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REVIEW

EDHF: an update

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ABSTRACT

The endothelium controls vascular tone not only by releasing NO and prostacyclin, but also by other pathways causing hyperpolarization of the underlying smooth muscle cells. This characteristic was at the origin of the term 'endothelium-derived hyperpolarizing factor' (EDHF). However, this acronym includes different mechanisms. Arachidonic acid metabolites derived from the cyclo-oxygenases, lipoxygenases and cytochrome P450 pathways, H_2O_2, CO, H_2S and various peptides can be released by endothelial cells. These factors activate different families of K^+ channels and hyperpolarization of the vascular smooth muscle cells contribute to the mechanisms leading to their relaxation. Additionally, another pathway associated with the hyperpolarization of both endothelial and vascular smooth muscle cells contributes also to endothelium-dependent relaxations (EDHF-mediated responses). These responses involve an increase in the intracellular Ca^{2+} concentration of the endothelial cells, followed by the opening of SK_{Ca} and IK_{Ca} channels (small and intermediate conductance Ca^{2+}-activated K^+ channels respectively). These channels have a distinct subcellular distribution: SK_{Ca} are widely distributed over the plasma membrane, whereas IK_{Ca} are preferentially expressed in the endothelial projections toward the smooth muscle cells. Following SK_{Ca} activation, smooth muscle hyperpolarization is preferentially evoked by electrical coupling through myoendothelial gap junctions, whereas, following IK_{Ca} activation, K^+ efflux can activate smooth muscle Kir2.1 and/or Na^+/K^+-ATPase. EDHF-mediated responses are altered by aging and various pathologies. Therapeutic interventions can restore these responses, suggesting that the improvement in the EDHF pathway contributes to their beneficial effect. 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.

INTRODUCTION

ECs (endothelial cells) synthesize and release factors that regulate angiogenesis, inflammatory responses and haemostasis, as well as vascular tone and permeability. The endothelium maintains the balance between inhibition and promotion of the proliferation and migration of SMCs (smooth muscle cells), between prevention and stimulation of the adhesion and aggregation of platelets and between thrombogenesis and fibrinolysis, as well

Key words: cell membrane potential, endothelium, endothelium-derived hyperpolarizing factor (EDHF), myoendothelial gap junction, potassium channel.

Abbreviations: AngII, angiotensin II; Ca_V channel, voltage-dependent Ca^{2+} channel; CNP, C-type natriuretic peptide; COX, cyclo-oxygenase; CSE, cystathionine γ-lyase; Cx, connexin; EBIO ethyl-2-benzimidazolinone; EC, endothelial cell; EDHF, endothelium-derived hyperpolarizing factor; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HO, haem oxygenase; K_{ATP} channel, ATP-sensitive K^+ channel; K_{Ca} channel, Ca^{2+}-activated K^+ channel; BK_{Ca} channel, large conductance K_{Ca} channel; IK_{Ca} channel, intermediate conductance K_{Ca} channel; IP receptor, PGI_2 receptor; K_{IR} channel, inward rectifier K^+ channel; LOX, lipoxygenase; NOS, NO synthase; eNOS, endothelial NOS; NPR, natriuretic peptide receptor; O_2^-, superoxide anion; PGI_2, prostacyclin; ROS, reactive oxygen species; SK_{Ca} channel, small conductance K_{Ca} channel; SMC, smooth muscle cell; SOD, superoxide dismutase; THETA, trihydroxyeicosatrienoic acid; TRPC, transient receptor potential canonical channel; TRPV, transient receptor potential vanilloid channel; VSMC, vascular SMC.

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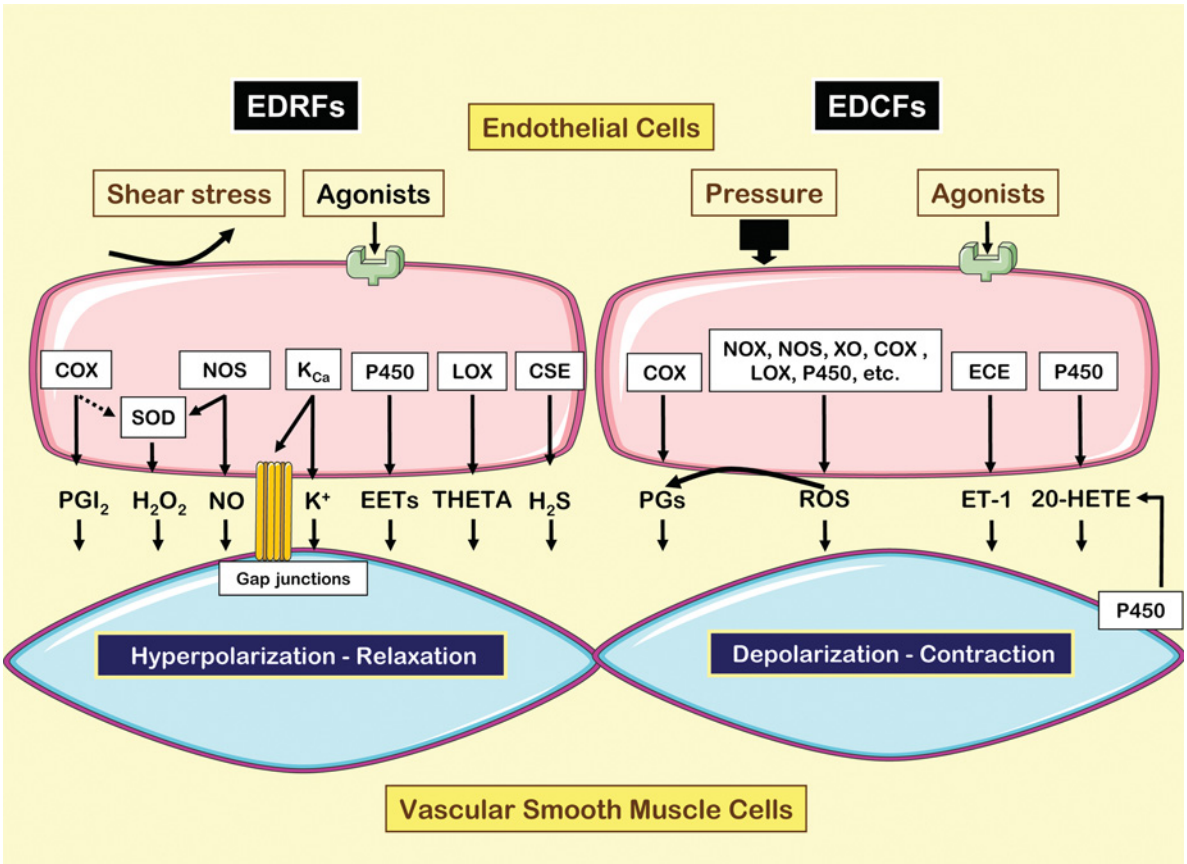

Figure 1 Endothelium-dependent relaxations and contractions

ECs synthesize and release various vasoactive factors and, therefore, maintain the balance between vasodilation and vasoconstriction. Upsetting this tightly regulated balance contributes to endothelial dysfunction [2]. EDCF, endothelium-derived contracting factors; ECE, endothelin-converting enzyme; ET-1, endothelin-1; P450, cytochrome P450; PGs, prostaglandins; XO, xanthine oxidase.

as between vasodilation and vasoconstriction. Upsetting this tightly regulated balance leads to endothelial dysfunction [1,2] (Figure 1).

Endothelium-dependent relaxations/vasodilations in response to neurohumoral mediators and physical forces, such as the shear stress exerted by the flowing blood, are generally attributed to the release of NO and/or PGI_2 (prostacyclin) [3–6]. However, in numerous blood vessels from different species, including humans, these responses cannot be totally explained by the release of these two mediators. The relaxations observed in the presence of COX (cyclo-oxygenase) and NOS (NO synthase) inhibitors are often associated with hyperpolarization of VSMCs (vascular SMCs) and were first attributed to a non-characterized endothelial factor termed EDHF (endothelium-derived hyperpolarizing factor). The acronym 'EDHF' turned out to be confusing because it implies that a single diffusible substance mediates this type of endothelium-dependent relaxation. In fact NO itself, but also numerous identified putative endothelium-derived factors including, CO (carbon monoxide), H_2S (hydrogen sulfide), ROS (reactive oxygen species), peptides and arachidonic acid metabolites derived from the COX, LOX (lipoxygenase) and cytochrome P450 mono-oxygenase pathways, can hyperpolarize the underlying SMCs [1].

Hyperpolarization decreases Ca^{2+} influx, either by reducing the open probability of Ca_V (voltage-dependent Ca^{2+}) channels or the Ca_V channel-dependent activation of the sarcoplasmic reticulum, which is a powerful means to produce the relaxation of VSMCs [7–9].

Another pathway, which does not involve the synthesis and the release of a factor, is associated with the hyperpolarization of both ECs and VSMCs (EDHF-mediated responses) and also contributes to endothelium-dependent relaxations. These responses involve an increase in the intracellular Ca^{2+} concentration of ECs, followed by the opening of SK_{Ca} and IK_{Ca} channels [small and intermediate conductance K_{Ca} (Ca^{2+}-activated K^+) channels respectively], and the subsequent hyperpolarization of these cells. Then, the endothelium-dependent hyperpolarization of the underlying SMCs can be evoked by direct electrical coupling through myoendothelial junctions and/or the accumulation of K^+ ions in the intercellular

space between the two cell types [1]. The present review will briefly summarize the hyperpolarizing effects of the various endothelium-derived factors and will focus on this latter mechanism, i.e. EDHF-mediated responses.

ARACHIDONIC ACID METABOLITES

COXs

In ECs, PGI_2 is the principal COX-derived metabolite of arachidonic acid. When activating its preferential receptor, the IP receptor (PGI_2 receptor), PGI_2 is a potent antithrombotic and antiplatelet agent, and is generally a vasodilator substance [3]. The deletion of PGI_2 synthase generates hypertensive mice with arterial sclerosis, whereas, in response to stress or injury, IP-receptor-knockout animals have enhanced platelet activation, thrombosis, intimal hyperplasia, atherosclerosis and restenosis, and are prone to ischaemia/reperfusion injury [10].

The vascular relaxation to PGI_2, or its synthetic analogues, is often associated with the concomitant hyperpolarization of the SMCs, which, depending on the blood vessels and the species, can involve the opening of various populations of K^+ channels [11,12]. Therefore, in numerous vascular beds, PGI_2 can act as an endothelium-derived hyperpolarizing substance [13]. Since COX inhibitors abolish the basal and stimulated generation of PGI_2, and potent and specific antagonist of the IP receptor block its vasodilator responses [14,15], the contribution of PGI_2 in endothelium-dependent responses can reasonably be assessed. This prostaglandin plays a role in flow-mediated vasodilation [16,17]; however, its contribution to acute endothelium-dependent relaxations in response to neurohumoral mediators is often minimal [1] or can only be observed when the other endothelial pathways have been inhibited [18,19]. The contribution of PGI_2 to endothelium-dependent responses is increased in eNOS (endothelial NOS)-knockout mice [20,21]. Similarly, in humans with cardiovascular diseases, COX-2-derived prostaglandins can play a compensatory role in the decreased NO bioavailability [22,23], possibly explaining some of the detrimental cardiovascular effects associated with COX-2 inhibitors [24].

However, in aging and in the course of some cardiovascular diseases, PGI_2, along with other prostaglandins, can also act as an endothelium-derived contracting factor by activating smooth muscle TP receptors (thromboxane/endoperoxide receptors) and, thus, contribute to endothelial dysfunction [14,15,25] (Figure 1).

Cytochrome P450 mono-oxygenases

EETs (epoxyeicosatrienoic acids), derived from the endothelial cytochrome P450 2C or 2J epoxygenases, are generally, but not necessarily, vasodilator agents [26,27], whereas 20-HETE (20-hydroxyeicosatetraenoic acid; a metabolite of the cytochrome P450 4A and 4F family preferentially located in VSMCs) is a potent endogenous vasoconstrictor of renal, cerebral, coronary, mesenteric and skeletal muscle arteries [28] (Figure 1).

EETs play an important role in endothelium-dependent relaxations either as diffusible factors or as essential endothelial intracellular second messenger(s) [29]. When released, they can act as an autocrine agent eliciting endothelium- and NO-dependent relaxations [30] or, more generally, diffuse toward the underlying VSMCs and produce their relaxation [31,32]. In the latter case, 11,12-EET and 14,15-EET, the two predominant diffusible isoforms, activate BK_{Ca} channels (large conductance K_{Ca} channels or $K_{Ca}1.1$) of VSMCs [33–36]. Experiments performed both *in vitro* and *in vivo* on human blood vessels suggest a contribution of EETs to endothelium-dependent relaxations/vasodilations in coronary and mammary arteries, as well as in peripheral muscular and subcutaneous arterioles [37–40].

Cytochrome P450 metabolites also play an important role in the regulation of the kidney circulation, and contribute to the long-term regulation of blood pressure and Na^+ homoeostasis. EETs regulate not only regional blood flow, but activate K_{ATP} channels in cardiomyocytes, limit platelet aggregation, exert anti-inflammatory actions, and improve insulin sensitivity and lipid metabolism. Thus they can protect the kidney vasculature from injury during renal and cardiovascular diseases, exert cardioprotection and prevent/delay metabolic syndrome and atherogenesis [41–43].

