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INVITED REVIEW

Connexins and gap junctions in the EDHF phenomenon and conducted vasomotor responses

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Abstract It is becoming increasingly evident that electrical signaling via gap junctions plays a central role in the physiological control of vascular tone via two related mechanisms (1) the endothelium-derived hyperpolarizing factor (EDHF) phenomenon, in which radial transmission of hyperpolarization from the endothelium to subjacent smooth muscle promotes relaxation, and (2) responses that propagate longitudinally, in which electrical signaling within the intimal and medial layers of the arteriolar wall orchestrates mechanical behavior over biologically large distances. In the EDHF phenomenon, the transmitted endothelial hyperpolarization is initiated by the activation of Ca^{2+}-activated K^+ channels channels by $InsP_3$-induced Ca^{2+} release from the endoplasmic reticulum and/or store-operated Ca^{2+} entry triggered by the depletion of such stores. Pharmacological inhibitors of direct cell-cell coupling may thus attenuate EDHF-type smooth muscle hyperpolarizations and relaxations, confirming the participation of electrotonic signaling via myoendothelial and homocellular smooth muscle gap junctions. In contrast to isolated vessels, surprisingly little experimental evidence argues in favor of myoendothelial coupling acting as the EDHF mechanism in arterioles in vivo. However, it now seems established that the endothelium plays the leading role in the spatial propagation of arteriolar responses and that these involve poorly understood regenerative mechanisms. The present review will focus on the complex interactions between the diverse cellular signaling mechanisms that contribute to these phenomena.

Keywords Gap junction · Connexin · EDHF

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Introduction

In addition to the ability of the endothelium to release NO and vasodilator prostanoids when stimulated pharmacologically by agonists such as acetylcholine (ACh) or fluid shear stress, it can promote arterial relaxation through a mechanism that evokes smooth muscle hyperpolarization, a phenomenon first described by Bolton and colleagues [8]. A wide range of agents, including NO itself, H_2O_2, eicosanoids, K^+ ions, and even species such as C-type natriuretic peptide and H_2S have subsequently been postulated to serve as endothelium-derived hyperpolarizing factors (EDHFs) that modulate smooth muscle membrane potential following release from the endothelium [59, 168]. However, none has emerged as a "universal" EDHF, and bioassay experiments with apposed endothelium-intact and -denuded arteries in which the donor endothelium and detector smooth muscle are electrically uncoupled, do not consistently demonstrate relaxation in the presence of inhibitors of NO synthase and cyclooxygenase. An alternative hypothesis is therefore that the EDHF phenomenon is primarily electrotonic in origin and driven by an endothelial hyperpolarization that spreads radially into the vessel wall via myoendothelial gap junctions [75].

Vascular cells are also coupled homocellularly (i.e., to neighbors of the same cell type) thereby creating a

syncytium of interconnected cells that ultimately allows concerted mechanical activity. In particular, endothelial cells are tightly coupled, thereby creating a preferential pathway for longitudinal transmission of electrical signals along the vascular wall. The spread of dilator and constrictor responses from a confined site of initiation to distant remote upstream and downstream sites in the microcirculation was initially identified by Duling [47], is independent of blood flow [87, 164, 165], and related to changes in membrane potential [57, 171]. Such so-called conducted or propagated responses can be transmitted over distances greater than 2 mm, thus suggesting the involvement of amplification or regenerative mechanisms, as will be also discussed.

Vascular gap junctions

Gap junctions are formed by the docking of two apposing hemichannels or connexons, which are constructed from six connexin (Cx) protein subunits that each traverse the cell membrane four times. Interdigitation and rotation of the extracellular loops of the contributing connexins creates an aqueous pore that allows electrical continuity and diffusion of ions and polar molecules <1 kilodalton (kDa) in size between coupled cells [143]. In the case of small molecules (e.g., tetraethylammonium) the electrical conductance and permeability of gap junctions are directly related, whereas for larger molecules (e.g., the fluorescent dye calcein) there is a more complex, nonlinear relationship that is influenced both by Stokes radius and charge [49, 192]. Intercellular coupling is enhanced by the aggregation of large numbers of individual gap junctions in plaques at points of cell–cell contact in which cooperative gating is thought to amplify channel opening [17]. The distinctive appearance of such plaques, which are abundant in the endothelial monolayer but relatively sparse in the media, can be demonstrated by immunostaining with antibodies targeted to the four vascular connexin subtypes, Cxs 37, 40, 43, and 45, which are classified according to their molecular mass in kilodaltons. Gap junctions constructed from a single connexin subtype are termed homotypic, whereas channels formed when each connexon contains a different connexin subtype or contains a mixture of subtypes, are termed heterotypic or heteromeric, respectively. Immunohistochemistry has demonstrated that patterns of Cx expression may vary widely between different arteries, with Cx45 being absent in most vessels and two connexin subtypes often co-localizing in the same gap junction plaque consistent with the formation of heterotypic or heteromeric channels [23, 28, 88, 113, 188, 207]. This has been confirmed by patch clamp analysis demonstrating the existence of gap junctions with complex conductances consistent with the presence of Cx40 and Cx43 in heterotypic/heteromeric channels in smooth muscle cells freshly isolated from the rat basilar artery [113].

Electron microscopy is necessary to detect the characteristic multilaminar appearance of gap junction plaques between endothelium and smooth muscle (myoendothelial gap junctions) because their inherently small size precludes visualization by conventional immunohistochemistry [103, 178]. However, the combination of electron microscopy and immunohistochemistry with antibodies conjugated to gold allows detection of specific connexins in gap junction plaques and has shown that Cx37 and Cx40 can be co-localized within myoendothelial gap junctions [79]. Fenestrations in the internal elastic lamina allow direct physical contact between endothelial and smooth muscle cells, but their presence and size does not correlate with the incidence of myoendothelial junctions since a high proportion of fenestrations are devoid of cellular elements [157]. Myoendothelial gap junctions are most numerous in distal vessels, suggesting that the larger EDHF-type relaxations observed as vessel size diminishes within the same microvascular bed might in part reflect stronger endothelial-smooth muscle coupling [90, 159, 169, 185]. By contrast, the incidence of smooth muscle gap junction plaques constructed from Cx43 appears to decrease with vessel size from elastic to muscular arteries [88, 121]. The relationship between function and the incidence and size of myoendothelial and medial gap junctions plaques thus remains to be clarified, as relaxation will ultimately depend on signaling via both. Furthermore, small arteries may be particularly responsive to electrically conducted hyperpolarization because their contractile function is highly dependent on Ca^{2+} influx via L-type voltage-dependent channels and therefore would be expected to be particularly sensitive to conducted hyperpolarizing signals originating in the endothelium that will promote channel closure [185].

The functional role of specific connexins in vascular signaling can be analyzed in genetically engineered mice. Connexin-deficient mice have been created for all cardiovascular connexins, and shown that global deficiency of Cx43 or Cx45 is lethal. Cx43-deficient mice develop a cardiac malformation and die soon after birth due to obstruction of the right ventricular outflow tract [147, 203]. Global loss of Cx45 results in death around embryonic day 10 and these embryos display a defect in vessel development with a lack of smooth muscle cells and impaired vessel maturation [104], although a cardiac malformation may also be present [105]. Simultaneous knockout of Cx40 and Cx37 results in a phenotype with abnormal vascular channels and markedly distended vessels and is lethal in the perinatal period [172, 173]. Detailed insights into connexin function have nevertheless been obtained in mice lacking Cx40 or Cx37 individually, which

are viable and fertile, and animals with a cell-specific connexin deficiency whose vascular phenotype has already been partly characterized (see below).

