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The endothelium in compliance and resistance vessels

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1. ABSTRACT

The endothelium is a single layer of cells lining all blood vessels. Although the endothelium is not an organ it does, nonetheless have autocrine, paracrine and endocrine-like functions that affect the cardiovascular system. Until the description in 1980 by Nobel Laureate Robert Furchgott of endothelium-derived relaxing factor (EDRF), later identified as nitric oxide (NO), the endothelium was considered to be a semi-permeable barrier between the blood and the smooth muscle cell layers of the blood vessel. Heterogeneity exists in the functions of the endothelium with differences evident between species and between the large conduit compliance vessels and the resistance vessels of the microcirculation. Endothelial dysfunction, defined as a reduction in the ability of the endothelium to transmit a vasodilatation influence on blood flow, has prognostic significance and serves as an early indicator of the development of vascular disease as well as a therapeutic target. This review compares the role of the endothelium in the regulation of vascular tone in conduit versus resistance vessels and how alterations in endothelial function may lead to vascular disease.

2. INTRODUCTION

Close to 500 years after the description of the circulation in 1628 by William Harvey considerable effort is now being focused on the importance of the endothelium – the single layer of cells that interfaces with the blood and is present in all blood vessels. The endothelium was first described by Theodor Shwann in 1847 as a "distinctly perceptible membrane", but the name "endothelium" first appears in 1865 and is attributed to Willem His (1,2). Use of electron microscopy in the 1950s and 1960s provided details of the structural aspects of the endothelium, but the importance of the endothelium in the regulation of blood flow was not realized until 1980. Pre-1980 the endothelium was viewed as functioning primarily, if not solely, as an inert, selectively permeable barrier between the blood in the circulation and the vessel wall.

In an adult human the endothelial cell layer consists of approximately ten trillion (10^{13}) cells that contribute approximately 1.4% to total body mass (3). Although pre-1980 the role of neuronal and endocrine influences on vascular tone were well known it was the

publication by Robert Furchgott's laboratory in 1980 that resulted in considerable attention being focused on the role of the endothelium in the regulation of cardiovascular function (4). Furchgott & Zawadzki described the essential role played by the endothelium in mediating the vasodilatation action of acetylcholine in rabbit aortic rings with the endothelium-derived relaxing factor (EDRF) eventually being identified as nitric oxide (NO) (4).

In the past 30 years it has become apparent that, in addition to NO, a multitude of endothelium-derived products as well as endothelium-dependent regulatory processes play critical roles in maintaining a balance between vasoconstriction and vasodilatation. The endothelium produces, or plays a key role in the production of, vasoconstrictors that include products of arachidonic acid metabolism such as thromboxane A2 and also peptides that include endothelin-1 and angiotensin II as well as superoxide anions. Vasoconstriction is offset by the generation or synthesis of endothelium-derived relaxing factors with NO and to a lesser extent prostacyclin (PGI_2) being "universal" vasodilator substances important throughout the circulation. Following pharmacological inhibition or genetic ablation of the contribution of endothelial nitric oxide synthase (eNOS) and cyclooxygenase (COX) an important non-NO/PGI_2 endothelium-dependent vasodilatation (EDV) mechanism is evident and involves endothelium-dependent hyperpolarization (EDH). EDH may involve an endothelium-derived "chemical mediator" termed "endothelium-derived hyperpolarizing factor", EDHF, and/or low-resistance electrical coupling between the endothelial cell and vascular smooth muscle cell (VSMC) layers that is facilitated by myo-endothelial gap junctions (MEGJs). In some vascular beds eicosanoids, notably epoxyeicosatrienoic acids (frequently abbreviated as "EETs"), are involved in endothelial cell-VSMC communication; however, evidence has also been presented that, among other putative candidates, a small increase in extracellular potassium (K^+), hydrogen peroxide (H_2O_2), or C-type natriuretic peptide (CNP) are suggested mediators of EDH. There is, however, evidence for considerable diversity in the relative importance of endothelium-dependent vasodilator versus vasoconstrictor influences in the vasculature suggesting some degree at least of specialization within different vascular beds with NO being the sole, or primary, EDRF in conduit elastic vessels and non-NO-mediated mechanisms seemingly taking on important roles in the microcirculation.

The influence of disease on endothelium-dependent regulation of vascular tone is also an area of considerable interest with both angiotensin II and endothelin-1 already being therapeutic targets for angiotensin converting enzyme inhibitors (ACEI) and angiotensin and endothelin receptor antagonists.

3. ENDOTHELIUM-DEPENDENT REGULATION OF BLOOD VESSELS

An accumulation of evidence convincingly supports the argument that the contribution of EDHF to EDV is considerably more important in the resistance vasculature than in conduit (elastic) compliance vessels. Thus, in a comparison of EDV in large versus small arteries from the rat mesenteric arcade the relative contribution of the EDHF-mediated component is much greater in the distal resistance arteries (5). In mice genetically deficient in the enzyme responsible for the generation of NO in the endothelium, namely eNOS, acetylcholine-mediated vasodilatation is completely absent in large elastic conduit (aorta) vessels, but partially maintained by EDHF-mediated EDV in mesenteric resistance arteries (6). Furthermore the expression level of eNOS protein and the contribution of NO is most prominent in the aorta, whereas that of EDHF is most prominent in the distal mesenteric arteries (7). Such data stress the importance of EDHF in the microcirculation and suggest that EDHF plays only a minimal role, if any at all, in conduit vessels. These findings raise important questions concerning the contribution of the endothelium to the regulation of vascular tone and regional specialization. Thus:

1. Is there an anatomical and/or physiological basis for the absence of an apparent significant contribution of EDHF to the regulation of vascular tone in conduit vessels?

A partial answer to this question may relate to the relationship between internal elastic lamina (IEL) and VSMC layers (8,9,10).

2. In the absence, or reduction in the bioavailability, of NO is there an up-regulation in the contribution of non-NO endothelium-dependent vasodilatation?

A partial answer to this question is provided by data indicating that at least in large conduit arteries such as the aorta, neither PGI_2 nor EDHF compensate for the absence of NO that results from genetic ablation of eNOS (6). However, spreading vasodilatation in the microcirculation is dependent on the integrity of the endothelial cell layer, but independent of NO.

3. Does the contribution of EDHF to endothelium-dependent vasodilatation demonstrate age-dependency?

There is an age-dependent progressive reduction in the participation of both NO and EDHF to endothelium-dependent regulation of vascular tone that can be associated with an increase in the contribution of endothelium-derived contracting factors (EDCFs) that include peptides in the endothelin-1 and angiotensin II pathways as well as oxygen-derived free radicals and COX-derived prostanoids (11,12). MEGJs and EDHF-mediated regulation of vascular tone have also been shown to be more important in the early stages of development, as evident from a study of the presence of MEGJs and relative importance of EDHF in saphenous arteries from juvenile and adult rats (13).

In disease states that affect cardiovascular function, such as diabetes, changes in the contribution of

both NO and non-NO-, including EDHF-, mediated vasodilatation and changes in the contribution of EDCFs has also been reported and the literature has been extensively reviewed (14,15,16).

To better understand the differences between the conduit and resistance vessels it is important to review in more detail the nature and the relative importance of NO versus non-NO mediated EDV.

4. ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION (EDH)

There is still no clear cut consensus on the cellular basis for EDH, but, based on pharmacological data, the hyperpolarizing influence of the endothelium on VSMC membrane potential results from closure of smooth muscle voltage-gated calcium (Ca^{2+}) channels subsequent to the activation in endothelial cells of small and intermediate calcium-activated potassium channels (SK, specifically SK3, KCa2.3, and IK, KCa3.1, respectively). In the greater number of studies concerning EDHF it has been reported that EDH is inhibited by a combination of inhibitors for the KCa2.3 and KCa3.1 channels – apamin and charybdotoxin (or TRAM-34 respectively) – an observation first reported in 1994 by Waldron & Garland (17,18). Thus, just as an increase in endothelial cell intracellular Ca^{2+} is a prerequisite for the activation of eNOS and the subsequent generation of NO so also is an increase in endothelial cell intracellular Ca^{2+} required for the initiation of EDH.

4.1. What is EDHF?

Since the field of EDHF research has been comprehensively analysed this review will only summarize key aspects and the reader is referred to several reviews for current views on the cellular mechanisms as well as putative candidate molecules that may mediate EDH (19,20,21,22,23). In brief, EDH may be mediated by a small increase in endothelial cell K^+ in the intercellular space between the endothelial cell and VSMC that results from the opening of endothelial KCa2.3 and KCa3.1 channels (24). Another potential candidate mediator of EDH, notably in the coronary circulation, is a cytochrome P450 (CYP) product of arachidonic acid metabolism, namely an epoxyeicosatrienoic acid (EET) with 14,15-EET and 11,12-EET derived likely from CYP2C and CYP2J9 (25,26). A confounding property of epoxyeicosatrienoic acids is that they mediate vascular smooth muscle hyperpolarization via an iberiotoxin-sensitive large conductance calcium-activated K^+ channel (BK, or KCa1.1) – a property that does not fit with the "classic" apamin + charybdotoxin (or TRAM-34)-sensitive pathway. Epoxyeicosatrienoic acids, however, may play key roles in homocellular signalling in the endothelial cell layer and regulation of store-operated calcium channels (27,28). Another candidate molecule for mediating EDHF is H_2O_2 with supportive evidence presented for rodent, porcine and human blood vessels (29,30,31). CNP has also been proposed and vigorously debated as a potential EDHF (32,33,34). Finally, evidence also exists that the EDHF phenomenon is primarily mediated by low-resistance electronic coupling between the endothelial cell and VSMC layers MEGJs (20,35).

