Endothelium-Derived Hyperpolarizing Factor
Where Are We Now?
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Abstract—The endothelium controls vascular tone not only by releasing nitric oxide (NO) and prostacyclin but also by other pathways causing hyperpolarization of the underlying smooth muscle cells. This characteristic was at the origin of the denomination endothelium-derived hyperpolarizing factor (EDHF). We know now that this acronym includes different mechanisms. In general, EDHF-mediated responses involve an increase in the intracellular calcium concentration, the opening of calcium-activated potassium channels of small and intermediate conductance and the hyperpolarization of the endothelial cells. This results in an endothelium-dependent hyperpolarization of the smooth muscle cells, which can be evoked by direct electrical coupling through myo-endothelial junctions and/or the accumulation of potassium ions in the intercellular space. Potassium ions hyperpolarize the smooth muscle cells by activating inward rectifying potassium channels and/or Na⁺/K⁺-ATPase. In some blood vessels, including large and small coronary arteries, the endothelium releases arachidonic acid metabolites derived from cytochrome P450 monoxygenases. The epoxyeicosatrienoic acids (EET) generated are not only intracellular messengers but also can diffuse and hyperpolarize the smooth muscle cells by activating large conductance calcium-activated potassium channels. Additionally, the endothelium can produce other factors such as lipoxigenases derivatives or hydrogen peroxide (H₂O₂). These different mechanisms are not necessarily exclusive and can occur simultaneously. (Arterioscler Thromb Vasc Biol. 2006;26:1215-1225.)

Key Words: CNP ■ cytochrome P450 ■ cyclooxygenase ■ EDHF ■ gap junction ■ lipoxygenase ■ NO ■ potassium channels ■ prostacyclin

Endothelial cells control the tone of the underlying vascular smooth muscle cells by releasing various relaxing and contracting factors.¹ The former include nitric oxide (NO) and prostacyclin.²⁻⁴ However, early experimental evidence suggested that, beside the cyclooxygenase and the NO synthase pathways, an additional endothelial pathway(s) had to be involved to fully explain endothelium-dependent relaxations.⁵ This third endothelium-dependent pathway(s) has been observed in numerous blood vessels from different species, including the human. It is associated with the hyperpolarization of the vascular smooth muscle cells and was then attributed to a noncharacterized endothelial factor termed endothelium-derived hyperpolarizing factor (EDHF).⁶⁻⁷ The coining of this acronym “EDHF” turned out to be
confusing because it implies that a single diffusible substance mediates this type of endothelium-dependent relaxation. In fact, numerous endothelium-derived factors, including NO and prostanoyl themselves, can hyperpolarize the underlying smooth muscle. The purpose of this review is to highlight the different pathways that can lead to endothelium-dependent hyperpolarization of the vascular smooth muscle cells. To clarify the nomenclature of non-NO-non-PGI2-mediated responses, we propose that once an endothelial mediator has been identified properly, it should be adequately named (ie, epoxyeicosatrienoic acid [EET], H2O2) and no longer denominated with the acronym EDHF.6,7

### Prostacyclin

Prostacyclin is the major metabolite of arachidonic acid produced by cyclooxygenase in endothelial cells.3 It activates IP receptors on vascular smooth muscle and, in most normal arteries, produces relaxation. Depending on the artery and/or the species, a hyperpolarization can occur, which involves the opening of one or more of types of potassium channels. Thus, ATP-sensitive potassium channels (K-ATP) (Figure 1), large conductance calcium-activated potassium channels (BKCa), inwardly rectifying potassium channels (Kir) and/or voltage-activated potassium channels ( Kv) can be associated with the prostacyclin-induced relaxation.7 Therefore, in numerous vascular beds, the prostanooid can be regarded as an endothelium-derived hyperpolarizing substance. Because most of the available inhibitors of cyclooxygenase abolish the production of prostaglandins in vascular tissues, any endothelium-dependent hyperpolarization observed in the presence of one of these inhibitors is unlikely to involve prostacyclin.7