EETs are rapidly metabolized by soluble epoxide hydrolase to form the generally less active DHETs (dihydroxyeicosatrienoic acids). In mice, disruption of the soluble epoxide hydrolase gene produces no or minor decreases in basal arterial blood pressure [44], but protects against myocardial ischaemia/reperfusion, heart failure and ischaemic stroke [45–47]. In rats, soluble epoxide hydrolase plays an essential role in AngII (angiotensin II)-induced cardiac hypertrophy [48], which, in patients with cardiovascular disease, is the most common cause of heart failure. Polymorphisms of *EPHX2* (the gene encoding soluble epoxide hydrolase) have been associated with coronary artery diseases, ischaemic stroke and insulin resistance [43,49,50]. Potent and selective inhibitors of this enzyme have been designed and are currently undergoing clinical trials for the treatment of hypertension. The additional anti-inflammatory properties of soluble epoxide hydrolase inhibitors also make them attractive for the treatment of chronic kidney disease in patients with cardiometabolic syndrome [51].

LOXs

In rat and porcine coronary arteries, the 12-LOX metabolite 12-*S*-HETE [12-(*S*)-hydroxyeicosatetraenoic acid] can be released by ECs and evoke relaxation of the vascular smooth muscle by activating BK_{Ca} channels [52].

In rabbit arteries, the generation of 11,12,15-THETA (11,12,15-trihydroxyeicosatrienoic acid) by reticulocyte 15-LOX-I contributes to acetylcholine-induced endothelium-dependent relaxations [53]. In this species, the age-related decrease in hypotension and in endothelium-dependent relaxations induced by acetylcholine are mediated by a decreased synthesis of 11,12,15-THETA, associated with a down-regulation of 15-LOX expression and a reduced activity of the enzyme [54,55]. By contrast, short-term hypercholesterolaemia, in the absence of atherosclerotic lesions, and chronic hypoxia increase endothelial 15-LOX expression and 11,12,15-THETA production, as well as acetylcholine-induced endothelium-dependent relaxations and hypotension [56,57] (Figure 1).

However, although a strong case for LOX derivatives acting as endothelium-derived hyperpolarizing substances can be built in specific arteries and especially in those of the rabbit, most of the EDHF-mediated responses, including that in human arteries, do not appear to involve metabolites of arachidonic acid produced by this pathway [1,58].

Endocannabinoids

In isolated blood vessels or *in vivo* in anaesthetized animals, endogenous and exogenous cannabinoids usually have vasodilator properties and are likely to play a role in cardiovascular homoeostasis [59]. However, the suggestion that anandamide could be an endothelium-derived hyperpolarizing substance has not been substantiated [1,60].

NOSs

Three different isoforms of an enzyme are able to synthesize NO from L-arginine and molecular oxygen: NOS-I [nNOS (neuronal NOS) or NOS-1], NOS-II [iNOS (inducible NOS) or NOS-2] and NOS-III (eNOS or NOS-3). Under various circumstances, such as the presence of NOS inhibitors, low levels of L-arginine or oxidized BH_4 (tetrahydrobiopterin) but also under physiological conditions, NOS can generate O_2^- (superoxide anion), which is reduced to H_2O_2 either spontaneously or enzymatically by SOD (superoxide dismutase). H_2O_2 can act locally close to its site of production or, as it is an uncharged molecule, diffuse through the cell membrane and act on neighbouring cells [5,61–63] (Figure 1).

NO

NO is a potent vasodilator and a powerful inhibitor of platelet adhesion and aggregation. The relaxations elicited by NO are generally associated with the stimulation of the cytosolic soluble guanylate cyclase and the subsequent activation of PKG (cGMP-dependent protein kinase) [5]. NO regulates the activity of various K^+ channels and, depending on the vascular beds, the hyperpolarizing effects of NO on VSMCs can substantially contribute to their relaxation [1]. The activation of BK_{Ca} channels by NO contributes to the beneficial effects of currently prescribed drugs associated with the L-arginine/NOS/cGMP pathway, for instance NO donors and phosphodiesterase inhibitors such as sildenafil [64].

In contrast with COX inhibitors, inhibitors of NOS do not necessarily fully inhibit the production of NO. Thus, in their presence, residual NO can still be produced by ECs and contributes to the relaxation and/or hyperpolarization of the underlying vascular smooth muscle [65]. Furthermore, NO can also be stored and released independently of the activation of NOS [66–68]. Therefore it appears likely that the role of NO as an endothelium-derived hyperpolarizing substance may be underestimated by assuming that the presence of an inhibitor of NOS rules out its contribution. A non-NO/non-PGI_2-mediated response should be reported not only when a relaxation and/or hyperpolarization is recorded in the combined presence of COX and NOS inhibitors, but when this response is still observed with the additional presence of an NO scavenger [69]. In *in vivo* studies in humans, the complete blockade of NOS is difficult to achieve (or to demonstrate) and, for obvious ethical reasons, many of the pharmacological tools used to study EDHF-mediated responses cannot be administrated. The limitation of these studies should always be kept in mind when interpreting these findings.

In NOS-3-knockout mice, EDHF-mediated responses play a compensatory role for the absence of endothelial NO [70] and this adaptation to NOS deletion is gender-specific [71]. Similarly, in resistance arteries from female double knockout mice for NOS-3 and COX-1, endothelium-dependent relaxations are preserved by an EDHF-mediated mechanism, whereas, in arteries from the double knockout males, the endothelium-dependent relaxations are impaired severely. In genetically modified female mice, the double deletion of NOS-3 and COX-1 does not affect mean arterial blood pressure, whereas the corresponding males are hypertensive [72].

H_2O_2

In the mesenteric arteries of mice with disruption of the various NOS isoform genes (single NOS-3-knockout, double NOS-1/NOS-3-knockout and triple NOS-1/NOS-2/NOS-3-knockout), the endothelium-dependent relaxations and hyperpolarizations resistant to NOS and COX inhibitors are progressively reduced as the number of the disrupted NOS genes is increased [73]. The dependency of the responses resistant to NOS and COX inhibitors towards the NOS systems could not be explained by residual NO release, but has been attributed to the production of H_2O_2 [73] (Figure 1).

Depending on the tissue, the experimental conditions or the concentrations studied, H_2O_2 possesses dilator

or constrictor properties, and can hyperpolarize or depolarize VSMCs [74]. For instance, in the isolated murine mesenteric artery, H_2O_2, at concentrations lower than 50 μmol/l, produces an endothelium-independent relaxation providing that K_{Ca} channels are operational, but, at the same concentrations, elicits a potent contractile response if the activity of these channels is compromised [75].

In NOS-disrupted murine arteries, relaxations resistant to NOS and COX inhibitors are sensitive to catalase, the enzyme that dismutates H_2O_2 into water and oxygen, and have been attributed to the NOS-3/Cu,Zn-SOD-dependent formation of H_2O_2 [76,77] (Figure 1). The maintenance of these endothelium-dependent relaxations/hyperpolarizations in NOS-3-knockout mice was explained by the compensatory endothelial expression of other NOS genes, the production of H_2O_2 being preserved up to the total disruption of the three NOS genes [73]. Nevertheless, whether or not the decrease in the EDHF-mediated responses is directly associated with the disruption of the NOS genes or is independently associated with the severe phenotype of these mice, especially with the one observed in the triple knockout mice (hypertension, dyslipidaemia, myocardial infarction and nephrogenic diabetes insipidus), remains to be fully assessed [also see the phenotype of mice with a CSE (cystathionine γ-lyase) deletion described below].

The involvement of H_2O_2 in agonist- and flow-induced endothelium-dependent relaxations/vasodilations has been suggested in other vascular beds [78], including human mesenteric [79] and coronary [80] arteries. In addition, without actually being released by ECs, H_2O_2 can enhance EDHF-mediated responses by potentiating Ca^{2+} release from endothelial stores [81]. In various experimental models or in arteries from patients with cardiovascular diseases, H_2O_2 generation can partially compensate for the decreased NO production, at least in terms of endothelium-dependent relaxations, but in the long term this production of ROS may contribute to vascular oxidative injury [82–84].

Depending on the blood vessels, besides NOSs, several other endothelial enzymes, such as COXs, LOXs, xanthine oxidases, cytochrome P450 epoxygenases, NADPH oxidases and mitochondrial respiratory enzymes, can generate O_2^- and be at the origin of H_2O_2 production [78]. In murine arteries, endothelial oxidases other than NOS do not appear to be involved in H_2O_2-mediated responses [85]; however, in human coronary arterioles, flow-induced endothelium-dependent dilation is associated with H_2O_2 generated by the mitochondrial respiratory chain, whereas bradykinin-induced endothelium-dependent relaxation requires NADPH-oxidase-derived H_2O_2 [86,87].

Therefore, in the presence of NOS and COX inhibitors, residual NO and/or H_2O_2, both potentially derived from various endothelial NOS isoforms, can act as endothelium-derived hyperpolarizing substances. However, in many arteries, EDHF-mediated responses cannot be attributed to the generation of residual NO or to that of H_2O_2 [1,74,88].

OTHER GASEOUS MEDIATORS

Besides NO, CO and H_2S are also water-soluble low-molecular-mass gases that readily cross lipid membranes and, therefore, diffuse homogeneously and in a non-polarized manner from their production site acting as autocrine and paracrine substances [89].

CO

The predominant biological source of CO is from haem degradation by HO (haem oxygenase), either from the constitutive (HO-2) or the inducible (HO-1) isoform, both being expressed in VSMCs and ECs [90,91]. In many pathophysiological situations, the HO/CO pathway compensates for the decrease in NO bioavailability [91]. CO is a potent vasodilator in most, but not all, vascular beds. The mechanisms of CO-induced vasodilation involve the stimulation of soluble guanylate cyclase, the inhibition of cytochrome P450-dependent production of 20-HETE and/or the activation of various populations of K^+ channels [91]. However, CO is also a tonic inhibitor of NOS, by binding to its prosthetic haem, and can contribute to endothelial dysfunction [92].

HO-1-knockout mice, although normotensive, when subjected to stress or injury have exacerbated responses. In contrast, overexpression of HO-1 plays a protective role in hypoperfusion and ischaemia/reperfusion injury, and the induction of HO-1 expression by transient haemin administration produces a long-lasting normalization of arterial blood pressure in SHRs (spontaneously hypertensive rats) [93,94]. HO-2-knockout mice are also normotensive, but stroke damage in response to injuries is accentuated in these animals, indicating that HO-2 plays an endogenous neuroprotective role in the brain [95]. CO-releasing molecules have vasodilator, anti-ischaemic and anti-inflammatory effects, and may present some therapeutic interest in cardiovascular diseases [96].

An endothelial production of CO, contributing to endothelium-dependent relaxations in response to neurohumoral substances, has been demonstrated only in a limited number of arteries and is, therefore, unlikely to explain most EDHF-mediated responses [1,97,98].

H_2S

Two main enzymes are responsible for the production of H_2S, CBS (cystathionine β-synthase) and CSE, and both use L-cysteine as a substrate. The physiological cardiovascular effects of H_2S, which are generally linked to the activation of the latter enzyme, involve anti-inflammatory and antioxidant properties, vasodilation and a decrease in arterial blood pressure [89,99] (Figure 1).

Mice with the deletion of CSE, an enzyme expressed in multiple tissues and recently identified in ECs, are hypertensive and the endothelium-dependent relaxations of their mesenteric artery in response to methacholine is virtually abolished [100]. H_2S is produced and released by ECs, in a Ca^{2+}-dependent manner, following neurohumoral stimulation and evokes relaxation and hyperpolarization of VSMCs by activating K_{ATP} (ATP-sensitive K^+) channels [100,101]. Thus these results suggest that H_2S is an endothelium-derived relaxing and hyperpolarizing factor. However, the precise role of this mediator needs to be substantiated further. In most studies, the endothelium-dependent relaxations of the murine mesenteric artery involve NO release and EDHF-mediated responses, which are not necessarily associated with the activation of K_{ATP} channels [1]. The disappearance of both the NO- and EDHF-mediated component of the endothelium-dependent relaxation in CSE-knockout mice is unexplained at present, but could be attributed to the increase in homocysteine levels [102,103]. Again, whether or not the decrease in endothelium-dependent responses (NO-mediated and EDHF-mediated responses) is directly associated with the disruption of the CSE gene or is independently associated with the phenotype of these mice remains to be determined (also see multiple NOS-knockout mice described above).

H_2S donors are currently being synthesized and have therapeutic potential in cardiovascular diseases associated with inflammatory processes, such as reperfusion injury, circulatory shock, atherosclerosis, diabetes and possibly hypertension [97,104,105].

CNP (C-TYPE NATRIURETIC PEPTIDE)

ECs can theoretically synthesize numerous vasoactive peptides. Among them CNP, a member of the natriuretic peptide family, evokes relaxations and hyperpolarizations of VSMCs, including those of human forearm resistance vessels. The vasodilator effects of CNP are generally attributed to the activation of the NPR-B (natriuretic peptide receptor-B) subtype on the smooth muscle, followed by the stimulation of particulate guanylate cyclase, leading to accumulation of cGMP and the subsequent opening of BK_{Ca} and K_{ATP} channels [106–108].