5. IS THERE AN ANATOMICAL AND/OR PHYSIOLOGICAL BASIS FOR THE ABSENCE OF AN APPARENT SIGNIFICANT CONTRIBUTION OF EDHF IN CONDUIT VESSELS?

An important consideration is the diffusion barrier that is presented by the IEL. The IEL, together with the single layer of endothelial cells, make up the tunica intima and the IEL consists of a network of elastic fibers that have small perforations giving it a fenestrated-like appearance. In small arteries the fenestrated IEL is quite thin, but in larger arteries, such as the aorta, it is comparatively thick. Thus, the IEL presents a barrier for both chemical and electrotonic communication between the endothelial cell and VSMC layers that, in itself, could explain the lack of a significant role for EDHF-mediated EDV in conduit vessels such as the aorta. However, the small holes, or perforations, in the IEL may provide for the passage of not only chemical mediators but also cell projections that could facilitate endothelial cell-VSMC communication and this is an area of active research. Protrusions of endothelial cells through the IEL are termed endothelial cell protusions (ECPs) and where there is plasma membrane juxtaposition between the two cell types a MEGJ is described. MEGJs were first observed in small arteries in the canine heart with the use of electron microscopy by Moore and Ruska (36) and the importance of the MEGJs, at least in terms of their frequency, can be attributed to the descriptions by Rhodin (37) in electron microscopic studies of rabbit kidney arterioles. A link between MEGJs and EDH was made by Sandow and Hill (38) who provided ultrastructural evidence for an increasing density of small MEGJs, the majority being <100 nm in diameter, moving from proximal to distal arterioles in the rat mesenteric vascular bed. The MEGJ is now the focus for considerable attention both with respect to the role of the MEGJ in the regulation of vascular tone as well as with respect to whether changes in MEGJ structure and/or function contribute to cardiovascular pathologies (39). Figure 1 illustrates the anatomical relationship, together with putative signalling mechanisms, that facilitates communication between the endothelium, through the IEL, and the underlying VSMC layer.

The differing contributions of ECPs in conduit versus resistance vessels are likely an important consideration not only for our understanding of the physiology but also the pathophysiology of blood vessels. Furthermore, not all IEL holes have associated MEGJ projections and this may also provide an additional explanation as to the vessel-to-vessel and species-to-species variability between the contribution of EDHF to EDV (9). It can also be speculated that the MEGJ projections are dynamic and subject not only to the effects of changes in the level of contraction of the blood vessel, but also the modulatory effects following activation of cell signalling pathways.

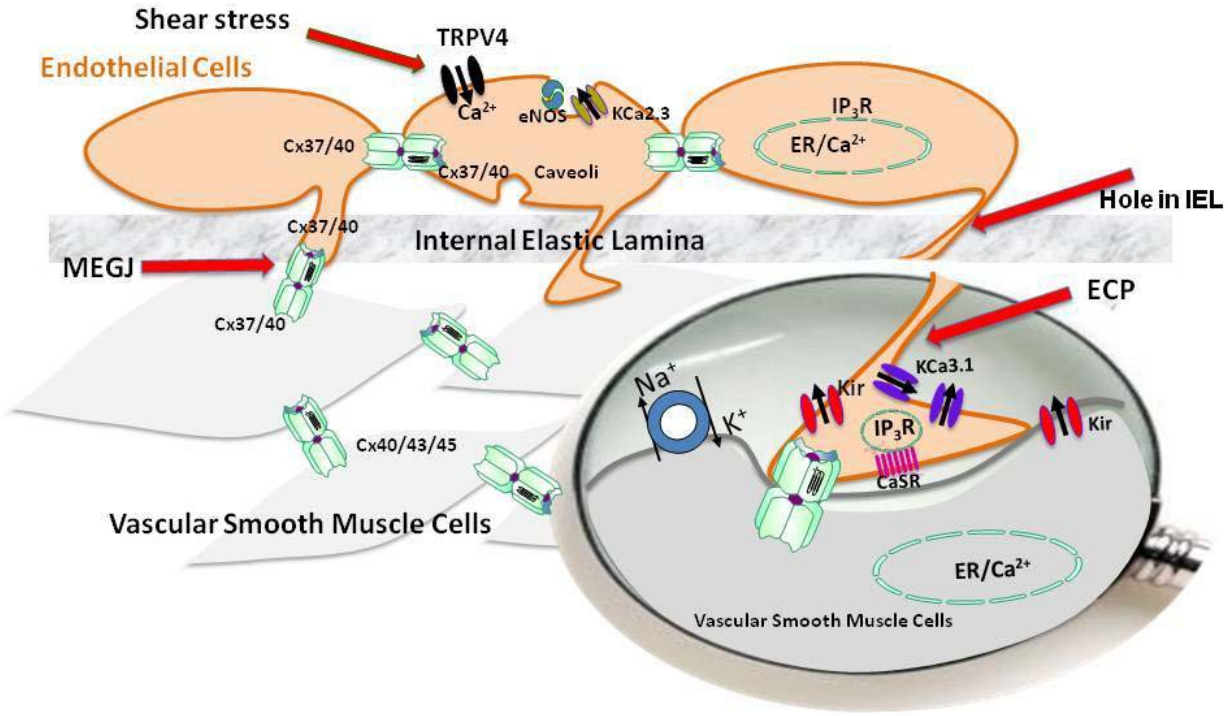

Figure 1. A schematic that illustrates the association between endothelial cells and vascular smooth muscle cells in a small artery in the microcirculation. Endothelial cell protusions (ECPs) extend through holes in the internal elastic lamina (IEL) and the juxtaposition of the plasma membranes from the two cell types form a myoendothelial gap junction (MEGJ) with connexins (Cx) 37 and Cx40 as the key proteins in these heterocellular contacts. On the luminal surface of the endothelial cell and in endothelial cell contact points KCa2.3 channels are associated with caveoli and also with eNOS and the shear stress activated TRPV4 cation channel. A magnifying glass view of an ECP at the junction of the IEL and the underlying VSMC reveals a microdomain where the MEGJ is in close association with Na+/K+ - ATPase and Kir on vascular smooth muscle cell membranes and KCa3.1, the calcium sensor receptor (CaSR) and Kir on the endothelial cell membranes. Strategically within the ECP endoplasmic reticulum. (ER) is also located together with inositol trisphosphate receptors (IP3). Outside of the microdomain endoplasmic reticulum is also seen in VSMC and in endothelial cells.

Emerson and Segal (40) demonstrated the importance of integrity of the endothelium for the conduction of the hyperpolarization response following acetylcholine application to the VSMC layer of the hamster retractor feed artery. Diep *et al* (41) and Tran *et al* (42) have proposed models based on a combination of computational modeling and simulations that also favour the endothelium as the principal pathway for conduction along the blood vessel. Based on modeling data the comparatively smaller role, or indeed absence, of a non-NO, non-PGI_2-mediated EDH in conduit vessels may simply reflect not only anatomical obstacles, but also dissipation of the hyperpolarization stimulus within the much greater smooth muscle cell mass in the medial layer of conduit vessels.

5.1. Importance of MEGJs in the regulation of vascular tone

Homocellular and heterocellular contact between endothelial cell and VSMC occurs via intercellular channels formed from the key gap junction proteins, or connexins (Cx), that are expressed in the vasculature: Cx37, Cx40, Cx43, Cx45. A gap junction is formed from two hemichannels, or connexons, which are themselves formed from six connexin molecules. Homotypic channels will thus be made from 12 identical connexins whereas heterotypic channels are formed from two hemichannels that are homomeric for different connexins.

In mouse blood vessels Cx37, Cx40 and Cx43 are expressed in endothelial cells and notably Cx43, but also Cx37 in VSMC. Chaytor *et al* (44) reported that Cx43 was the major connexin protein expressed in endothelium-denuded rabbit superior mesenteric arteries. Expression of connexins is not uniform and species differences have been reported with evidence that Cx37 does not participate in mouse MEGJs, but is present in rat MEGJs (8,20,43,45). Connexins may also play an important role in repair mechanisms as, following injury, gap junctions decrease, but return to normal following repair (46). The global genetic knock out of Cx43 or Cx45 in mice is lethal and can be related to cardiac abnormalities and impaired blood vessel development (47,48). In contrast, mice lacking either Cx40 or Cx37 remain viable and fertile and have provided opportunities to study the role of these two connexins in relation to endothelial cell-VSMC regulation (49). Cx40

deficient mice have a hypertensive phenotype and display impaired vasomotion (50). In contrast, when the genes for both Cx37 and Cx40 are ablated the embryo is lethal in the perinatal period as a result of pronounced vascular abnormalities that includes vessel dilatation, congestion and localized hemorrhages (51).

In the microcirculation MEGJs allow for changes in the membrane potential of endothelial cells to be electronically transmitted to the VSMC layer. The ability, however, of the endothelial cell layer to influence the VSMC layers of large conduit vessels is greatly limited not only by anatomical restrictions of the IEL, but also as the electrical signal will be readily dissipated in the multiple muscle layers.