### NO

NO also can hyperpolarize, or repolarize, vascular smooth muscle cells by activating, in either a cyclic-GMP–dependent or cyclic-GMP–independent manner, potassium channels such as K-ATP (Figure 1), BKCa, Kir, and/or Kv. NO interacts with other ionic channels of the smooth muscle, including chloride and cationic channels and also influences the membrane potential of the smooth muscle cells indirectly in an autocrine fashion.7

In contrast to inhibitors of cyclooxygenase, inhibitors of NO synthase do not necessarily fully inhibit the production of NO. Thus, in their presence, residual NO can be produced by
the endothelial cells and contribute to the relaxation and/or hyperpolarization of the underlying vascular smooth muscle.8,9

NO can contribute in another way to responses resistant to inhibitors of cyclooxygenases and NO synthases. NO can also be stored and released independently of the activation of NO synthase. Two such stores have been described. The first one is the so-called photosensitive store that involves the release of NO by ultraviolet light.10 The mechanism of NO release by this store in the absence of ultraviolet light is unknown. Nevertheless, in rabbit and rat mesenteric arteries, the same pool of NO that is released during photoactivation may contribute to those responses.11 The NO-dependent responses are sensitive to guanylyl cyclase inhibitors or NO scavengers but not to NO synthase inhibitors. The second store is located in the adventitia. It is generated by the formation of protein-bound dinitrosyl non-heme iron complexes and S-nitrosated proteins, when elevated concentrations of NO are produced, particularly when NOS-2 has been induced by lipopolysaccharides.12 Low-molecular-weight thiols displace NO from these stores and transfer it to various target membrane proteins including potassium channels and may therefore function as hyperpolarizing factors.13

Figure 2. IKCa in freshly nonenzymatically dissociated porcine coronary endothelial cells (outside-out configuration of the patch-clamp technique). A, Single-channel activity was recorded at the indicated holding potentials under asymmetrical K+ gradients with Ca2+ fixed at 250 nmol in the pipette solution. Representative traces are shown. B, Mean unitary currents were plotted against holding potential and fitted with a linear function. The slope conductance computed was 171.1±0.4 pS. C, Effect of Ca2+ on the open probability of IKCa. Distributions of unitary conductance amplitude recorded at 0 mV are shown, together with representative traces. A Gaussian fit was performed for every single channel experiment, and the amplitude of single channels was determined with the best fit. Then, the mean of the obtained amplitudes was calculated. The effect of Ca2+ was determined using pipette solutions with free Ca2+ fixed at 100, 250, and 500 nmol. D, The effects of iberiotoxin (10−7 mol; left panel) and the subsequent addition of charybdotoxin (10−7 M; right panel) were recorded. Distributions of unitary conductance amplitude (recorded at 0 mV and using 250 nmol free Ca2+ in the pipette solution) are shown, together with representative traces. These data indicate that the current is insensitive to iberiotoxin (10−7 mol) but blocked by charybdotoxin (10−7 mol) (modified with permission from Bychkov et al).33

Non-Prostanoid–Dependent, Non-NO–Dependent Endothelium-Dependent Hyperpolarizations

In NOS-3 knockout and NOS-3/COX-1 double knockout mice, EDHF-mediated responses compensate for the absence of endothelial NO.15–18 The importance of this compensation depends on the gender. In resistance arteries of NOS-3/COX-1 double knockout female mice, endothelium-dependent relaxations are preserved whereas in arteries from corresponding males they are impaired. In female mice, the deletion of NOS-3 and COX-1 does not affect mean arterial blood pressure, whereas the males with these deletions are hypertensive.18 These results suggest that EDHF-mediated responses contribute to the overall regulation of arterial blood pressure.
Characterization of EDHF-Mediated Responses

