The endothelial saga: the past, the present, the future

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Abstract Endothelium-dependent changes in vasomotor tone, whether evoked by vasoactive agents or physical forces, are recognized as essential for the local hemodynamic control in various normal and pathological circumstances. They are based on a complex signaling network within the vascular wall. In recent years, substantial efforts have been made to analyze how such signals are generated and used in the endothelium-dependent control of vascular smooth muscle. The underlying mechanisms vary with species, age, sex, hormonal status, vascular bed studied, caliber of the blood vessels, triggering stimuli, pre-existing vascular tone, oxidative stress, and pathology. Such aspects and many others will be addressed specifically by the authors contributing to this volume.

Keywords Endothelium · Nitric oxide · EDHF · KCa · TRP · Oxidative stress

The endothelial saga: the past

The endothelial saga started with Robert Furchgott [18, 61], who demonstrated that endothelial cells play an essential role in the relaxation evoked by acetylcholine in isolated arteries, which is mediated by activation of endothelial muscarinic receptors. His simple pharmacological experiments have revolutionized not only vascular pharmacology and physiology but science in general, as they lead to the discovery of the role of nitric oxide (NO) in biology [61]. Using “sandwich” bioassay preparations (a layering of arterial strips with and without endothelium whereby the contractile responses are measured only in the strip without endothelium), he demonstrated that the endothelium-dependence of the response to acetylcholine is due to the diffusion of a vasodilator substance from the endothelial cells to the vascular smooth muscle cells [18]. Having ruled out prostacyclin, which is produced by endothelial cells [41], he called the unknown mediator “endothelium-derived relaxing factor” (EDRF). The existence of endothelium-dependent responses was rapidly confirmed in different laboratories around the world [see 37]. More sophisticated superfusion-bioassay systems permitted to apply pharmacological inhibitors to either the endothelial cells or the effector vascular smooth muscle cells [e.g., 50]. The biological half-life of EDRF was found to be disappointingly brief (in the order of seconds), making identification by conventional chemical techniques impossible. Early pharmacological studies indicated that endothelial cells can generate several other signals leading to endothelium-dependent relaxations [8]. The latter multiple signals (Fig. 1) eventually became known as “endothelium-derived hyperpolarizing factor(s)” (EDHF), which play a prominent role in smaller arteries and resistance vessels [7, 15]. In addition, it soon became obvious that, in veins [9], and in
Fig. 1 EDRFs in 2009. Substances such as acetylcholine (ACh), bradykinin (BK), and substance P (SP), through the activation of M3 muscarinic, B2-bradykinin, and NK1-neurokinin receptor subtypes, respectively, and agents that increase intracellular calcium, such as the calcium ionophore A23187, release EDRFs. CaM calmodulin, COX cyclooxygenase, EET epoxyeicosatrienoic acid, IP3 inositol triphosphate, GC guanylate cyclase, NAPe N-acylphosphaticylethanolamine, Hyperpol hyperpolarization, NOS NO synthase, O2 superoxide anions, PG12 prostacyclin, P450 cytochrome P450 monoxygenase, R receptor, X putative EDHF synthase. SR141716 is an antagonist of the cannabinoid receptor subtype CB1. Glibenclamide (Glib) is a selective inhibitor of ATP-sensitive potassium channels (KATP). Tetraethylammonium (TEA) and tetrabutylammonium (TBA) are nonspecific inhibitors of potassium channels when used at high concentrations (>5 mM), while at lower concentrations (1–3 mM), these drugs are selective for calcium-activated potassium channels (KCa). I berotoxin (IBX) is a specific inhibitor of large conductance KCa (BKCa). Charybdotoxin (CTX) is an inhibitor of BKCa, intermediate conductance KCa (IKCa), and voltage-dependent potassium channels. Apamin is a specific inhibitor of small conductance KCa (SKCa). Barium (Ba2+), in the micromolar range, is a specific inhibitor of the inward rectifier potassium channel (Kir). GAP 27 is an 11-amino acid peptide possessing conserved sequence homology to a portion of the second extracellular loop of connexin. 18α-glycyrrhetinic acid (αGA), and heptanol are gap junction uncouplers (from Vanhoutte et al., 2009. By permission).
The use of NOS inhibitors in vivo rapidly lead to the conclusion that NO not only is a key player in vasomotor control but it affects almost every bodily function. The next breakthrough came when Salomon Snyder and his group isolated NOS from the brain [e.g., 6]. We now know that there are three isoforms of the enzyme: neuronal NOS (nNOS, NOS 1), inducible NOS (iNOS, NOS 2), and endothelial NOS (eNOS, NOS 3). Paul Huang and colleagues genetically engineered mice with deletion of the eNOS gene [25]. These animals have an increased arterial blood pressure, illustrating the role of NO in the control of cardiovascular homeostasis.