A clearer understanding of the role of MEGJs in the regulation of vascular tone and, specifically, endothelium-dependent regulation of vascular tone has been hampered by the lack of specific pharmacological inhibitors. Heptanol and octanol are very non-specific and glycrrhetinic acid and derivatives, such as carbenoxolone also have been reported to have a variety of actions that result in difficulties in analyzing the results (52,53). The use of gap peptides that have amino acid sequences corresponding to regions of the extracellular loops of a connexin have been used with interesting results. For instance Gap27, an 11 amino acid peptide with sequence homology to a portion of the second extracellular loop, contains the critical conserved sequence SRPTEK and thus can be predicted to inhibit junctions constructed from Cx40 and Cx43 in vascular tissue (54). Gap27 has been reported to reversibly inhibit rhythmic contractile activity in rabbit superior mesenteric arteries thus inferring the importance of gap junctions involving Cx40 and Cx43 in the oscillatory control of vascular tone (44). Gap27 has also been reported to attenuate the assumed EDHF-mediated component (resistant to inhibition by the eNOS inhibitor, L-NAME and a COX inhibitor such as indomethacin) of EDV in the rabbit superior mesenteric artery thus supporting the emerging view at that time that bidirectional communication through MEGJs may facilitate the coordinated regulation of vascular tone and, at least partially, contribute to EDH (55). Gap peptides have been extensively used to evaluate the contribution of gap junctions to EDV (35). Nonetheless there are limitations to the interpretation of the data from the use of gap peptide inhibitors owing to the high concentrations that are needed. In addition, questions have also been raised concerning the effectiveness of gap peptides at MEGJs, versus homocellular communication between endothelial cells, or versus homocellular communication between VSMCs as well as the cellular basis of their inhibitory actions (39,56,57).

Mather *et al* (58) utilized connexin-specific antibodies that targeted the intracellular regions of Cx37, 40 or 43 as well as the mimetic peptide for the intracellular loop of Cx37 and were loaded into the endothelial cells of pressurized small mesenteric arteries of the rat. Depression of the EDHF-mediated EDV in third order rat mesenteric arteries was only observed with the antibody that targeted the intracellular carboxyl-terminal region of Cx40 (residues 340 to 358), or the mimetic peptide for the cytoplasmic loop (Cx40; residues 130 to 140). High-resolution immunohistochemistry also localized Cx40 to the MEGJs in the ECPs that have also been associated with IELs thus supporting the critical role for Cx40 in EDHF-mediated vasodilatation of rat mesenteric arteries as well as the importance of a microdomain location of the key proteins that modulate EDH. Arguably data using a specific antibody approach to the interruption of connexin function provides more reliable data concerning the physiological function(s) of a connexin whereas, in the case of genetic ablation studies, the loss of a specific connexin may be offset by long-term compensatory changes.

VSMC to endothelial cell gap junction communication can also contribute to the regulation of vascular tone and, for instance, depolarization of VSMC can indirectly inhibit EDV via MEGJs. This has been demonstrated in rat basilar arteries where the delayed rectifier potassium channel, K_{DR}, blocker, 4-aminopyridine, 4-AP, was reported to inhibit EDH and EDV via a process that was reversed by the, albeit non-specific, MEGJ inhibitor, 18beta-glycyrrhetinic acid (59).

5.2. The role of microdomains

Crane *et al* (60) studied acetylcholine-mediated EDH of third order rat mesenteric arteries and reported that EDH is attributable to apamin-sensitive KCa2.3 and the repolarization phase due to TRAM-34 sensitive KCa3.1 channels thus suggesting to the authors that these KCa channels are activated independently and have different cellular locations. Similarly, Crane *et al* (61) demonstrated, also in rat small mesenteric arteries, that functional, inwardly rectifying Ba^{2+}-sensitive K_{IR} channels are restricted to the endothelial cell layer and therefore may serve to amplify hyperpolarization in both cell types; in other vessels K_{IR} may be associated with VSMCs and/or both endothelial cells and VSMCs (Figure 1).

Anatomical data also supports the argument that the ion channels and connexins that have been associated with EDH are spatially separated. Thus, Sandow *et al* (8), using conventional confocal and high-resolution ultrastructural immunocytochemistry to study the location of KCa2.3, KCa3.1 and connexins in association with the IEL and endothelial cells and VSMCs in first-order branches of the male Wistar rat mesenteric artery, provided evidence that the potassium channels and connexins were co-localised in an apparent functional manner. Holes in the IEL providing potential sites for MEGJs also demonstrated strong immunocytochemical evidence for Cx37, Cx40 and the TRAM-34-sensitive KCa3.1 channel whereas Cx37, Cx40, Cx43 and the apamin-sensitive KCa2.3 channels co-localized in areas of endothelial cell gap junctions (8,9). An association between KCa3.1 channels and the ECPs through the IEL to form the MEGJs was also reported by Dora *et al* (34) in their study of EDHF-mediated signalling in rat small mesenteric arteries thus supporting the concept of an association between the activation of endothelial cell KCa3.1 and arterial relaxation via the activation of Na^{+}/K^{+}-ATPase alpha2/alpha3 subunits associated with VSMCs.

The critical importance of the KCa3.1 channel for EDHF-mediated events is also clearly shown by studies with mice in which the either KCa2.3 or KCa3.1, or both channels, have been genetically ablated (62).

Absi *et al* (63) studied the effects of methyl beta-cyclodextrin, a cholesterol depleting and caveolae-disrupting agent, on EDHF-mediated responses in rat mesenteric second-order arteries and immunofluorescence studies of KCa2.3, KCa3.1 and caveolin-1 localisation in porcine coronary arteries and concluded that KCa2.3 is associated with the caveolin-rich membrane compartment of the endothelial cell. Further evidence that the EDHF-mediated regulation of vascular tone may also be dependent on caveolae-linked events comes from studies of caveolin-1 deficient mice in which EDHF-mediated relaxation is impaired in rat mesenteric arteries and is associated with lower expression levels of connexins 37, 40 and 43 as well as fewer gap junctions (64). Since Cx43 partially co-localises with caveolin-1 these data suggest that, similar to eNOS and NO-mediated relaxation, components of the EDHF-mediated events are also regulated in cellular microdomains in the caveolar compartment of the endothelial cell plasma membrane (64,65,66). Similarly, eNOS in endothelial cells is primarily associated with cell surface caveolae and Golgi (67) as well as in association with protein kinase A (PKA) in distinct endothelial cell junctions (68). Furthermore, luminal-sided caveoale have been argued to be the mechanical sensors for shear stress activation of eNOS in the rat pulmonary vasculature (69). The association of KCa2.3 with the caveolin-rich compartment of the membrane suggests that this KCa channel may also be linked with the calcium-dependent regulation of eNOS activation with the opening of KCa2.3 providing the electrochemical driving force for the entry of extracellular Ca^{2+}. The importance of the KCa2.3 channel in the regulation of eNOS is supported by data from the study of acetylcholine and shear-stress mediated relaxation of carotid arteries from genetic ablation studies. Thus, studies of EDV in carotid arteries from KCa2.3 and KCa3.1 deficient mice indicate that it is the KCa2.3 that is particularly crucial for both chemical- and shear-stress-mediated NO-dependent EDV (62). This conclusion is supported by data indicating that the KCa2.3 inhibitor, apamin, but not the KCa3.1 blocker, charybdotoxin, reduced NO-mediated EDV, but not relaxation mediated by authentic NO, in porcine retinal arterioles; however, this is not a universal finding as, under comparable experimental conditions, in the rat superior mesenteric artery a combination of both apamin and charybdotoxin is required to inhibit EDV (70,71).

6. CALCIUM SIGNALLING AND ENDOTHELIUM-DEPENDENT VASODILATATION

An increase in intracellular Ca^{2+} is a requirement for the activation of the three major pathways EDV pathways that involve NO, PGI_2 and EDHF (72). Endothelial cell Ca^{2+} homeostasis is disrupted in caveolin-1 deficient mice and linked to a decreased activity of the Ca^{2+} permeable transient receptor potential vallinoid type 4 (TRPV4) cation channel that, based on studies with TRPV4-deficient mice, participates in NO- and EDHF-mediated relaxation and possibly contributes to epoxyeicosatrienoic acid-mediated EDH (64,73,74). Since the TRPV4 channel has been demonstrated to be expressed not only in mouse aortic endothelial cells but also mouse mesenteric resistance vessels these data suggest that the signalling mechanisms for shear-stress activation of EDV do not differ between compliance and resistance vessels of the mouse. (73,75).

6.1. Role of calcium sensing receptors in the regulation of endothelial cell function

Bohr (76) reported that raising extracellular Ca^{2+}, seemingly paradoxically given its role in muscle contraction, relaxes VSMCs and later provided evidence that high extracellular Ca^{2+}-mediated vasodilatation was linked to the activity of Na^+/K^+-ATPase (77). Based upon our current knowledge Ca^{2+}-mediated relaxation of smooth muscle most likely involves activation of the calcium-sensing receptor (CaSR). The CaSR is a G protein-coupled receptor belonging to the superfamily C class coupling to Galphaq or Galpha11, which was first cloned from bovine parathyroid glands (78). CaSR has now been shown to be expressed in many cell types including human and rodent endothelial cells and VSMCs from both resistance and conduit arteries and also argued to play an important role in the regulation of blood pressure (78,79,80,81,82,83). The CaSR agonist, AMG-73, induces VSMC relaxation via both endothelium-dependent and independent mechanisms and, in cell cultures of human aortic endothelial cells, has been linked to the generation of NO (80,83). The CaSR may play also an important role in at least partially explaining the epidemiological positive correlation between low dietary calcium intake and high blood pressure (84). Changes in CaSR expression and function may be important contributing factors to the development of vascular disease as a reduction in CaSR expression is associated with increased calcification *in vivo* and *in vitro* thus suggesting a role of calcimimetics for the treatment of vascular calcification and also decreased expression is evident in vascular tissue from diabetic rats (85,86). Conversely, high levels of Ca^{2+} (3mM), as may be associated within an atherosclerotic plaque, activate CaSR, or a related receptor, leading to mitogen-activated protein kinase, MAPK, activation and VSMC proliferation (79).