In addition, it has been suggested that CNP could contribute to EDHF-mediated responses. CNP would activate the NPR-C receptor subtype and evoke hyperpolarization of SMCs via the cGMP-independent activation of GIRK channels [G-protein-regulated K_{IR} (inward-rectifier K^+) channels] [109,110]. However, this hypothesis had not been confirmed [111–113], and in mice deficient for the NPR-C gene EDHF-mediated responses are not altered [114].

Therefore CNP is unlikely to act as an endothelium-derived relaxing/hyperpolarizing substance and contribute to moment-to-moment endothelium-dependent regulation of vascular tone. Nevertheless, this peptide plays a key role in preventing smooth muscle proliferation, leucocyte recruitment and platelet reactivity. As such, CNP is likely to exert an anti-atherogenic influence on the blood vessel walls [115,116].

EDHF-MEDIATED RESPONSES

EDHF-mediated responses are endothelium-dependent relaxations resistant to NOS and COX inhibitors, which do not involve one of the identified mediators mentioned above (arachidonic acid metabolites, and residual or stored NO, H_2O_2, CO, H_2S or CNP) and which require the activation of endothelial K_{Ca} channels.

K_{Ca} channels

The three subtypes of K_{Ca} channels of large (BK_{Ca}), intermediate (IK_{Ca} or $K_{Ca}3.1$) and small conductance (SK_{Ca} or $K_{Ca}2.3$ isoform) are present in the vascular wall, but with very specific cellular and subcellular localization (Figure 2).

BK_{Ca} channels are expressed in virtually all VSMCs [1], whereas, in most ECs, when freshly isolated, BK_{Ca} channels are at best poorly expressed and iberiotoxin-sensitive currents are observed only at very positive potentials [117–119]. In SMCs, BK_{Ca} channels, often clustered in groups of 20–100 units, are activated by a general increase in intracellular Ca^{2+} or by Ca^{2+} sparks, localized elemental Ca^{2+} release events from internal Ca^{2+} stores, which then generate STOCs (spontaneous transient outward currents) [120,121]. BK_{Ca} channels are often co-localized in discrete smooth muscle areas with endoplasmic reticulum and form a signal complex physically associated with cationic channels, such TRPC1 (transient receptor potential canonical channel 1) [122] or indirectly associated with TRPV4 (transient receptor potential vanilloid channel 4). The Ca^{2+} influx through TRPV4 preferentially stimulates ryanodine receptors located on the endoplasmic reticulum increasing the frequency of Ca^{2+} sparks. In some arteries, EET-induced hyperpolarization involves the latter Ca^{2+} signalling complex [36] (Figure 2). Conversely, there is little evidence for a functional role of SK_{Ca} channels in VSMCs, although a non-identified apamin-sensitive conductance has been reported in some arteries [123–125]. Similarly, in healthy and freshly isolated VSMCs, IK_{Ca} channels are not or very poorly expressed. However, in proliferating cells, as observed in culture or after vascular injury, the expression of this channel increases dramatically [126,127].

By contrast, the IK_{Ca} and SK_{Ca} channels (especially the SK3 α subunit) are constitutively expressed in ECs [117,118,128,129], but have a very different spatial distribution. SK_{Ca} are diffusely distributed over the plasma membrane with preferential locations at sites of

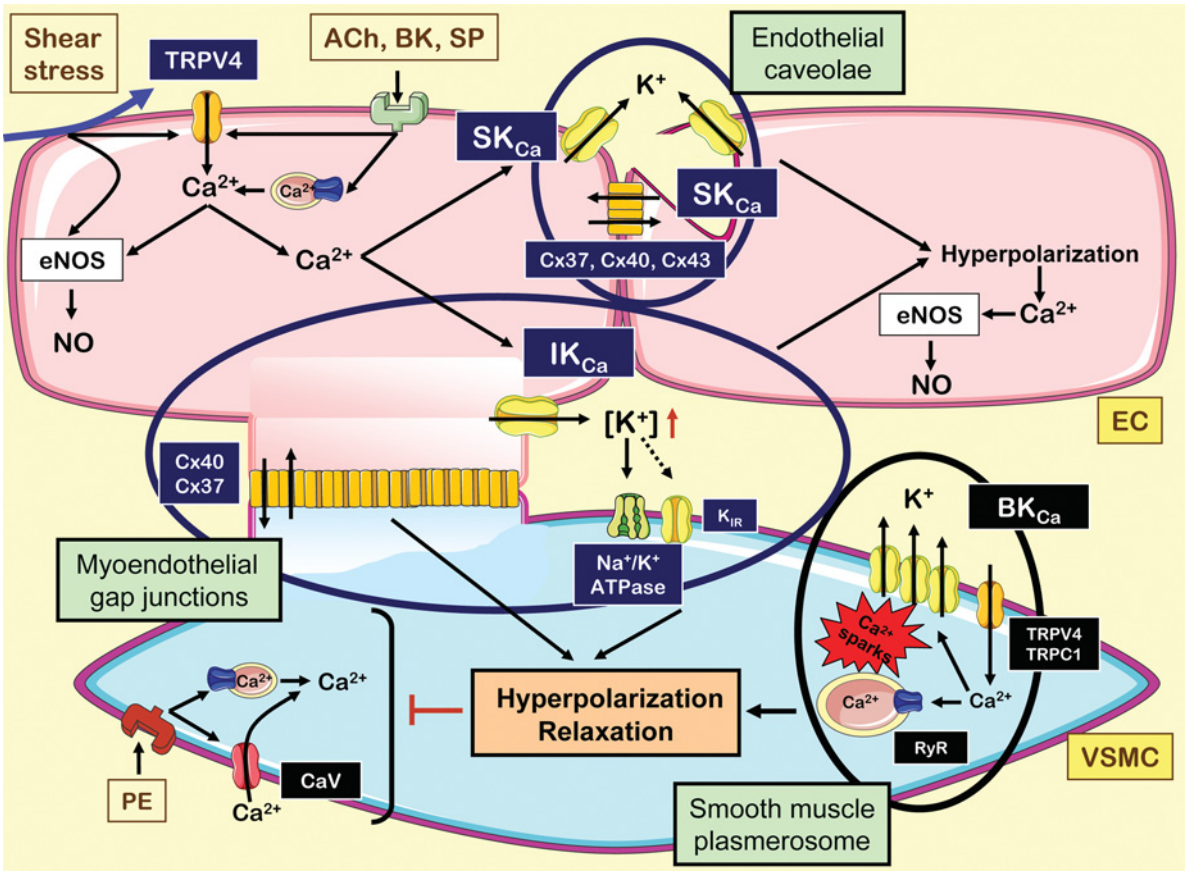

Figure 2 K_{Ca} **channels in the vascular wall and EDHF-mediated responses**

The three subtypes of K_{Ca} channels, BK_{Ca}, IK_{Ca} and SK_{Ca} channels, are present in the vascular wall, but with very specific cellular and subcellular localization. BK_{Ca} channels are expressed preferentially in discrete vascular smooth muscle areas, smooth muscle plasmerosome, associated with endoplasmic reticulum. They form signal complexes with TRPC1 or TRPV4. The IK_{Ca} and SK_{Ca} channels (especially the SK3 α subunit) are constitutively expressed in ECs. SK_{Ca} are diffusely distributed over the plasma membrane with preferential locations at sites of homocellular endothelial gap junctions and caveolin-rich domains, and are associated with various Cxs (Endothelial caveolae). IK_{Ca} are preferentially localized within the endothelial projections through the internal elastic lamina (myoendothelial gap junctions). The activation of endothelial receptors and the shear stress exerted by the flowing blood increase endothelial intracellular Ca^{2+} concentrations and activates eNOS, as well as SK_{Ca} and IK_{Ca} channels. The subsequent endothelial hyperpolarization favours the entry of Ca^{2+} as a positive-feedback loop. The hyperpolarization can be conducted through myoendothelial gap junctions composed of Cx40 and possibly Cx37 to the underlying vascular smooth muscle. Additionally, accumulation of K^+ ions in the intercellular space can hyperpolarize the SMCs by activating Na^+/K^+-ATPase and K_{IR} channels. ACh, acetylcholine; BK, bradykinin; PE, phenylephrine; RyR, ryanodine receptor; SP, substance P.

homocellular endothelial gap junctions and caveolin-rich domains, and are associated with various Cxs (connexins). IK_{Ca} are localized preferentially within endothelial projections through the internal elastic lamina at the sites of myoendothelial gap junctions [113,130–133] (Figure 2).

Agonists that stimulate GPCRs (G-protein-coupled receptors) and compounds such as the Ca^{2+} ionophore A23187, thapsigargin and cyclopiazonic acid evoke EDHF-mediated responses. These substances share the property of increasing the endothelial intracellular Ca^{2+} concentration and activating endothelial SK_{Ca} channels (blocked by apamin, scyllatoxin or UCL 1684) and/or IK_{Ca} channels (blocked by charybdotoxin or TRAM-34) [1]. 1-EBIO (1-ethyl-2-benzimidazolinone), a non-specific activator of K_{Ca} channels [134], which activates endothelial IK_{Ca} and SK_{Ca} channels [118], but not BK_{Ca} channels of vascular smooth muscle, hyperpolarizes ECs and produces endothelium-dependent hyperpolarization [135,136]. Similar results were obtained with more potent analogues of 1-EBIO, such as DC-EBIO (5,6-dichloro-EBIO) or NS-309 [137,138], and with derivatives of the neuroprotective agent riluzole such as SK-20 or SK-31 [139], indicating that activation of endothelial K_{Ca} channels and/or EC hyperpolarization elicit(s) EDHF-mediated responses.

The hyperpolarization of the ECs in turn favours the entry of Ca^{2+} by increasing the driving force for this ion [140,141]. Therefore endothelial K_{Ca} channels are not only key players in EDHF-mediated responses, but also

contribute to the activation of Ca^{2+}-sensitive enzymes, such as eNOS, and thus to the generation of NO [142,143] (Figure 2).

Hyperpolarization of vascular smooth muscle

The involvement of two populations of endothelial K_{Ca} channels in EDHF-mediated responses has been puzzling for a long time, but the discrete role of each channel is now better appreciated.

In the blood vessel wall, gap junctions link SMCs with other SMCs, ECs with other ECs and, in many blood vessels, SMCs with ECs. Cx37, Cx40 and Cx43 are the predominant isoforms of gap-junction proteins expressed in the vascular wall and, in rodents, the Cx37 and Cx40 isoforms are involved preferentially in myoendothelial gap-junction communication [144–146] (Figure 2). The number of myoendothelial gap junctions increases with a reduction in the size of the artery [147], a phenomenon that parallels the contribution of the EDHF-mediated responses with endothelium-dependent relaxations [148,149]. Endothelium and SMCs can communicate via these myoendothelial gap junctions physically, as Ca^{2+} and Ins(1,4,5)P_3 can diffuse from one cell type to another [150–152], and electrically, as depolarization and hyperpolarization are conducted bi-directionally from one cell type to the other [151–156]. However, endothelium-dependent dilations do not simply propagate electronically, but involve a regenerative mechanism [155,157]. Blockers of gap junctions abolish or partially inhibit EDHF-like responses in many arteries and, in the rat mesenteric artery, antibodies directed against Cx40, when loaded selectively in ECs, block EDHF-mediated responses [144,158–161]. Furthermore, in mice, Cx40 is essential for the acetylcholine-activated regenerative endothelium-dependent vasodilation [157,162]. Activation of either SK_{Ca} or IK_{Ca} channels leads to endothelium-dependent hyperpolarizations and relaxations of VSMCs, but, in quiescent arteries (in the absence of vasoconstrictor stimulation), EDHF-mediated responses are associated with the preferential activation of SK_{Ca} channels and the contribution of myoendothelial gap junctions [113,131,133,163].

Taken in conjunction, the results of these *in vitro* experiments provide compelling evidence for a major contributing role of myoendothelial gap junctions in EDHF-mediated responses [164]. However, experiments performed *in vivo* have generally failed to demonstrate such a significant role for myoendothelial gap junctions [165,166]. The origin of this discrepancy is unknown, but may involve the type and size of arteries studied *in vivo*, the presence of shear stress, sympathetic innervation and circulating hormones, as well as confounding factors such as the use of anaesthetics which inhibit gap junctions [131,164].

Additionally, the efflux of K^+ ions associated with the activation of endothelial K_{Ca} can contribute to EDHF-mediated responses [166]. The resultant moderate increase in the extracellular K^+ concentration (from 1 to 15 mmol/l) can provoke the relaxation of VSMCs [167] by activating K_{IR} channels [168] and the Na^+/K^+ pump [169]. The activation of K_{IR} channels and the Na^+/K^+ pump overcomes the small depolarizing effects linked to the increase in K^+ ions and the net resultant is hyperpolarization and, thus, relaxation of the SMCs. This hypothesis was first demonstrated successfully in the hepatic and mesenteric arteries of the rat [166], and has been observed in many other blood vessels including human arteries [170–177].