Agonists stimulating G protein–coupled receptors as well the calcium ionophore A23187, thapsigargin, and cyclopiazonic acid increase the endothelial intracellular calcium concentration ([Ca\(^{2+}\)], hyperpolarize the endothelial cells, and in some blood vessels evoke endothelium-dependent hyperpolarization of the underlying vascular smooth muscle cells.\(^{19-23}\) In most arteries, such EDHF-mediated responses involve the activation of SK\(^{\text{Ca}}\) (blocked by apamin, scyllatoxin, tubocurarine, or UCL 1684) and/or IK\(^{\text{Ca}}\) (blocked by charybdotoxin, TRAM-34 or TRAM-39),\(^{23-27}\) but not that of BK\(^{\text{Ca}}\) (blocked by charybdotoxin and iberiotoxin).\(^{28,29}\)

SK\(^{\text{Ca}}\) and IK\(^{\text{Ca}}\) channels are present in freshly isolated endothelial cells from various arteries (Figures 2, 3).\(^{36,30-34}\) In porcine coronary endothelial cells, messenger RNAs encoding IK1 as well as the SK2 and SK3 subunits of SK\(^{\text{Ca}}\) can be detected by RT-PCR. Western blot analysis indicates that the SK3 protein is abundant, whereas immunofluorescent labeling confirms that IK1 and SK3 (Figure 3) are expressed at the plasmalemma of endothelial cells but not in that of vascular smooth muscle. Substance P and bradykinin, both of which initiate EDHF-mediated responses in the porcine coronary artery, activate an outward current sensitive to the combination of blockers of IK\(^{\text{Ca}}\) and SK\(^{\text{Ca}}\). By contrast, in freshly isolated vascular smooth muscle cells from various species, IK\(^{\text{Ca}}\) and SK\(^{\text{Ca}}\) are not or very poorly expressed, whereas functional BK\(^{\text{Ca}}\) are constitutively present.\(^{35,36}\) These observations demonstrate that the 2 Ca\(^{2+}\)-activated potassium channels involved in EDHF-mediated responses are located on the endothelial plasma membrane.\(^{26,32-34}\) In the mouse, the level of expression of SK3 channels on the endothelial cells correlates with the cell membrane potential of both endothelial and vascular smooth muscle cells, with the tone of isolated mesenteric arteries and the diameter of these arteries in situ, as well as with the arterial blood pressure of the animals.\(^{37}\)

Depolarization with KCl and inhibition of SK\(^{\text{Ca}}\) and/or IK\(^{\text{Ca}}\) 2 different maneuvers that fully prevent the hyperpolarization of both the endothelial and the smooth muscle cells, produce no or only minimal inhibition in the sustained phase of the increase in endothelial [Ca\(^{2+}\)].\(^{38-40}\) Conversely, 1-ethyl-2-benzimidazolinone (1-EBIO, an activator of SK\(^{\text{Ca}}\) and/or IK\(^{\text{Ca}}\) but not BK\(^{\text{Ca}}\))\(^{41}\) evokes EDHF-mediated responses without increasing endothelial [Ca\(^{2+}\)].\(^{23}\) Thus, the increase in [Ca\(^{2+}\)], after endothelial receptors activation, stimulates K\(^{\text{Ca}}\) channels to hyperpolarize the endothelial cells.

This endothelial hyperpolarization is a prerequisite to obtain the endothelium-dependent hyperpolarization of the underlying vascular smooth muscle.

Beyond Endothelial Hyperpolarization

Two mechanisms have been proposed to explain how the activation of endothelial SK\(^{\text{Ca}}\) and/or IK\(^{\text{Ca}}\) leads to the hyperpolarization of the smooth muscle cells: (1) hyperpolarization of the endothelial cell transmitted directly to the vascular smooth muscle by means of gap junctions; and (2) accumulation in the intercellular cleft of K\(^{+}\) ions released from the endothelial cells through opening of K\(_{\text{ATP}}\) channels leading to hyperpolarization of the smooth muscle by activating K\(^{+}\) conductances and/or Na\(^{+}\)/K\(^{+}\)-ATPase in the latter.