6.2. Calcium signalling and endothelial cell microdomains

Ca^{2+} signalling is associated with cellular microdomains in many cell types and this is also the case for endothelial cells. The CaSR has been shown to be present on the vascular endothelium of both rat second and third order mesenteric arteries and pig coronary arteries and activation of the CaSR linked to the opening of endothelial cell KCa3.1 channels that initiate EDH of the of the VSMC with the physical proximity of the key plasmalemmal proteins (82).

Ca^{2+} signalling is also associated with cellular microdomains and, based on data from pressurized small mesenteric arteries, this would also seem to be the case for endothelial cells with Ca^{2+} events in endothelial cells being

regulated not only by Ca^{2+} release from within endothelial cells, but also via MEGJs following the release of inositol trisphosphate (IP_3) in adjoining VSMCs and entry into endothelial cells via MEGJs (87). Ledoux *et al* (88) suggest a role for endothelial cell "Ca Pulsars" that originate from endoplasmic reticulum Ca^{2+} stores following activation of xestospongin C-sensitive IP_3 receptors as demonstrated in mouse third order mesenteric arteries. Both the Kansui *et al* (87) and Ledoux *et al* (88) studies indicate that IP_3 receptor-, rather than ryanodine receptor-, regulated intracellular Ca^{2+} stores in the endoplasmic reticulum serve as the source of spontaneous Ca^{2+} events within the endothelial cell. Thus, there is a clear distinction between endothelial cells and VSMCs as to the respective roles of IP_3 versus ryanodine receptors in the generation of "Ca Sparks" in VSMC (89).

In resistance vessels, ECP microdomains play an important role in the regulation of NO-independent EDH in VSMC and the regulation of conducted (or spreading) vasodilatation in the microcirculation (88,90). The ECP microdomain, as depicted in Figure 1, contains endothelial cell KCa3.1 channels, CaSR, IP_3 receptors associated with endoplasmic reticulum and also MEGJ-forming connexins that are in close association with VSMC and endothelial cell Kir. KCa3.1 activity is increased by lowering extracellular Ca^{2+}, thus suggesting modulation by CaSR, whereas KCa3.1 is blocked following activation of PKA by forskloin (34). However, endothelial cell Ca^{2+} does not seem to increase in response to local or spreading hyperpolarisation and associated vasodilatation (90,91).

In the absence of any strong contradictory data one can tentatively conclude that although the signalling events described for the luminal activation of endothelial cells are the same or similar for conduit versus resistance vessels the contribution and importance of the ECP microdomain dominates in the microcirculation, but contributes minimally in the regulation of vascular tone in large conduit vessels.

7. DISEASE PROGRESSION

A healthy endothelium is required for cardiovascular health and endothelial dysfunction is an important contributor to the clinical expression of vascular disease (92). Endothelial dysfunction is an important and strategic target for the treatment of vascular diseases and notably in hypertension and diabetes (14,16).

Endothelial dysfunction is frequently defined as a reduced EDV response to either a chemical mediator, such as acetylcholine or bradykinin, or to flow- (shear stress-) mediated vasodilatation and such a definition can be equally applied to large conduit or microvessels (15). However, a broader definition of endothelial dysfunction is appropriate as, in addition to a reduction in EDV, there are a number of molecular changes that occur in vascular function that include an elevation in the expression of adhesion molecules, enhanced VSMC proliferation and the development of a hypercoagulatory state (15). Cardiovascular disease is associated with early changes in both functional and molecular properties of the endothelium with considerable direct evidence, as well as a number of recent reviews, providing support for this view point (14,15,16,93,94,95,96). Assessment of endothelial dysfunction in humans has considerable potential prognostic significance for risk prediction in cardiovascular disease (97).

From a clinical perspective coronary atherosclerotic plaque severity has been linked to local low endothelial cell shear stress and thus considerable interest is being focused on the benefit of evaluating shear stress as a predictor of coronary events (98,99,100). In conduit vessels arterial stiffness plays an important role in maintaining optimal arterial mechanics and cardiovascular function and arterial stiffening results in elevated systolic blood pressure and systolic hypertension and heart failure. Both NO and EDHF have been reported to reduce arterial wall stiffness via hyperpolarization of VSMCs (101).

7.1. eNOS and endothelial dysfunction

It is generally accepted that a reduction in the bioavailability of NO is associated with the development of atherosclerosis as well as microvascular disease. Endothelial dysfunction can be linked to elevated oxidative stress and the oxidation of the eNOS co-factor, tetrahydrobiopeterin, and an uncoupling of eNOS from a dimeric to a monomeric state that promotes further superoxide generation (15,102). Thus, therapeutic interventions that enhance NO- and EDHF-mediated processes in the vascular system might be expected to be beneficial; however, caution is required. The overexpression of eNOS has been reported to accelerate atherosclerosis in apoE-deficient mice indicating that it is eNOS function that is important rather than the levels of eNOS protein (15,103,104). Elevated formation of reactive oxygen species and the production of peroxynitrite via the interaction of superoxide and NO, are key events in the aetiology of endothelial dysfunction (105). Vascular oxidative stress has been linked to the development of atherosclerosis, hypertension, diabetic vascular disease and a reduction in the bioavailability of NO results in an elevation in the oxidation of lipoproteins as well as the activation of pro-inflammatory genes, such as vascular cell adhesion molecule-1 (VCAM-1), as well as the proliferation of VSMCs (106,107,108). Thus, targeting oxidative stress would seem to be a logical avenue for the prevention of both microvascular and macrovascular disease; however, the results from large randomized, double-blinded trials with antioxidants have been disappointing resulting in a reevaluation of such approaches (109).

7.2. EDHF and endothelial dysfunction

As stated by Feletou and Vanhoutte (22): *"A better characterization of EDHF-mediated responses should allow the determination of whether or not new drugable targets can be identified for the treatment of cardiovascular diseases."* Thus, based upon our current knowledge of the importance of EDHF in the microcirculation as well as the key ion channels involved in the modulation of EDH, multiple potential protein targets can be identified for study (15).

Selemidis & Cocks (110) proposed that EDH not only served an important role in the regulation of blood flow, but also by virtue of spreading hyperpolarization along the vascular tree served an anti-inflammatory role that also retarded atherosclerosis. A hyperpolarizing influence, most likely via the endothelial cell production of epoxyeicosatrienoic acids, has been shown to attenuate platelet aggregation and atherogenesis (111,112).

Impairment in the expression and/or function of the KCa channels that are involved in the regulation of shear stress and EDHF-mediated EDV can be predicted to have profound effects on vascular function and this is supported by studies with mice deficient in both KCa2.3 and KCa3.1 channels (113,114). Altering the expression level of KCa2.3 channels significantly affects vascular tone and blood pressure and KCa2.3-deficient mice have elevated blood pressure whereas an overexpression of KCa2.3 results in an increase in arterial diameters (115). Genetic ablation of KCa3.1 in the mouse notably affects EDHDF-mediated events as well as results in hypertension that is exacerbated during exercise and the combined loss of both KCa2.3 and KCa3.1 resulted in mice with a higher mean blood pressure than ablation of either channel alone (62,116). Interestingly an upregulation of KCa3.1 in VSMC, where in non-proliferating VSMC expression of KCa3.1 is absent, is associated with mitogen-induced proliferation of VSMCs (117). Polymorphisms in KCa channels have also been associated with cardiovascular disease, and notably for the KCa3.1 for vascular disease in a Japanese population study, suggesting a role for the KCNN4, which encodes for KCa3.1, as a susceptibility gene for myocardial infarction (118,119). Collectively, the results from studies of the link between KCa channel function/dysfunction indicate that pharmacological manipulation of vascular KCa channels may prove valuable as adjunct therapy in the treatment of vascular diseases linked to diabetes, hypertension, restenosis and atherosclerosis, with the KCa2.3 and KCa3.1 openers targeting hypertension, and blockers of KCa3.1 utlilised for the treatment of pathological states such as restenosis that involve vascular remodelling (120). The likely lack of specificity of such drugs for the vasculature would require local administration and limiting the diffusion by, for instance, the use of stents. Alterations in the calcium sensor, CaSR, have been linked to diabetic vascular disease in a study of mesenteric arteries from Zucker diabetic fatty rats wherein reduced CaSR-mediated hyperpolarizing and vasodilator responses were linked to a decrease in the expression of CaSR, but not KCa3.1 channels (86).

Studies of vascular development and vascular function in various connexin-deficient mice indicate that Cx40 deficient mice have a hypertensive phenotype and display impaired vasomotion and in the absence of the genes for both Cx37 and Cx40 profound vascular abnormalities are evident and the embryo is lethal in the perinatal period (50,51). ApoE-/- mice that also lack Cx37 demonstrate an accelerated rate of atherosclerotic lesion development (122). Conversely Cx40 and Cx43 expression has been shown to be upregulated in atherosclerotic plaques in a rabbit model of a high cholesterol diet, but expression changes were normalized by treatment with the statins, lovastatin or fluvastatin (123). Gap junction frequency and size was also higher in neointimal smooth muscle in atherosclerotic vessels and again was normalized by treatment with statins suggesting that the increase in Cx40 and Cx43 gap junctions is linked to VSMC activation and intimal growth (123).