However, this phenomenon is likely to occur only in specialized microdomains situated in the endothelial projections associated with myoendothelial gap junctions. Sections of endoplasmic reticulum densely expressing Ins(1,4,5)P_3 receptors, Cx40, IK_{Ca} channels and Ca^{2+}-sensing receptors are co-located in these endothelial projections [113,130–133,178]. Repetitive localized Ca^{2+} events (pulsars), driven by Ins(1,4,5)P_3 and/or Ca^{2+} ions (Ca^{2+}-induced Ca^{2+} release), originate from these endothelial Ca^{2+} stores. Ins(1,4,5)P_3 can be generated by ECs (for instance following acetylcholine stimulation) or SMCs (for instance following phenylephrine stimulation). In the latter case, Ins(1,4,5)P_3 would diffuse toward ECs (possibly with Ca^{2+} ions) through the myoendothelial gap junctions [113,133,179,180]. The closely situated IK_{Ca} channels are activated by these Ca^{2+} pulsars and the resultant endothelial hyperpolarization can be transmitted to SMCs either via the myoendothelial gap junctions, as described previously, or be elicited by K^+ ions accumulating in the restricted extracellular space surrounding these endothelial projections (Figure 3). In the rat mesenteric artery, K^+ ion accumulation preferentially activates the Na^+/K^+ pump [113,181].

The precise role of the Ca^{2+}-sensing receptor at the site of these endothelial projections is not completely understood. Stimulation of the Ca^{2+}-sensing receptor results in selective IK_{Ca} channel-dependent endothelial hyperpolarization and endothelium-dependent vascular smooth muscle hyperpolarization [178,182,183]. In quiescent arteries (in the absence of vasoconstrictor stimulation), the Ca^{2+}-sensing receptor would be fully stimulated by the concentration of Ca^{2+} bathing the ECs and IK_{Ca} channel inactivated [113]. Stimulation of smooth muscle (for instance by phenylephrine) opens the Ca_V channel and, in the small extracellular space surrounding myoendothelial projections, could create a localized Ca^{2+} sink. The endothelial Ca^{2+}-sensing receptor would detect the changes in extracellular Ca^{2+} and allow the recruitment of endothelial IK_{Ca} channels. The subsequent endothelium-dependent hyperpolarization would restrain excessive activation of the smooth muscle [113] (Figure 3). Indeed, reducing the extracellular Ca^{2+} concentration

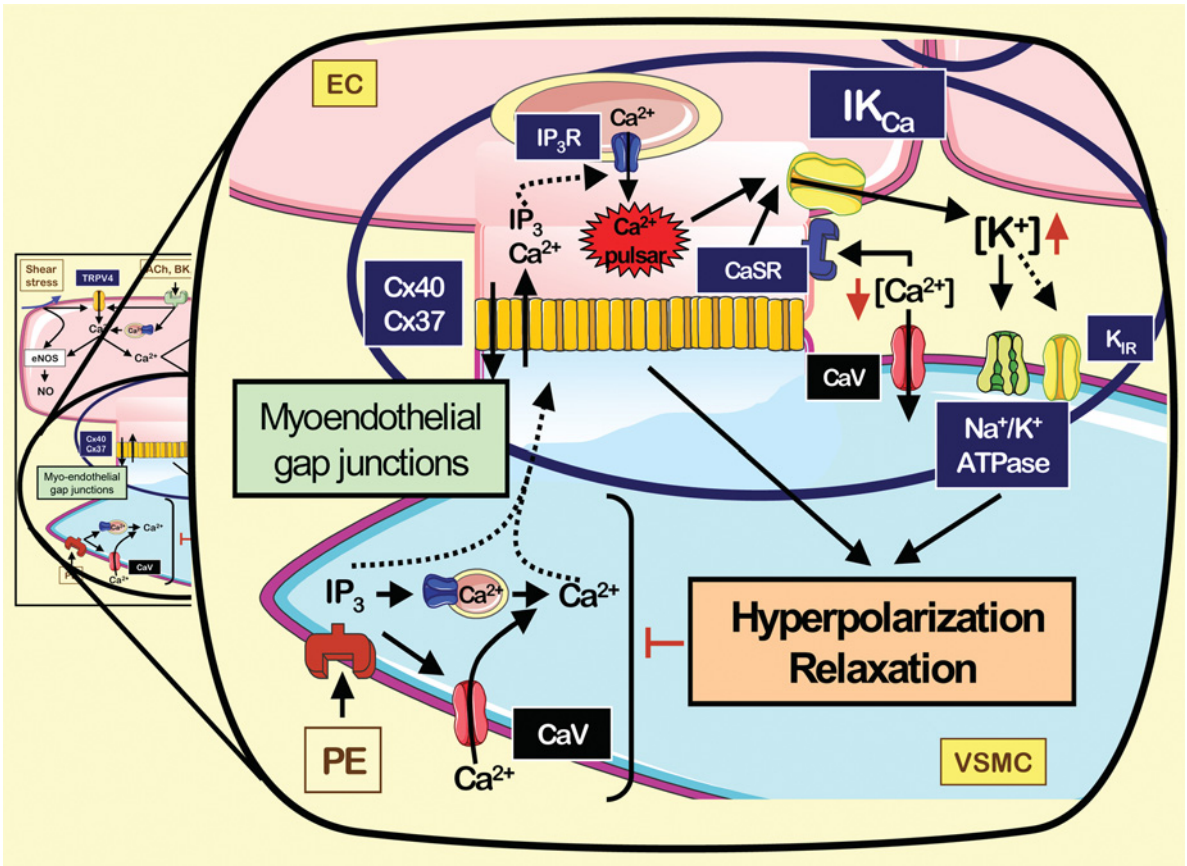

Figure 3 Myoendothelial projections

Stimulation of VSMCs by contractile agonists, for instance phenylephrine (PE), increases the intracellular Ca^{2+} concentration via the release of Ca^{2+} from internal stores, through the production of Ins(1,4,5)P_3, and via the entry of Ca^{2+} through Ca_V channels. In order to prevent excessive contractions, various negative-feedback mechanisms leading to smooth muscle hyperpolarization can operate, i.e. the increase in Ca^{2+} can activate SMC BK_{Ca} channels, Ca^{2+} and Ins(1,4,5)P_3 can diffuse to the ECs through myoendothelial gap junctions and activate IK_{Ca} channels (and possibly SK_{Ca} channels) either directly or via the generation of Ca^{2+} pulsars, depletion of Ca^{2+} in the intercellular space at the sites of myoendothelial projections, via the activation of local Ca_V channels, can be sensed by the Ca^{2+}-sensing receptor (CaSR) and, thus, enable the activation of IK1 channels. Therefore intracellular Ca^{2+} concentrations and Ins(1,4,5)P_3, as well as intercellular concentrations of Ca^{2+} and K^+ as surrogates of cellular activation, could finely regulate the membrane potential of ECs and VSMCs and, therefore, vascular tone. The diagram is based on the findings presented in [113] and [133]. IP_3R, Ins(1,4,5)P_3 receptor.

enables IK_{Ca} channel activation by acetylcholine [113,163]. Therefore endothelial projections and myoendothelial gap junctions are key structures intrinsically associated with extracellular and intracellular Ca^{2+} homoeostasis in both ECs and VSMCs [113,131,133].

However, in some blood vessels, K^+ does not evoke, or inconsistently produces, relaxations and hyperpolarizations [136,159,160,184,185], indicating that, in these blood vessels, the contribution of K^+ ions in EDHF-mediated responses must be, if anything, minimal. The involvement of gap junctions and K^+ ions are not necessarily mutually exclusive. The relative proportion of each mechanism almost certainly depends on numerous parameters, including the extracellular concentrations in K^+ and Ca^{2+} ions associated with the state of activation of the underlying VSMCs, the density of myoendothelial gap junctions and the level of the expression of the appropriate isoforms of Na^+/K^+-ATPase and/or K_{IR} channels [1,131].

Genetically modified animals

In transgenic mice with SK3 gene expression under the control of dietary doxycycline, the suppression of SK3 expression in ECs depolarizes both ECs and VSMCs, reduces the diameter of resistance vessels *in situ* and increases arterial blood pressure, a reversible phenotype upon restoration of endothelial SK3 expression [186]. Disruption of the IK1 gene reduces the hyperpolarization of ECs and SMCs in response to acetylcholine and decreases the associated vasodilation, because of a substantial reduction in EDHF-mediated responses. Moreover, the IK1 deletion also led to a significant increase in arterial blood pressure and to mild left ventricular hypertrophy [187]. In double knockout mice

lacking both SK3 and IK1, an addition of the detrimental effects provoked by the deletion of either gene is observed [188,189]. These results confirm that, in mice, endothelial SK_{Ca} and IK_{Ca} channels are fundamental determinants of endothelial hyperpolarization and EDHF signalling, and indicate that they actively control vascular tone and contribute to the overall regulation of the circulation.

Cx37 and Cx40 are the predominant gap-junction proteins in murine ECs [190]. Cx40 is involved in endothelial homocellular gap junctions and also in heterocellular gap junctions linking ECs not only to SMCs, but also to renin-producing juxtaglomerular cells. The presence of the latter gap-junction communication is required in order to maintain the Ca^{2+}-dependent inhibitory effects of AngII and that of intrarenal pressure on renin secretion and synthesis, suggesting that the endothelium is strongly involved in the regulation of the renin system. Mice deficient for Cx40 are hypertensive. However, alteration in the control of renin release only partially explained the hypertension observed in Cx40-knockout mice [191]. The arterioles of these animals also had a reduced spread of dilation in response to endothelium-dependent vasodilators and irregular arteriolar vasomotion [192–194].

These results show that the deletion of each key molecular component of EDHF-mediated responses is associated with haemodynamic alterations, suggesting that this endothelial pathway contributes to the overall regulation of arterial blood pressure.

Endothelial dysfunction and therapeutic interventions

Endothelial dysfunction, observed in various cardiovascular diseases, is associated with a decrease in NO synthesis and/or a loss of its biological activities. However, alterations in the EDHF pathway can also contribute to these endothelial dysfunctions or conversely compensate for the loss in NO bioavailability. Alterations of EDHF-mediated responses have been reported with aging and under various pathological conditions (hypertension, atherosclerosis, hypercholesterolaemia, heart failure, ischaemia/reperfusion, angioplasty, eclampsia, diabetes and sepsis) [1,2,195].

No drug is available which has been designed to target EDHF-mediated responses. Nevertheless, therapeutic interventions, with beneficial effects on the cardiovascular system, such as angiotensin-converting enzyme inhibitors, AngII type 1 receptor blockers and phosphodiesterase 3 inhibitors [1,195,196], can restore these responses, suggesting that the improvement in the EDHF pathway contributes to the observed beneficial effect. Similarly, various so-called non-pharmacological therapeutic strategies, including exercise and supplementation with oestrogens, omega-3 polyunsaturated fatty acids, polyphenol derivatives, potassium and/or calcium, help to reverse endothelial dysfunction such as blunted EDHF-mediated responses [1,195].

The improvement or restoration in EDHF responses has not yet been the direct purpose of any pharmaceutical effort. SKA-31, a preferential activator of murine IK_{Ca} channels, potentiates EDHF-mediated responses *in vitro* and lowers mean arterial blood pressure in normotensive and in AngII-hypertensive mice [139]. However, IK_{Ca} channels are required for the differentiation of VSMCs, as well as for their proliferation and migration [126,127,197,198]. Selective blockade of IK_{Ca} with TRAM-34 [199] prevents phenotypic changes of smooth muscle and coronary artery neointimal formation in two different models of post-angioplasty restenosis and the development of atherosclerosis in ApoE (apolipoprotein E)$^{-/-}$ mice [126,198,200]. IK_{Ca} channels are also involved in the proliferation of ECs [201] and various cancerous cells [202,203]. Therefore activators of IK_{Ca} channels may have some unwanted detrimental effects.

Additionally, activation of endothelial TRPCs and SK_{Ca} channels, Ca^{2+}-sensing receptors, smooth muscle K_{IR} channels and/or specific isoform(s) of Na^+/K^+-ATPase, as well as facilitating myoendothelial communication and increasing the expression of appropriate Cxs, channels and receptors, may represent new potential targets. However, the precise role of these various molecular elements is far from being completely understood. For instance, the TRPV4 channel could appear as a promising target in cardiovascular diseases as this cationic channel is involved in Ca^{2+} entry following endothelial stimulation. [204]. Indeed, the arterial responses to shear stress critically depend on the activation of this endothelial channel, and both the NO- and EDHF-mediated components of acetylcholine-induced vasodilation are attenuated in TRPV4-deficient mice [205,206]. However, GSK1016790A, a specific and potent agonist, which as expected increases endothelial intracellular Ca^{2+} concentration and produces endothelium-dependent relaxations, also causes endothelial failure, circulatory collapse and death [207]. Rotigaptide (ZP123), an anti-arrhythmic peptide that prevents uncoupling of Cx43-mediated gap-junction communication [208], has no effect on basal vascular tone and does not enhance endothelium-dependent or -independent vasodilation in the forearm arterial circulation of healthy subjects [209]. Whether or not augmenting Cx43 communication would improve endothelial function in patients with vascular disease and whether or not Cx40 would be a more appropriate target than Cx43 remain to be determined.