Gap Junctions

Gap junction channels are formed by the docking of 2 connexons present in adjacent cells leading to the creation of an aqueous central pore that permits the transfer of ions and polar molecules. This provides an electrical continuity allowing a uniform membrane potential among coupled cells. Endothelium and smooth muscle cells communicate via myo-endothelial gap junctions.\(^{42-48}\)

In arteries exhibiting EDHF-mediated responses, the endothelium-dependent hyperpolarization of vascular smooth muscle cells and the hyperpolarization of the endothelial cells follow the same time course.\(^{49,50}\) A close correlation exists between the expression of myo-endothelial gap junctions and
the occurrence of EDHF-mediated responses.\textsuperscript{51–53} Furthermore, the number of myo-endothelial gap junctions increases as the size of the artery decreases,\textsuperscript{54,55} a phenomenon that parallels the contribution of the EDHF-mediated responses in endothelium-dependent relaxations.\textsuperscript{56,57} In many blood vessels, blockers of gap junctions (glycyrrhetinic acid and some of its derivative such as carbenoxolone, HEPES and other taurine-based buffers and Gap peptides), partially inhibit or abolish EDHF-mediated responses.\textsuperscript{45,58,59} However, many questions remain unanswered. To date, what flows through myo-endothelial gap junction is basically unknown. The fact that a single layer of endothelial cell can drive the hyperpolarization of a multiple layers of smooth muscle cells suggests the involvement of an undefined active membrane process in the conducted hyperpolarization.\textsuperscript{7,60} Some EDHF-mediated responses are associated with a small but significant early and transient endothelium-dependent increase in the cyclic-AMP content of the smooth muscle cells. The stimulation of a calcium-sensitive adenyl cyclase isoform results in an increase in the production of cyclic-AMP with subsequent enhancement of gap junctional communication.\textsuperscript{61,62}

Connexins 37 and 40 are the predominant gap junction proteins in the endothelial cells of the mouse. Mice deficient for connexin 40 are hypertensive and exhibit a reduced spread of dilatation in response to endothelium-dependent vasodilators and electrical stimulation, as well as irregular arteriolar vasomotion.\textsuperscript{63,64} Antibodies directed against connexin 40, when loaded selectively in the endothelial cells of the murine mesenteric artery, block EDHF-mediated responses.\textsuperscript{65} By contrast, mice subjected to specific deletion of endothelial connexin 43 do not present any major alteration in blood pressure,\textsuperscript{66} possibly because this connexin is not the major one expressed in their endothelial cells.

Taken in conjunction, the available data show that transmission of the hyperpolarization via gap junctions contributes to many EDHF-mediated responses whereby, at least in the mouse, connexin 40 appears to play a predominant role. However, in a number of EDHF-mediated responses, the involvement of gap junctions appears minimal implying the occurrence of other mechanisms.\textsuperscript{7,67}

**Potassium Ions**

Another mechanism to achieve hyperpolarization and relaxation of the underlying vascular smooth muscle cells is linked directly to the hyperpolarization of the endothelial cells. Indeed, the activation of endothelial $\text{IK}_{Ca}$ and/or $\text{SK}_{Ca}$ causes an efflux of potassium ions from the intracellular compartment toward the extracellular space. Potassium is an endogenous metabolic vasodilator involved in exercise hyperemia and in active hyperemia of the brain due to increased neuronal activity. A moderate increase in the extracellular potassium concentration (1 to 15 mmol/L) causes relaxation of vascular smooth muscle.\textsuperscript{68} This observation is counterintuitive because, as a result of such an increase in the extracellular potassium ion concentration, the Nernst equation predicts depolarization and a subsequent contraction. However, small increases in the extracellular concentration of potassium ions can also activate $\text{K}_{IR}$ and the Na’/K’ pump.\textsuperscript{69,70} The activation of these 2 systems overcomes the minor depolarizing effects linked to the increase in potassium ions per se. The net result is hyperpolarization and thus relaxation of the smooth muscle cells. The endothelium is a thin monolayer and any efflux of potassium from this small cell mass into the lumen of the blood vessel is washed away by the flowing blood. However, an efflux of potassium in the abluminal direction could lead to accumulation of those ions in the small intercellular space between endothelial and smooth muscle cells and to reach levels sufficient to activate $\text{K}_{IR}$ and the Na’/K’ pump on the latter. Therefore, potassium ions could contribute to EDHF-mediated responses.

