The EDHF Story
The Plot Thickens
Helena C. Parkington, Marianne Tare, Harold A. Coleman

The dilator function of the vascular endothelium is critical for optimal tissue perfusion, and this is brought into stark reality in a number of diseases, such as hypertension, diabetes, and preeclampsia, in which endothelium-dependent vasodilator function is impaired. Prostacyclin and nitric oxide (NO) were the earliest identified endothelium-dependent vasodilators, but it was soon found that in many vascular beds, particularly in resistance vessels, vasorelaxation persisted when production of these autacoids was suppressed. This residual vasorelaxation was invariably associated with hyperpolarization of the vascular smooth muscle and the entity was dubbed “endothelium-derived hyperpolarizing factor” (EDHF). From the outset, the identity of EDHF and mechanism(s) mediating EDHF remained unresolved, and these can be different in the various vascular systems where resistance is primarily determined. The subject of vigorous debate. Nonetheless, that the “EDHF effect” is blocked by agents that target calcium-activated K+ channels is among the few aspects of EDHF on which there is apparent consensus. There was also the debate on the significance, or otherwise, of EDHF. Is it just the poor cousin of NO, stepping into the breach when the latter is compromised, especially under conditions of enhanced oxidative stress? Then a beacon of light shone out from Japan, with the demonstration that EDHF assumed greater importance as vessel diameter decreased. This was a key observation, supporting the importance of EDHF in the region of the vascular system where resistance is primarily determined. This was hotly followed by the revelation that EDHF may be targeted in diseases, diabetes,3–5 hypertension,6 and apolipoprotein E-deficient mice.7 Thus, EDHF, suggested to provide vasodilation when NO was compromised, was also under attack in disease. Despite its Cinderella status, investigation into the nature of EDHF has continued with breakneck intensity, with the conviction that EDHF has an important role in tissue perfusion and blood flow. The fact that the acronym has been retained to describe the NO- and prostacyclin-insensitive relaxation reflects the fact that the identity of and mechanism(s) mediating EDHF remain unresolved, and these can be different in the various vascular beds.

Small-conductance, calcium-activated K+ channels (KCa2.3, blocked by apamin) and intermediate-conductance, calcium-activated K+ channels (KCa3.1, blocked by charybdotoxin or TRAM-34) are located on the endothelial cells (ECs) and play a pivotal role in the smooth muscle cell (SMC) hyperpolarization that underpins EDHF. A hotly debated issue surrounding the nature of EDHF is whether the hyperpolarization generated in ECs by activation of these channels spreads directly to subjacent SMCs via myoendothelial gap junctions.8–9 Myoendothelial gap junctions arise predominantly on EC projections passing through holes in the internal elastic lamina to contact medial SMCs. An alternative view is that K+ exiting ECs activates inwardly rectifying K+ channels and Na+/K+/ATPase pumps to induce hyperpolarization secondarily in the SMCs, although their contribution to the EDHF current, recorded under voltage clamp, are minimal in guinea pig submucosal arterioles.10 It has been demonstrated previously that KCa3.1 channels are localized to the holes in the internal elastic lamina and are therefore on EC projections.11 In this issue of Circulation Research, Dora et al show that Na+/K+/ATPase pumps are also localized to EC projections, suggesting an intimate link between the pump and the KCa3.1 channel, and they hypothesize that this amplifies EC hyperpolarization.12 In arteries that had been treated with carbamoxolone to block gap junctions, the remaining TRAM-34-sensitive relaxation was significantly reduced by ouabain, consistent with previous observations that K+ released from KCa3.1 on EC projections activated Na+/K+/ATPase pumps located on SMC.10 However, in the new scheme, would not transfer of the amplified hyperpolarization component to EC be expected to be blocked in carbamoxolone, leaving the ouabain-sensitive component that results from extracellular K+ stimulation of SMC pumps? Results with ouabain must be interpreted with caution, because it also has uncoupling effects at gap junctions.14–15 Progress in defining EDHF mechanisms is hampered by the nonselective actions of currently available pharmacological tools.