CONCLUSION AND PERSPECTIVES

ECs control the tone of the underlying vascular smooth muscle by releasing numerous vasoactive substances, including NO, ROS, K^+ ions and metabolites of

arachidonic acid (e.g. prostacyclin, EETs and LOX derivatives). Furthermore, the endothelial monolayer behaves as a conductive tissue propagating an electrical signal along the axis of the blood vessel by means of homocellular gap junctions and throughout the vascular wall itself by means of myoendothelial gap junctions. Endothelium-dependent relaxations, independent of the production of NO and PGI_2, probably play an important role in cardiovascular physiology in numerous animal species and in humans. They can act as a back up system when NO is inhibited or reduced, but this is not necessarily the case.

However, it is often difficult to reach a conclusion as to the true importance of endothelium-dependent hyperpolarizations because of the use of unspecific pharmacological tools and the lack of electrophysiological measurements. The mechanisms underlying endothelium responses must be carefully dissected in order to be properly identified. The synthesis of more selective compounds, such as non-peptidic inhibitors and activators of SK_{Ca} and IK_{Ca} channels, may in the future allow the selective blockade/activation of EDHF-mediated responses and, hence, the proper determination of their physiological role in the human circulation. The limited information available suggests that if better (i.e. more potent, more specific and, if possible, orally active) pharmacological tools are developed to modulate the role of the various molecular constituents underlying EDHF-mediated responses, it may be possible to determine whether or not putative cardiovascular targets identified within this pathway are drugable.

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REFERENCES

1 Félétou, M. and Vanhoutte, P. M. (2005) EDHF: the Complete Story, CRC Press, Taylor & Francis, Boca Raton, FL
2 Félétou, M. and Vanhoutte, P. M. (2006) Endothelial dysfunction: a multifaceted disorder. Am. J. Physiol. Heart Circ. Physiol. **291**, H985–H1002
3 Moncada, S. and Vane, J. R. (1979) Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2 and prostacyclin. Pharmacol. Rev. **30**, 293–331
4 Furchgott, R. F. and Zawadzki, J. V. (1980) The obligatory role of the endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature **288**, 373–376
5 Moncada, S., Palmer, R. J. M. and Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. **43**, 109–142
6 Furchgott, R. F. and Vanhoutte, P. M. (1989) Endothelium-derived relaxing and contracting factors. FASEB J. **3**, 2007–2018
7 Nelson, M. T., Huang, Y., Brayden, J. E., Hescheler, J. and Standen, N. B. (1990) Arterial dilations in response to calcitonin gene related peptide involve activation of K^+ channels. Nature **344**, 770–773
8 Bolton, T. B., Gordienko, D. V., Pucovsky, V., Parsons, S. and Povstyan, O. (2002) Calcium release events in excitation-contraction coupling in smooth muscle. Novartis Found. Symp. **246**, 154–168
9 Del Valle-Rodriguez, A., Lopez-Barneo, J. and Urena, J. (2003) Ca^{2+} channel-sarcoplasmic reticulum coupling: a mechanism of arterial myocyte contraction without Ca^{2+} influx. EMBO J. **22**, 4337–4345
10 Smyth, E. M. and FitzGerald, G. A. (2002) Human prostacyclin receptor. Vitam. Horm. **65**, 149–165
11 Félétou, M. and Vanhoutte, P. M. (2007) Endothelium-dependent hyperpolarizations: past beliefs and present facts. Ann. Med. **39**, 495–516
12 Corriu, C., Félétou, M., Edwards, G., Weston, A. H. and Vanhoutte, P. M. (2001) Differential effects of Prostacyclin and Iloprost in the isolated carotid artery of the guinea-pig. Eur. J. Pharmacol. **426**, 89–94
13 Parkington, H., C., Coleman H. A. and Tare, M. (2004) Prostacyclin and endothelium-dependent hyperpolarization. Pharmacol. Res. **49**, 509–514
14 Gluais, P., Lonchampt, M., Morrow, J. D., Vanhoutte, P. M. and Félétou, M. (2005) Acetylcholine-induced endothelium-dependent contractions in the SHR aorta: the Janus face of prostacyclin. Br. J. Pharmacol. **146**, 834–845
15 Gomez, E., Schwendemann, C., Roger, S., Simonet, S., Paysant, J., Courchay, C., Verbeuren, T. J. and Félétou, M. (2008) Aging and prostacyclin responses in aorta and platelets from WKY and SHR rats. Am. J. Physiol. Heart Circ. Physiol. **295**, H2198–H2211
16 Koller, A., Sun, D. and Kaley, G. (1993) Role of shear stress and endothelial prostaglandins in flow- and viscosity-induced dilation of arterioles *in vitro*. Circ. Res. **72**, 1276–1284
17 Duffy, S. J., Tran, B. T., New, G., Tudball, R. N., Esler, M. D., Harper, R. W. and Meredith, I. T. (1998) Continuous release of vasodilator prostanoids contributes to regulation of resting forearm blood flow in humans. Am. J. Physiol. **274**, H1174–H1183
18 Corriu, C., Félétou, M., Canet, E. and Vanhoutte, P. M. (1996) Endothelium-derived factors and hyperpolarisations of the isolated carotid artery of the guinea-pig. Br. J. Pharmacol. **119**, 959–964
19 Zygmunt, P. M., Plane, F., Paulsson, M., Garland, C. J. and Högestätt, E. D. (1998) Interactions between endothelium-derived relaxing factors in the rat hepatic artery: focus on regulation of EDHF. Br. J. Pharmacol. **124**, 992–1000
20 Chataigneau, T., Félétou, M., Huang, P. L., Fishman, M. C., Duhault, J. and Vanhoutte, P. M. (1999) Acetylcholine-induced relaxation in blood vessels from endothelial nitric oxide synthase knockout mice. Br. J. Pharmacol. **126**, 219–226
21 Sun, D., Huang, A., Smith, C. J., Stackpole, C. J., Connetta, J. A., Shesely, E. G., Koller, A. and Kaley, G. (1999) Enhanced release of prostaglandins contributes to flow-induced arteriolar dilation in eNOS knockout mice. Circ. Res. **85**, 288–293
22 Bulut, D., Liaghat, S., Hanefeld, C., Koll, R., Miebach, T. and Mügge, A. (2003) Selective cyclo-oxygenase-2 inhibition with parecoxib acutely impairs endothelium-dependent vasodilatation in patients with essential hypertension. J. Hypertens. **21**, 1663–1667
23 Szerafin, T., Erdei, N., Fülöp, T., Pasztor, E. T., Edes, I., Koller, A. and Bagi, Z. (2006) Increased cyclooxygenase-2 expression and prostaglandin-mediated dilation in coronary arterioles of patients with diabetes mellitus. Circ. Res. **99**, e12–e17
24 Andersohn, F., Suissa, S. and Garbe, E. (2006) Use of first- and second-generation cyclooxygenase-2-selective nonsteroidal antiinflammatory drugs and risk of acute myocardial infarction. Circulation **113**, 1950–1957
25 Vanhoutte, P. M. and Tang, E. H. (2008) Endothelium-dependent contractions: when a good guy turns bad! J. Physiol. **586**, 5295–5304

26 Fleming, I. (2004) Cytochrome P450 epoxygenases as EDHF synthase(s). Pharmacol. Res. **49**, 525–533
27 Chataigneau, T., Félétou, M., Duhault, J. and Vanhoutte, P. M. (1998) Epoxyeicosatrienoic acids, potassium channel blockers and endothelium-dependent hyperpolarisation in the guinea-pig carotid artery. Br. J. Pharmacol. **123**, 574–580
28 Miyata, N. and Roman, R. J. (2005) Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in vascular system. J. Smooth Muscle Res. **41**, 175–193
29 Fleming, I. (2008) Vascular cytochrome p450 enzymes: physiology and pathophysiology. Trends Cardiovasc. Med. **18**, 20–25
30 Hercule, H. C., Schunck, W. H., Gross, V., Seringer, J., Leung, F. P., Weldon, S. M., da Costa Goncalves, A. C., Huang, Y., Luft, F. C. and Gollasch, M. (2009) Interaction between P450 eicosanoids and nitric oxide in the control of arterial tone in mice. Arterioscler. Thromb. Vasc. Biol. **29**, 54–60
31 Campbell, W. B., Gebremedhin, D., Pratt, P. F. and Harder, D. R. (1996) Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factor. Circ. Res. **78**, 415–423
32 Fisslthaler, B., Popp, R., Kiss, L., Potente, M., Harder, D. R., Fleming, I. and Busse, R. (1999) Cytochrome P4540 2C is an EDHF synthase in coronary arteries. Nature **401**, 493–497
33 Gauthier, K. M., Edwards, E. M., Falck, J. R., Reddy, D. S. and Campbell, W. B. (2005) 14,15-Epoxyeicosatrienoic acid represents a transferable endothelium-dependent relaxing factor in bovine coronary arteries. Hypertension **45**, 666–671
34 Huang, A., Sun, D., Jacobson, A., Carroll, M. A., Kalck, J. R. and Kaley, G. (2005) Epoxyeicosatrienoic acids are released to mediate shear stress-dependent hyperpolarization of arteriolar smooth muscle. Circ. Res. **96**, 376–383
35 Weston, A. H., Félétou, M., Vanhoutte, P. M., Falck, J. R., Campbell, W. B. and Edwards, G. (2005) Endothelium-dependent hyperpolarizations induced by bradykinin in the vasculature; clarification of the role of epoxyeicosatrienoic acids. Br. J. Pharmacol. **145**, 775–784
36 Earley, S., Heppner, T. J., Nelson, M. T. and Brayden, J. E. (2005) TRPV4 forms a novel Ca^{2+}-signaling complex with ryanodine receptors and BK_{Ca} channels. Circ. Res. **97**, 1270–1279
37 Miura, H. and Gutterman, D. D. (1998) Human coronary arteriolar dilation to arachidonic acid depends on cytochrome P450 monooxygenase and Ca^{2+}-activated K^+ channels. Circ. Res. **83**, 501–507
38 Archer, S. L., Gragasin, F. S., Wu, X., Wang, S., McMurthry, S., Kim, D. H., Platonov, M., Koshal, A., Hashimoto, K., Campbell, W. B. et al. (2003) Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxieicosatrienoic acid and causes relaxation by activating BK_{Ca} channels. Circulation **107**, 769–776
39 Coats, P., Johnston, F., MacDonald, J., McMurray, J. J. and Hillier, C. (2001) Endothelium-derived hyperpolarizing factor: identification and mechanisms of action in human subcutaneous resistance arteries. Circulation **103**, 1702–1708
40 Bellien, J., Thuillez, C. and Joannides, R. (2008) Contribution of endothelium-derived hyperpolarizing factors to the regulation of vascular tone in humans. Fundam. Clin. Pharmacol. **22**, 363–377
41 Imig, J. D. (2005) Epoxide hydrolase and epoxygenase metabolites as therapeutic targets for renal diseases. Am. J. Physiol. Renal Physiol. **289**, F496–F503
42 Larsen, B. T., Campbell, W. B. and Gutterman, D. D. (2007) Beyond vasodilatation: non-vasomotor roles of epoxyeicosatrienoic acids in the cardiovascular system. Trends Pharmacol. Sci. **28**, 32–38
43 Gauthier, K. M., Yang, W., Gross, G. J. and Campbell, W. B. (2007) Roles of epoxyeicosatrienoic acids in vascular regulation and cardiac preconditioning. J. Cardiovasc. Pharmacol. **50**, 601–608
44 Luria, A., Weldon, S. M., Kabcenell, A. K., Ingraham, R. H., Matera, D., Jiang, H., Gill, R., Morisseau, C., Newman, J. W. and Hammock, B. D. (2007) Compensatory mechanism for homeostatic blood pressure regulation in Ephx2 gene-disrupted mice. J. Biol. Chem. **282**, 2891–2898
45 Motoki, A., Merkel, M. J., Packwood, W. H., Cao, Z., Liu, L., Iliff, J. J., Alkayed, N. J. and Van Winkle, D. M. (2008) Soluble epoxide hydrolase inhibition and gene deletion are protective against myocardial ischemia-reperfusion injury *in vivo*. Am. J. Physiol. Heart Circ. Physiol. **295**, H2128–H2134
46 Monti, J., Fischer, J., Paskas, S., Heinig, M., Schulz, H., Gösele, C., Heuser, A., Fischer, R., Schmidt, C., Schirdewan, A. et al. (2008) Soluble epoxide hydrolase is a susceptibility factor for heart failure in a rat model of human disease. Nat. Genet. **40**, 529–537
47 Zhang, W., Otsuka, T., Sugo, N., Ardeshiri, A., Alhadid, Y. K., Iliff, J. J., DeBarber, A. E., Koop, D. R. and Alkayed, N. J. (2008) Soluble epoxide hydrolase gene deletion is protective against experimental cerebral ischemia. Stroke **39**, 2073–2078
48 Ai, D., Pang, W., Li, N., Xu, M., Jones, P. D., Yang, J., Zhang, Y., Chiamvimonvat, N., Shyy, J. Y., Hammock, B. D. and Zhu, Y. (2009) Soluble epoxide hydrolase plays an essential role in angiotensin II-induced cardiac hypertrophy. Proc. Natl. Acad. Sci. U.S.A. **106**, 564–569
49 Gschwendtner, A., Ripke, S., Freilinger, T., Lichtner, P., Müller-Myhsok, B., Wichmann, H. E., Meitinger, T. and Dichgans, M. (2008) Genetic variation in soluble epoxide hydrolase (EPHX2) is associated with an increased risk of ischemic stroke in white Europeans. Stroke **39**, 1593–1596
50 Ohtoshi, K., Kaneto, H., Node, K., Nakamura, Y., Shiraiwa, T., Matsuhisa, M. and Yamasaki, Y. (2005) Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients. Biochem. Biophys. Res. Commun. **331**, 347–350
51 Imig, J. D. (2008) Eicosanoids and renal damage in cardiometabolic syndrome. Expert Opin. Drug Metab. Toxicol. **4**, 165–174
52 Zink, M. H., Oltman, C. L., Lu, T., Katakam, P. V., Kaduce, T. L., Lee, H., Dellsperger, K. C., Spector, A. A., Myers, P. R. and Weintraub, N. L. (2001) 12-Lipoxygenase in porcine coronary circulation: implications for coronary vasoregulation. Am. J. Physiol. Heart Circ. Physiol. **280**, H693–H704
53 Tang, X., Holmes, B. B., Nithipatikom, K., Hillard, C. J., Kuhn, H. and Campbell, W. B. (2006) Reticulocyte 15-lipoxygenase-I is important in acetylcholine-induced endothelium-dependent vasorelaxation in rabbit aorta. Arterioscler. Thromb. Vasc. Biol. **26**, 78–84
54 Aggarwal, N. T., Gauthier, K. M. and Campbell, W. B. (2008) 15-Lipoxygenase metabolites contribute to age-related reduction in acetylcholine-induced hypotension in rabbits. Am. J. Physiol. Heart Circ. Physiol. **295**, H89–H96
55 Tang, X., Aggarwal, N., Holmes, B. B., Kuhn, H. and Campbell, W. B. (2008) Age-related decrease in 15-lipoxygenase contributes to reduced vasorelaxation in rabbit aorta. Am. J. Physiol. Heart Circ. Physiol. **294**, H679–H687
56 Aggarwal, N. T., Pfister, S. L. and Campbell, W. B. (2008) Hypercholesterolemia enhances 15-lipoxygenase-mediated vasorelaxation and acetylcholine-induced hypotension. Arterioscler. Thromb. Vasc. Biol. **28**, 2209–2215
57 Aggarwal, N. T., Pfister, S. L., Gauthier, K. M., Chawengsub, Y., Baker, J. E. and Campbell, W. B. (2009) Chronic hypoxia enhances 15-lipoxygenase mediated vasorelaxation in rabbit arteries. Am. J. Physiol. Heart Circ. Physiol. **296**, H678–H688
58 Quignard, J. F., Chataigneau, T., Corriu, C., Edwards, G., Weston, A. H., Félétou, M. and Vanhoutte, P. M. (2002) Endothelium-dependent hyperpolarization and lipoxygenase derived metabolites of arachidonic acid in the carotid artery of the guinea-pig. J. Cardiovasc. Pharmacol. **40**, 467–477

