or presence of the gap junction channel. The disparity may be due to MEJ structure (flat apposition versus club shaped interface) or closing of the gap junction channel due to phosphorylation or other factors (e.g., NO). Despite the ongoing debate regarding the full role of gap junctions at the MEJ, there are several lines of evidence demonstrating the potential for signaling between EC and VSMC at the MEJ.

Intercellular communication from vascular smooth muscle to endothelium

The MEJ potentially allows for the transfer of signals from VSMC to EC and it has been hypothesized that this signaling may be an important component to the control of vessel tone. For example, application of phenylephrine (PE) causes a vascular constriction, quickly

followed by a small vasodilation and it was thought that a signaling molecule transferred from VSMC to the EC induced the dilatory response. If increases in VSMC intracellular calcium concentrations ($[Ca^{2+}]_i$) do stimulate the production of vasodilators in EC, the increase in EC $[Ca^{2+}]_i$ should result in EC derived NO. In experiments by Dora et al, NO production was inhibited with L-name, which attenuated the vasodilatory response after application of PE, implying a transmitted signal from VSMC to EC (15). Additional evidence showed that loading EC with BAPTA was able to significantly reduce the vasodilatory response after PE stimulation, indicating a diffusible molecule from VSMC could induce an increase in EC $[Ca^{2+}]_i$ (106). In support of this, transient increases in EC $[Ca^{2+}]_i$ after VSMC stimulation showed a distinct time interval between the point of VSMC stimulation and subsequent increases in EC $[Ca^{2+}]_i$, where increases in PE concentrations caused a decreased time interval between stimulation and EC $[Ca^{2+}]_i$ increase (47). Treatment with gap junction un-couplers demonstrated a decrease in EC $[Ca^{2+}]_i$, suggesting gap junctional coupling of EC to VSMC via the MEJs (42;47). Taken together, these data strongly suggest a chemical mediator transferred at the MEJ causes an increase in EC $[Ca^{2+}]_i$.

Phenylephrine acts on the VSMC by binding to $\alpha 1$ -adrenergic receptors on the VSMC membrane, activating phospholipase C, which produces an increase in IP_3 (44). Initial experiments demonstrated that by blocking phospholipase C production, there is a decrease in EC $[Ca^{2+}]_i$ following VSMC stimulation with vasoconstrictors (50). In vitro studies went on to show that both IP_3 and Ca^{2+} can move from VSMC to EC (42). This work was confirmed by the observation of IP_3 -based signaling in EC originating from holes in the IEL (47;55). The inositol 1,4,5-trisphosphate-receptor (IP_3 -R) specific isoform responsible for this signaling event was determined to be IP_3 -R1 on the EC side of the MEJ based on immuno-TEM and functional studies of IP_3 movement from VSMC to EC (37). These data suggest it is the binding of IP_3 (and possibly the priming of IP_3 -R1 by Ca^{2+} (4)) that mediates the increase in EC $[Ca^{2+}]_i$ (37;47;50). The IP_3 molecule is a ubiquitous cellular second messenger responsible for internal Ca^{2+} mobilization and it is possible that IP_3 in EC may be capable of activating eNOS (indirectly through Ca^{2+} release at the ER) or directly by store-operated channels, e.g., TRPV4, which has been shown to be necessary for initiation of the EDHF response (74;96). Taken together, the increase in EC $[Ca^{2+}]_i$ is considered an important component in the induction of vessel dilation and so the rapid activation of Ca^{2+} stores at the MEJ by VSMC derived IP_3 hypothetically ensure continuous and rapid control of vessel tone.

Despite indications of signal transfer from the VSMC to EC, early studies were unable to demonstrate robust dye transfer (e.g., (56;84)). Although dye transfer is not always indicative of cell coupling, the lack of transfer suggests caution should be used when interpreting intercellular communication from the VSMC to EC. The discrepancy in the literature regarding the presence of dye transfer between EC and VSMC again highlights the difficulty commonly encountered when studying the MEJ.

Intercellular communication from endothelial cells to vascular smooth muscle cells

It has also been suggested that MEJs may play a role in the dissemination of a gap junction mediated signal from the EC to the VSMC, with experiments showing dye transfer from EC to VSMC (56;63). Although a number of endothelium derived dilatory signaling molecules such as arachidonic acid, EETs and NO exist (2;7;8), one of the most studied forms of EC to VSMC communication is known as EDHF and it has been extensively reviewed (e.g., (2;7)). Gap junctions have been implicated in the EDHF response, specifically in conjunction with endothelial K_{Ca} channels. When gap junctions were uncoupled, $K_{Ca}3.1$ channels appeared to provide the predominant input for EDHF mediation (16). This response is seen with application of acetylcholine (ACh; presumably EC specific) and an increase in calcium via TRPV4

channels occurs, hyperpolarizing the EC (96). The hyperpolarizing current from EC to VSMC is shown to be attenuated by K^+ channel blockers, including apamin, TRAM-34 (16), charybdotoxin, iberiotoxin (17;84), TEA (26), and clotrimazole (61). By inhibiting K^+ channels throughout the vessel in the additional presence of gap junction uncouplers, there is a near complete abolition of the hyperpolarizing response in VSMC, however the inability to specifically target gap junctions at the MEJ remains problematic (16). With the recent identification of calcium activated potassium channels ($K_{Ca3.1}$ and $K_{Ca2.3}$; (16;55;76)) and ER (42;55) at the MEJ, there is a strong implication that these channels may be important components in the EDHF mechanism.

Although the movement of signal through gap junctions at the MEJ is commonly used to explain the EDHF phenomenon, this may not always be the case. It has been shown that treating rat mesenteric EC with Cx40 antibodies causes an inhibition of the EDHF dilatory response (63). However, these experiments were performed at both high and low PE stimulation and the variance in PE concentration resulted in two different dilatory responses (63). In vessels stimulated with low PE, there was an increase in the time necessary to return to basal diameter and high PE stimulation completely attenuated vessel dilation, suggesting control of the dilatory response may also be regulated by non gap junction mediators (e.g., EETs (2;64)). In addition, the abundance of MEJ structures may not correlate with magnitude of the EDHF response (88;93). In pulmonary arteries, there is a prevalence of MEJs; however, there is no corresponding EDHF response (93). Differences in EDHF responses in male and female rats have also been shown, however no significant difference in the number of MEJs between the two sexes were apparent (88). While there may be a role for the MEJ in the EDHF response, more research is required to better understand the possible contribution of the MEJ to the EDHF phenomena (14).

The conducted response: integration of EC and VSMC intercellular communication?