This hypothesis was demonstrated successfully in the hepatic and mesenteric arteries of the rat.\textsuperscript{71} This study provided evidence that the $\text{K}_{Ca}$ channels involved were located on the endothelial cells, that potassium ions indeed accumulate in the intercellular space between endothelial and smooth muscle cells and that the potassium efflux associated with the activation of endothelial $\text{IK}_{Ca}$ and/or $\text{SK}_{Ca}$ channels produces hyperpolarization by activating both $\text{K}_{IR}$ and the Na’/K’ pump on the vascular smooth muscle cells.\textsuperscript{71} The contribution of K’ in EDHF-mediated responses was demonstrated in many blood vessels including human arteries.\textsuperscript{72}

Reverse-transcription polymerase chain reaction and immunohistochemistry studies indicate the $\text{K}_{IR}$ channel in vascular smooth muscle involved in potassium ion-induced hyperpolarization is composed of the Kir2.1 $\alpha$-subunits.\textsuperscript{73,74} In cerebral arteries of mice (an artery in which potassium-induced relaxation does not involve the activation of the Na’/K’-ATPase) potassium-induced dilation are no longer observed in mice subjected to the deletion of Kir2.1 whereas these responses are preserved Kir2.2 knockout in mice,\textsuperscript{75} supporting a predominant role of Kir2.1 $\alpha$-subunits.

However, in many arteries potassium does not consistently produces a relaxation and/or a hyperpolarization, suggesting that in these blood vessels EDHF-mediated responses do not rely on the accumulation of interstitial potassium.\textsuperscript{6}

The involvement of gap junctions and K’ ions are not necessarily mutually exclusive. They can occur simultaneously or sequentially and also may act synergistically (Figure 4).

**Other Identified Endothelium-Derived Hyperpolarizing Substances**

Besides the production of NO, prostacyclin and potassium ions, the endothelial cells can release other relaxing factors. The contribution of these depends on the species and/or the vascular bed studied. The hyperpolarization of the smooth muscle cells evoked by these other endothelium-derived hyperpolarizing factors can be the major mechanism underlying the relaxation or only contribute partially to it.

**Metabolites of Arachidonic Acid**

**Products of Cytochrome P450 Monoxygenase**

EETs, derived from cytochrome P450 2C or 2J epoxygenases, and possibly their epoxide hydrofase metabolites dihydroxyeicosatrienonic acids, contribute to endothelium-dependent relaxations of various blood vessels, including large and small coronary arteries.\textsuperscript{76–79}
The presence of a “potassium cloud” can markedly affect the endothelium-dependent hyperpolarization. The relative proportions of each mechanism almost certainly depend on numerous parameters including the state of activation of the vascular smooth muscle, the density of myo-endothelial gap junctions and the level of the expression of the appropriate isoforms of Na+/K+-ATPase and/or K$_\text{ir}$. R indicates receptor; Bk, bradykinin; Sp, substance P; IP$_3$, inositol trisphosphate; SR, sarcoplasmic reticulum; TRP, transient receptor potential channel; AC, adenyl cyclase; cAMP, cyclic AMP; uGA, glycyrrhetinic acid derivatives; CK, connexin; 4-AP, 4-aminopyridine; IbTX, iberiotoxin; SK3, small conductance calcium-activated potassium channel formed by SK$_\text{3a}$-subunits; IK$_\text{1}$, intermediate conductance calcium-activated potassium channel formed by IK$_\text{1a}$-subunits; Kir$_\text{2.1}$, inward rectifying potassium channel; Kir$_\text{3}$.2, large conductance calcium-activated potassium channels.

