Clinical assessment of human vascular endothelial function in vivo involves use of specific antagonists such as l-NMMA and cyclooxygenase inhibition to establish the contribution of NO and prostaglandins, respectively, to either resting vascular tone, agonist-stimulated vasodilation, or physiological and metabolic vasodilation.\textsuperscript{1–4} Even after complete inhibition of NO and prostaglandin (PG) synthesis, endothelium-dependent vasodilation persists, revealing the existence of a substantial NO- and PG-independent component that has been attributed to endothelium-derived hyperpolarizing factor (EDHF) release. Prime candidate EDHFs that often differ by species and circulatory beds have been extensively reviewed (Figure).\textsuperscript{5,6} In the human vasculature, endothelium-dependent hyperpolarization is at least partly caused by the release of epoxyeicosatrienoic acids (EETs) from the cytochrome P450 (CYP450)-dependent metabolism of arachidonic acid that promote vasodilation by stimulation of small and large calcium-dependent potassium channels (K\textsubscript{Ca}) on endothelial cells (Figure).\textsuperscript{7} Agonists such as bradykinin stimulate endothelial G-protein-coupled receptors, provoking an increase in endothelial cellular calcium [Ca\textsuperscript{2+}], causing opening endothelial K\textsubscript{Ca} channels and triggering processes that explain the EDHF phenomena, including: synthesis of EETs,\textsuperscript{2} transmission of endothelial cell hyperpolarization to the vascular smooth muscle via gap junctions,\textsuperscript{3} and release of K\textsuperscript{+} from the endothelial cells via K\textsubscript{Ca} channels that, in turn, induces smooth muscle hyperpolarization by activating several other K\textsuperscript{+} channels (Figure).\textsuperscript{5,6} The hallmark of the EDHF-mediated responses is its abolition by the combination of apamin (a specific inhibitor of K\textsubscript{Ca} channels of small conductance) plus charybdoxin (a nonselective inhibitor of large-conductance and intermediate-conductance K\textsubscript{Ca} channels), and of some voltage-dependent K\textsuperscript{+} channels, but not ATP-dependent K\textsuperscript{2+} channels.

Hydrogen peroxide can also activate K\textsubscript{Ca} channels and remains a contender as another EDHF (Figure).\textsuperscript{8} Various endothelial oxidases, including NO synthase, lipoxigenases, P-450 epoxyenases, NAD(P)H oxidases, and xanthine oxidase generate superoxide anions that are degraded to hydrogen peroxide spontaneously or through superoxide dismutase–dependent dismutation.\textsuperscript{9} Gap junctions couple endothelial cells to other endothelial cells and smooth muscle cells provide a low-resistance electrical pathway between these 2 cell layers. Gap junctions are formed by the docking of 2 connexons present in adjacent cells that creates an aqueous pore permitting the transfer of ions and electrical continuity that establishes a uniform membrane potential across cells (Figure).\textsuperscript{10} Their number increases with diminution in the size of the artery, paralleling the importance of EDHF to vessel size. Finally, a moderate increase in the myoendothelial K\textsuperscript{+} concentration can in some species induce hyperpolarization of vascular smooth muscle cells by activating the inwardly rectifying K\textsuperscript{+} (K\textsubscript{ir}) channels and the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, but is an unlikely candidate as EDHF in people.

The absence of a consensus regarding the precise identity of EDHFs and a consequent lack of specific inhibitors has long hampered clinical translation of this phenomenon. Recently, with improved understanding of the major signaling mechanisms underlying vascular hyperpolarization, the role of EDHF in the human circulation in vivo has begun to be dissected, but experimental pitfalls remain: the nature of the antagonists that is often used is nonspecific, the concentrations and duration of action of these blockers are variable, and complete blockade cannot be achieved in vivo, even with high doses, because of the competitive nature of the antagonism. Nevertheless, an impressive body of knowledge has already emerged regarding the role of EDHF in the human circulation, including the article in this issue of Hypertension.\textsuperscript{13}