The conducted response in the vasculature is a coordinated constriction or dilation down the length of an arteriole after stimulation with an EC or VSMC agonist (for review, see (3)). This event involves a coordinated change in cell-to-cell membrane potential from the point of agonist stimulation. Early work regarding the conducted response indicated that heterocellular gap junctions may have been crucial to the spread of such a signal (104). Near identical electromechanical responses in the EC and VSMC at the point of stimulation and at points distal from the stimulation site, indicated continual electrical coupling between the two cell types, presumably through gap junctions at the MEJ (104). Although the start of the conduction response may rely on cell specific stimulation, electromechanical responses could spread in an intercellular pattern back and forth across MEJs down the length of a vessel, (e.g., a signal from EC to VSMC, and back to EC from VSMC), essentially coordinating the two cell types in an overall vasomotor response.

An accumulation of data now suggests that conduction may not be conducted by gap junctions at the MEJ in some vascular beds (e.g., (6;84)). Budel et al demonstrated in mouse cremaster that disruption of the EC layer abrogated the vasodilatory responses to bradykinin and potassium chloride (KCl), but the dilatory response to ACh was unchanged, suggesting the two responses were separately mediated (6). Further studies in the same vessel type demonstrated that the equilibration of membrane potentials between EC and VSMC was weak (84). From the same vascular bed, phosphorylation of Cx43 at S368 was shown to be present between EC and VSMC, indicating that although gap junctions may be localized at the MEJ, the channels may be closed (38). It should be noted that these experiments do not disprove homocellular coupling among EC and VSMC, which is likely a central component to the conducted response (e.g., (24); reviewed (13)). Additional studies suggest that although the

VSMC and EC layers may not be functionally coupled (82;98), the hyperpolarization of EC following ACh stimulation may be sufficient to induce the conduction response in the VSMC (99). While evidence suggests that gap junctions at the MEJ may not be central to the conducted response, this does not eliminate the MEJ as a location where other signaling components, such as eNOS (28) and caveolae (74) may be sequestered as part of a paracrine-based cell signaling domain.

The myoendothelial junction as a cell signaling microdomain

Signaling domains are localized areas within a cell that organize proteins and structural components in order to facilitate a particular cellular function (for review, see (54)). Rhodin's 1967 ultrastructure paper refers to the MEJ as a potential "nexus" for signaling between the two cell types (70). As identification of proteins at the MEJ begins to highlight potential signaling domains, this description continues to hold true (e.g., (77)). However, identifying possible proteins at the MEJ is difficult using standard immunocytochemistry, due largely to the small size of the structure and its location between two monolayers of cells.

The two main methods for identifying proteins at the MEJ have been the use of immunolabeling on TEM sections and immunofluorescence on holes in the IEL. Although immunolabeling on TEM sections is considered the most definitive, it is a process that can be both time consuming and difficult to quantify. Immunofluorescence on holes in the IEL is a more time efficient process with results that are easier to interpret, but has an inherent difficulty in proving the holes represent the sites of cellular MEJ structures. Recently, a new technique to observe protein localization between EC and VSMC (presumably the MEJ) utilized confocal microscopy on intact arterioles by measuring the intensity of fluorescently tagged proteins on actin bridges between EC and VSMC monolayers and the proteins localized to this region (38). Although fluorescently tagged proteins on the actin bridges correlate with TEM images of MEJs in the same tissue, there is currently no definitive evidence to confirm the visualized actin bridges are MEJs (similar to above). Despite the difficulties, all of these techniques have provided preliminary evidence supporting the targeting of specific proteins to the MEJ.

The expression of IP₃-Rs as seen using immunofluorescence at holes in the IEL, suggests the localization of these receptors at the MEJ (55). Using immunotechniques on actin bridges as well as in TEM images, the specific IP₃-R1 isoform was shown to be selectively localized to the EC side of the MEJ (37). In comparison to the selective localization of IP₃-R1, type 1 5-phosphatase (5-P) appears to be concentrated in the VSMC extension of the MEJ (37). Because 5-P can rapidly breakdown IP₃, the localization of 5-P and IP₃-R1 suggest IP₃ may preferentially act on the EC layer following stimulation of the VSMC, while any IP₃ produced from EC stimulation is likely broken down before it can bind to IP₃-Rs in the VSMC (37). Based on these observations, the specific localizations of IP₃-R1 and 5-P may serve to direct the IP₃ mediated response in a highly coordinated manner between the EC and VSMC.

In adult rat myocytes there is a suggested interaction between the Na⁺/K⁺ ATPase and the IP₃-Rs (92). The ATPase pump has been implicated in a variety of protein interactions crucial to the control of IP₃ and IP₃-Rs as well as Ca²⁺ signaling (92). It has been hypothesized that this binding may be crucial for the regulation of the controlled release of Ca²⁺ from the ER (92). The pump has also been shown to be localized within caveolae at the plasma membrane, which when considered in concert with recent immunofluorescence data from rat mesenteric arteries, suggests a possible localization of the ATPase pump to the MEJ. Localization of the Na⁺/K⁺ ATPase with K⁺ channels in the MEJ may help to explain the suggested role of this molecule in the EDHF phenomenon (16;17).

Pharmacological and descriptive evidence has highlighted the possible presence of K_{Ca}3.1 and K_{Ca}2.3 at the MEJ (16;55;80). In 2006, K_{Ca}3.1 channels were shown to localize in holes of

the IEL as well as at the MEJ in the rat mesentery whereas the $K_{Ca2.3}$ channels were only shown to localize between adjacent EC (80). Localization of the $K_{Ca3.1}$ channels was confirmed in separate experiments (16;55) and in addition, Dora et al also presented evidence for localization of the $K_{Ca2.3}$ channels at the MEJ (16), which may suggest a difference in antigenicity of antibodies used or a heterogeneity in expression of the channels at the MEJ. The $K_{Ca3.1}$ channel has been associated with a calcium sensing receptor (CaSR), although neither were localized in caveolin enriched fractions from EC (100). The CaSR is a member of the G-protein coupled receptor family and is sensitive to changes in the extracellular Ca^{2+} concentrations (9;10). It has a proposed role for maintaining Ca^{2+} homeostasis through activation of a variety of intracellular pathways and may be functionally coupled to K_{Ca} channels (107;108). Currently there is no direct pharmacological or descriptive evidence to support specific MEJ localization. However, the potential targeting of the CaSR to the MEJ and suggested interaction with K^{+} channels coincides with the hypothesized role of K^{+} channels at the MEJ to moderate the EDHF response as previously described from within this microdomain (16).