Endothelial hyperpolarization as the initiating step: different roles for SK_{Ca} and IK_{Ca} channels

The key driving force for the smooth muscle hyperpolarization that underpins EDHF-type relaxation is an elevation in endothelial $[Ca^{2+}]_i$, which in the case of G protein-coupled agonists such as ACh is generated by (1) inositol-1,4,5-trisphosphate ($InsP_3$)-induced Ca^{2+} release from the endoplasmic reticulum (ER) and (2) secondary store-operated Ca^{2+} entry (SOCE) through Ca^{2+} channels whose opening is linked to $InsP_3$-evoked depletion of the ER [66, 135]. Indeed, inhibitors of the sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump such as cyclopiazonic acid (CPA), which stimulate SOCE by blocking ER Ca^{2+} uptake directly, evoke receptor-independent EDHF-type responses that may be more sustained than those evoked by ACh [60, 66, 186]. Notably, the threshold in $[Ca^{2+}]_i$ necessary to elicit the EDHF phenomenon may be higher than that required to activate NO-dependent relaxation at 340 vs. 220 nM [119]. The resulting endothelial hyperpolarization is mediated principally by the opening of small- and intermediate-conductance Ca^{2+}-activated K^+ channels channels (K_{Ca}), which are designated as SK_{Ca}/KCa2.3 or IK_{Ca}/KCa3.1, respectively, and typically consists of a rapid change in membrane potential towards the reversal potential for K^+ (~−80 mV), sometimes followed by a slow rise towards a plateau [59, 117, 130, 136]. Both K_{Ca} channel subtypes are constitutively expressed in the endothelium, their currents are evident in freshly isolated endothelial cells, and there is limited evidence for their functional expression in vascular smooth muscle [59, 73]. Furthermore, selective pharmacological activation of SK_{Ca} and IK_{Ca} channels induces endothelium-dependent smooth muscle hyperpolarization, and combined SK_{Ca}/IK_{Ca} inhibition attenuates EDHF-type relaxations when applied to the arterial intima but not the adventitia, thus confirming the importance of the initiating endothelial hyperpolarization [46, 112].

In theory, endothelial hyperpolarization should itself elevate endothelial $[Ca^{2+}]_i$ by increasing the electrochemical force for Ca^{2+} entry, and some studies have indeed demonstrated that agonist-induced elevations in $[Ca^{2+}]_i$ are reduced by SK_{Ca} and IK_{Ca} inhibitors in isolated endothelial cells [98, 116, 135, 167]. In intact arterial preparations, however, such inhibitors may attenuate endothelial hyperpolarization without affecting the initiating rise in endothelial $[Ca^{2+}]_i$ [9, 71, 129, 179]. In some arteries, specific SK_{Ca} and IK_{Ca} inhibitors may be individually ineffective, whereas substantial or complete inhibition of EDHF-type responses is usually evident with their combination. In rat mesenteric arteries, for example, endothelium-dependent smooth muscle hyperpolarizations are abolished by the SK_{Ca} channel inhibitor apamin under resting conditions, whereas in arteries initially depolarized by phenylephrine the combination of apamin and the IK_{Ca} inhibitor TRAM-34 is required to abolish relaxation, thereby suggesting a specific role for IK_{Ca} channels following depolarization [30]. This may reflect distinct functional roles and activating mechanisms because IK_{Ca} may localize in proximity to myoendothelial gap junctions, whereas SK_{Ca} channels are diffusely distributed over the plasma membrane with preferential localization at sites of homocellular endothelial gap junctions and caveolin rich domains [59, 158]. This distribution may explain why fluid shear stress evokes an EDHF-type dilation that relies solely on the activation of SK_{Ca} in the mouse carotid artery [12].

Differential roles for SK_{Ca} and IK_{Ca} have also been highlighted in knockout mice deficient for one or both of these channel subtypes. In freshly isolated endothelial cells, combined SK_{Ca}/IK_{Ca} deficiency abolishes the overall K_{Ca}-current stimulated by Ca^{2+} dialysis, whereas in single channel knockouts K_{Ca} conductance is halved (when normalized to cell capacitance), indicating that SK_{Ca} and IK_{Ca} normally contribute equally but are unable to compensate for each other [12, 170]. Despite their equivalent contributions to the overall K_{Ca} current, however, IK_{Ca} was more important for smooth muscle hyperpolarization and dilation in the carotid artery following agonist stimulation, and this divergence was further accentuated in arterioles where EDHF-type dilations to ACh were strongly attenuated by the lack of IK_{Ca}, but only slightly reduced in SK_{Ca}-deficient mice [12, 170, 201].

Conditional overexpression strategies using a genetic switch [10] have provided further insights into the contribution of SK_{Ca} to endothelial function in mice [12, 184]. In isolated small mesenteric arteries, endothelial K_{Ca} currents were elevated by tenfold and endothelial membrane potential shifted by 15 mV towards more negative values compared to the same vessels from SK_{Ca}-down-regulated mice. This concurred with a reduced vascular tone at a given pressure in isolated vessels [184] although it remains to be determined whether this reflects enhancement of both the EDHF phenomenon and NO synthase activity. In larger vessels (isolated carotid arteries), ACh-evoked dilation was unaltered in mice overexpressing SK_{Ca} despite strongly enhanced endothelial K_{Ca} currents. However, high SK_{Ca} expression levels were able to compensate for deletion of IK_{Ca} and effectively normalized ACh-induced relaxations [12]. As noted above, differential functional roles

of SK_{Ca} and IK_{Ca} channels may reflect their localization in specific membrane domains and such localisation may be lost when SK_{Ca} is overexpressed.

BK_{Ca} channels in smooth muscle and endothelial cells

Large conductance BK_{Ca} channels (also designated as KCa1.1) are widely expressed in vascular smooth muscle cells and can be activated by global elevations in $[Ca^{2+}]_i$, by local Ca^{2+} sparks arising from adjacent sarcoplasmic reticulum and by Ca^{2+} influx via transient receptor potential (TRP) channels with which they may co-localize in the cell membrane [109, 195]. Notably, the four pore-forming central α-subunits of the channel are gated by voltage, as well as Ca^{2+}, and this sensitivity is enhanced by four β-subunits so that channel activity is regulated physiologically both by membrane potential and Ca^{2+}, rather than just Ca^{2+} as in the case of SK_{Ca} and IK_{Ca} [111]. The extent to which BK_{Ca} activity contributes to the EDHF phenomenon exhibits considerable experimental variability. For example, the selective BK_{Ca} blocker iberiotoxin attenuates EDHF-type responses in rabbit iliac and renal but not mesenteric arteries, and attentuates EDHF-type relaxations in first order but not third order branch arteries from the rat mesenteric bed [52, 64, 85, 96, 132]. In general, BK_{Ca} channels are not detectable in native endothelium, either immunohistochemically or electrophysiologically [70, 156]. However, exceptions do exist [13, 152], thus raising the possibility that differences in the endothelial expression of this K_{Ca} channel subtype contribute to the heterogeneous effects of iberiotoxin across vessels and species. Differences in underlying mechanistic pathways might also be a contributory factor. In arteries where relaxation involves a freely diffusible EDHF that activates smooth muscle BK_{Ca} channels, as proposed for epoxyeicosatrienoic acids (EETs) and H_2O_2, responses should be particularly sensitive to iberiotoxin. By contrast, in arteries where the EDHF phenomenon depends on gap junctions, electrotonic spread of endothelial hyperpolarization would be expected to close BK_{Ca} channels, both as a consequence of their intrinsic voltage sensitivity and reduced Ca^{2+} influx via L-type channels. It is also likely that BK_{Ca} blockade will antagonize relaxation by removing the negative feedback mechanism that normally allows BK_{Ca} channels to limit smooth muscle constriction by opposing depolarization and elevations in $[Ca^{2+}]_i$ [14, 111, 160]. These complexities remain to be clarified experimentally and cannot be dissociated simply on the basis of observations that rapid reductions in arterial and arteriolar smooth muscle $[Ca^{2+}]_i$ occur during EDHF-type relaxations, as these will reflect the closure of L-type Ca^{2+} channels by hyperpolarization per se [9, 118, 137].