Changes in vascular structure are important contributors to the development of elevated peripheral resistance and hypertension and thus the question arises as to whether there are alterations in IELs and MEGJs in blood vessels from hypertensives? Sandow *et al* (10), utilising conventional, ultrastructural and confocal microscopy methodology, have studied the relationship between IELs, vessel size in control rats and spontaneously hypertensive (SHR), Dahl and DOCA-salt hypertension models as well as C57, BALB/c and ApoE knockout mice, and examined the thoracic aorta, proximal and distal mesenteric, caudal, saphenous, middle cerebral and caudal cerebellar arteries. These studies demonstrated a positive correlation between the size of the resistance vessels and the density of MEGJs; however, no such correlation is apparent in conduit vessels, and nor is there a clear correlation between IEL holes and MEGJ density in either conduit or resistance vessels. Furthermore, although there was a higher density of IELs in vessels from normotensive rats when compared to SHR and DOCA-salt rats, the density of IELs was comparable between Dahl and control rats and the authors concluded that IEL density was independent of blood pressure. Nonetheless, as the authors also argued, the lower density of IEL holes reported in vessels from SHR versus their normotensive control WKY may, as a result of a reduction in endothelial-VSMC contact, be one of the contributing factors to the development of an elevated peripheral resistance. Additional studies of immunocytochemical analysis of vascular tissue from the SHR has also reported lower expression levels of Cx40 as well as reduced endothelial cell size in the caudal artery from 3 week old prehypertensive as well as 12 week old hypertensive SHR when compared to tissue from age matched Kyoto Wistar, WKY, normotensive control rats (123). Inhibition of angiotensin converting enzyme, ACE, with enalapril decreased blood pressure and restored connexin expression levels in the endothelium, but not in the media of the caudal artery and also the aorta from SHR (124). Of additional interest is that data from the same group indicates that the cellular mechanisms mediating EDH in the small first order mesenteric arteries from SHR differ from that for the WKY with, based on the use of gap peptides, gap junctions dominating in tissues from the WKY and H_2O_2 and epoxyeicosatrienoic acid mediating a reduced level of EDH in the SHR (125). Enalapril treatment of the SHR enhanced epoxyeicosatrienoic acid, but not gap junction, mediated EDH. Of additional interest is that comparable data indicating a greater role for epoxyeicosatrienoic acid mediated EDH has also been reported for small mesenteric vessels from the type 2 diabetic db/- mouse thus suggesting that changes in the nature of EDHF-mediated regulation of vascular function may either contribute and/or serve to compensate for the loss of normal physiological control

Table 1. Endothelial dysfunction, vascular disease and molecular targets

Target	Functional change	Reference
eNOS	Uncoupled eNOS with an increase in monomer to dimer ratio linked to reduced NO bioavailability, oxidative stress, atherosclerosis and microvascular disease	15
EDH/EDHF	EDHF has an anti-inflammatory/anti-atherogenic function Changes in EDHF in hypertension and diabetes	110,111,112 125, 126
KCa2.3	Reduced expression (mice) results in hypertension. Enhanced expression (mice) results in increase in arterial diameter.	115 115
KCa3.1	Genetic ablation results in hypertension (mice). Enhanced expression in VSMC associated with proliferation and potential therapeutic target for restenosis. Polymorphisms in humans linked to increase risk of myocardial infarction.	62 116, 120 118,119
CaSR	Reduced expression and/or coupling with KCa3.1 linked to reduced EDV in diabetic rat	86
MEGJs	No correlation between density and blood pressure, but greater density in microvasculature versus conduit vessels	10
Connexins	Global ablation of Cx43 or Cx45 is lethal. Global ablation of Cx37+Cx40 is lethal. Global ablation of Cx37 results in accelerated atherosclerosis in ApoE-/- mice. Global ablation of Cx40 results in hypertensive phenotype with impaired vasomotion. Cx40 and Cx43 expression upregulated in atherosclerotic plaques and lowered by statins. Reduced Cx40 in hypertension	47,48 49 121 50 122 123, 124
EDCFs	Increase in contribution of EDCFs linked to vascular disease, hypertension in animal and human studies.	16, 129, 130

processes in cardiovascular disease (126). Collectively these data do suggest that EDHF-mediated regulation of vascular tone is altered in disease states such as hypertension and diabetes and may involve both changes in connexin-regulated and epoxyeicosatrienoic-regulated EDH. It is also worthy of stressing that Cx40 has been implicated as playing a critical role in EDHF-mediated regulation of vasodilatation in rat mesenteric arteries – see section 5.1 (58).

7.3. Endothelium-derived contracting factors (EDCFs) and vascular disease

Shortly after the recognition in 1980 that the endothelium was the source of a vascular relaxing factor it was also recognised that the presence of the endothelium could, notably in veins rather than arteries, also enhance vascular contraction in canine blood vessels (128). In a review article in 1989 Furchgott and Vanhoutte discussed that not only did the endothelium produce relaxing factors such as NO, but it also produced contracting factors that included peptides such as endothelin (129). We now know that in addition to endothelin the endothelium can also produce a number of other contracting factors including vasoconstrictor prostanoids and that changes in the production of EDCFs, likely linked to a decrease in the bioavailability of NO, contributes to the development of vascular disease (16,130,127). Table 1 provides a summary, with key references, of the contribution of changes in endothelial cell protein and/or signalling functions that are believed to contribute to cardiovascular disease.

8. SUMMARY

There are both functional and anatomical differences between conduit and resistance arteries that determine the importance and nature of endothelium-dependent regulation of vascular tone. Whereas the bioavailability of NO likely is important for maintaining vascular function and health throughout the vasculature the importance of EDHF dominates in the microvasculature. EDHF-mediated regulation of vascular tone is highly dependent on the association of several key signalling proteins/channels within 'microdomains' that are determined by ECP through the IEL and their close association with the plasma membrane of VSMC via MEGJs. A key difference between the large conduit vessels versus the resistance vessels of the microcirculation may simply be that a significant number of ECPs do not occur and/or the comparatively much larger cell mass of VSMCs in the conduit vessel readily dissipates any hyperpolarizing influence from the endothelial cell layer. An important question remains unanswered and that is: "What is the role of EDHF in the progression of vascular disease?" Thus, does EDHF help protect the vasculature from disease progress and therefore will targeting EDHF and enhancing its' contribution in the vasculature have a positive therapeutic effect?

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10. REFERENCES

1. T. Schwann*:* Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants. London: *Sydenham Society* (1847)

2. W. His: *Die* Haute und Hohlen des Korpers. Basel: *Schwighauser*, Basel (1865)

3. H. F. Galley, N. R. Webster: Physiology of the endothelium. *Br J Anaesth* 93, 105-113 (2004)

4. R. F. Furchgott, J. V. Zawadzki: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288, 373-376 (1980)

5. J. J. Hwa, L. Ghibaudi, P. Williams, M. Chatterjee: Comparison of acetylcholine-dependent relaxation in large and small arteries of rat mesenteric vascular bed. *Am J Physiol* 266, H952-H958 (1994)

6. G. J. Waldron, H. Ding, F. Lovren, P. Kubes, C. R. Triggle: Acetylcholine-induced relaxation of peripheral arteries isolated from mice lacking endothelial nitric oxide synthase. *Br J Pharmacol* 128, 653-658 (1999)

7. H. Shimokawa, H. Yasutake, K. Fujii, M. K. Owada, R. Nakaike, Y. Fukumoto, T. Takayanagi, T. Nagao, K. Egashira, M. Fujishima, A. Takeshita: The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric. *J Cardiovasc Pharmacol* 28, 703-711 (1996)

8. S. L. Sandow, C. B. Neylon, M. X. Chen, C. J. Garland: Spatial separation of endothelial small- and intermediate-conductance calcium-activated potassium channels (K(Ca)) and connexins: possible relationship to vasodilator function? *J Anat* 209, 689-698 (2006)

9. S. L. Sandow, R. E. Haddock, C. E. Hill, P. S. Chadha, P. M. Kerr, D. G. Welsh, F. Plane: What's where and why at a vascular myoendothelial microdomain signalling complex. *Clin Exp Pharmacol Physiol* 36, 67-76 (2009a)

10. S. L. Sandow, D. J. Gzik, R. M. Lee: Arterial internal elastic lamina holes: relationship to function? *J Anat* 214, 258-266 (2009b)

11. S. Krummen, A. Drouin, M. E. Gendron, J. R. Falck, E. Thorin: ROS-sensitive cytochrome P450 activity maintains endothelial dilatation in ageing but is transitory in dyslipidaemic mice. *Br J Pharmacol* 147, 897-904 (2006)

12. O. Yildiz: Vascular smooth muscle and endothelial functions in aging. *Ann N Y Acad Sci* 1100, 353-360 (2007)

13. S. L. Sandow, K. Goto, N. M. Rummery, C. E. Hill: Developmental changes in myoendothelial gap junction mediated vasodilator activity in the rat saphenous artery. *J Physiol* 556, 875-886 (2004)

14. H. Ding, C. R. Triggle: Endothelial dysfunction in diabetes: multiple targets for treatment. *Pflugers Arch* 459, 977-994 (2010)

15. C. R. Triggle, H. Ding: A review of endothelial dysfunction in diabetes: a focus on the contribution of a dysfunctional eNOS. *J Am Soc Hypertens* 4, 102-115 (2010)

16. E. H. Tang, P. M. Vanhoutte: Endothelial dysfunction: a strategic target in the treatment of hypertension? *Pflugers Arch* 459, 995-1004 (2010)

17. G. J.Waldron, C. J. Garland: Effect of potassium channel blockers on L-NAME insensitive relaxations in rat small mesenteric artery. *Can J Physiol Pharmacol* 72 (Suppl. 1), P1.7.26 (1994)

18. C. J. Garland, F. Plane, F: Relative importance of endothelium-derived hyperpolarizing factor for the relaxation of vascular smooth muscle in different arterial beds. In: *Endothelium-Derived Hyperpolarizing Factor*, editor Vanhoutte P. M. Vol 1, pp 173-179. Amsterdam: *Harwood Academic Publishers* (1996)