Within areas of cell-to-cell contact such as the MEJ, it is probable that some form of linker proteins stabilize these interactions. Besides connexins, the current most likely candidate appears to be N-cadherin. In co-culture models of EC and VSMC, N-cadherin was identified as a protein that enabled EC and VSMC to mediate adhesions and form tight junctions (27; 72). N-cadherin has also been identified in vivo on actin bridges linking EC and VSMC in the mouse cremaster arterioles (38). With the intimate link between N-cadherin and connexins (97), it is possible the interaction between these two proteins at the MEJ may act to stabilize the EC-VSMC interaction.

The localization of ER in very close proximity to plasma membranes is indicative of specific signaling events and likely represents a localization of specific signaling molecules (e.g., (35)). Endoplasmic reticulum was first identified at the MEJ in TEM sections from mouse cremaster arterioles (42) and further pharmacological and immunocytochemistry evidence from in vivo preparations has confirmed its existence at the MEJ (55). Based on in vitro cell culture models, it was hypothesized that this structure was a reservoir for intracellular Ca^{2+} , to be released upon activation of IP_3 -R by IP_3 derived from VSMC (37;42;47;50). The reservoir of Ca^{2+} , especially in proximity to caveolae and the plasma membrane, again provides strong circumstantial evidence for the MEJ acting as a signaling domain. Although an abundance of evidence currently suggests targeting of different proteins to the MEJ, a question that remains to be answered is how these proteins come to be organized at the MEJ.

Caveolae are lipid rich invaginations of the plasma membrane that play a major role in the mobilization and organization of signaling proteins. They are found in especially high concentrations in EC and are thought to play a role in a variety of cell signaling pathways and the trafficking of proteins to localized regions of the cell (69). Recently, these structures were identified using TEM in mouse mesenteric arteries at the MEJ, and were co-localized with connexins and caveolin-1 (cav-1) (74). The localization of caveolae at the MEJ provides circumstantial evidence that there is signaling domain organization occurring at the MEJ. Caveolin-1 has been associated with each of the IP_3 -Rs (43;74), Cx43 (74), Na^{+}/K^{+} ATPase (57;92) and $K_{Ca2.3}$ channels (1;74), all of which have been shown to localize at the MEJ. Beyond caveolae, there is a requirement for both structural organization and additional transport of proteins to the MEJ. Both actin and microtubules have been identified as cytoskeletal components within in vitro models of the MEJ (38;41). These basic cellular structures would not only be required for specific trafficking to the MEJ, but are in many cases required to organize signaling domains (e.g., (34)). Evidence supports an interaction between cav-1 and microtubules, which could provide for the rapid movement of proteins to and from

the MEJ (34). However, there is currently a lack of in vivo evidence for any cytoskeletal components at the MEJ.

When taken together, there is promising evidence for a signaling microdomain at the MEJ (Fig 2). This includes the observed localization of protein receptors, channels and signal mediators such as 5-P. Furthermore, the proposed interaction of cav-1 with several of these proteins suggests they may be selectively organized at the MEJ. Much research is still required to understand how the localized proteins interact, and due to the inaccessibility of the MEJ in vivo and the difficulties associated with protein identification and isolation, it is possible that a cell culture model of the MEJ will be required to extend these studies.

Models of the Myoendothelial Junction

In order to better understand the pathologies and potential cellular physiology that occurs at the MEJ, a mechanistic approach to studying the structure is required. Unfortunately the need for such a study of the MEJ is hindered by the structure's size and location in an intact vessel, making in vivo interpretations of its function almost entirely reliant on pharmacologically induced effects on a vessel. While these experiments can be both insightful and provocative, they can only suggest the cellular biology at the MEJ, and therefore other methods to study the MEJ may need to be considered.

Although cell culture represents an artificial model of tissue integration, the advantage of utilizing cell culture lies in the capacity to understand what may occur at the cellular level, coupled with the ability for an ever-expanding range of experimental manipulations. Several studies have utilized a monolayer mixture of cultured EC and VSMC to examine connexin expression and by using dye transfer were the first to show that gap junctional coupling and connexin expression may be unique between the two cell types (62;81). It should be noted that such a system does not allow for the direct study of the MEJ, only the actual interface between the two cell types. In a more recent model of the MEJ, a vascular cell co-culture (VCCC) allows for the growth of EC and VSMC monolayers on opposite sides of a porous Transwell insert, facilitating the formation of cellular extensions between the two cell types, mimicking an in vivo environment ((40); Fig 3). This model allows for protein identification at the MEJ that has thus far been similar to that observed in vivo (40) and has also allowed the study of heterocellular Ca^{2+} signaling, (42), the results of which have also been replicated in isolated vessels (47;50). The VCCC offers the versatility of using cells cultured from any number of animal models, providing a means for studying a variety of phenotypic and pathological conditions.

As with all cell culture systems, the VCCC model of the MEJ is not without problems. As a co-culture model, it can be difficult to relate cell culture data to in vivo equivalents. The VCCC system contains pore lengths that are approximately 10 μm in distance, a range of up to 10 times longer than that observed in vivo (40). In addition, the VCCC model originally did not incorporate the flow typically seen in in vivo models, but this has recently been addressed by Hastings et al in which the VCCC model was modified to introduce flow (33). These modifications highlight the versatility of the VCCC and its ability to be adapted per experimental need.

As demonstrated above, cell culture models can show potential cellular mechanisms at the MEJ that would otherwise be difficult to initiate or observe on an intact system. Therefore, the coupling of traditional in vivo experiments on vessels with cell culture models can serve to enhance the studies of signaling mechanisms and physiological functions associated with the MEJ.

Possible pathologies associated with myoendothelial junctions

Because of the potential of the MEJ to facilitate chemical and/or electrical signals between EC and VSMC, it is also being studied as a potential mediator of vascular disease. At the ultrastructural level, the total number of MEJs in the vasculature increases during hypertension in SHR rats (75). However, subcutaneous fat arteries biopsied from hypertensive preeclamptic women confirmed a morphological obstruction of intracellular contacts through the IEL and a lack of MEJs (59). In another study, experimentally induced hypoxia also increased the number of MEJ structures in a vessel (87). Here again, the difficulty in studying the MEJ can be seen in the directly opposing nature of data regarding changes in the MEJ in pathological conditions. Ultrastructural observations also indicated that extensive projections from the VSMCs established contact with ECs rather than ECs establishing contact with VSMC (87). In sum, the data have led investigators to suggest that MEJs may play a role in the promotion of cell growth, especially in response to vascular remodeling (65), possibly angiogenesis, and the progression of disease development.