The role of EETs as potential EDHFs can be studied using azoles such as miconazole that selectively inhibit epoxidation (EET generation) of arachidonic acid and are partly responsible for non-NO and non–prostacyclin-mediated, endothelium-dependent vasodilation in the human microcirculation.\textsuperscript{7,11,12} In vivo, CYP450 inhibition does not alter conductance vessel diameter or resting blood flow,\textsuperscript{11,14} but after inhibition of NO and prostacyclin, inhibition of EET synthesis further decreases radial arterial blood flow and diameter as shown by Bellien.\textsuperscript{11} Thus, although it appears that under resting conditions in the healthy human forearm conductance and resistance vessel tone is not modulated by tonic activity of CYP450-derived epoxides, their role becomes evident after inhibition of NO and prostacyclin synthesis, illustrating the compensatory role of NO and EETs on maintenance of basal tone. Future studies need to investigate whether this contribution is altered in patients with endothelial dysfunction.

Because K\textsubscript{Ca} channel activation on the endothelial or smooth cells is a prerequisite for hyperpolarization and their...
Mechanisms of potential endothelial cell mediated relaxation/hyperpolarization. Agonist (bradykinin) or shear stress increases the activity of endothelial nNO synthase (eNOS) and cyclooxygenase (COX), providing NO and prostacyclin-mediated dilation. There are multiple potential EDHF pathways. Agonist (bradykinin) or shear stress-mediated increases in intracellular calcium activates phospholipase A2 (PLC) to produce arachidonic acid. Its metabolism by cytochrome P450 2C (CYP4502c) generates EETs that can stimulate KCa channels in endothelial and smooth muscle cells. EETs may also directly activate gap junctions (Gap). The increase in K+ in the interstitium may activate KCa, channels, KIR, or the Na+/K+ pump on smooth muscle cells and cause hyperpolarization. The action of eNOS (with cofactor tetrahydrobiopterin [BH4]) and oxidases on oxygen (O2) produces the reactive oxygen species superoxide (O2·−). Hydrogen peroxide (H2O2) generated by dismutation of superoxide anions (O2·−) by superoxide dismutase (SOD) can also cause hyperpolarization by activating endothelial and smooth muscle KCa channels or by gap junctions. AC indicates adenylyl cyclase; cAMP, cAMP; cGMP, cyclic guanosine monophosphate; sGC, soluble guanylyl cyclase; IP, prostacyclin receptor.

Blockade with charybdotoxin and apamin is too toxic in humans, tetrathydroammonium chloride (TEA), which selectively antagonizes KCa channels, has been used. Whereas TEA at a lower dose did not alter resting forearm blood flow,15 at a higher dose of 1 mmol/L used in the accompanying article,12,13 it clearly decreased resting forearm blood flow by 23% and radial artery diameter by 5%. TEA also inhibits bradykinin- and C-type natriuretic peptide–induced vasodilation after inhibition of NO and PGs in the healthy forearm microcirculation, demonstrating release of EDHF with these agonists. Moreover, there appears to be a synergistic effect of a combined blockade of NO synthesis and KCa channels on resting radial arterial blood flow and diameter.12,13 These observations demonstrate the contribution of both NO and KCa channel activation to resting conductance artery and microvascular tone in the healthy human forearm circulation. The contribution of KCa channels is potentially greater in the microvessels compared with the larger conductance arteries. Because inhibition of EETs had a lesser effect than inhibition of KCa channels on resting tone, it can be speculated that sources of KCa channel activation other than EETs may well be contributing to this phenomenon or that there are stores of EETs in the endothelial layer that may not be inhibited by these antagonists.