Myoendothelial gap junctions allow transfer of charge and small molecules

In isolated hamster and guinea pig arterioles, endothelial hyperpolarizations induced by direct injection of electrical current are conducted electrotonically (i.e., passively) to subjacent smooth muscle cells and action potentials originating in the arterial media are rapidly conducted to the endothelium [57, 205]. Signals transmitted in either direction are reduced by just 10-20% in amplitude, without change in form, suggesting that myoendothelial gap junctions behave as simple ohmic resistors that mediate the radial spread of endothelial hyperpolarization without rectification [205]. In conduit vessels, both longitudinal and circumferential spread of hyperpolarization within the media has also been demonstrated in strips of rabbit iliac and porcine coronary artery from which the endothelium has been partly removed, following hyperpolarization of the residual endothelium [77, 166] (Fig. 1). In the converse sense, smooth muscle hyperpolarization induced by K_{ATP} channel openers has been shown to conduct to the endothelium via myoendothelial gap junctions in the rabbit mesenteric artery [131]. Direct communication between the endothelium and subjacent smooth muscle cells can also be visualized by perfusing isolated arterial segments with the cell-permeant agent calcein AM. Following cleavage by esterases within the endothelium, the fluorescent tracer calcein then diffuses into the media via gap junctions [5, 77, 100, 155]. The ability of molecules <1 kDa in size to diffuse through gap junctions also allows the possibility that non-electrotonic mechanisms contribute to endothelium-dependent relaxation. Indeed, in endothelial/smooth

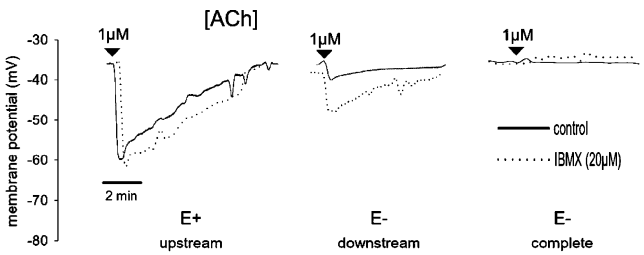

Fig. 1 Cyclic AMP modulates electrical coupling. Smooth muscle potential recordings in iliac artery strips from which the endothelium had been partially or completely removed by abrasion. The cAMP phosphodiesterase inhibitor IBMX did not affect hyperpolarizations evoked by acetylcholine within regions where the anatomically upstream endothelium remained intact (*E+ upstream*), whereas small conducted hyperpolarizations detected 1.5 mm from the edge of the residual endothelium were potentiated by IBMX (*E- downstream*). No electrical response was observed in endothelium-denuded strips (*E- complete*). These findings are consistent with enhanced coupling between smooth muscle cells. Cyclic AMP can also modulate inter-endothelial and myoendothelial coupling (see text)

muscle cell cultures, myoendothelial channels allow diffusion of $InsP_3$ and possibly Ca^{2+} into the endothelium from activated smooth muscle cells, thereby elevating endothelial $[Ca^{2+}]_i$ [93]. This mechanism has been postulated to promote dilation by stimulating NO production and K_{Ca} channel-mediated hyperpolarization in isolated arteries and arterioles [16, 42, 206]. Other vasoactive signaling molecules that diffuse through gap junctions include cAMP (see below).

Targeting specific connexins with peptides, antibodies, and knockout strategies

Studies with short peptides that block gap junctional communication by targeting specific connexins have confirmed the contribution of electrical coupling to the EDHF phenomenon. These agents attenuate NO- and prostanoid-independent relaxations and subintimal hyperpolarizations induced by agonists such as ACh, ATP, UTP, substance P and bradykinin and by the SERCA inhibitor CPA in a range of rabbit, rat, pig, guinea pig, and human arteries, without depressing the initiating endothelial hyperpolarization or affecting smooth muscle membrane conductance [4, 23, 24, 26, 28, 29, 43, 45, 51, 53, 76, 110, 121, 159, 176, 188]. Connexin-mimetic peptides appear to act by modulating the gating of gap junction channels because they do not perturb the structural stability of pre-existing myoendothelial gap junction plaques and do not affect connexin trafficking or the de novo formation of gap junction plaques in endothelial and smooth muscle co-cultures [120]. Indeed, their ability to interrupt electrical coupling between isolated pairs of rat aortic A7r5 myocytes (~5 min) is much more rapid than the turnover of connexin protein in plaques [120, 121]. The EDHF phenomenon can also be inhibited by "general" inhibitors of gap junctional communication such as the lipophilic 18α- and 18β-isoforms of glycyrrhetinic acid (GA) and carbenoxolone, a water-soluble derivative of 18β-GA [27, 45, 183, 204]. In cells expressing Cx43, 18α-GA causes connexin dephosphorylation and irreversible time- and concentration-dependent plaque disassembly and internalization after >30 min incubation, thus contrasting with the reversibility of connexin-mimetic peptides [26, 91, 120]. Dye transfer studies have confirmed that connexin-mimetic peptides and carbenoxolone block myoendothelial dye coupling in rabbit, rat, mouse, and porcine arteries [5, 77, 100, 155].

Peptide inhibitors can target specific connexins because of differences in the amino acid sequences of the Gap 26 and Gap 27 domains of Cxs 37, 40, and 43, which are located on the first and second extracellular loops of these connexins and are conserved in man, rat, and mouse [75]. They therefore allow some insight into the contribution of different connexins to the EDHF phenomenon in isolated tissues. In the rabbit iliac artery, for example, peptides targeted against Cx37 and/or Cx40 attenuate endothelium-dependent subintimal smooth muscle hyperpolarization, whereas peptides targeted against Cx43 attenuate the spread of hyperpolarization through the media, which corresponds to the specific pattern of connexin expression found in this vessel [23]. In some arteries, however, inhibition of EDHF-type responses may require double or triple peptide combinations to target Cxs 37, 40, and 43 collectively [28, 188]. Speculatively, this may reflect the presence of homotypic, heterotypic and/or heteromeric gap junction channels in the vascular wall, because one or more peptides may be necessary to abolish dye coupling in cultured fibroblasts, endothelial and smooth muscle cells according to the specific connexins expressed and the degree of their immunohistochemical overlap [43, 91, 120].