Endothelium derived hyperpolarization factor, as mentioned in previous sections, may play an important role in the regulation of vessel tone. When this regulation is compromised, disease states such as hypertension may occur. Because MEJs may help to mediate the EDHF response, it is possible that they may act in a compensatory manner in vascular disease states as well. In spontaneously hypertensive rats (SHR), the observed VSMC hyperplasia and reduction in VSMC coupling in the caudal artery correlated with an increased incidence of MEJs (75). Because of the decreases in homocellular VSMC coupling, the ability to maintain a dilatory response may be compromised, which may suggest the observed increases in heterocellular coupling via the MEJ could be compensatory and could allow for EDHF mediated relaxation to counteract the pathological state (75). However, hypertensive preeclamptic women, there is an observed decrease in MEJ occurrence in myometrial arteries, which coincides with a decrease in EDHF response, compared to normal pregnant women (48;53). There is also an observed significant reduction of EDHF response in obese and diabetic rat models (25;60; 101), however, in a mouse model of type II diabetes, no change in the overall contribution of EDHF-induced relaxation was apparent (68). Taken together, these studies demonstrate that EDHF induced relaxation may be important in a variety of pathological conditions and warrants a more mechanistic approach in order to understand the role of MEJs in regards to the EDHF phenomena in the development of hypertension.

Based on these observations, there are several questions that remain to be answered: why is there a variation of MEJ frequency in some disease states? Is the increase or decrease of MEJs an adaptive response to pathological states and if so, do various model systems of disease or different diseases (e.g. preeclampsia, diabetes, and hypertension) contribute to differential regulation of MEJs during the disease? What triggers the MEJ changes during vessel remodeling and what regulates the increased invagination through the IEL to initiate this structural formulation? While many of these questions have not been answered, it is likely that in a normal tissue, the process would require localized degradation of the IEL and a reorganization of the cell membrane to allow for cellular extensions from the EC or VSMC to form a MEJ.

Mechanistic studies into this particular area have not yet been attempted, but some potential insight into the formation and retraction of MEJs may be garnered from EC podosomes. Podosomes are formed as part of an inflammatory response and are visualized as small holes (~0.5 μm in diameter) in EC (95). Indeed, inflammatory stimulation of podosomes promotes MMP activation to generate holes in the IEL (29;95), which may allow for cellular infiltration and an increase in MEJs. If so, it would suggest that vascular inflammation may be an important

part of myoendothelial communication and that the actual MEJ structure may retract or form in response to particular stimuli.

Since there is currently little data concerning an initiating role of MEJs in vascular pathologies, these observations suggest that MEJs may form as an adaptive or contributive response during disease development. However, given that there is little known about the MEJ overall, more detailed, mechanistic studies of the structures may expose a novel role in both the physiology and pathology associated with the several different vascular disease states already alluded to.

Conclusions

The MEJ has emerged as an exciting area of study in the field of vascular physiology and it could be concluded that the initial hypothesis put forward by Rhodin over 40 years ago concerning the physiological function of the MEJ may be correct. However, it is obvious that for the evidence citing the MEJ in a particular cellular function, there is also evidence against it. Differences in number of MEJs, response of the MEJ to various stimuli, functionality of the MEJ, even location of the MEJ are still in question. As such, further work, including, but not limited to, mechanistic studies of the MEJ, a clear determination of whether the MEJ is a cell signaling domain, and the potential role of gap junctions is still required to understand the function of the MEJ and its role in vascular disease. Only with the combination of traditional pharmacological methods and novel molecular and cell biology approaches can we elucidate how the MEJ functionally integrate the EC and VSMC.

Acknowledgments

We thank Angela Best, Scott Johnstone, Michael Rizzo, Peter W. Kluge, Calley Troutman, Jenny Han and Manoj Patel for their help in preparation of the manuscript. We are especially grateful to Brian Duling for critical feedback, enlightening discussions, and TEM micrograph selection. This work is supported by NIH HL088554 (BEI) and an American Heart Association Scientist Development Grant (BEI).

Reference List

1. Absi M, Burnham MP, Weston AH, Harno E, Rogers M, Edwards G. Effects of methyl beta-cyclodextrin on EDHF responses in pig and rat arteries; association between SK(Ca) channels and caveolin-rich domains. *Br J Pharmacol* 2007;151:332–340. [PubMed: 17450174]
2. Bellien J, Thuillez C, Joannides R. Contribution of endothelium-derived hyperpolarizing factors to the regulation of vascular tone in humans. *Fundam Clin Pharmacol* 2008;22:363–377. [PubMed: 18705747]
3. Beny JL. Information Networks in the Arterial Wall. *News Physiol Sci* 1999;14:68–73. [PubMed: 11390823]
4. Bosanac I, Michikawa T, Mikoshiba K, Ikura M. Structural insights into the regulatory mechanism of IP3 receptor. *Biochim Biophys Acta* 2004;1742:89–102. [PubMed: 15590059]
5. Brisset AC, Isakson BE, Kwak BR. Connexins in Vascular Physiology and Pathology. *Antioxid Redox Signal* 2008;11:267–282. [PubMed: 18834327]
6. Budel S, Bartlett IS, Segal SS. Homocellular conduction along endothelium and smooth muscle of arterioles in hamster cheek pouch: unmasking an NO wave. *Circ Res* 2003;93:61–68. [PubMed: 12791708]
7. Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH. EDHF: bringing the concepts together. *Trends Pharmacol Sci* 2002;23:374–380. [PubMed: 12377579]
8. Campbell WB, Falck JR. Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. *Hypertension* 2007;49:590–596. [PubMed: 17200437]
9. Chattopadhyay N. Biochemistry, physiology and pathophysiology of the extracellular calcium-sensing receptor. *Int J Biochem Cell Biol* 2000;32:789–804. [PubMed: 10940638]