The development of antibodies to proteins of interest (ion channels, connexins, etc) has provided valuable insights into their spatial distribution. An elegant study in rat small mesenteric arteries demonstrated that KCa2.3, colocalized with EC–EC connexons around the periphery of these cells, are clearly spatially separate from KCa3.1, present on EC projections.12 Dora et al suggest that KCa2.3 are also localized on EC projections, along with the KCa3.1 channels.13 The high level of sequence homology between KCa2.3 and KCa3.1 channels16 makes it wise to use more than one antibody source, preferably made to separate epitopes targeting the same channel. Consistent with previous reports, not all internal elastic lamina holes are associated with KCa2 expression, and this reflects the absence of myoendothelial gap junctions.
junctions from some holes. It is intriguing that close to 100% of holes appear to be associated with EC Na⁺/K⁺-ATPase pump expression in this article.

The scheme proposed by Dora et al. sets out the relationship between the important ion channels, gap junctions, and pumps involved in the generation of EC hyperpolarization and its transfer to the SMC in rat small mesenteric artery (Figure). It is envisaged that hyperpolarization resulting from KCa2.3 activation spreads through myoendothelial gap junctions. Thus, it is intriguing that, in unstimulated arteries at rest, the hyperpolarization recorded in SMCs is ≈20 mV in amplitude but in ECs, is <10 mV. Additional hyperpolarization generated in SMCs by Na⁺/K⁺-ATPase pump activity would be expected to flow back and be recorded in the ECs. Despite the comprehensive nature of the proposed scheme, it is clear that the nature and mechanisms of EDHF remain unresolved.

Another piece of the puzzle that needs to be incorporated into the overall scheme is the role of inositol trisphosphate–mediated Ca²⁺ release. In mouse mesenteric artery, Ledoux et al elegantly showed evidence for functional focal Ca²⁺ release at sites associated with myoendothelial gap junctions, where localization of endoplasmic reticulum and associated inositol trisphosphate receptors occur in EC projections in close proximity to myoendothelial gap junctions and KCa3.1. Membrane potential clearly plays a significant role in vascular tone in the smallest arteries/arterioles. Although NO and prostanoid can evoke hyperpolarization, their impact on membrane potential is modest, shifting this burden onto EDHF. EDHF is implicated in spreading vasodilation, important in recruiting vasodilation in larger arteries feeding blood to regions of greatest cellular activity. There is also the issue of the importance of EDHF in vivo. Deletion of either KCa2.3 or KCa3.1 results in elevated blood pressure. However, a significant volume of elegant data provides a serious challenge to the notion that direct current spread from ECs to SMCs via myoendothelial gap junctions operates in healthy arteries supplying skeletal muscles of laboratory species in vivo. A previous study by Ledoux et al and the present work by Dora et al. indicate that subtle, localized differences in calcium may have profound implications for rapid recruitment of endothelial KCa channels. Myoendothelial gap junctions and KCa channels are also involved. Thus, we must strive harder to resolve how the pieces of this tricky jigsaw puzzle fit together. These issues, together with the fate of EDHF in disease, render the gaps in our understanding of the nature of EDHF and how it operates unacceptable if we are serious about improving treatment of vascular disease.

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None.

References


Figure. At 2.5 mmol/L extracellular calcium, the EC calcium-sensing receptor is activated and KCa3.1 is nonconducting and not available. Increasing EC cytoplasmic calcium activates KCa2.3, hyperpolarizing (Vₘ) ECs, which is transmitted to SMCs via myoendothelial gap junctions. Opening of SMC voltage-gated calcium channels reduces extracellular calcium, removing calcium-sensing receptor activation and making KCa3.1 available. Potassium efflux through KCa3.1 stimulates local Na⁺/K⁺ pumps. The additional hyperpolarization resulting from the activity of both KCa3.1 and the pumps amplifies EC hyperpolarization and hence EDHF-mediated responses in SMCs.
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