Morphological studies have identified Cx40 and Cx37 in proximity to intimal fenestrations in small arteries from certain vascular beds (e.g., in rat mesenteric and cerebral) and it has been suggested that myoendothelial gap junctions are constructed from these connexins in these vessels [79, 122, 158]. More specifically, EDHF-type dilations have also been suggested to depend exclusively on Cx40 in isolated rat small mesenteric arteries because endothelial loading with antibodies targeted against the cytoplasmic tail of Cx40 abrogated responses without affecting increases in endothelial $[Ca^{2+}]_i$, whereas antibodies targeted against Cx37 and Cx43 were inactive [122]. However, there is evidence that a connexin-mimetic peptide targeted against Cx43 and Cx37 inhibits EDHF-type responses in rat small mesenteric arteries [45], and that Cx43 participates in myoendothelial communication in conduit arteries [28, 121, 188] and co-culture models [91, 120].

Further evidence that Cx40 is not universally required for EDHF-type signaling has been obtained in anesthestized Cx40-deficient mice in which intravital microscopy has shown that ACh-induced dilations are intact in skeletal muscle arterioles [36], and in awake Cx40-deficient mice intraarterial ACh reduces blood pressure to the same absolute level as wildtype controls, despite their initially elevated arterial pressure [37]. This hypotensive response can be attributed to the EDHF phenomenon because it is unaffected by L-NA and is preserved in knockout mice with targeted disruption of the NO-cGMP pathway [102]. Furthermore, resistance changes in the renal circulation in response to ACh are comparable in wildtype and Cx40-deficient mice [95]. While it might be anticipated that other connexins are upregulated to compensate for the loss of Cx40 in such mice, Cx37 expression is actually down-regulated in the aortic endothelium [174] and cannot be detected immunohistochemically in arterioles [33].

Intrinsic modulation of the EDHF phenomenon

Cyclic AMP Agonist-evoked EDHF-type relaxations of isolated rabbit and rat arteries can be potentiated by a cell permeant analog of cAMP (8-bromo-cAMP) and by elevating endogenous cAMP levels with a phosphodiesterase inhibitor (IBMX) that limits cyclic nucleotide hydrolysis [77, 125, 182]. Dye transfer studies also demonstrate that 8-bromo-cAMP and IBMX enhance the diffusion of the fluorescent tracer dye calcein from the endothelium into and through the arterial media via gap junctions [77]. While IBMX does not affect the subintimal smooth muscle hyperpolarization initiated by endothelial stimulation, it potentiates medial hyperpolarization in regions remote from the endothelium (Fig. 1). In the converse sense, pharmacological inhibition of adenylyl cyclase or protein kinase A (PKA) attenuates both EDHF-type relaxations and subintimal hyperpolarizations [77, 125, 182]. Cyclic AMP has also been demonstrated to enhance inter-endothelial cell coupling [144]. Taken together, these observations suggest that cAMP can be an important physiological regulator of direct myoendothelial and homocellular communication in the vascular wall, consistent with evidence that elevations in intracellular [cAMP] can enhance dye and electrical coupling via channels constructed from Cx40 or Cx43 [19, 144, 177, 190]. More than one cellular mechanism may be involved because cAMP/PKA-dependent events not only promote rapid transport of connexins to the cell membrane and their subsequent incorporation into gap junction plaques, but may also directly enhance channel opening via connexin phosphorylation [19, 144, 177, 190].

In isolated rabbit arteries, EDHF-type relaxations are associated with NO- and prostanoid-independent elevations in smooth muscle cAMP levels that peak ~15-30 s after exposure to ACh, require the presence of an intact endothelium and can be attenuated by blockade of gap junctions with connexin-mimetic peptides or 18α-GA [77, 182]. Measurements of [cAMP] levels in the effluent from buffer-perfused rabbit and rat vascular beds have shown that ACh and the SERCA inhibitor cyclopiazonic acid both stimulate prostanoid-independent efflux of cAMP from the endothelium [97, 182]. Collectively, these observations suggest that smooth muscle cAMP levels might in part reflect diffusion of endothelium-derived cAMP into the arterial media via myoendothelial gap junctions containing Cx43 or Cx40 [99]. Candidate pathways leading to activation of endothelial adenylyl cyclase include (1) the formation of eicosanoids from arachidonic acid (see below) and (2) activation of specific Ca^{2+}-stimulated isoforms of adenylyl cyclase (e.g., type 8) by store-operated Ca^{2+} entry which can reflect the co-localization of this isoenzyme with store-operated Ca^{2+} channels in membrane caveolae [194]. It is also conceivable that transmitted endothelial hyperpolarization might influence smooth muscle [cAMP] by reducing $[Ca^{2+}]_i$ and disinhibiting the Ca^{2+}-sensitive type 5/6 adenylyl cyclase isoforms that can be coupled to L-type Ca^{2+} channels [133].

Reduced PKA activity has been hypothesized to contribute to the dysfunctional EDHF-type relaxations evident in rodent models of type 1 and 2 diabetes (streptozotocin-induced and OLEFT rats, respectively) in which responses can be normalized by IBMX [125, 126]. A single abnormality seems unlikely, however, because there may be increased cAMP hydrolysis by type 3 phosphodiesterase, reduced generation of cAMP secondary to impaired activity of adenylate cyclases types 5/6, and impaired PKA function, possibly resulting from altered catalytic and regulatory subunit expression [125–128]. Furthermore, abnormalities in other essential components of the EDHF pathway such as impaired SK_{Ca} channel function and reduced Cx40 expression have also been described [20, 208].

Eicosanoids Endothelial cells express specific cytochrome P-450 epoxygenases that synthesize four epoxyeicosatrienoic acid regioisomers (5,6-, 8,9-, 11,12- and 14,15-EET) whose pleiotrophic cellular actions are increasingly recognized to participate in the regulation of vascular tone. In some arteries, EETs closely mimic the endothelial effects of agonists and CPA, including the ability to stimulate NO production and initiate EDHF-type hyperpolarizations that conduct into the media [67, 84, 89, 182]. EDHF-type relaxations induced by exogenous 5,6- or 11,12-EET may thus be attenuated by inhibitors of SK_{Ca}/IK_{Ca} channels, gap junctional communication and adenylyl cyclase [54, 67, 89, 182]. As with agonists and CPA, these diverse effects may be interrelated. Early studies thus demonstrated that depletion of the ER Ca^{2+} store promotes the synthesis of 5,6-EET which then elevates endothelial $[Ca^{2+}]$ by stimulating store-operated Ca^{2+} entry [72, 86]. More recently, 11,12-EET generated endogenously in response to bradykinin has been shown to promote endothelial cAMP synthesis by activating adenylyl cyclase [144], and to contribute to store-operated Ca^{2+} entry and endothelial hyperpolarization by promoting cAMP/PKA-dependent translocation of TRP channels to the plasma membrane [63]. Endogenous synthesis of 11,12-EET also enhances gap junctional communication through mechanisms that involve cAMP/PKA-mediated phosphorylation events that can be mimicked by exogenous 11,12-EET [144].