10. Chattopadhyay N, Brown EM. Role of calcium-sensing receptor in mineral ion metabolism and inherited disorders of calcium-sensing. *Mol Genet Metab* 2006;89:189–202. [PubMed: 16919492]
11. Chaytor AT, Martin PE, Edwards DH, Griffith TM. Gap junctional communication underpins EDHF-type relaxations evoked by ACh in the rat hepatic artery. *Am J Physiol Heart Circ Physiol* 2001;280:H2441–H2450. [PubMed: 11356596]
12. Conejo VA, De Hara R, Sosa-Melgarejo J, Mendez JD. New insights in endothelial and smooth muscle cell communication. *Biomed Pharmacother* 2007;61:173–179. [PubMed: 17383847]
13. de Wit C, Wolfle SE, Hopfl B. Connexin-dependent communication within the vascular wall: contribution to the control of arteriolar diameter. *Adv Cardiol* 2006;42:268–283. [PubMed: 16646597]
14. de Wit C, Boettcher M, Schmidt VJ. Signaling across myoendothelial gap junctions--fact or fiction? *Cell Commun Adhes* 2008;15:231–245. [PubMed: 18979293]
15. Dora KA, Doyle MP, Duling BR. Elevation of intracellular calcium in smooth muscle causes endothelial cell generation of NO in arterioles. *Proceedings of the National Academy of Sciences of the United States of America* 1997;94:6529–6534. [PubMed: 9177252]
16. Dora KA, Gallagher NT, McNeish A, Garland CJ. Modulation of Endothelial Cell KCa3.1 Channels During Endothelium-Derived Hyperpolarizing Factor Signaling in Mesenteric Resistance Arteries. *Circ Res* 2008;102:1247–1255. [PubMed: 18403729]
17. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH. K⁺ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* 1998;396:269–272. [PubMed: 9834033]
18. Elfgang C, Eckert R, Lichtenberg-Frate H, Butterweck A, Traub O, Klein RA, Hulser DF, Willecke K. Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells. *J Cell Biol* 1995;129:805–817. [PubMed: 7537274]
19. Emerson GG, Segal SS. Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: role in vasomotor control. *Circ Res* 2000;87:474–479. [PubMed: 10988239]
20. Evans WH, Leybaert L. Mimetic peptides as blockers of connexin channel-facilitated intercellular communication. *Cell Commun Adhes* 2007;14:265–273. [PubMed: 18392994]
21. Evans WH, Martin PEM. Gap junctions: structure and function (Review). *Molecular Membrane Biology* 2002;19:121–136. [PubMed: 12126230]
22. Fawcett, DW. *The Microcirculation*. Urbana, IL: University of Illinois Press; 1959.
23. Figueroa XF, Isakson BE, Duling BR. Connexins: gaps in our knowledge of vascular function. *Physiology (Bethesda)* 2004;19:277–284. [PubMed: 15381756]
24. Figueroa XF, Paul DL, Simon AM, Goodenough DA, Day KH, Damon DN, Duling BR. Central role of connexin40 in the propagation of electrically activated vasodilation in mouse cremasteric arterioles in vivo. *Circulation Research* 2003;92:793–800. [PubMed: 12637364]
25. Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A. Alterations in endothelium-dependent hyperpolarization and relaxation in mesenteric arteries from streptozotocin-induced diabetic rats. *Br J Pharmacol* 1997;121:1383–1391. [PubMed: 9257918]
26. Gebremedhin D, Ma YH, Falck JR, Roman RJ, VanRollins M, Harder DR. Mechanism of action of cerebral epoxyeicosatrienoic acids on cerebral arterial smooth muscle. *Am J Physiol* 1992;263:H519–H525. [PubMed: 1510149]
27. Gilbertson-Beadling SK, Fisher C. A potential role for N-cadherin in mediating endothelial cell-smooth muscle cell interactions in the rat vasculature. *Lab Invest* 1993;69:203–209. [PubMed: 8350598]
28. Goligorsky MS, Li H, Brodsky S, Chen J. Relationships between caveolae and eNOS: everything in proximity and the proximity of everything. *Am J Physiol Renal Physiol* 2002;283:F1–F10. [PubMed: 12060581]
29. Guegan F, Tatin F, Leste-Lasserre T, Drutel G, Genot E, Moreau V. p190B RhoGAP regulates endothelial-cell-associated proteolysis through MT1-MMP and MMP2. *J Cell Sci* 2008;121:2054–2061. [PubMed: 18505793]
30. Gustafsson F, Mikkelsen HB, Arensbak B, Thunberg L, Neve S, Jensen LJ, Holstein-Rathlou NH. Expression of connexin 37, 40 and 43 in rat mesenteric arterioles and resistance arteries. *Histochemistry and Cell Biology* 2003;119:139–148. [PubMed: 12610733]

31. Haas TL, Duling BR. Morphology favors an endothelial cell pathway for longitudinal conduction within arterioles. *Microvasc Res* 1997;53:113–120. [PubMed: 9143542]
32. Haddock RE, Grayson TH, Brackenbury TD, Meaney KR, Neylon CB, Sandow SL, Hill CE. Endothelial coordination of cerebral vasomotion via myoendothelial gap junctions containing connexins 37 and 40. *Am J Physiol Heart Circ Physiol* 2006;291:H2047–H2056. [PubMed: 16815985]
33. Hastings NE, Simmers MB, McDonald OG, Wamhoff BR, Blackman BR. Atherosclerosis-prone hemodynamics differentially regulates endothelial and smooth muscle cell phenotypes and promotes pro-inflammatory priming. *Am J Physiol Cell Physiol* 2007;293:C1824–C1833. [PubMed: 17913848]
34. Head BP, Patel HH, Roth DM, Murray F, Swaney JS, Niesman IR, Farquhar MG, Insel PA. Microtubules and actin microfilaments regulate lipid raft/caveolae localization of adenylyl cyclase signaling components. *J Biol Chem* 2006;281:26391–26399. [PubMed: 16818493]
35. Hisatsune C, Mikoshiba K. Novel compartment implicated in calcium signaling--is it an "induced coupling domain"? *Sci STKE* 2005;2005:e53.
36. Iacobas DA, Urban-Maldonado M, Iacobas S, Scemes E, Spray DC. Array analysis of gene expression in connexin-43 null astrocytes. *Physiol Genomics* 2003;15:177–190. [PubMed: 12928503]
37. Isakson BE. Localized expression of an Ins(1,4,5)P₃ receptor at the myoendothelial junction selectively regulates heterocellular Ca²⁺ communication. *J Cell Sci* 2008;121:3664–3673. [PubMed: 18946029]
38. Isakson BE, Best AK, Duling BR. Incidence of protein on actin bridges between endothelium and smooth muscle in arterioles demonstrates heterogeneous connexin expression and phosphorylation. *Am J Physiol Heart Circ Physiol* 2008;294:H2898–H2904. [PubMed: 18408134]
39. Isakson BE, Damon DN, Day KH, Liao Y, Duling BR. Connexin40 and connexin43 in mouse aortic endothelium: evidence for coordinated regulation. *Am J Physiol Heart Circ Physiol* 2006;290:H1199–H1205. [PubMed: 16284228]
40. Isakson BE, Duling BR. Heterocellular contact at the myoendothelial junction influences gap junction organization. *Circ Res* 2005;97:44–51. [PubMed: 15961721]
41. Isakson BE, Kronke G, Kadl A, Leitinger N, Duling BR. Oxidized phospholipids alter vascular connexin expression, phosphorylation, and heterocellular communication. *Arterioscler Thromb Vasc Biol* 2006;26:2216–2221. [PubMed: 16857951]
42. Isakson BE, Ramos SI, Duling BR. Ca²⁺ and inositol 1,4,5-trisphosphate-mediated signaling across the myoendothelial junction. *Circ Res* 2007;100:246–254. [PubMed: 17218602]
43. Isshiki M, Anderson RG. Function of caveolae in Ca²⁺ entry and Ca²⁺-dependent signal transduction. *Traffic* 2003;4:717–723. [PubMed: 14617355]
44. Jackson WF, Boerman EM, Lange EJ, Lundback SS, Cohen KD. Smooth muscle alpha(1D)-adrenoceptors mediate phenylephrine-induced vasoconstriction and increases in endothelial cell Ca (2+) in hamster cremaster arterioles. *Br J Pharmacol* 2008;155:514–522. [PubMed: 18604236]
45. Johnson RG, Herman WS, Preus DM. Homocellular and heterocellular gap junctions in *Limulus*: a thin-section and freeze-fracture study. *J Ultrastruct Res* 1973;43:298–312. [PubMed: 4575714]
46. Kameritsch P, Khandoga N, Nagel W, Hundhausen C, Lidington D, Pohl U. Nitric oxide specifically reduces the permeability of Cx37-containing gap junctions to small molecules. *J Cell Physiol* 2005;203:233–242. [PubMed: 15481066]
47. Kansui Y, Garland CJ, Dora KA. Enhanced spontaneous Ca(2+) events in endothelial cells reflect signalling through myoendothelial gap junctions in pressurized mesenteric arteries. *Cell Calcium* 2008;44:135–146. [PubMed: 18191200]
48. Kenny LC, Baker PN, Kendall DA, Randall MD, Dunn WR. Differential mechanisms of endothelium-dependent vasodilator responses in human myometrial small arteries in normal pregnancy and pre-eclampsia. *Clin Sci (Lond)* 2002;103:67–73. [PubMed: 12095405]
49. Kruger O, Beny JL, Chabaud F, Traub O, Theis M, Brix K, Kirchhoff S, Willecke K. Altered dye diffusion and upregulation of connexin37 in mouse aortic endothelium deficient in connexin40. *Journal of Vascular Research* 2002;39:160–172. [PubMed: 12011587]