EETs hyperpolarize coronary arterial smooth muscle cells and increase the open-state probability of BK$_\text{Ca}$. Although EETs activate BK$_\text{Ca}$ through a G protein–signaling cascade, the existence of a specific cell membrane receptor(s) for EETs in vascular smooth muscle has not been established. The observation that some EET analogues, 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE) and 14,15-epoxyeicosa-5(Z)-enoic-methylsulfonylimide (14,15-EEZE-mSi), act as inhibitors of the action of EETs support the existence of such receptors. Additionally, EETs activate smooth muscle vanilloid transient receptor potential channel (TRPV4), which increase the frequency of calcium sparks and subsequently that of spontaneous transient outward currents. This EET-dependent activation of a calcium-signaling complex (TRPV4–ryanodine receptors–BK$_\text{Ca}$) hyperpolarizes and relaxes the smooth muscle cells.

In many arteries, inhibitors of cytochrome P450 2C9 derived metabolites are not involved in the regulation of basal blood flow and in these non-NO–non-PGI$_2$–mediated responses that are unlikely to rely on this metabolite of arachidonic acid.
Figure 5. Bradykinin and substance P induced endothelium-dependent hyperpolarization in the porcine coronary artery. Both peptides stimulate endothelial SKCa and IKCa and evoke the subsequent hyperpolarization of the smooth muscle cells (see Figure 6). Additionally, bradykinin, but not substance P, activates the metabolism of arachidonic acid via cytochrome P450 monoxygenase. The endothelial formation of epoxyeicosatrienoic acids (EETs) can have multiple effects in the vascular wall. EETs are endothelium-derived hyperpolarizing substances activating smooth muscle BKCa by different means directly via a G protein-signaling cascade in bovine and porcine coronary arteries or indirectly by the calcium signaling complex, TRPV4 - ryanodine receptors - BKCa, in rat cerebral artery. Additionally, EETs can modulate EDHF-mediated responses by regulating the intracellular calcium concentration, potassium channel activity and gap junctional communications. NK1 indicates neurokinin 1 receptor subtype; B2, bradykinin B2 receptor subtype; PLC, phospholipase C; DAG, diacylglycerol; AA, arachidonic acid; SR, sarcoplasmic reticulum; TRPV4, transient receptor channel vanilloid-4; SOC, store-operated channel; AC, adenylyl cyclase; cAMP, cyclic-AMP; P450, cytochrome P450 monoxygenase; EETs, epoxyeicosatrienoic acids; SK3, small conductance calcium-activated potassium channel formed by SK3 subunits; IKCa, intermediate conductance calcium-activated potassium channel formed by IK1 α-subunits; BKCa, large conductance calcium-activated potassium channels; RyR, ryanodine receptors.

Products of Lipoxygenases
Under various physiological and/or pathophysiological conditions, endothelial cells express different lipoxygenases, which can metabolize arachidonic acid into relaxing and contracting substances. Thus, 12-(S)-HETE is released from the endothelium by different stimuli and activates BKCa on the smooth muscle cells.106–108 In rabbit arteries, the NO- and PGI2–mediated responses are partially or totally prevented by catalase. In those arteries the agonist-induced non-NO–non-PGI2–mediated responses are accompanied by the endothelial production of H2O2,111 which, depending on the tissue and the experimental conditions or the concentrations studied, possesses dilator or constrictor properties and hyperpolarizes or depolarizes smooth muscle.112

The role of H2O2 as an endothelium-derived hyperpolarizing factor comes from the observation that, in certain blood vessels, the agonist- and flow-induced responses attributed to non-NO–non-PGI2–mediated phenomenon are partially or totally prevented by catalase. In those arteries the agonist-induced non-NO–non-PGI2–mediated responses are accompanied by the endothelial production of H2O2,111 Endothelial Cu Zn superoxide dismutase probably plays a major role in producing H2O2 to elicit endothelium-dependent hyperpolarizations.114