19. W. B. Campbell, I Fleming I: Epoxyeicosatrienoic acids and endothelium-dependent responses. *Pflugers Arch* 459, 881-895 (2010)

20. C. de Wit, T. M. Griffith: Connexins and gap junctions in the EDHF phenomenon and conducted vasomotor responses. *Pflugers Arch* 459, 897-914 (2010)

21. G. Edwards, M. Feletou, A. H. Weston: Endothelium-derived hyperpolarising factors and associated pathways: a synopsis. *Pflugers Arch* 459, 863-879 (2010)

22. M. Feletou, P. M. Vanhoutte: EDHF: an update. *Clin Sci* 117, 139-155 (2009)

23. H. Shimokawa: Hydrogen peroxide as an endothelium-derived hyperpolarizing factor. *Pflugers Arch* 459, 915-922 (2010)

24. G. Edwards, K. A. Dora, M. J. Gardener, C. J. Garland, A. H. Weston: K+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* 396, 269-272 (1998)

25. D. C. Zeldin, R. N. DuBois, J. R. Falck, J. H. Capdevila: Molecular cloning, expression and characterization of an endogenous human cytochrome P450 arachidonic acid epoxygenase isoform. *Arch Biochem Biophys* 322, 76-86 (1995)

26. S. Wu, C. R. Moomaw, K. B. Tomer, J. R. Falck, D. C. Zeldin: Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. *J Biol Chem* 71, 3460-3468 (1996)

27. J. V. Mombouli, S. Holzmann, G. M. Kostner, W. F. Graier: Potentiation of Ca2+ signaling in endothelial cells by 11,12-epoxyeicosatrienoic acid. *J Cardiovasc Pharmacol* 33, 779-784 (1999)

28. H. Watanabe, J. Vriens, J. Prenen, G. Droogmans, T. Voets, B. Nilius: Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 424, 434-438 (2003)

29. T. Matoba, H. Shimokawa, M. Nakashima, Y. Hirakawa, Y. Mukai, K. Hirano, H. Kanaide, A. Takeshita: Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J Clin Invest* 106, 1521-1530 (2000)

30. T. Matoba, H. Shimokawa, H. Kubota, K. Morikawa, T. Fujiki, I. Kunihiro, Y. Mukai, Y. Hirakawa, A. Takeshita: Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. *Biochem Biophys Res Commun* 290, 909-913 (2002)

31. T. Matoba, H. Shimokawa, K. Morikawa, H. Kubota, I. Kunihiro, L. Urakami-Harasawa, Y. Mukai, Y. Hirakawa, T. Akaike, A. Takeshita: Electron spin resonance detection

of hydrogen peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary microvessels. *Arterioscler Thromb Vasc Biol* 23, 1224-1230 (2003)

32. S. D. Chauhan, H. Nilsson, A. Ahluwalia, A. J. Hobbs: Release of C-type natriuretic peptide accounts for the biological activity of endothelium-derived hyperpolarizing factor. *Proc Natl Acad Sci U S A* 100, 1426-1431 (2003)

33. S. L. Sandow, M. Tare: C-type natriuretic peptide: a new endothelium-derived hyperpolarizing factor? *Trends Pharmacol Sci* 28, 61-67 (2007)

34. K. A. Dora, N. T. Gallagher, A. McNeish, C. J. Garland: Modulation of endothelial cell KCa3.1 channels during endothelium-derived hyperpolarizing factor signaling in mesenteric resistance arteries. *Circ Res* 102(, 1247-1255 (2008)

35. T. M. Griffith, A. T. Chaytor, D. H. Edwards: The obligatory link: role of gap junctional communication in endothelium-dependent smooth muscle hyperpolarization. *Pharmacol Res* 49, 551-564 (2004)

36. D. H. Moore, H. Ruska: The fine structure of capillaries and small arteries. *J Biophys Biochem Cytol* 25, 457-462 (1957)

37. J. A. Rhodin: The ultrastructure of mammalian arterioles and precapillary sphincters. *J Ultrastruct Res* 18, 181-223 (1967)

38. S. L. Sandow, C. E. Hill: 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* 86, 341-346 (2000)

39. K. R. Heberlein, A. C. Straub, B. E. Isakson: The myoendothelial junction: breaking through the matrix? *Microcirculation* 16, 307-322 (2009)

40. G. G. Emerson, S. S. Segal: Endothelial cell pathway for conduction of hyperpolarization and vasodilation along hamster feed artery. *Circ Res* 86, 94–100 (2000)

41. H. K. Diep, E. J. Vigmond, S. S. Segal, D. G. Welsh: Defining electrical communication in skeletal muscle resistance arteries: a computational approach. *J Physiol* 568, 267-281 (2005)

42. C. H. Tran, E. J. Vigmond, F. Plane, D. G. Welsh: Mechanistic basis of differential conduction in skeletal muscle arteries. *J Physiol* 587, 1301-1318 (2009)

43. J. A. Haefliger, P. Nicod, P. Meda: Contribution of connexins to the function of the vascular wall. *Cardiovasc Res* 62, 345-356 (2004)

44. A. T. Chaytor, W. H. Evans, T. M. Griffith. Peptides homologous to extracellular loop motifs of connexin 43 reversibly abolish rhythmic contractile activity in rabbit arteries. *J Physiol* 503, 99-110 (1997)

45. B. E. Isakson, B. R. Duling: Heterocellular contact at the myoendothelial junction influences gap junction organization. *Circ Res* 97, 44-51 (2005)

46. H. I. Yeh, Y. J. Lai, H. M. Chang: Multiple connexin expression in regenerating arterial endothelial gap junctions. *Arterioscler Thromb Vasc Biol* 20, 1753– 1762 (2000)

47. A. G. Reaume, P. A. de Sousa, S. Kulkarni, B. L. Langille, D. Zhu, T. C. Davies, S. C. Juneja, G. M. Kidder, J. Rossant: Cardiac malformation in neonatal mice lacking connexin43. *Science* 267, 1831-1834 (1995)

48. O. Kruger, A. Plum, J. S. Kim, E. Winterhager, S. Maxeiner, G. Hallas, S. Kirchhoff, O. Traub, W. H. Lamers, K. Willecke: Defective vascular development in connexin 45-deficient mice. *Development* 127, 4179-4193 (2000)

49. C. de Wit, F. Roos, S. S. Bolz, S. Kirchhoff, O. Kruger, K. Willecke, U. Pohl: Impaired conduction of vasodilation along arterioles in connexin40-deficient mice. *Circ Res* 86, 649-655 (2000)

50. C. de Wit, F. Roos, S. S. Bolz, U. Pohl: Lack of vascular connexin 40 is associated with hypertension and irregular arteriolar vasomotion. *Physiol Genomics* 13, 169-177 (2003)

51. A. M. Simon, A. R. McWhorter: Role of connexin37 and connexin40 in vascular development. *Cell Commun Adhes* 10(4-6), 379-85 (2003)

52. M. Tare, H. A. Coleman, H. C. Parkington: Glycyrrhetinic derivatives inhibit hyperpolarization in endothelial cells of guinea pig and rat arteries. *Am J Physiol Heart Circ Physiol* 282, H335-H341 (2002)

53. G. D. Sagar, D. M. Larson: Carbenoxolone inhibits junctional transfer and upregulates Connexin43 expression by a protein kinase A-dependent pathway. *J Cell Biochem* 98, 1543-1551 (2006)

54. N. M. Kumar, N. B. Gilula: Molecular biology and genetics of gap junction channels. *Semin Cell Biol* 3, 3-16 (1992)

55. A. T. Chaytor, W. H. Evans, T. M. Griffith: Central role of heterocellular gap junctional communication in endothelium-dependent relaxations of rabbit arteries. *J Physiol* 508, 561-573 (1998)

56. G. Edwards, C. Thollon, M. J. Gardener, M. Félétou, J. Vilaine, P. M. Vanhoutte, A. H. Weston: Role of gap junctions and EETs in endothelium-dependent hyperpolarization of porcine coronary artery. *Br J Pharmacol* 129, 1145-1154 (2000)

57. W. H. Evans, S. Boitano: Connexin mimetic peptides: specific inhibitors of gap-junctional intercellular communication. *Biochem Soc Trans* 29, 606-612 (2001)

58. S. Mather, K.A. Dora, S. L. Sandow, P. Winter, C. J. Garland: Rapid endothelial cell-selective loading of connexin 40 antibody blocks endothelium-derived hyperpolarizing factor dilation in rat small mesenteric arteries. *Circ Res* 97, 399-407 (2005)

59. T. Allen , M. Iftinca , W. C. Cole , F. Plane: Smooth muscle membrane potential modulates endothelium-dependent relaxation of rat basilar artery via myo-endothelial gap junctions. *J Physiol* 545, 975-986 (2002)

60. G. J. Crane, N. Gallagher, K. A. Dora and C. J. Garland: Small- and intermediate-conductance calcium-activated K+ channels provide different facets of endothelium-dependent hyperpolarization in rat mesenteric artery. *J Physiol* 553, 183-189 (2003a)

61. G. J. Crane, S.D. Walker, K. A. Dora, C. J. Garland: Evidence for a differential cellular distribution of inward rectifier K channels in the rat isolated mesenteric artery. *J Vasc Res* 40, 159-168 (2003b)