50. Lamboley M, Pittet P, Koenigsberger M, Sauser R, Beny JL, Meister JJ. Evidence for signaling via gap junctions from smooth muscle to endothelial cells in rat mesenteric arteries: possible implication of a second messenger. *Cell Calcium* 2005;37:311–320. [PubMed: 15755492]
51. Lampe PD, Lau AF. Regulation of gap junctions by phosphorylation of connexins. *Arch Biochem Biophys* 2000;384:205–215. [PubMed: 11368307]
52. Lampe PD, TenBroek EM, Burt JM, Kurata WE, Johnson RG, Lau AF. Phosphorylation of connexin43 on serine368 by protein kinase C regulates gap junctional communication. *J Cell Biol* 2000;149:1503–1512. [PubMed: 10871288]
53. Lang NN, Luksha L, Newby DE, Kublickiene K. Connexin 43 mediates endothelium-derived hyperpolarizing factor-induced vasodilatation in subcutaneous resistance arteries from healthy pregnant women. *Am J Physiol Heart Circ Physiol* 2007;292:H1026–H1032. [PubMed: 17085540]
54. Laude AJ, Prior IA. Plasma membrane microdomains: organization, function and trafficking. *Mol Membr Biol* 2004;21:193–205. [PubMed: 15204627]
55. Ledoux J, Taylor MS, Bonev AD, Hannah RM, Solodushko V, Shui B, Tallini Y, Kotlikoff MI, Nelson MT. Functional architecture of inositol 1,4,5-trisphosphate signaling in restricted spaces of myoendothelial projections. *Proc Natl Acad Sci U S A* 2008;105:9627–9632. [PubMed: 18621682]
56. Little TL, Xia J, Duling BR. Dye tracers define differential endothelial and smooth muscle coupling patterns within the arteriolar wall. *Circ Res* 1995;76:498–504. [PubMed: 7859395]
57. Liu L, Mohammadi K, Aynafshar B, Wang H, Li D, Liu J, Ivanov AV, Xie Z, Askari A. Role of caveolae in signal-transducing function of cardiac Na⁺/K⁺-ATPase. *Am J Physiol Cell Physiol* 2003;284:C1550–C1560. [PubMed: 12606314]
58. Looft-Wilson RC, Payne GW, Segal SS. Connexin expression and conducted vasodilation along arteriolar endothelium in mouse skeletal muscle. *J Appl Physiol* 2004;97:1152–1158. [PubMed: 15169746]
59. Luksha L, Nisell H, Luksha N, Kublickas M, Hultenby K, Kublickiene K. Endothelium-derived hyperpolarizing factor in preeclampsia: heterogeneous contribution, mechanisms, and morphological prerequisites. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R510–R519. [PubMed: 18032472]
60. Makino A, Ohuchi K, Kamata K. Mechanisms underlying the attenuation of endothelium-dependent vasodilatation in the mesenteric arterial bed of the streptozotocin-induced diabetic rat. *Br J Pharmacol* 2000;130:549–556. [PubMed: 10821782]
61. Marrelli SP, Eckmann MS, Hunte MS. Role of endothelial intermediate conductance K_{Ca} channels in cerebral EDHF-mediated dilations. *Am J Physiol Heart Circ Physiol* 2003;285:H1590–H1599. [PubMed: 12805022]
62. Martin PE, Wall C, Griffith TM. Effects of connexin-mimetic peptides on gap junction functionality and connexin expression in cultured vascular cells. *Br J Pharmacol* 2005;144:617–627. [PubMed: 15678088]
63. Mather S, Dora KA, Sandow SL, Winter P, Garland CJ. Rapid endothelial cell-selective loading of connexin 40 antibody blocks endothelium-derived hyperpolarizing factor dilation in rat small mesenteric arteries. *Circ Res* 2005;97:399–407. [PubMed: 16037574]
64. McSherry IN, Sandow SL, Campbell WB, Falck JR, Hill MA, Dora KA. A Role for Heterocellular Coupling and EETs in Dilation of Rat Cremaster Arteries. *Microcirculation* 2006;13:119–130. [PubMed: 16459325]
65. Michel RP, Hu F, Meyrick BO. Myoendothelial junctional complexes in postobstructive pulmonary vasculopathy: a quantitative electron microscopic study. *Exp Lung Res* 1995;21:437–452. [PubMed: 7621779]
66. Miyoshi J, Takai Y. Structural and functional associations of apical junctions with cytoskeleton. *Biochim Biophys Acta* 2008;1778:670–691. [PubMed: 18201548]
67. Moore DH, Ruska H. The fine structure of capillaries and small arteries. *J Biophys Biochem Cytol* 1957;3:457–462. [PubMed: 13438930]
68. Pannirselvam M, Verma S, Anderson TJ, Triggle CR. Cellular basis of endothelial dysfunction in small mesenteric arteries from spontaneously diabetic (db/db ^{-/-}) mice: role of decreased tetrahydrobiopterin bioavailability. *Br J Pharmacol* 2002;136:255–263. [PubMed: 12010774]
69. Parton RG, Simons K. The multiple faces of caveolae. *Nat Rev Mol Cell Biol* 2007;8:185–194. [PubMed: 17318224]