In smooth muscle, EETs can induce relaxation by activating BK_{Ca} channels, possibly following the occupation of specific membrane receptors, and this mechanism has been suggested to confer a role for EETs as freely transferable EDHFs [21, 69, 83, 138, 175]. EET-induced hyperpolarization has also been suggested to involve a signaling complex in which Ca^{2+} influx via vanilloid

transient receptor potential (TRPV4) channels preferentially stimulates ryanodine receptors located on Ca^{2+} stores to increase the frequency of Ca^{2+} sparks and activate BK_{Ca} channels [48]. Observations that selective blockade of BK_{Ca} channels with iberiotoxin fails to inhibit authentic agonist-induced EDHF-type relaxations in some arteries, while attenuating smooth muscle hyperpolarizations evoked by exogenous EETs, nevertheless indicates that they cannot be universally equated with "EDHF" [21, 41, 48, 50, 54, 63, 65, 69, 72, 83, 86, 138, 146, 175]. Indeed, endogenous EET synthesis may not contribute consistently to the EDHF phenomenon because exogenous EETs fail to hyperpolarize some rat and guinea pig arteries, even in preparations with intact endothelium, thus seemingly excluding both autocrine and paracrine mechanisms of vasorelaxation [22, 191, 209]. Some reports also demonstrate that EETs may elevate $[Ca^{2+}]_i$ in vascular smooth muscle cells [58] and are potent vasoconstrictors in the pulmonary circulation [101].

Hydrogen peroxide The hypothesis that endothelium-derived H_2O_2 is a freely diffusible EDHF is predicated on evidence that NO- and prostanoid-independent relaxations depend on catalase-sensitive endothelial H_2O_2 production in murine and human arteries in which exogenous H_2O_2 activates smooth muscle BK_{Ca} channels [123, 124]. However, in rabbit iliac arteries without endothelium relaxations equivalent to those observed during EDHF-type, responses are only obtained with supraphysiological concentrations of H_2O_2 (≥300 μM) and are insensitive to inhibitors of BK_{Ca} and other K^+ channel subtypes [52, 68]. H_2O_2 may nevertheless participate in the EDHF phenomenon in this vessel by modulating endothelial function (Fig. 2). In preparations with intact endothelium EDHF-type relaxations evoked by ACh and CPA are strongly potentiated by preincubation with exogenous H_2O_2 at concentrations (30-100 μM) that exert only a small direct smooth muscle hyperpolarizing action [24, 52, 68]. In the converse sense, incubation with submaximal concentrations

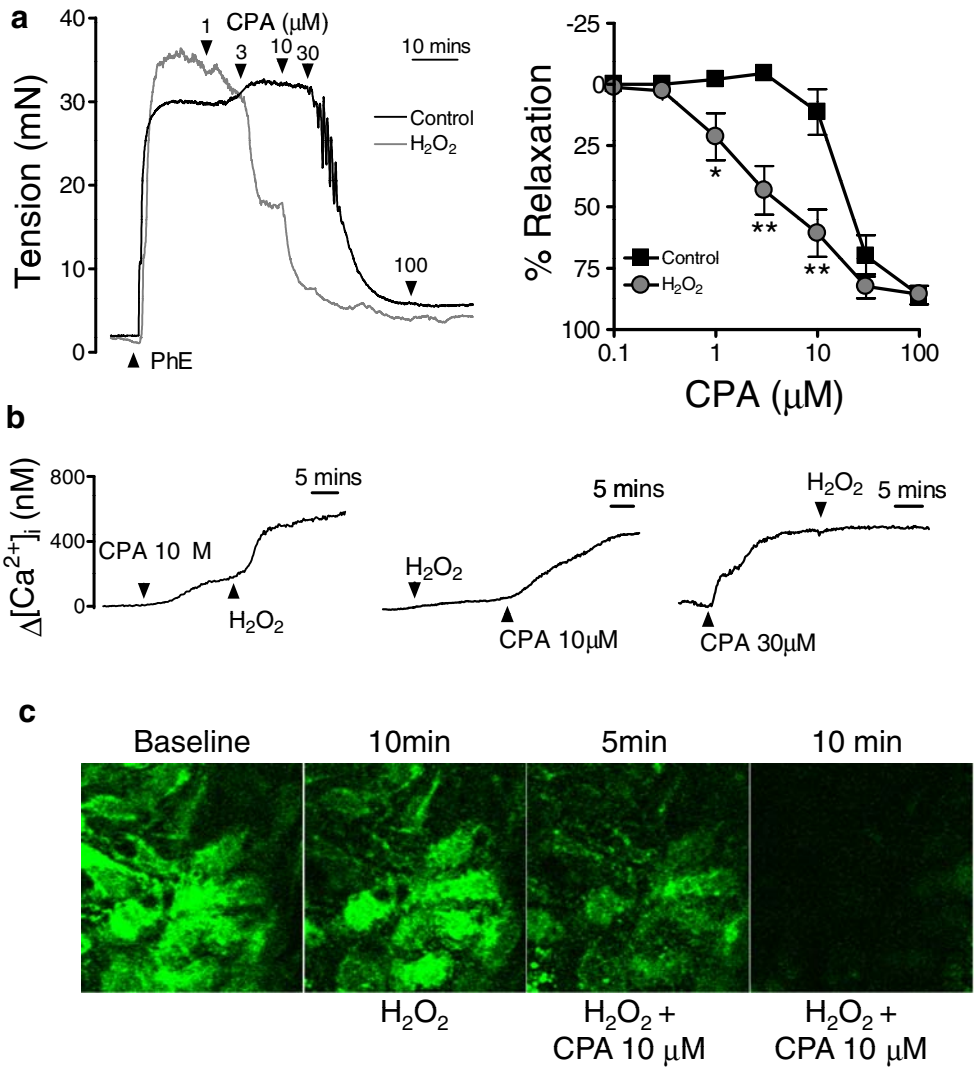

Fig. 2 Potentiating effects of H_2O_2 on the EDHF phenomenon. **a** Relaxations evoked by cyclopiazonic acid (CPA) in the rabbit iliac artery and **b** CPA-evoked mobilization of Ca^{2+} in rabbit aortic valve endothelial cells were both potentiated by 100 μM H_2O_2. **c** Confocal images of valvular endothelial Ca^{2+} stores loaded with Mag-fluo-4 demonstrated how incubation with 100 μM H_2O_2 minimally affected $[Ca^{2+}]_{ER}$ but facilitated complete store depletion in the presence of a normally submaximal concentration of CPA (10 μM)

of CPA unmasks an EDHF-type response to H_2O_2 that is sensitive to combined SK_{Ca}/IK_{Ca} blockade thereby confirming its endothelial origin [52]. Analysis of intracellular endothelial Ca^{2+} mobilization has shown that this synergism can be attributed to sensitization of the $InsP_3$ receptor such that at any given level of stimulation there is enhanced Ca^{2+} release from the ER Ca^{2+} store and consequently increased endothelial K_{Ca} channel activity [52]. EDHF-type relaxations and endothelial Ca^{2+} mobilization are similarly potentiated by the sulphydryl reagent thimerosal, which sensitizes ER Ca^{2+} release to both Ca^{2+} and $InsP_3$ by oxidizing critical thiol groups present in the $InsP_3$ receptor, suggesting that H_2O_2 and thimerosal may share a common molecular target [18, 52, 89].