59 Randall, M. D., Kendall, D. A. and O'Sullivan, S. (2004) The complexities of the cardiovascular actions of cannbinoids. Br. J. Pharmacol. **142**, 20–26
60 Chataigneau, T., Félétou, M., Thollon, C., Villeneuve, N., Vilaine, J-P., Duhault, J. and Vanhoutte, P. M. (1998) Cannabinoid CB_1 receptor and endothelium-dependent hyperpolarisation in guinea-pig carotid, rat mesenteric and porcine coronary arteries. Br. J. Pharmacol. **123**, 968–974
61 Vásquez-Vivar, J., Kalyanaraman, B. and Martásek, P. (2003) The role of tetrahydrobiopterin in superoxide generation from eNOS: enzymology and physiological implications. Free Radical Res. **37**, 121–127
62 Capettini, L. S., Cortes, S. F., Gomes, M. A., Silva, G. A., Pesquero, J. L., Lopes, M. J., Teixeira, M. M. and Lemos, V. S. (2008) Neuronal nitric oxide synthase-derived hydrogen peroxide is a major endothelium-dependent relaxing factor. Am. J. Physiol. Heart Circ. Physiol. **295**, H2503–H2511
63 Drouin, A., Thorin-Trescases, N., Hamel, E., Falck, J. R. and Thorin, E. (2007) Endothelial nitric oxide synthase activation leads to dilatory H_2O_2 production in mouse cerebral arteries. Cardiovasc. Res. **73**, 73–81
64 Nardi, A. and Olesen, S. P. (2008) BK channel modulators: a comprehensive overview. Curr. Med. Chem. **15**, 1126–1146
65 Cohen, R. A., Plane, F., Najibi, S., Huk, I., Malinski, T. and Garland, C. J. (1997) Nitric oxide is the mediator of both endothelium-dependent relaxation and hyperpolarisation of the rabbit carotid artery. Proc. Natl. Acad. Sci. U.S.A. **94**, 4193–4198
66 Chauhan, S., Rahman, A., Nilsson, H., Clapp, L., MacAllister, R. and Ahluwalia, A. (2003) NO contributes to EDHF-like responses in rat small arteries: role of NO stores. Cardiovasc. Res. **57**, 207–216
67 Muller, B., Kleschyov, A. L., Alencar, J. L., Vanin, A. and Stoclet, J. C. (2002) Nitric oxide transport and storage in the cardiovascular system. Ann. N.Y. Acad. Sci. **962**, 131–139
68 Batenburg, W. W., De Vries, R., Saxena, P. R. and Danser, A. H. (2004) L-S nitrosothiols: endothelium-derived hyperpolarizing factors in porcine coronary arteries. J. Hypertens. **22**, 1927–1936
69 Félétou, M. and Vanhoutte, P. M. (2006) EDHF: where are we now? Arterioscler. Thromb. Vasc. Biol. **26**, 1215–1225
70 Brandes, R. P., Schmitz-Winnenthal, F-H., Félétou, M., Gödecke, A., Huang, P-L., Vanhoutte, P. M., Fleming, I. and Busse, R. (2000) An endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild type and endothelial NO synthase knock-out mice. Proc. Natl. Acad. Sci. U.S.A. **97**, 9747–9752
71 Wu, Y., Huang, A., Sun, D., Falck, J. R., Koller, A. and Kaley, G. (2001) Gender-specific compensation for the lack of NO in the mediation of flow-induced arteriolar dilation. Am. J. Physiol. Heart Circ. Physiol. **280**, H2456–H2461
72 Scotland, R. S., Madhani, M., Chauhan, S., Moncada, S., Andresen, J., Nilsson, H., Hobbs, A. J. and Ahluwalia, A. (2005) Investigation of vascular responses in endothelial nitric oxide synthase/cyclooxygenase-1 double knock-out mice. Key role for endothelium-derived hyperpolarizing factor in the regulation of blood pressure *in vivo*. Circulation **111**, 796–803
73 Takaki, A., Morikawa, K., Tsutsui, M., Murayama, Y., Tekes, E., Yamagishi, H., Ohashi, J., Yada, T., Yanagihara, N. and Shimokawa, H. (2008) Crucial role of nitric oxide synthases system in endothelium-dependent hyperpolarization in mice. J. Exp. Med. **205**, 2053–2063
74 Ellis, A. and Triggle, C. R. (2003) Endothelium-derived reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and vascular tone. Can. J. Physiol. Pharmacol. **81**, 1013–1028
75 Lucchesi, P. A., Belmadani, S. and Matrougui, K. (2005) Hydrogen peroxide acts as both vasodilator and vasoconstrictor in the control of perfused mesenteric resistance arteries. J. Hypertens. **23**, 571–579
76 Matoba, Y., Shimokawa, H., Nakashima, M., Hirakawa, Y., Mukai, Y., Hirano, K., Kanaide, H. and Takeshita, A. (2000) Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J. Clin. Invest. **106**, 1521–1530
77 Morikawa, K., Shimokawa, H., Matoba, T., Kubota, H., Akaike, T., Talukder, M. A., Hatanaka, M., Fujiki, T., Maeda, H., Takahashi, S. and Takeshita, A. (2003) Pivotal role of Cu,Zn-superoxide dismutase in endothelium-dependent hyperpolarization. J. Clin. Invest. **112**, 1871–1879
78 Shimokawa, H. and Matoba, T. (2004) Hydrogen peroxide as an endothelium-derived hyperpolarizing factor. Pharmacol. Res. **49**, 543–549
79 Matoba, Y., Shimokawa, H., Kubota, H., Morikawa, K., Fujiki, T., Kunihiro, I., Mukai, Y., Hirakawa, Y. and Takeshita, A. (2002) Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric artery. Biochem. Biophys. Res. Commun. **290**, 909–913
80 Miura, H., Bosnjak, J. J., Ning, G., Salto, T., Miura, M. and Gutterman, D. D. (2003) Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. Circ. Res. **92**, e31–e40
81 Edwards, D. H., Li, Y. and Griffith, T. M. (2008) Hydrogen peroxide potentiates the EDHF phenomenon by promoting endothelial Ca^{2+} mobilization. Arterioscler. Thromb. Vasc. Biol. **28**, 1774–1781
82 Cosentino, F. and Katusic, Z. S. (1995) Tetrahydrobiopterin and dysfunction of endothelial nitric oxide synthase in coronary arteries. Circulation **91**, 139–144
83 Cosentino, F., Barker, J. E., Brand, M. P., Heales, S. J., Werner, E. R., Tippins, J. R., West, N., Channon, K. M., Volpe, M. and Lüscher, T. F. (2001) Reactive oxygen species mediate endothelium-dependent relaxations in tetrahydrobiopterin-deficient mice. Arterioscler. Thromb. Vasc. Biol. **21**, 496–502
84 Phillips, S. A., Hatoum, O. A. and Gutterman, D. D. (2007) The mechanism of flow-induced dilation in human adipose arterioles involves hydrogen peroxide during CAD. Am. J. Physiol. Heart Circ. Physiol. **92**, H93–H100
85 Takaki, A., Morikawa, K., Murayama, Y., Yamagishi, H., Hosoya, M., Ohashi, J. and Shimokawa, H. (2008) Roles of endothelial oxidases in endothelium-derived hyperpolarizing factor responses in mice. J. Cardiovasc. Pharmacol. **52**, 510–517
86 Liu, Y., Zhao, H., Li, H., Kalyanaraman, B., Nicolosi, A. C. and Gutterman, D. D. (2003) Mitochondrial sources of H_2O_2 generation play a key role in flow-mediated dilation in human coronary resistance arteries. Circ. Res. **93**, 573–580
87 Larsen, B. T., Bubolz, A. H., Mendoza, S. A., Pritchard, Jr, K. A. and Gutterman, D. D. (2009) Bradykinin-induced dilation of human coronary arterioles requires NADPH oxidase-derived reactive oxygen species. Arterioscler. Thromb. Vasc. Biol. **29**, 739–745
88 Gluais, P., Edwards, G., Weston, A. H., Vanhoutte, P. M. and Félétou, M. (2005) Hydrogen peroxide and the endothelium-dependent hyperpolarization of the guinea-pig isolated carotid artery. Eur. J. Pharmacol. **513**, 219–224
89 Wang, R. (2002) Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? FASEB J. **16**, 1792–1798
90 Durante, W. (2002) Carbon monoxide and bile pigments: surprising mediators of vascular function. Vasc. Med. **7**, 195–202
91 Wu, L. and Wang, R. (2005) Carbon monoxide: endogenous production, physiological functions, and pharmacological applications. Pharmacol. Rev. **57**, 585–630
92 Durante, W., Johnson, F. K. and Johnson, R. A. (2006) Role of carbon monoxide in cardiovascular function. J. Cell. Mol. Med. **10**, 672–686
93 Chen, Y. H., Yet, S. F. and Perrella, M. A. (2003) Role of heme oxygenase-1 in the regulation of blood pressure and cardiac function. Exp. Biol. Med. **228**, 447–453

94 Wang, R., Shamloul, R., Wang, X., Meng, Q. and Wu, L. (2006) Sustained normalization of high blood pressure in spontaneously hypertensive rats by implanted hemin pump. Hypertension **48**, 685–692

95 Doré, S., Sampei, K., Goto, S., Alkayed, N. J., Guastella, D., Blackshaw, S., Gallagher, M., Traystman, R. J., Hurn, P. D., Koehler, R. C. and Snyder, S. H. (1999) Heme oxygenase-2 is neuroprotective in cerebral ischemia. Mol. Med. **5**, 656–663

96 Motterlini, R. (2007) Carbon monoxide-releasing molecules (CO-RMs): vasodilatory, anti-ischaemic and anti-inflammatory activities. Biochem. Soc. Trans. **35**, 1142–1146

97 Zakhary, R., Gaine, S. P., Dinerman, J. L., Ruat, M., Flavahan, N. A. and Snyder, S. H. (1996) Heme oxygenase 2: endothelial and neuronal localization and role in endothelium-dependent relaxation. Proc. Natl. Acad. Sci. U.S.A. **93**, 795–798