70. Rhodin JA. The ultrastructure of mammalian arterioles and precapillary sphincters. *J Ultrastruct Res* 1967;18:181–223. [PubMed: 5337871]
71. Rozental R, Srinivas M, Spray DC. How to close a gap junction channel. Efficacies and potencies of uncoupling agents. *Methods Mol Biol* 2001;154:447–476. [PubMed: 11218664]
72. Sabatini PJ, Zhang M, Silverman-Gavrila R, Bendeck MP, Langille BL. Homotypic and endothelial cell adhesions via N-cadherin determine polarity and regulate migration of vascular smooth muscle cells. *Circ Res* 2008;103:405–412. [PubMed: 18617695]
73. Sagar GD, Larson DM. Carbenoxolone inhibits junctional transfer and upregulates Connexin43 expression by a protein kinase A-dependent pathway. *J Cell Biochem* 2006;98:1543–1551. [PubMed: 16552723]
74. Saliez J, Bouzin C, Rath G, Ghisdal P, Desjardins F, Rezzani R, Rodella LF, Vriens J, Nilius B, Feron O, Balligand JL, Dessy C. Role of caveolar compartmentation in endothelium-derived hyperpolarizing factor-mediated relaxation: Ca²⁺ signals and gap junction function are regulated by caveolin in endothelial cells. *Circulation* 2008;117:1065–1074. [PubMed: 18268148]
75. Sandow SL, Bramich NJ, Bandi HP, Rummery NM, Hill CE. Structure, function, and endothelium-derived hyperpolarizing factor in the caudal artery of the SHR and WKY rat. *Arterioscler Thromb Vasc Biol* 2003;23:822–828. [PubMed: 12649082]
76. Sandow SL, Garland CJ. Spatial association of K-Ca and gap junction connexins in rat mesenteric artery. *Faseb Journal* 2006;20:A275.
77. Sandow SL, Haddock RE, Hill CE, Chadha PS, Kerr PM, Welsh DG, Plane F. What's where and why at a vascular myoendothelial microdomain signalling complex. *Clin Exp Pharmacol Physiol*. 2008
78. Sandow SL, Hill CE. Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. *Circ Res* 2000;86:341–346. [PubMed: 10679487]
79. Sandow SL, Looft-Wilson R, Doran B, Grayson TH, Segal SS, Hill CE. Expression of homocellular and heterocellular gap junctions in hamster arterioles and feed arteries. *Cardiovasc Res* 2003;60:643–653. [PubMed: 14659810]
80. Sandow SL, Neylon CB, Chen MX, Garland CJ. Spatial separation of endothelial small- and intermediate-conductance calcium-activated potassium channels (K(Ca)) and connexins: possible relationship to vasodilator function? *J Anat* 2006;209:689–698. [PubMed: 17062025]
81. Sato T, Haimovici R, Kao R, Li AF, Roy S. Downregulation of connexin 43 expression by high glucose reduces gap junction activity in microvascular endothelial cells. *Diabetes* 2002;51:1565–1571. [PubMed: 11978657]
82. Segal SS, Beny JL. Intracellular recording and dye transfer in arterioles during blood flow control. *Am J Physiol* 1992;263:H1–H7. [PubMed: 1636748]
83. Severs NJ, Rothery S, Dupont E, Coppen SR, Yeh HI, Ko YS, Matsushita T, Kaba R, Halliday D. Immunocytochemical analysis of connexin expression in the healthy and diseased cardiovascular system. *Microsc Res Tech* 2001;52:301–322. [PubMed: 11180622]
84. Siegl D, Koeppen M, Wolfle SE, Pohl U, de WC. Myoendothelial coupling is not prominent in arterioles within the mouse cremaster microcirculation in vivo. *Circ Res* 2005;97:781–788. [PubMed: 16166558]
85. Simionescu, M.; Simionescu, N. Ultrastructure of the microvascular wall: functional correlations. In: Renkin, E.; Michel, C.; Geiger, S., editors. *Handbook of Physiology: The Cardiovascular System*. Bethesda, MD: 1984. p. 41-101.
86. Simon AM, McWhorter AR. Decreased intercellular dye-transfer and downregulation of non-ablated connexins in aortic endothelium deficient in connexin37 or connexin40. *Journal of Cell Science* 2003;116:2223–2236. [PubMed: 12697838]
87. Smith P, Heath D, Padula F. Evagination of smooth muscle cells in the hypoxic pulmonary trunk. *Thorax* 1978;33:31–42. [PubMed: 644538]
88. Sokoya EM, Burns AR, Marrelli SP, Chen J. Myoendothelial gap junction frequency does not account for sex differences in EDHF responses in rat MCA. *Microvasc Res* 2007;74:39–44. [PubMed: 17490692]
89. Sosa-Melgarejo JA, Berry CL. Myoendothelial contacts in the human fetal aorta. *Arch Med Res* 1995;26:431–435. [PubMed: 8555740]