Buffer concentrations of $H_2O_2 \leq 100$ μM would be expected to generate intracellular H_2O_2 levels within the suggested physiological range (1-10 μM) because intrinsic antioxidant mechanisms are thought to limit cytosolic [H_2O_2] to 1-15% of that applied extracellularly [161]. It nevertheless remains to be established if the principal endothelial sources of H_2O_2 (eNOS, NADPH oxidase and mitochondria) modulate the EDHF phenomenon under physiological and/or pathophysiological conditions by promoting intracellular Ca^{2+} release. If so, the increased endothelial oxidant stress and H_2O_2 production from superoxide anions that is a feature of diseases such as hypertension, hypercholesterolemia, and diabetes, might offset reductions in the bioavailability of NO resulting from its direct chemical interaction with superoxide. It also remains to be established whether H_2O_2 generated by smooth muscle and/or adventitial cells in disease states can modulate the EDHF phenomenon because selective adventitial application of H_2O_2 is capable of potentiating NO-independent dilations to CPA and ACh in perfused rabbit iliac artery segments [68].

Ascorbic acid (AA) and R-5,6,7,8-tetrahydrobiopterin (BH_4) can both reverse endothelial dysfunction by preventing eNOS uncoupling and the production of superoxide anions by the oxygenase component of the enzyme. Although AA and BH_4 are generally regarded as antioxidants, both agents are also able to generate H_2O_2 in the presence of molecular oxygen via metal-catalyzed oxidation and autoxidation, respectively [68]. This pro-oxidant activity can potentiate EDHF-type relaxations evoked by ACh and CPA in the rabbit iliac artery via a mechanism that correlates with the extracellular generation of H_2O_2 [68]. Species-specific effects of H_2O_2 nevertheless remain to be evaluated because EDHF-type dilations are inhibited rather than potentiated by exogenous H_2O_2 and AA-derived H_2O_2 in the buffer-perfused rat mesentery [134]. Indeed, H_2O_2 has been reported both to inhibit and to enhance cell-cell coupling by altering the phosphorylation/oxidation status of residues present in the intracellular cytoplasmic tail of Cx43 in non-vascular cells [150, 153, 189].

Myoendothelial coupling in vivo—no evidence yet?

In contrast to the findings with isolated arteries and arterioles described above, a number of studies have failed to demonstrate tight myoendothelial coupling in arterioles in vivo [34]. Thus, electrophysiological measurements obtained during intravital microscopy have demonstrated differences in the resting membrane potential of endothelial and smooth muscle cells of the order of 10 mV in murine skeletal muscle arterioles [171]. Additionally, BK_{Ca} blockade with iberiotoxin inhibits ACh-induced smooth muscle hyperpolarization in such vessels without affecting endothelial hyperpolarization [171], and adenosine induces dilation by activating smooth muscle K_{ATP} channels but does not affect endothelial cell membrane potential [33], thus contrasting with isolated conduit arteries in which K_{ATP} mediated hyperpolarization is transmitted to the endothelium via gap junctions [131]. In hamster cheek pouch arterioles also, smooth muscle depolarizations fail to affect endothelial membrane potential in vivo, hyperpolarizations initiated by ACh differ between endothelial and smooth muscle cells [196], and endothelial and smooth muscle hyperpolarization exhibit differential sensitivity to 17-octadecynoic acid, a putative inhibitor of cytochrome *P*-450 [197]. Furthermore, the effect of selective endothelial or smooth muscle destruction on conducted dilatory responses suggests that hyperpolarization is not freely transferred between the two cell types, as detailed below [15].

These discrepant results obtained in vivo vs. in vitro are puzzling. The most obvious differences concern the experimental approaches. Firstly, vessels studied in vitro are usually larger in diameter (>80 μm) than those studied in vivo (30-80 μm). Secondly, in many cases, vessels studied in vitro have been harvested from the mesenteric vascular bed which are technically easy to obtain, whereas in vivo arterioles have been most frequently studied in the hamster cheek pouch or skeletal muscle (e.g., the cremaster microcirculation) because such tissues are readily accessible for intravital microscopy. Other possible explanations are that physiologic stimuli that act in vivo but not in vitro may influence myoendothelial coupling in a specific manner. These include the presence of blood, shear stress and blood flow with NO release [149], or inhibitory effects of sympathetic innervation [81]. Specifically, mechanical stimuli may alter the membrane potential of vascular cells which reflects an alteration of ion channel activity and in turn may affect myoendothelial coupling. Blood-borne substances such as oxidized phospholipids have also been found to affect the expression and phosphorylation status of connexins at myoendothelial junctions in an in vitro co-culture model [92]. Another important variable may be anesthesia which is obviously required in in vivo

studies. Indeed, certain anesthetics can impair EDHF-type relaxations [35] and may also exhibit effects on connexin gating and therefore presumably on myoendothelial coupling [198].

Longitudinal signaling through gap junctions—conducted responses

Gap junctions facilitate longitudinal signaling because they interconnect neighboring endothelial cells and similarly link together smooth muscle cells. Consequently, local stimulation of resistance vessels with agents that modulate membrane potential cause local changes in diameter at the stimulation site that literally conduct or propagate along the vessel [32, 38, 163]. Depending on the stimulus used and the resulting directional change in membrane potential (hyperpolarization or depolarization) conducted dilations or constrictions are initiated. In case of dilations, remote responses are not due to shear stress-induced dilations because not every dilator initiates such a conducted response (specifically NO is unable to elicit a conducted response) and shear rate does not increase at remote sites [87, 165]. In contrast, membrane potential changes are crucial at the local site and such changes can also be retrieved at remote sites [55, 56, 171, 197].

The abundant connexin expression found in the endothelium suggests a preferential low-resistance longitudinal pathway for electrotonic signaling via endothelial cells. However, in most resistance vessels, the smooth muscle layer provides a second conducting pathway (Fig. 3), although this is not always the case, e.g., in small arteries and arterioles in the hamster retractor skeletal muscle [165]. As noted in the previous section, the two pathways may not be tightly coupled heterocellularly in vivo [15, 33] and may exhibit distinct properties (length constants, amplification mechanisms, connexins involved, etc.). The idea of separate pathways is most evident from in vivo experiments with skeletal muscle arterioles in which either the smooth muscle or the endothelial pathway was selectively destroyed in succession along the vessel [15]. Under these conditions, the dilation conducted always up to the second destroyed site regardless of whether this was in the endothelial or the smooth muscle layer, but never beyond this second site. These observations suggest that the vasodilator signal is initiated at the stimulation site in both layers and conducts in each layer up to the position of destruction but that the signal does not actually cross between layers while traveling [15]. Further evidence for the idea that separate pathways exist in vivo comes from their dependency on distinct connexins as will be outlined below. It should be noted, however, that in rat mesenteric arteries studied in vitro, the endothelium may be crucial for the longitudinal propagation of hyperpolarizing signals initiated in smooth muscle cells by activation of K_{ATP} channels [180].

The distances over which a vasomotor response is able to conduct should be related to the resistance of the electrical communication pathway and thus determined by gap junction resistivity and number of cell membranes passed. These theoretical considerations predict that the electrotonic spread of locally initiated membrane potential changes will differ between the endothelial and smooth muscle layers, as the latter exhibits a larger resistance due to the anatomical position of the smooth cell cells which are wrapped with their long axis circularly around the vessel and connexin expression is less abundant in the media than the endothelium. Indeed, the amplitude of conducted vasoconstrictions decreases exponentially with distance from their point of origin, consistent with electrotonic conduction via the smooth muscle cell layer [2, 149]. The decay in the amplitude is enforced by NO which may reflect an ability to attenuate gap junction conductance [149]. In contrast to vasoconstrictions, dilations which supposedly travel along the smooth muscle layer in response to certain agonists (e.g., adenosine) cover larger distances and augmentation by active membrane processes such as ion channel openings have been suggested [31]. A likely candidate to sustain a propagating hyperpolarization regeneratively is the inward rectifier K^+-channel (K_{IR}) which may serve as an amplifier because blockade of K_{IR} by barium ions attenuated remote but not local dilations [33, 94, 148]. K_{IR} channels are expressed in vascular smooth muscle [11] and are activated by hyperpolarization [202] and by increased extracellular K^+ concentrations (for review on K_{IR} channels in smooth muscle, see [139]).