62. S. Brahler, A. Kaistha, V. J. Schmidt, S. E. Wolfle, C. Busch, B. P. Kaistha, M. Kacik, A. L. Hasenau, I. Grgic, H. Si, C. T. Bond, J. P. Adelman, H. Wulff, C. de Wit, J. Hoyer, Kohler R: Genetic deficit of SK3 and IK1 channels disrupts the endothelium-derived hyperpolarizing factor vasodilator pathway and causes hypertension. *Circulation* 119, 2323-2332 (2009)

63. M. Absi, M. P. Burnham, A. H. Weston, E. Harno, M. Rogers, G. Edwards: 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* 151, 332-340 (2007)

64. J. Saliez, C. Bouzin, G. Rath, P. Ghisdal, F. Desjardins, R. Rezzani, L. F. Rodella, J. Vriens, B. Nilius, O. Feron, J. L. Balligand, C. Dessy: 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* 117, 1065-1074 (2008)

65. A. L. Schubert, W. Schubert, D. C. Spray, M. P. Lisanti: Connexin family members target to lipid raft domains and interact with caveolin-1. *Biochemistry* 41, 5754 –5764 (2002)

66. C. Dessy, O. Feron, J. L. Balligand: The regulation of endothelial nitric oxide synthase by caveolin: a paradigm validated *in vivo* and shared by the 'endothelium-derived hyperpolarizing factor. *Pflugers Arch* 459, 817-827 (2010)

67. W. C. Sessa, G. Garcia-Cardena, J. Liu, A. Keh, J. S. Pollock, J. Bradley, S. Thiru, I. M. Braverman, K. M. Desai: The Golgi association of endothelial nitric oxide synthase is necessary for the efficient synthesis of nitric oxide. *J Biol Chem* 270, 17641-17644 (1995)

68. H. F. Heijnen, S. Waaijenborg, J. D. Crapo, R. P. Bowler, J. W. Akkerman, J. W. Slot: Colocalization of eNOS and the catalytic subunit of PKA in endothelial cell junctions: a clue for regulated NO production. *J Histochem Cytochem* 52, 1277-1285 (2004)

69. V. Rizzo, D. P. McIntosh, P. Oh, J. E. Schnitzer: *In situ* flow activates endothelial nitric oxide synthase in luminal caveolae of endothelium with rapid caveolin dissociation and calmodulin association. *J Biol Chem* 273, 34724-34729 (1998)

70. T. Dalsgaard, C. Kroigaard, T. Bek, U. Simonsen: Role of calcium-activated potassium channels with small conductance in bradykinin-induced vasodilation of porcine retinal arterioles. *Invest Ophthalmol Vis Sci* 50, 3819-3825 (2009)

71. E. Stankevicius, V. Lopez-Valverde, L. Rivera, A. D. Hughes, M. J. Mulvany, U. Simonsen: Combination of Ca^{2+}-activated K^+ channel blockers inhibits acetylcholine-evoked nitric oxide release in rat superior mesenteric artery. *Br J Pharmacol* 149, 560–572 (2006)

72. M. Feletou, P. M. Vanhoutte: Endothelium-derived hyperpolarizing factor: where are we now? *Arterioscler Thromb Vasc Biol* 26, 1215-1225 (2006)

73. S. A. Mendoza , J. Fang , D. D. Gutterman , D. A. Wilcox , A. H. Bubolz , R. Li , M. Suzuki, D. X. Zhang : TRPV4-mediated endothelial Ca2+ influx and vasodilation in response to shear stress. *Am J Physiol Heart Circ Physiol* 298, H466-H476 (2010)

74. J. Vriens, G. Owsianik, B. Fisslthaler, M. Suzuki, A. Janssens, T. Voets, C. Morisseau, B. D. Hammock, I. Fleming, R. Busse and B. Nilius. Modulation of the Ca2+ permeable cation channel TRPV4 by cytochrome P450 epoxygenases in vascular endothelium. *Circ Res* 97, 908–915 (2005)

75. H. Watanabe, J. Vriens, S. H. Suh, C. D. Benham, G. Droogmans, B. Nilius: Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. *J Biol Chem* 277, 47044-47051 (2002)

76. D. F. Bohr: Vascular Smooth Muscle: Dual Effect of Calcium. *Science* 1139, 597-599 (1963)

77. R. C. Webb, D. F. Bohr: Mechanism of membrane stabilization by calcium in vascular smooth muscle. *Am J Physiol* 235, C227-C232 (1978)

78. E. M. Brown, G. Gamba, D. Riccardi, M. Lombardi, R. Butters, O. Kifor, A. Sun, M. A. Hediger J. Lytton, S. C. Hebert: Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature* 366, 575-580 (1993)

79. S. Smajilovic, J. L. Hansen, T. E. Christoffersen, E. Lewin, S. P. Sheikh, E. F. Terwilliger, E. M. Brown, S.

Haunso and J. Tfelt-Hansen: Extracellular calcium sensing in rat aortic vascular smooth muscle cells. *Biochem Biophys Res Commun* 348, 1215-1223 (2006)

80. S. Smajilovic, M. Sheykhzade, H. N. Holmegard, S. Haunso, J. Tfelt-Hansen: Calcimimetic, AMG 073, induces relaxation on isolated rat aorta. *Vascul Pharmacol* 47, 222-228 (2007)

81. S. Smajilovic and J. Tfelt-Hansen: Novel role of the calcium-sensing receptor in blood pressure modulation. *Hypertension* 52, 994-1000 (2008)

82. A. H. Weston, M. Absi, D. T. Ward, J. Ohanian, R. H. Dodd, P. Dauban, C. Petrel, M. Ruat, G. Edwards: Evidence in favor of a calcium-sensing receptor in arterial endothelial cells: studies with calindol and Calhex 231.*Circ Res* 97, 391-398 (2005)

83. R. C. Ziegelstein, Y. Xiong, C. He, Q. Hu: Expression of a functional extracellular calcium-sensing receptor in human aortic endothelial cells. *Biochem Biophys Res Commun* 342, 153-163 (2006)

84. D. A. McCarron, C. D. Morris, C. Cole: Dietary calcium in human hypertension. *Science* 217, 267-269 (1982)

85. M. U. Alam, J. P. Kirton, F. L. Wilkinson, E. Towers, S. Sinha, M. Rouhi, T. N. Vizard, A. P. Sage, D. Martin, D. T. Ward, M. Y. Alexander, D. Riccardi, A. E. Canfield: Calcification is associated with loss of functional calcium-sensing receptor in vascular smooth muscle cells. *Cardiovasc Res* 81, 260-268 (2009)

86. A. H. Weston, M. Absi, E. Harno, A. R. Geraghty, D. T. Ward, M. Ruat, R. H. Dodd, P. Dauban, G. Edwards: The expression and function of Ca(2+)-sensing receptors in rat mesenteric artery; comparative studies using a model of type II diabetes. *Br J Pharmacol* 154, 652-662 (2008)

87. Y. Kansui, C. J. Garland, K. A. Dora: Enhanced spontaneous Ca2+ events in endothelial cells reflect signalling through myoendothelial gap junctions in pressurized mesenteric arteries. *Cell Calcium* 44, 135-146 (2008)

88. J. Ledoux, M. S. Taylor, A. D. Bonev, R. M. Hannah, V. Solodushko, B. Shui, Y. Tallini, M. I. Kotlikoff and M. T. Nelson. Functional architecture of inositol 1,4,5-trisphosphate signaling in restricted spaces of myoendothelial projections. *Proc Natl Acad Sci U S A* 105, 9627-9632 (2008)

89. M. T. Nelson, H. Cheng, M. Rubart, L. F. Santana, A. D. Bonev, H. J. Knot, W. J. Lederer: Relaxation of arterial smooth muscle by calcium sparks. *Science* 270, 633-637 (1995)

90. K. A. Dora: Coordination of vasomotor responses by the endothelium. *Circ J* 74, 226-232 (2010)

91. H. Takano, K. A. Dora, M. M. Spitaler, C. J. Garland: Spreading dilatation in rat mesenteric arteries associated with calcium-independent endothelial cell hyperpolarization. *J Physiol* 556, 887-903 (2004)

92. G. A. Mensah: Healthy endothelium: The scientific basis for cardiovascular health promotion and chronic disease prevention. *Vascul Pharmacol* 46, 310-314 (2007)

93. M. I. Cybulsky, M. A. Gimbrone Jr: Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* 251, 788–791(1991)

94. D. S. McLeod, D. J. Lefer, C. Merges, G. A. Lutty: Enhanced expression of intracellular adhesion molecule-1 and P-selectin inthe diabetic human retina and choroid. *Am J Pathol* 147, 642–653 (1995)

95. A. Schafer, N. J. Alp, S. Cai, C. A. Lygate, S. Neubauer, M. Eigenthaler, J. Bauersachs, K. M. Channon: Reduced vascular NO bioavailability in diabetes increases platelet activation *in vivo*. *Arterioscler Thromb Vasc Biol* 24, 1720–1726 (2004)

96. C. R. Triggle, A. Howarth, Z. J. Cheng, H. Ding: Twenty-five years since the discovery of endothelium-derived relaxing factor (EDRF): does a dysfunctional endothelium contribute to the development of type 2 diabetes? *Can J Physiol Pharmacol* 83, 681–700 (2005)

97. B. J. Martin, T. J. Anderson: Risk prediction in cardiovascular disease: the prognostic significance of endothelial dysfunction. *Can J Cardiol* 25, Suppl A, 15A-20A (2009)

98. Y. S. Chatzizisis, A. U. Coskun, M. Jonas, E. R. Edelman, C. L. Feldman, P. H. Stone: Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular and vascular behavior. *J Am Coll Cardiol* 49, 2379 –2393 (2007)