90. Sosinsky GE, Solan JL, Gaietta GM, Ngan L, Lee GJ, Mackey MR, Lampe PD. The C-terminus of connexin43 adopts different conformations in the Golgi and gap junction as detected with structure-specific antibodies. *Biochem J* 2007;408:375–385. [PubMed: 17714073]
91. Taugner R, Kirchheim H, Forssmann WG. Myoendothelial contacts in glomerular arterioles and in renal interlobular arteries of rat, mouse and Tupaia belangeri. *Cell Tissue Res* 1984;235:319–325. [PubMed: 6705035]
92. Tian J, Xie ZJ. The Na-K-ATPase and Calcium-Signaling Microdomains. *Physiology (Bethesda)* 2008;23:205–211. [PubMed: 18697994]
93. Tolsa JF, Marino M, Peyter AC, Beny JL. Role of membrane potential in endothelium-dependent relaxation of isolated mouse main pulmonary artery. *J Cardiovasc Pharmacol* 2006;47:501–507. [PubMed: 16680062]
94. van Kempen MJ, Jongsma HJ. Distribution of connexin37, connexin40 and connexin43 in the aorta and coronary artery of several mammals. *Histochem Cell Biol* 1999;112:479–486. [PubMed: 10651100]
95. Varon C, Tatin F, Moreau V, Van Obberghen-Schilling E, Fernandez-Sauze S, Reuzeau E, Kramer I, Genot E. Transforming growth factor beta induces rosettes of podosomes in primary aortic endothelial cells. *Mol Cell Biol* 2006;26:3582–3594. [PubMed: 16611998]
96. Vriens J, Owsianik G, Fisslthaler B, Suzuki M, Janssens A, Voets T, Morisseau C, Hammock BD, Fleming I, Busse R, Nilius B. Modulation of the Ca₂ permeable cation channel TRPV4 by cytochrome P450 epoxygenases in vascular endothelium. *Circ Res* 2005;97:908–915. [PubMed: 16179585]
97. Wei CJ, Francis R, Xu X, Lo CW. Connexin43 associated with an N-cadherin-containing multiprotein complex is required for gap junction formation in NIH3T3 cells. *J Biol Chem* 2005;280:19925–19936. [PubMed: 15741167]
98. Welsh DG, Segal SS. Endothelial and smooth muscle cell conduction in arterioles controlling blood flow. *Am J Physiol* 1998;274:H178–H186. [PubMed: 9458866]
99. Welsh DG, Segal SS. Role of EDHF in conduction of vasodilation along hamster cheek pouch arterioles in vivo. *Am J Physiol Heart Circ Physiol* 2000;278:H1832–H1839. [PubMed: 10843879]
100. Weston AH, Absi M, Ward DT, Ohanian J, Dodd RH, Dauban P, Petrel C, Ruat M, Edwards G. Evidence in favor of a calcium-sensing receptor in arterial endothelial cells: studies with calindol and Calhex 231. *Circ Res* 2005;97:391–398. [PubMed: 16037572]
101. Wigg SJ, Tare M, Tonta MA, O'Brien RC, Meredith IT, Parkington HC. Comparison of effects of diabetes mellitus on an EDHF-dependent and an EDHF-independent artery. *Am J Physiol Heart Circ Physiol* 2001;281:H232–H240. [PubMed: 11406490]
102. Xia J, Duling BR. Electromechanical coupling and the conducted vasomotor response. *Am J Physiol* 1995;269:H2022–H2030. [PubMed: 8594912]
103. Xia J, Duling BR. Patterns of excitation-contraction coupling in arterioles: dependence on time and concentration. *Am J Physiol* 1998;274:H323–H330. [PubMed: 9458883]
104. Xia J, Little TL, Duling BR. Cellular pathways of the conducted electrical response in arterioles of hamster cheek pouch in vitro. *Am J Physiol* 1995;269:H2031–H2038. [PubMed: 8594913]
105. Yao J, Hiramatsu N, Zhu Y, Morioka T, Takeda M, Oite T, Kitamura M. Nitric oxide-mediated regulation of connexin43 expression and gap junctional intercellular communication in mesangial cells. *J Am Soc Nephrol* 2005;16:58–67. [PubMed: 15537869]
106. Yashiro Y, Duling BR. Integrated Ca(2+) signaling between smooth muscle and endothelium of resistance vessels. *Circ Res* 2000;87:1048–1054. [PubMed: 11090551]
107. Ye C, Chattopadhyay N, Brown EM, Vassilev PM. Defective extracellular calcium (Ca(o))-sensing receptor (CaR)-mediated stimulation of a Ca(2+)-activated potassium channel in glioblastoma cells transfected with a dominant negative CaR. *Brain Res Mol Brain Res* 2000;80:177–187. [PubMed: 11038250]
108. Ye CP, Yamaguchi T, Chattopadhyay N, Sanders JL, Vassilev PM, Brown EM. Extracellular calcium-sensing-receptor (CaR)-mediated opening of an outward K(+) channel in murine MC3T3-E1 osteoblastic cells: evidence for expression of a functional CaR. *Bone* 2000;27:21–27. [PubMed: 10865205]

Figure 1.

Ultrastructural images of the MEJ in different arterioles. In A, a TEM image of a mouse cremaster arteriole clearly shows red blood cells in the lumen (R), as well as endothelial cell (E) and vascular smooth muscle cell (S) monolayers. The box in A highlights cellular extensions between the two monolayers. In the image from B, an endothelial extension from a mouse cremasteric endothelial cell is observed penetrating the IEL and making contact with a smooth muscle cell. Note the flat apposition of the MEJ, as seen in arterioles, rather than the club-like formation seen in arteries (e.g., (91)). The box in B highlights one location where the membranes of endothelial cells and smooth muscle cells meet. Although it is possible that not all MEJs contain gap junctions, the image from C is the only described freeze fractured MEJ published and is from the rat afferent arteriole in kidney. The original description claimed to have demonstrated gap junction “particles” (91) on the endothelial cell side of the cellular extension in the P-face; magnification $\times 46,000$; image taken from Taugner, et al (91). In panel A, bar is 5 μm and in panel B the same bar is 1 μm .

Figure 2.

Schematic of proteins localized to the MEJ. Image portrays identified structural and signaling components at the MEJ in both endothelial (blue) and vascular smooth muscle (gray) cells. Components are categorized into proteins identified in vivo (caveolae (74), Na⁺/K⁺ ATPase (16), K_{Ca}2.3 channel (16), K_{Ca}3.1 channel (16;55;80)), proteins identified in vitro and in vivo (IP₃-R1 (37), connexins (32;38;80), N-Cadherin (27;38), endoplasmic reticulum (42;55)) and proteins identified in vitro (microtubules (41), actin (38), type-1 5 phosphatase (37)).

Figure 3.

An in vitro model of the MEJ. In A, TEM image of a mouse cremaster arteriole with an endothelial cell monolayer on the top (E) and smooth muscle cell (S) monolayer on the bottom of the image. Separation of the monolayers by the internal elastic lamina (IEL) reveals multiple locations where cellular extensions from endothelium or smooth muscle penetrate the matrix, presumably MEJs (arrow). In B, a vascular cell co-culture stained for phalloidin with separated endothelial (E) and smooth muscle cell (S) monolayers showing cellular extensions within the porous Transwell insert (arrow). Bar in A is 3 μm and is 9 μm in B.