Conducted dilations initiated by endothelium-dependent agonists such as ACh or bradykinin propagate at 2 mm/s along the endothelial layer several millimeter with minimal decrement although the decay of the amplitude with distance varies between species. The decay is more pronounced in hamsters [40, 87, 106] than in mice [3, 36, 200]. Moreover, decay increases with age [3] and is enhanced by hypertension [107] but not changed in hypercholesteremic animals [199]. The reasons for increased decay are unknown but may involve alterations in possible amplifier mechanisms or sympathetic nerves whose activation decreases conduction [82]. Cyclic AMP levels may also be altered under pathophysiological conditions, and as in the case of myoendothelial coupling, cAMP modulates homocellular coupling in cultured endothelial cells as indicated by its ability to prevent inhibitory effects of lipopolysaccharide or reoxygenation on gap junctional coupling in a Cx40-dependent manner [6, 7]. In mice and hamsters, Ca^{2+}-activated K^+-channels initiate the response [40, 87] and may also act as amplifiers along the

conduction pathway [201]. Although amplification of the initiating signal helps to cover larger distances, the signal which initiates the response will concomitantly be disturbed and hence the response at remote sites may not be related to or reflect the initiating signal amplitude.

Intracellular calcium may contribute and act as another player in conducting responses [40, 187]. In mice, the endothelial $[Ca^{2+}]_i$ increase evoked by agonists such as ACh can itself propagate slowly at 110 μm/s although it decays relatively rapidly from the point of stimulation [181]. This wave of elevated $[Ca^{2+}]_i$ can promote release of NO whose dilator effect may superimpose on electrotonically mediated dilation as shown in hamster arterioles, albeit only for short distances (Fig. 3) [15]. Indeed, the contribution of NO to conducted responses is even more questionable in other species (mice) [141, 171, 199] and may also be related to the agonist used (ACh vs. bradykinin), vessel studied, or experimental conditions. For example, in isolated larger vessels (small arteries) from the hamster cheek pouch or the rat mesentery $[Ca^{2+}]_i$ increases are restricted to the local site [44, 180]. Increases in $[Ca^{2+}]_i$ may contribute to the hyperpolarization through activation of K_{Ca} and in turn the hyperpolarization may further augment the $[Ca^{2+}]_i$ increase and thus the role of a propagating Ca^{2+} wave warrants further study.

Simple resistive/capacitative models suggest how localized electrical events might conduct robustly along the endothelium while eliciting a parallel response in underlying smooth muscle because loss of charge is minimized by low endothelial coupling resistance (3 MΩ) and high myoendothelial coupling resistance (1,800 MΩ) [39]. Small movements of charge from the endothelium are able to effect substantial changes in the membrane potential of smooth muscle cells because the input resistance of the media is high (thus explaining the EDHF phenomenon in terms of Ohm's law). By contrast, localized electrical events in smooth muscle conduct poorly to neighboring cells because their high coupling resistance (90 MΩ)

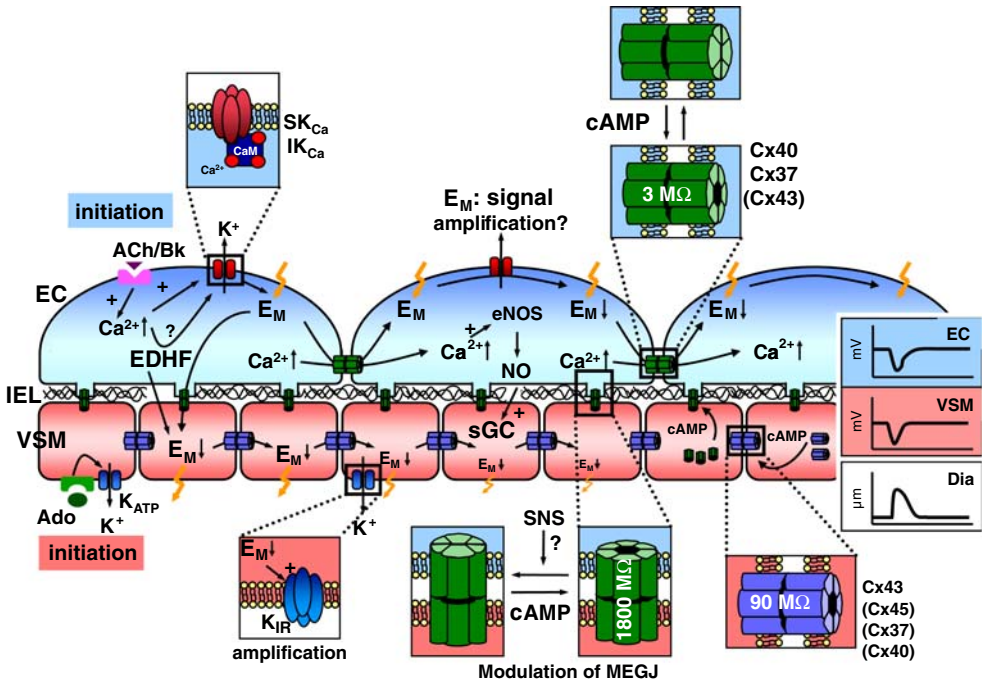

Fig. 3 Longitudinal coupling coordinates vascular behavior. Local stimulation using agonists (initiation) acting on the endothelium (*ACh* acetylcholine, *Bk* bradykinin) or the smooth muscle (*Ado*, adenosine) initiates a dilation that conducts along the vessel. Such a conducted response requires a hyperpolarization, e.g., through the activation of K^+-channels (endothelial Ca^{2+}-activated K^+-channels, SK_{Ca} and IK_{Ca}, or smooth muscle ATP-dependent K^+-channels, K_{ATP}). The hyperpolarization is transmitted along the vessel wall through gap junctions interconnecting either endothelial or smooth muscle cells homocellularly that are composed of different connexins (Cx) as indicated. Cx subtypes shown in brackets may contribute in some vessels. During conduction the signal is suggestedly amplified by inward rectifier K^+-channels (K_{IR}) in smooth muscle, whereas the amplification mechanism in the endothelium is unknown. Nevertheless the signal dissipates with distance which is pronounced in smooth muscle in part due to higher intercellular resistances (coupling resistances are taken from [39]). In some vessels a localized rise in Ca^{2+} and its conduction may contribute to the dilation which involves the release of NO. An EDHF or myoendothelial coupling elicits the transfer of charge from the endothelium to smooth muscle. Note, that myoendothelial resistance was estimated to be quite large but subject to modulation by cAMP and proposedly by the sympathetic nerve system (*SNS*). In addition, cAMP enhances conductance of homocellular gap junctions and insertion of connexons into the membrane. Further details see text. *EC* endothelial cells, *IEL* internal elastic lamina, *VSM* vascular smooth muscle, E_M membrane potential, *MEGJ* myoendothelial gap junctions, *sGC* soluble guanylate cyclase, *Dia* diameter

promotes signal dissipation, and when charge does conduct to the endothelium, its effects on membrane potential are minimized by the low endothelial input resistance unless a large number of smooth muscle cells are stimulated simultaneously [39]. This attractive model has been used to explain data generated from isolated feed arteries, but does not fully accord with experimental findings in respect of myoendothelial in vivo coupling, as outlined above.