A critical appraisal of the available evidence linking H2O2 to EDHF-mediated responses requires a word of caution. H2O2 is a rather weak relaxing and hyperpolarizing substance. One of the major achievements that has improved the understanding of non-NO–non-PGI2–mediated responses has been the identification and localization of the potassium channels involved in these responses. In the various arteries where the hyperpolarizations in response to H2O2 and non-NO–non-PGI2–mediated responses were compared, none of the specific potassium channels blockers has been studied, and it is not yet known which population(s) of potassium inhibitors of cytochrome P450100 and of the cellular targets of EETs (14,15-EEZE and 14,15-EEZE-mSi)81 as well as specific blockers of IKCa that are devoid of activity toward either BKCa or cytochrome P450 (TRAM 34 and TRAM 39)101 allows the proper definition of the contribution of EET to non-NO–non-PGI2–mediated responses.

EETs can be involved in non-NO–non-PGI2–mediated responses without being a diffusible factor per se. The hyperpolarization of the endothelial cells may be partly modified by the activation of cytochrome P450. For example, EETs can regulate endothelial calcium homeostasis, by modulating store-operated Ca2+ channels in response to calcium store depletion,102 and thus have been identified as the elusive “calcium influx factor.”103 The endothelial [Ca2+]i controls the activation of endothelial K+ channels. In addition, independently of their role in calcium homeostasis, EETs may regulate the activity of endothelial KCa.104 Finally, EETs also cause a biphasic change in gap junctional communication between endothelial cells,105 suggesting that these metabolites of arachidonic acid, if they produce similar effects on myo-endothelial gap junctions, will favor transmission of the endothelial hyperpolarization toward the smooth muscle cells (Figure 5).
channels are activated by H$_2$O$_2$ or whether they are situated on the endothelial or the smooth muscle cells. The absence of this information undermines any conclusion as to the potential role of H$_2$O$_2$ in non-NO–non-PGI$_2$–mediated responses. In particular, the questions remain unanswered whether H$_2$O$_2$ is a diffusible factor activating potassium channels on the smooth muscle or an intracellular messenger involved in the activation of endothelial potassium channels. Likewise, although H$_2$O$_2$ is produced in response to an increase in endothelial [Ca$^{2+}$], it is uncertain whether this is truly a pathway linked to activation of endothelial IK$_C_{Ca}$ and SK$_C_{Ca}$ or whether this is an independent (epi)phenomenon. Finally, catalase does not inhibit non-NO–non-PGI$_2$–mediated responses in all arteries, and H$_2$O$_2$ does not relax or hyperpolarize all vascular smooth muscle cells.

C-Type Natriuretic Peptide

C-type natriuretic peptide (CNP) causes relaxation and hyperpolarization of arterial and venous smooth muscle cells and opens BK$_C_{Ca}$ This endothelium-derived natriuretic peptide has therefore been proposed as a putative hyperpolarizing factor. However, at least in the porcine coronary artery, the characteristics and the amplitude of the hyperpolarizations evoked by exogenous CNP are by no means comparable to those observed during EDHF-mediated responses. Experiments in the rat mesenteric artery suggest that acetylcholine releases CNP from the endothelial cells, which in turn activates NPR-C receptors on vascular smooth muscle. Hyperpolarization of the smooth muscle cell is obtained by the cyclic-GMP–independent activation of a G-protein regulated inward-rectifier potassium channel (GIRK).

The hypothesis proposing that CNP is an endothelium-derived hyperpolarizing substance requires the validation of various concepts. Whether the CNP-dependent activation of NPR-C in vascular smooth muscle cells produces a cyclic GMP-independent, pertussis toxin-sensitive signaling is unknown. The expression and activity of GIRK has been well-characterized in neurons and cardiac myocytes, but the protein expression and the complete characterization of this channel in vascular smooth muscle cells await full demonstration. Finally, there is no evidence to date, in any cell type, that CNP can activate GIRK.