98 Baragatti, B., Brizzi, F., Barogi, S., Laubach, V. E., Sodini, D., Shesely, E. G., Regan, R. F. and Coceani, F. (2007) Interactions between NO, CO and an endothelium-derived hyperpolarizing factor (EDHF) in maintaining patency of the ductus arteriosus in the mouse. Br. J. Pharmacol. **151**, 54–62

99 Szabó, C. (2007) Hydrogen sulphide and its therapeutic potential. Nat. Rev. Drug Discovery **6**, 917–935

100 Yang, G., Wu, L., Jiang, B., Yang, W., Qi, J., Cao, K., Meng, Q., Mustafa, A. K., Mu, W., Zhang, S. et al. (2008) H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine γ-lyase. Science **322**, 587–590

101 Zhao, W., Zhang, J., Lu, Y. and Wang, R. (2001) The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. EMBO J. **20**, 6008–6016

102 Powers, R. W., Gandley, R. E., Lykins, D. L. and Roberts, J. M. (2004) Moderate hyperhomocysteinemia decreases endothelial-dependent vasorelaxation in pregnant but not nonpregnant mice. Hypertension **44**, 327–333

103 De Vriese, A. S., Blom, H. J., Heil, S. G., Mortier, S., Kluijtmans, L. A., Van de Voorde, J. and Lameire, N. H. (2004) Endothelium-derived hyperpolarizing factor-mediated renal vasodilatory response is impaired during acute and chronic hyperhomocysteinemia. Circulation **109**, 2331–2336

104 Wang, Y., Zhao, X., Jin, H., Wei, H., Li, W., Bu, D., Tang, X., Ren, Y., Tang, C. and Du, J. (2009) Role of hydrogen sulfide in the development of atherosclerotic lesions in apolipoprotein E knockout mice. Arterioscler. Thromb. Vasc. Biol. **29**, 173–179

105 Brancaleone, V., Roviezzo, F., Vellecco, V., De Gruttola, L., Bucci, M. and Cirino, G. (2008) Biosynthesis of H_2S is impaired in non-obese diabetic (NOD) mice. Br. J. Pharmacol. **155**, 673–680

106 Suga, S., Itoh, H., Komatsu, Y., Ogawa, Y., Hama, N., Yoshimasa, T. and Nakao, K. (1993) Cytokine-induced c-type natriuretic peptide (CNP) secretion from vascular endothelial cells – evidence for CNP as a novel autocrine/paracrine regulator from endothelial cells. Endocrinology **133**, 3038–3041

107 Wei, C. M., Hu, S., Miller, V. M. and Burnett, Jr, J. C. (1994) Vascular actions of C-type natriuretic peptide in isolated porcine coronary arteries and coronary vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **205**, 765–771

108 Honing, M. L., Smits, P., Morrison, P. J., Burnett, Jr, J. C. and Rabelink, T. J. (2001) C-type natriuretic peptide-induced vasodilation is dependent on hyperpolarization in human forearm resistance vessels. Hypertension **37**, 1179–1183

109 Chauhan, S. D., Nilsson, H., Ahluwalia, A. and Hobbs, A. J. (2003) 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

110 Villar, I. C., Panayiotou, C. M., Sheraz, A., Madhani, M., Scotland, R. S., Nobles, M., Kemp-Harper, B., Ahluwalia, A. and Hobbs, A. J. (2007) Definitive role for natriuretic peptide receptor-C in mediating the vasorelaxant activity of C-type natriuretic peptide and endothelium-derived hyperpolarising factor. Cardiovasc. Res. **74**, 515–525

111 Barton, M., Beny, J. L., D'uscio, L. V., Wyss, T., Noll, G. and Luscher, T. F. (1998) Endothelium-independent relaxation and hyperpolarization to C-type natriuretic peptide in porcine coronary arteries. J. Cardiovasc. Pharmacol. **31**, 377–383

112 Leuranguer, V., Vanhoutte, P. M., Verbeuren, T. and Félétou, M. (2008) C-type natriuretic peptide and endothelium-dependent hyperpolarization in the guinea-pig carotid artery. Br. J. Pharmacol. **153**, 57–65

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

114 McGuire, J. J., Hollenberg, M. D., Bennett, B. M. and Triggle, C. R. (2004) Hyperpolarization of murine small caliber mesenteric arteries by activation of endothelial proteinase-activated receptor 2. Can. J. Physiol. Pharmacol. **82**, 1103–1112

115 Ahluwalia, A. and Hobbs, A. J. (2005) Endothelium-derived C-type natriuretic peptide: more than just a hyperpolarizing factor. Trends Pharmacol. Sci. **26**, 162–167

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

117 Marchenko, S. M. and Sage, S. O. (1996) Calcium-activated potassium channels in the endothelium of intact rat aorta. J. Physiol. **492**, 53–60

118 Bychkov, R., Burnham, M. P., Richards, G. R., Edwards, G., Weston, A. H., Félétou, M. and Vanhoutte, P. M. (2002) Characterization of a charybdotoxin-sensitive intermediate conductance Ca^{2+}-activated K^+ channel in porcine coronary endothelium: relevance to EDHF. Br. J. Pharmacol. **137**, 1346–1354

119 Gauthier, K. M., Liu, C., Popovic, A., Albarwani, S. and Rusch, N. J. (2002) Freshly isolated bovine coronary endothelial cells do not express the BK Ca channel gene. J. Physiol. **545**, 829–836

120 Ledoux, J., Werner, M. E., Brayden, J. E. and Nelson, M. T. (2006) Calcium-activated potassium channels and the regulation of vascular tone. Physiology **21**, 69–78

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

122 Kwan, H. Y., Shen, B., Ma, X., Kwok, Y. C., Huang, Y., Man, Y. B., Yu, S. and Yao, X. (2009) TRPC1 associates with BK_{Ca} channel to form a signal complex in vascular smooth muscle cells. Circ. Res. **104**, 670–678

123 Gauthier, K. M., Spitzbarth, N., Edwards, E. M. and Campbell, W. B. (2004) Apamin-sensitive K^+ currents mediate arachidonic acid-induced relaxations of rabbit aorta. Hypertension **43**, 413–419

124 Gebremedhin, D., Kaldunski, M., Jacobs, E. R., Harder, D. R. and Roman, R. J. (1996) Coexistence of two types of calcium activated potassium channels in rat renal arterioles. Am. J. Physiol. **270**, F69–F81

125 Quignard, J. F., Félétou, M., Edwards, G., Duhault, J., Weston, A. H. and Vanhoutte, P. M. (2000) Role of endothelial cell hyperpolarization in EDHF-mediated responses in the guinea-pig carotid artery. Br. J. Pharmacol. **129**, 1103–1112

126 Kohler, R., Wulff, H., Eichler, I., Kneifel, M., Neumann, D., Knorr, A., Grgic, I., Kampfe, D., Si, H., Wibawa, J. et al. (2003) Blockade of the intermediate-conductance calcium-activated potassium channel as a new therapeutic strategy for restenosis. Circulation **108**, 1119–1125

127 Tharp, D. L., Wamhoff, B. R., Turk, J. R. and Bowles, D. K. (2006) Upregulation of intermediate-conductance Ca^{2+}-activated K^+ channel (IKCa1) mediates phenotypic modulation of coronary smooth muscle. Am. J. Physiol. Heart Circ. Physiol. **291**, H2493–H2503

128 Burnham, M. P., Bychkov, R., Félétou, M., Richards, G. R., Vanhoutte, P. M., Weston, A. H. and Edwards, G. (2002) Characterization of an apamin-sensitive small-conductance Ca^{2+}-activated K^+ channel in porcine coronary artery endothelium: relevance to EDHF. Br. J. Pharmacol. **135**, 1133–1143

129 Kohler, R., Brakemeier, S., Kuhn, M., Behrens, C., Real, R., Degenhardt, C., Orzechowski, H. D., Pries, A. R., Paul, M. and Hoyer, J. (2001) Impaired hyperpolarization in regenerated endothelium after balloon catheter injury. Circ. Res. **89**, 174–179

130 Sandow, S. L., Neylon, C. B., Chen, M. X. and Garland, C. J. (2006) 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

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

132 Absi, M., Burnham, M. P., Weston, A. H., Harno, E., Rogers, M. and Edwards, G. (2007) Effects of methyl β-cyclodextrin on EDHF responses in pig and rat arteries; association between SK_{Ca} channels and caveolin-rich domains. Br. J. Pharmacol. **151**, 332–340

133 Ledoux, J., Taylor, M. S., Bonev, A. D., Hannah, R. M., Solodushko, V., Shui, B., Tallini, Y., Kotlikoff, M. I. and Nelson, M. T. (2008) 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

134 Syme, C. A., Gerlach, A. C., Singh, A. K. and Devor, D. C. (2000) Pharmacological activation of cloned intermediate and small conductance Ca^{2+}-activated K^+ channels. Am. J. Physiol. Cell Physiol. **278**, C570–C581

135 Edwards, G., Gardener, M. J., Félétou, M., Brady, G., Vanhoutte, P. M. and Weston, A. H. (1999) Further investigation of endothelium-derived hyperpolarizing factor (EDHF) in rat hepatic artery: studies using 1-EBIO and ouabain. Br. J. Pharmacol. **128**, 1064–1070

136 Coleman, H. A., Tare, M. and Parkington, H. C. (2001) EDHF is not K^+ but may be due to spread of current from the endothelium in guinea-pig arterioles. Am. J. Physiol. Heart Circ. Physiol. **280**, H2478–H2483

137 Sokoya, E. M., You, J. and Chen, J. (2007) DCEBIO-mediated dilations are attenuated in the female rat middle cerebral artery. J. Vasc. Res. **44**, 169–174

138 Leuranguer, V., Gluais, P., Vanhoutte, P. M., Verbeuren, T. J. and Félétou, M. (2008) Openers of calcium-activated potassium channels and endothelium-dependent hyperpolarizations in the guinea pig carotid artery. Naunyn Schmiedebergs Arch. Pharmacol. **377**, 101–109

139 Sankaranarayanan, A., Raman, G., Busch, C., Schultz, T., Zimin, P. I., Hoyer, J., Kohler, R. and Wulff, H. (2009) SKA-31, a new activator of KCa2 and KCa3.1 potassium channels, potentiates the EDHF response and lowers blood pressure. Mol. Pharmacol. **75**, 281–295

140 Busse, R., Fichtner, H., Luckhoff, A. and Kohlhardt, M. (1988) Hyperpolarization and increased free calcium in acetylcholine-stimulated endothelial cells. Am. J. Physiol. **255**, H965–H969

141 Luckhoff, A. and Busse, R. (1990) Activators of potassium channels enhance calcium influx into endothelial cells as a consequence of potassium currents. Naunyn Schmiedebergs Arch. Pharmacol. **342**, 94–99

142 Stankevicius, E., Lopez-Valverde, V., Rivera, L., Hughes, A. D., Mulvany, M. J. and Simonsen, U. (2006) 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

143 Sheng, J. Z. and Braun, A. P. (2007) Small- and intermediate-conductance Ca^{2+}-activated K^+ channels directly control agonist-evoked nitric oxide synthesis in human vascular endothelial cells. Am. J. Physiol. Cell Physiol. **293**, C458–C467

144 Griffith, T. M. (2004) Endothelium-dependent smooth muscle hyperpolarization: do gap junctions provide a unifying hypothesis? Br. J. Pharmacol. **141**, 881–903

145 Lang, N. N., Luksha, L., Newby, D. and Kublickiene, K. (2006) Connexin 43 mediates endothelium-derived hyperpolarising factor induced vasodilatation in subcutaneous resistance arteries from healthy pregnant women. Am. J. Physiol. Heart Circ. Physiol. **292**, H1026–H1032

146 Isakson, B. E., Best, A. K. and Duling, B. R. (2008) Incidence of protein on actin bridges between endothelium and smooth muscle in arterioles demonstrates heterogeneous connexin expression and phosphorylation. Am. J. Physiol. Heart Circ. Physiol. **294**, H2898–H2904

147 Sandow, S. L. and Hill, C. E. (2000) 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

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

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

150 Dora, K. A., Doyle, M. P. and Duling, B. R. (1997) Elevation of intracellular calcium in smooth muscle causes endothelial cell generation of NO in arterioles. Proc. Natl. Acad. Sci. U.S.A. **94**, 6529–6534

151 Yashiro, Y. and Duling, B. R. (2000) Integrated Ca^{2+} signaling between smooth muscle and endothelium of resistance vesels. Circ. Res. **87**, 1048–1054

152 Dora, K. A. (2001) Intercellular Ca^{2+} signalling: the artery wall. Semin. Cell. Dev. Biol. **12**, 27–35

153 Beny, J.-L. and Pacicca, C. (1994) Bidirectional electrical communication between smooth muscle and endothelial cells in the pig coronary artery. Am. J. Physiol. **266**, H1465–H1472

154 Marchenko, S. M. and Sage, S. O. (1994) Smooth muscle cells affect endothelial membrane potential in rat aorta. Am. J. Physiol. **267**, H804–H811