99. Y. S. Chatzizisis, M. Jonas, A. U. Coskun, R. Beigel, B. V. Stone, C. Maynard, R. G. Gerrity, W. Daley, C. Rogers, E. R. Edelman, C. L. Feldman, P. H. Stone: Prediction of the localization of high-risk coronary atherosclerotic plaques on the basis of low endothelial shear stress: an intravascular ultrasound and histopathology natural history study. *Circulation* 117, 993-1002 (2008)

100. K. C. Koskinas, C. L. Feldman, Y. S. Chatzizisis, A. U. Coskun, M. Jonas, C. Maynard, A. B. Baker, M. I. Papafaklis, E. R. Edelman and P. H. Stone: Natural history of experimental coronary atherosclerosis and vascular remodeling in relation to endothelial shear stress: a serial, *in vivo* intravascular ultrasound study. *Circulation* 121, 2092-101 (2010)

101. J. Bellien, J. Favre, M. Iacob, J. Gao, C. Thuillez, V. Richard, R. Joannides: Arterial stiffness is regulated by nitric oxide and endothelium-derived hyperpolarizing

factor during changes in blood flow in humans. *Hypertension* 55, 674-680 (2010)

102. R. M. Wever, T. van Dam, H. J. van Rijn , F. de Groot, T. J. Rabelink: Tetrahydrobiopterin regulates superoxide and nitric oxide generation by recombinant endothelial nitric oxide synthase. *Biochem. Biophys. Res. Commun.* 237, 340–344 (1997)

103. M. Ozaki, S. Kawashima, T. Yamashita, T. Hirase, M. Namiki, N. Inoue, K. Hirata, H. Yasui, H. Sakurai, Y. Yoshida, M. Masada, M. Yokoyama: Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *J Clin Invest* 110, 331-340 (2002)

104. M. Aljofan, D. Ding: High glucose increases expression of cyclooxygenase-2, increases oxidative stress and decreases the generation of nitric oxide in mouse microvessel endothelial cells. *J Cell Physiol* 222, 669-675 (2010)

105. P. Pacher, J. S. Beckman, L. Liaudet: Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87, 315–424 (2007)

106. K. K. Griendling, M. Ushio-Fukai: Redox control of vascular smooth muscle proliferation. *J Lab Clin Med* 132**,** 9–15 (1998)

107. N. Marui, M. K. Offermann, R. Swerlick, C. Kunsch, C. A. Rosen, M. Ahmad, R. W. Alexander, R. M. Medford: Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 92**,** 1866–1874 (1993)

108. D. Steinberg, A. Lewis: Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. *Circulation* 95, 1062–1071 (1997)

109. MRC/BHF Heart Protection Study: Antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomized placebo-controlled trial. *Lancet* 360, 23–33 (2002)

110. S. Selemidis, T. M. Cocks: Endothelium-dependent hyperpolarization as a remote anti-atherogenic mechanism. *Trends Pharmacol Sci* 23, 213–220 (2002)

111. F. Krotz, T. Riexinger, M. A. Buerkle, K. Nithipatikom, T. Gloe, H. Y. Sohn: Membrane-potential-dependent inhibition of platelet adhesion to endothelial cells by epoxyeicosatrienoic acids. *Arterioscler Thromb Vasc Biol* 24,595–600 (2004)

112. F. Krotz, N. Hellwig, M. A. Buerkle, S. Lehrer, T. Riexinger, H. Mannell: A sulfaphenazole- sensitive EDHF opposes platelet-endothelium interactions *in vitro* and in the hamster microcirculation *in vivo*. *Cardiovasc Res* 85, 542–550 (2010)

113. I. Grgic, B. P. Kaistha, J. Hoyer, R. Kohler: Endothelial Ca+-activated K+ channels in normal and impaired EDHF-dilator responses--relevance to cardiovascular pathologies and drug discovery. *Br J Pharmacol* 157, 509-526 (2009)

114. R. Kohler, P Ruth: Endothelial dysfunction and blood pressure alterations in K+-channel transgenic mice. *Pflugers Arch* 459, 969-976 (2010)

115. M. S. Taylor, A. D. Bonev, T. P. Gross, D. M. Eckman, J. E. Brayden, C.T. Bond, J. P. Adelman, M. T. Nelson: Altered expression of small-conductance Ca2+-activated K+ (SK3) channels modulates arterial tone and blood pressure. *Circ Res* 93, 124-131 (2003)

116. H. Si, W. T. Heyken, S. E. Wölfle, M. Tysiac, R. Schubert, I. Grgic, L. Vilianovich, G. Giebing, T. Maier, V. Gross, M. Bader, C. de Wit, J. Hoyer, R. Kohler: Impaired endothelium-derived hyperpolarizing factor-mediated dilations and increased blood pressure in mice deficient of the intermediate-conductance Ca2+-activated K+ channel. *Circ Res* 99, 537-544 (2006a)

117. H. Si, I. Grgic, W. T. Heyken, T. Maier, J. Hoyer, H. P. Reusch, R. Kohler: Mitogenic modulation of Ca2+ -activated K+ channels in proliferating A7r5 vascular smooth muscle cells. *Br J Pharmacol* 148, 909-917 (2006b)

118. M. Yamaguchi, T. Nakayama, Z. Fu, T. Naganuma, N. Sato, M. Soma, N. Doba, S. Hinohara, A. Morita, T Mizutani: Relationship between haplotypes of KCNN4 gene and susceptibility to human vascular diseases in Japanese. *Med Sci Monit* 15, CR389-CR397 (2009)

119. R. Kohler: Single-nucleotide polymorphisms in vascular Ca2+-activated K+-channel genes and cardiovascular disease. *Pflugers Arch* 460, 343-351(2010)

120. R. Kohler, B. P. Kaistha, H. Wulff: Vascular KCa-channels as therapeutic targets in hypertension and restenosis disease. *Expert Opin Ther Targets* 14, 143-155 (2010)

121. C. W. Wong, T. Christen, I. Roth, C. E. Chadjichristos, J. P. Derouette, B. F. Foglia, M. Chanson, D. A. Goodenough, B. R. Kwak: Connexin37 protects against atherosclerosis by regulating monocyte adhesion. *Nat Med* 12, 950-954 (2006)

122. L. H. Wang, J. Z. Chen, Y. L. Sun, F. R. Zhang, J. H. Zhu, S. J. Hu, D. H. Wang: Statins reduce connexin40 and connexin43 expression in atherosclerotic aorta of rabbits. *Int J Cardiol* 100, 467-475 (2005)

123. N. M. Rummery, K. U. McKenzie, J. A. Whitworth, C. E. Hill: Decreased endothelial size and connexin expression in rat caudal arteries during hypertension. *J Hypertens* 20, 247-253 (2002)

124. N. M. Rummery, T. H. Grayson, C. E. Hill: Angiotensin-converting enzyme inhibition restores

endothelial but not medial connexin expression in hypertensive rats. *J Hypertens* 23, 317-328 (2005)

125. A. Ellis, K. Goto, D. J. Chaston, T. D. Brackenbury, K. R. Meaney, J. R. Falck, R. J. Wojcikiewicz, C. E. Hill. Enalapril treatment alters the contribution of epoxyeicosatrienoic acids but not gap junctions to endothelium-derived hyperpolarizing factor activity in mesenteric arteries of spontaneously hypertensive rats. *J Pharmacol Exp Ther* 330, 413-422 (2009)

126. M. Pannirselvam, H. Ding, T. J. Anderson, C. R. Triggle. Pharmacological characteristics of endothelium-derived hyperpolarizing factor-mediated relaxation of small mesenteric arteries from db/db mice. *Eur J Pharmacol* 551, 98-107 (2006)

127. J. G. De Mey, P. M. Vanhoutte: Heterogeneous behavior of the canine arterial and venous wall. Importance of the endothelium. *Circ Res* 51, 439-447 (1982)

128. R. F. Furchgott, P. M.Vanhoutte: Endothelium-derived relaxing and contracting factors. *FASEB J* 3, 2007-2018 (1989)

129. M. Feletou, Y. Huang, P. M. Vanhoutte: Vasoconstrictor prostanoids. *Pflugers Arch* 459, 941-950 (2010)

130. A. Virdis, L. Ghiadoni, S. Taddei: Human endothelial dysfunction: EDCFs. *Pflugers Arch* 459, 1015-1523 (2010)

Abbreviations: Ca^{2+}: calcium, CaSR: calcium sensing receptor, CNP: C-type natriuretic peptide, COX: cyclooxygenase, Cx: connexins, CYP: cytochrome P450, ECP(s): endothelial cell protusion(s), EDH: endothelium-dependent hyperpolarization, EDHF: endothelium-derived hyperpolarizing factor, EDV: endothelium-dependent vasodilatation, EET: epoxyeicosatrienoic acid, eNOS: endothelial nitric oxide synthase, H2O2: hydrogen peroxide, IEL: internal elastic lamina, IP3: inositol trisphosphate, K+: potassium ion, KCa: calcium-activated potassium channels, KCa1.1: large conductance calcium-activated potassium channel, KCa2.3: small conductance calcium-activated potassium channel, KCa3.1: intermediate conductance calcium-activated potassium channel, KDR: delayed rectifier potassium channel, Kir; inward rectifying potassium channel,

Key Words: Endothelium, Endothelial Cell, Endothelial Dysfunction, Nitric Oxide, Endothelial Nitric Oxide Synthase, Endothelium-Dependent Hyperpolarization, Endothelium-Derived Hyperpolarizing Factor, Endothelial Dysfunction, Myo-Endothelial Gap Junctions, Connexins, Calcium-Activated Potassium Channels, Calcium Sensing Receptor, Calcium Pulsars, Microdomains, Review

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