Conclusion

Endothelial cells control the tone of the underlying vascular smooth muscle by releasing numerous vasoactive substances, including NO, reactive oxygen species, potassium ions, and metabolites of arachidonic acid (eg, prostacyclin, EETs, lipoxygenase 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 myo-endothelial gap junctions.

Endothelium-dependent relaxations, independent of the production of NO and prostacyclin, probably play an important role in cardiovascular physiology in the animal and in the human. They can act as a backup system when NO is inhibited or reduced but this is not necessarily the case. On the one hand, the production of superoxide anion and subsequently H$_2$O$_2$ is increased when the eNOS is dysfunctional (decrease substrate and/or cofactor concentration) and the generation of EETs can be enhanced if the tonic repression of the cytochrome P450 activity is alleviated by a reduced NO availability. On the other hand, EDHF-mediated responses, dependent on endothelial IK$_C_{Ca}$ and SK$_C_{Ca}$ activation, appear independent of the activity of eNOS. 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. Clarity could be improved by applying simple rules.

Demonstrate the Existence of a Non-NO–Non-PGI$_2$–Mediated Responses Unequivocally

A non-NO–non-PGI$_2$–mediated responses response should be reported only when the evidence for a third pathway, besides the L-arginine-NO-synthase and the arachidonic acid-cyclooxygenase pathways, is demonstrated beyond any doubt. Demonstration of resistance to the combined presence of inhibitors of NO synthases and cyclooxygenases does not necessarily prove the existence of an EDHF-mediated response. Ideally, the proof that NO generation has been abolished should be brought. However, if a relaxation and/or hyperpolarization is still observed in the combined presence of inhibitors of cyclooxygenase and NO synthase, as well as an NO scavenger (eg, oxyhemoglobin, carboxy-PTIO), it is reasonable to consider that the blood vessel studied exhibits non-NO–non-PGI$_2$–mediated responses. These experiments are needed to rule out the possible involvement of residual NO production, either from NO synthase itself or from NO synthase–independent sources (NO stores).

Determine the Mechanism Underlying the Suspected Non-NO–Non-PGI$_2$–Mediated Responses

These responses often encompass different endothelial mediators or pathways that occur independently or in some cases coexist. The generation of H$_2$O$_2$ and the production of EETs by cytochrome P450 or that of CNP do not necessarily require the hyperpolarization of endothelial cells, although in a similar manner to the release of NO itself, the membrane potential of the endothelial cells can be a regulatory factor. H$_2$O$_2$, EETs, CNP, or adenosine, again like NO, are released by the endothelial cells and activate potassium channels on the vascular smooth muscle cells. Appropriate tools are now available to properly identify these mediators. Once their involvement is confirmed in a given vascular bed, they must be referred to by their proper name, ie, endothelium-derived NO, prostacyclin, H$_2$O$_2$, EETs, and CNP, and no longer should be termed “EDHF.” Only the responses requiring the activation of endothelial SK$_C_{Ca}$, and/or IK$_C_{Ca}$ and hyperpolarization of the endothelial cells should be referred to as “EDHF-mediated” responses because there is, at present, no better way to name them. Obviously, such EDHF-mediated responses must be characterized and the involvement of gap junction and/or potassium ions must be determined whenever possible. To implement this simple nomenclature will simplify the interpretation of incoming studies.
Use the Most Specific Pharmacological Tools Available

The use of nonselective drugs, particularly inhibitors of cytochrome P450, which also inhibit calcium-activated potassium channels, and charybdoxin, which inhibits both IKCa and BKCa, has clouded the EDHF field. The use of more specific inhibitors of cytochrome P450 and of antagonists of the EETs such as the EEZE compounds, iberiotoxin to specifically inhibit BKCa and TRAM 34 or TRAM 39 to will allow proper identification of the non-NO–non-PGI2–mediated responses.

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Endothelium-Derived Hyperpolarizing Factor