155 Emerson, G. G. and Segal, S. S. (2000) Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: role in vasomotor control. Circ. Res. **87**, 474–479

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

157 De Wit, C., Roos, F., Bolz, S. S., Kirchhoff, S., Kruge, O., Willecke, K. and Pohl, U. (2000) Impaired conduction of vasodilatation along arterioles in connexin 40-deficient mice. Circ. Res. **86**, 649–655

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

159 Edwards, G., Félétou, M., Gardener, M. J., Thollon, C., Vanhoutte, P. M. and Weston, A. H. (1999) Role of gap junctions in the responses to EDHF in rat and guinea-pig small arteries. Br. J. Pharmacol. **128**, 1788–1794

160 Edwards G., Thollon C., Gardener M.J., Félétou M., Vilaine, J-P., Vanhoutte, P. M. and Weston, A. H. (2000) Role of gap junctions and EETs in endothelium-dependent hyperpolarization of porcine coronary artery. Br. J. Pharmacol. **129**, 1145–1162

161 Mather, S., Dora, K. A., Sandow, S. L., Winter, P. and Garland, C. J. (2005) 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

162 Figueroa, X. F. and Duling, B. R. (2008) Dissection of two Cx37-independent conducted vasodilator mechanisms by deletion of Cx40: electrotonic versus regenerative conduction. Am. J. Physiol. Heart Circ. Physiol. **295**, H2001–H2007

163 Gluais, P., Edwards, G., Weston, A. H., Falck, J. R., Vanhoutte, P. M. and Félétou, M. (2005) SK_{Ca} and IK_{Ca} in the endothelium-dependent hyperpolarization of the guinea-pig isolated carotid artery. Br. J. Pharmacol. **144**, 477–485

164 De Wit, C., Boettcher, M. and Schmidt, V. J. (2008) Signaling across myoendothelial gap junctions–fact or fiction? Cell. Commun. Adhes. **15**, 231–245

165 De Vriese, A. S., Van de Voorde, J. and Lameire, N. H. (2002) Effects of connexin-mimetic peptides on nitric oxide synthase- and cyclooxygenase-independent renal vasodilatation. Kidney Int. **61**, 177–185

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

167 Hendrickx, H. and Casteels, R. (1974) Electrogenic sodium pump in arterial smooth muscle cells. Pflugers Arch. **346**, 299–306

168 Nelson, M. T. and Quayle, J. M. (1995) Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. **268**, C799–C822

169 Prior, H. M., Webster, N., Quinn, K., Beech, D. J. and Yates, M. S. (1998) K^+-induced dilation of a small renal artery: no role for inward rectifier K^+ channels. Cardiovasc. Res. **37**, 780–790

170 Edwards, G., Gardener, M. J., Félétou, M., Brady, G., Vanhoutte, P. M. and Weston, A. H. (1999) Further investigation of endothelium-derived hyperpolarizing factor (EDHF) in rat hepatic artery: studies using 1-EBIO and ouabain. Br. J. Pharmacol. **128**, 1064–1070

171 Dora, K. A. and Garland, C. J. (2001) Properties of smooth muscle hyperpolarization and relaxation to K^+ in the rat isolated mesenteric artery. Am. J. Physiol. Heart Circ. Physiol. **280**, H2424–H2429

172 McGuire, J. J., Hollenberg, M. D., Andrade-Gordon, P. and Triggle, C. R. (2002) Multiple mechanisms of vascular smooth muscle relaxation by the activation of proteinase-activated receptor 2 in mouse mesenteric arterioles. Br. J. Pharmacol. **135**, 155–169

173 Beny, J.-L. and Schaad, O. (2000) An evaluation of potassium ions as endothelium-derived hyperpolarizing factor in porcine coronary arteries. Br. J. Pharmacol. **131**, 965–973

174 Nelli, S., Wilson, W. S., Laidlaw, H., Llano, A., Middleton, S., Price, A. G. and Martin, W. (2003) Evaluation of potassium ions as the endothelium-derived hyperpolarizing factor (EDHF) in the bovine coronary artery, Br. J. Pharmacol. **139**, 982–988

175 Randriamboavonjy, V., Busse, R. and Fleming, I. (2003) 20-HETE-induced contraction of small coronary arteries depends on the activation of Rho-kinase. Hypertension **41**, 801–806

176 Büssemaker, E., Popp, R., Binder, J., Busse, R. and Fleming, I. (2003) Characterization of the endothelium-derived hyperpolarizing factor (EDHF) response in the human interlobar artery. Kidney Int. **63**, 1749–1755

177 Torondel, B., Vila, J. M., Segarra, G., Lluch, P., Medina, P., Martinez-Leon, J, Ortega, J. and Lluch, S. (2004) Endothelium-dependent responses in human isolated thyroid arteries from donors. J. Endocrinol. **181**, 379–384

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

179 Kansui, Y., Garland, C. J. and Dora, K. A. (2008) Enhanced spontaneous Ca^{2+} events in endothelial cells reflect signalling through myoendothelial gap junctions in pressurized mesenteric arteries. Cell Calcium **44**, 135–146

180 Isakson, B. E., Ramos, S. I. and Duling, B. R. (2007) Ca^{2+} and inositol 1,4,5-trisphosphate-mediated signaling across the myoendothelial junction. Circ. Res. **100**, 246–254

181 Richards, G. R., Weston, A. H., Burnham, M. P., Félétou, M., Vanhoutte, P. M. and Edwards, G. (2001) Suppression of K^+-induced hyperpolarization by phenylephrine in rat mesenteric artery: relevance to studies of endothelium-derived hyperpolarizing factor. Br. J. Pharmacol. **134**, 1–5

182 Weston, A. H., Absi, M., Harno, E., Geraghty, A. R., Ward, D. T., Ruat, M., Dodd, R. H., Dauban, P. and Edwards, G. (2008) 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

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

184 Quignard, J.-F., Félétou, M., Duhault, J. and Vanhoutte, P. M. (1999) Potassium ions as endothelium-derived hyperpolarizing factors in the isolated carotid artery of the guinea-pig. Br. J. Pharmacol. **127**, 27–34

185 Dong, H., Jiang, Y., Cole, W. C. and Triggle, C. R. (2000) Comparison of the pharmacological properties of EDHF-mediated vasorelaxation in the guinea-pig cerebral and mesenteric resistance vessels. Br. J. Pharmacol. **130**, 1983–1991

186 Taylor, M. S., Bonev, A. D., Gross, T. P., Eckman, D. M., Brayden, J. E., Bond, C. T, Adelman, J. P. and Nelson, M. T. (2003) Altered expression of small-conductance Ca^{2+}-activated K^+ (SK3) channels modulate arterial tone and blood pressure. Circ. Res. **93**, 124–131

187 Si, H., Heyken, W. T., Wolfle, S. E., Tysiac, M., Schubert, R., Grgic, I., Vilianovich, L., Giebing, G., Maier, T., Gross, V. et al. (2006) Impaired endothelium-derived hyperpolarizing factor-mediated dilations and increased blood pressure in mice deficient of the intermediate-conductance Ca^{2+}-activated K^+ channel. Circ. Res. **99**, 537–544

188 de Wit, C. (2008) Calcium-activated potassium channels and EDHF-mediated responses. Basic Clin. Pharmacol. Toxicol. **102** (Suppl. 1), 8

189 Brähler, S., Grgic, I., Busch, C., Kacik, M., Hoyer, J. and Köhler, R. (2008) EDHF-signalling and arterial blood pressure in mice deficient of endothelial KCa-channels. Basic Clin. Pharmacol. Toxicol. **102** (Suppl. 1), 9

190 Simon, A. M. and McWhorter, A. R. (2003) Decreased intercellular dye-transfer and downregulation of non-ablated connexins in aortic endothelium deficient in connexin37 or connexin40. J. Cell Sci. **116**, 2223–2236

191 Wagner, C., de Wit, C., Kurtz, L., Grunberger, C., Kurtz, A. and Schweda, F. (2007) Connexin40 is essential for the pressure control of renin synthesis and secretion. Circ. Res. **100**, 556–563

192 Simon, A. M. and McWhorter, A. R. (2002) Vascular abnormalities in mice lacking the endothelial gap junction protein connexin37 and connexin40. Dev. Biol. **251**, 206–220

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

194 Figueroa, X. F., Paul, D. L., Simon, A. M., Goodenough, D. A., Day, K. H., Damon, D. N. and Duling, B. R. (2003) Central role of connexin 40 in the propagation of electrically activated vasodilation in mouse cremasteric arterioles *in vivo*. Circ. Res. **92**, 793–800

195 Félétou, M. and Vanhoutte, PM. (2004) EDHF: new therapeutic targets? Pharmacol. Res. **49**, 565–580

196 Matsumoto, T., Kobayashi, T., Wakabayashi, K. and Kamata, K. (2005) Cilostazol improves endothelium-derived hyperpolarizing factor-type relaxation in mesenteric arteries from diabetic rats. Am. J. Physiol. Heart Circ. Physiol. **289**, H1933–H1940

197 Neylon, C. B., Lang, R. J., Fu, Y., Bobik, A. and Reinhart, P. H. (1999) Molecular cloning and characterization of the intermediate-conductance Ca^{2+}-activated K^+ channel in vascular smooth muscle: relationship between K_{Ca} channel diversity and smooth muscle function. Circ. Res. **85**, 33–43
198 Tharp, D. L., Wamhoff, B. R., Wulff, H., Raman, G., Cheong, A. and Bowles, D. K. (2008) Local delivery of the KCa3.1 blocker, TRAM-34, prevents acute angioplasty-induced coronary smooth muscle phenotypic modulation and limits stenosis. Arterioscler. Thromb. Vasc. Biol. **28**, 1084–1089
199 Wulff, H., Miller, M. J., Haensel, W., Grissner, S., Cahalan, M. D. and Chandy, K. G. (2000) Design of potent and selective inhibitor of the intermediate-conductance Ca^{2+}-activated K^+ channel, IKCa1: a potential immunosuppressant. Proc. Natl. Acad. Sci. U.S.A. **97**, 8151–8156
200 Toyama, K., Wulff, H., Chandy, K. G., Azam, P., Raman, G., Saito, T., Fujiwara, Y., Mattson, D. L., Das, S., Melvin, J. E. et al. (2008) The intermediate-conductance calcium-activated potassium channel KCa3.1 contributes to atherogenesis in mice and humans. J. Clin. Invest. **118**, 3025–3037
201 Grgic, I., Eichler, I., Heinau, P., Si, H., Brakemeier, S., Hoyer, J. and Kohler, R. (2005) Selective blockade of the intermediate-conductance Ca^{2+}-activated K^+ channel suppresses proliferation of microvascular and macrovascular endothelial cells and angiogenesis *in vivo*. Arterioscler. Thromb. Vasc. Biol. **25**, 704–709
202 Jäger, H., Dreker, T., Buck, A., Giehl, K., Gress, T. and Grissmer, S. (2004) Blockage of intermediate-conductance Ca^{2+}-activated K^+ channels inhibit human pancreatic cancer cell growth *in vitro*. Mol. Pharmacol. **65**, 630–638
203 Wang, Z. H., Shen, B., Yao, H. L., Jia, Y. C., Ren, J., Feng, Y. J. and Wang, Y. Z. (2007) Blockage of intermediate-conductance-Ca^{2+}-activated K^+ channels inhibits progression of human endometrial cancer. Oncogene **26**, 5107–5114
204 Nilius, B. and Voets, T. (2004) Diversity of TRP channel activation. Novartis Found. Symp. **258**, 140–149
205 Hartmannsgruber, V., Heyken, W. T., Kacik, M., Kaistha, A., Grgic, I., Harteneck, C., Liedtke, W., Hoyer, J. and Köhler, R. (2007) Arterial response to shear stress critically depends on endothelial TRPV4 expression. PLoS ONE **2**, e827
206 Zhang, D. X., Mendoza, S. A., Bubolz, A. H., Mizuno, A., Ge, Z. D., Li, R., Warltier, D. C., Suzuki, M. and Gutterman, D. D. (2009) Transient receptor potential vanilloid type 4-deficient mice exhibit impaired endothelium-dependent relaxation induced by acetylcholine *in vitro* and *in vivo*. Hypertension **53**, 532–538
207 Willette, R. N., Bao, W., Nerurkar, S., Yue, T. L., Doe, C. P., Stankus, G., Turner, G. H., Ju, H., Thomas, H., Fishman, C. E. et al. (2008) Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: part 2. J. Pharmacol. Exp. Ther. **326**, 443–452
208 Kjølbye, A. L., Knudsen, C. B., Jepsen, T., Larsen, B. D. and Petersen, J. S. (2003) Pharmacological characterization of the new stable antiarrhythmic peptide analog Ac-D-Tyr-D-Pro-D-Hyp-Gly-D-Ala-Gly-NH_2 (ZP123): *in vivo* and *in vitro* studies. J. Pharmacol. Exp. Ther. **306**, 1191–1199
209 Lang, N. N., Myles, R. C., Burton, F. L., Hall, D. P., Chin, Y. Z., Boon, N. A. and Newby, D. E. (2008) The vascular effects of rotigaptide *in vivo* in man. Biochem. Pharmacol. **76**, 1194–1200

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