KIR or Na+/K+ ATPase activation may mediate smooth muscle vasodilation once K+ is released into the interstitial space as a result of endothelial KCa activation. Blockade of KCa channels with barium or Na+/K+ ATPase with ouabain reduced forearm blood flow in healthy subjects,16 a process that may compensate for the lack of NO in hypertension.17

Endothelial cells are sensitive to shear stress and respond by synthesizing factors that regulate vascular smooth muscle tone (Figure). Flow-mediated vasodilation (FMD) is a physiologically important mechanism regulating vascular tone1 and is an endothelium-dependent phenomenon, although the mediators vary depending on species, vascular bed, and vessel size.5,6 In isolated human coronary microvessels, flow induces potent endothelium-dependent vasodilation that requires CYP450 metabolites, membrane hyperpolarization, and the opening of KCa channels, but not PGs. NO contributes modestly to FMD in arterioles from patients without coronary artery disease, but not in those with coronary artery disease. Finally, shear stress appears to also elicit endothelial generation and the release of hydrogen peroxide.18

Measuring FMD after a brief period of ischemia, reperfusion, and hyperemia has been widely adopted to assess endothelial NO activity in vivo, and FMD is often abnormal in those with atherosclerosis or its risk factors.4,19 Although
NO is the predominant contributor to FMD of conductance arteries, its role in the microcirculation appears minimal.\(^3\)\(^,\)\(^7\) Whereas radial arterial FMD after a brief period of ischemia (5 minutes) followed by hyperemia was almost completely abolished by NO inhibition, after prolonged hyperemia produced by hand warming for 15 minutes or longer, diameter remained unchanged after inhibition of NO synthase, cyclooxygenase, and the autonomic nervous system.\(^4\) Using the same model of sustained hyperemia in the article by Bellien et al, \(^l\)-NMMA used in a higher concentration did indeed inhibit radial FMD by 39%, but importantly, it was also inhibited by the blockade of EET synthesis with fluniconazole (18%) and \(K\text{Ca}_3\) channels with TEA (14%).\(^13\) Moreover, combined inhibition of NO and either EETs or \(K\text{Ca}_3\) channels resulted in >70% inhibition of FMD, demonstrating that both NO and EETs, the latter acting via stimulation of \(K\text{Ca}_3\) channels, contribute to physiological vasodilation during sustained hyperemia.\(^13\) Although the study is complicated because of the multiple infusions used in a small number of subjects, it provides important insights into the regulation of blood flow in humans and the heterogeneity of responses, depending on the characteristics of the flow stimulus, vessel size, and location. There is also evidence that hypercholesterolemic subjects who have reduced FMD in response to transient hyperemia have normal vasodilation with sustained hyperemia, indicating potentially intact EDHF pathways in disease states.\(^4\)

Mechanisms underlying skeletal muscle vasodilation during exercise include the release of local metabolites including lactate, adenine nucleotides \(K^+\), and others. The role for both NO and prostacyclin to metabolic forearm microrcirculatory vasodilation appears to be minor, accounting for <15% of the peak increase.\(^2\) Additional CYP-2C9 blockade during exercise resulted in a further 16% reduction in flow, illustrating the contribution of epoxide-mediated hyperpolarization to metabolic skeletal muscle vasodilation.

Apart from its contribution to normal vascular physiology, the accentuated role of EDHF in diseased states is worthy of further investigation because CYP450 expression and EET generation are increased in hypertension, during salt loading, and in hypercholesterolemia. Vasodilation in essential hypertension\(^17\) and in atherosclerotic coronary arteries is largely secondary to CYP and \(K\text{Ca}_3\) channel stimulation. There are also potential implications regarding disease susceptibility, with some polymorphisms within CYP epoxidases being associated with an enhanced risk of developing coronary artery disease and hypertension.\(^6\) What may ultimately be of even greater interest is the development of specific agents targeting EDHF synthesis and the understanding of other biological effects of EETs such as angiogenesis and modulation of cell growth and their potential role in human disease.\(^6\)

**Disclosures**

None.

**References**

Vasodilation by Hyperpolarization: Beyond NO
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