Connexins connecting vascular cells that promote conducted responses

Cx37 and Cx40 are abundantly expressed in the endothelium of arterioles in many species [80, 88, 115, 200] and Cx43 may be additionally present in the endothelium of larger vessels in rats, hamsters, and rabbits (Fig. 3) [4, 88, 114, 121, 188]. Because it is difficult to block a specific connexin in vivo, the role of different connexin subtypes in conducted responses can only be rigorously evaluated in animals deficient in the appropriate connexin. Cx40-deficent mice exhibit a strong impairment of the conduction of dilations initiated by endothelium-dependent agonists (ACh, bradykinin) as indicated by an attenuation of the dilatory amplitude at remote sites compared to wildtype mice despite a similar local dilation [36]. Notably, remote dilations were attenuated but not delayed in these mice suggesting that amplification mechanisms are not able to compensate for the lack of Cx40. Conducted dilations induced by electrical activation of endothelial cells were attenuated at remote sites in a similar fashion in Cx40-deficient mice [62]. The crucial role of Cx40 in conducting endothelium-dependent dilations was further confirmed in mice in which Cx40 was replaced by Cx45 using homologous recombination (Cx40KI45 mice). These mice express Cx45 instead of Cx40 in endothelial cells as well as in other tissues that normally express Cx40 [1] and the conduction deficit was indistinguishable between these Cx40KI45 mice and animals carrying a global deficit of Cx40. This demonstrates that Cx45 expressed in endothelial cells is not sufficient to support the endothelial function of Cx40 [200]. However, other Cx40 functions related to hypertension and renin secretion can be at least partially rescued by Cx45 highlighting a distinct tissue specificity of connexin function which possibly reflects distinct requirements for channel gating or conductance [162].

In contrast, conducted constrictions initiated by K^+-depolarization remained intact in both mice (deletion of Cx40 or its replacement by Cx45). This observation supports the idea that constrictions are initiated in smooth muscle and are conducted along the smooth muscle layer in a Cx40-independent manner. As noted above, adenosine initiates conducted dilations by activation of K_{ATP} channels and adenosine, in contrast to ACh, does not elicit an endothelial hyperpolarization in skeletal muscle arterioles in vivo [33]. Moreover, the amplitude of conducted dilations initiated by adenosine decayed with distance and notably remained intact in Cx40-deficient and Cx40KI45 mice [33, 200]. This suggests that the dilator signal evoked by adenosine conducts independently of Cx40 along the smooth muscle layer. Strikingly, the decay of the amplitude of ACh-evoked dilations in Cx40-deficient mice resembled those of adenosine in wildtype animals suggesting that the signal of the dilation in response to ACh also conducts along the smooth muscle cell layer in the absence of Cx40, and thus exhibits a similar decay of the amplitude as seen with adenosine [33].

A key question is why Cx37 is unable to support endothelial conduction in the absence of Cx40 because Cx37 is abundantly expressed in endothelial cell membranes in cremasteric arterioles. This surprising observation may be related to the downregulation of Cx37 expression in Cx40-deficient arterioles to a level that cannot be detected by immunohistochemistry [33]. Likewise, Cx37 was not found in renin-producing cells in Cx40-deficient mice although physiologically expressed in these cells [108] and Cx37 expression is also reduced in aortic endothelial cells [33, 174]. This suggests that Cx40 is crucial to support the insertion of Cx37 into the cell membrane although the mechanisms for this dependency remain obscure. In contrast to deletion of Cx40, Cx37-deficiency does not impair the conduction of dilations [61] and these mice are likewise not hypertensive or exhibit defects in renin regulation [193]. Cx40 expression is not altered in kidneys from Cx37-deficient mice [193] but has not yet been studied in endothelial cells in these mice.

Role of gap junctions in vasomotion

Spontaneous fluctuations in vascular caliber (the phenomenon of vasomotion) are ubiquitous in the microcirculation and provide an example of emergent chaotic behavior that ultimately reflects the entrainment of nonlinear oscillatory activity in large populations of coupled smooth muscle cells [74, 78]. Physiologically, arterial diameter oscillations around a fixed mean are thought to increase local perfusion by increasing time-averaged hydraulic conductance, and oscillatory perfusion has been shown to enhance the clearance of tracer dyes from the tissue interstitium [140, 154]. Furthermore, irregular chaotic flow accelerates diffusion-limited processes, and may therefore be particularly effective in enhancing the delivery of oxygen [74, 145]. Vasomotion may thus serve to optimize the distribution of microcirculatory flow and mass transport processes.

At the cellular level, imaging studies have shown that the emergence of vasomotion in isolated arteries coincides with the synchronization of intracellular Ca^{2+} waves that propagate through the cytosol of individual smooth muscle cells in an apparently random fashion under resting conditions [142]. Entrainment is critically dependent on gap junctional communication because connexin-mimetic peptides rapidly suppress the synchronization of Ca^{2+} oscillations in intact arteries and cultured smooth muscle cells [120, 121], and in endothelium-denuded rabbit arteries peptides targeted against Cx43 abolish rhythmic contractile activity without affecting average force development, suggesting that the uncoupling of smooth muscle cells allows uncoordinated fluctuations to summate as a non-oscillatory response [25]. The endothelium may also play an important role in regulating vasomotion because focal regions of spasm that propagate slowly along the arteriolar wall appear spontaneously in Cx40-deficient mice, and are able to stop flow completely [37]. Impaired gap junctional communication may therefore have major implications for microvascular function in disease states [74, 151].

Conclusions

There is growing evidence that mechanisms central to the EDHF phenomenon include (1) an initiating endothelial hyperpolarization that is dependent on the opening of K_{Ca} channels and (2) hyperpolarization of the arterial media following electrotonic relay of this change in membrane potential via myoendothelial and homocellular smooth muscle gap junctions. The contributions of both components of this pathway can be modulated by endogenous factors. For example, cAMP can enhance homocellular and myoendothelial vascular communication via gap junctions; and in some vessels, endothelial production of EETs can exert autocrine effects that stimulate capacitative Ca^{2+} entry, open K_{Ca} channels, and enhance cAMP synthesis. Endothelial Ca^{2+} mobilization and hyperpolarization can also be modulated by H_2O_2 suggesting that endothelial redox signaling may play a novel role in the EDHF phenomenon under normal and pathophysiological conditions. While the role of myoendothelial coupling in the EDHF phenomenon seems established in isolated vessels, experimental data collected on smaller arterioles have so far failed to clarify its role in vivo. However, longitudinal coupling via gap junctions allows the relay of dilatory signals over biologically significant distances almost without decrement. A major effort is now required to identify the amplification mechanisms that underpin the propagation of such responses and define the roles of specific connexin subtypes using specific blockers and connexin-deficient mice. Increased understanding of highly complex intra- and inter-cellular interactions will be essential in the quest for future vascular therapies that ameliorate disease resulting from alterations in gap junctional coupling.

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