The endothelial saga: the present

The advent of genetically modified animals and of inhibitors of NOS permits the systematic exploration of the role of NO in vascular health and disease, considerably increasing our knowledge (Fig. 2). In a given blood vessel, the level of activity of eNOS and the amounts of endothelium-derived NO released are not constant. They can be upregulated by chronic increases in shear stress (exercise), hormones (estrogens), and diet (ω3-unsaturated fatty acids or polyphenols of red wine, green tea, and dark chocolate). The endothelial production of NO is reduced by high glucose (diabetes) and increased oxidative stress (hypertension) [see 3, 63]. NO not only affects the tone of vascular smooth muscle, but also inhibits platelet aggregation, in synergy with endothelium-derived prostacyclin [47], and the growth of the media [55]. It reduces the endothelial production of endothelin-1 [62] and of cyclooxygenase-derived EDCF [14]. NO inhibits the expression of endothelial adhesion molecules and, thus, the adhesion of platelets and white blood cells [47, 63]. It modulates angiogenesis [3, 68]. The signaling cascade, in particular, the role of Akt, in the phosphorylation that leads to activation of eNOS is unraveled [3, 16, 29, 33]. The original concept that the eNOS is a strictly Ca2+-dependent enzyme, and, thus, that endothelium-dependent relaxations rely entirely on an increase in intracellular Ca2+-concentration, has been challenged [3, 16]. Moreover, in vivo responses to acetylcholine in arterioles consist of two phases: (a) a rapidly conducted vasodilatation initiated by a local rise in endothelial Ca2+ but independent of endothelial Ca2+-signaling at remote sites and (b) a slower complementary dilatation associated with a Ca2+-wave that propagates along the endothelium [57]. In the mouse aorta, calcium-imaging shows that only some clusters of endothelial cells respond to acetylcholine, which represent only one third of the total number of cells, but this is enough for endothelium-dependent relaxation [4]. The importance of the caveolae for the activity of eNOS is now established [20, 40]. The formation of NO-metabolites constitutes a non-enzymatic source of activators of soluble guanylyl cyclase [38]. Beyond NO itself, derivatives such as nitroxyl (HNO) and nitrosothiols have also emerged as EDRFs and non-enzymatic source of activators of soluble guanylyl cyclase [38]. Beyond NO itself, derivatives such as nitroxyl (HNO) and nitrosothiols have also emerged as EDRFs and non-enzymatic source of activators of soluble guanylyl cyclase [38].
We now appreciate better the importance and the complexity of endothelium-dependent hyperpolarization in the local control of vascular tone [7]. Although EDHF has been considered to be of particular importance in smaller arteries, we have to recognize that its contribution to vasodilatation may be merely transient [22]. Nevertheless, coordinated increases in small artery diameter occur by means of flow-mediated vasodilatation (shear-stress-induced and NO-dependent) combined with the conducted vasodilatation resulting from electroronic propagation of hyperpolarization in the endothelium [56]. At the level of endothelial protrusions, functional cooperation ensures the EDHF-component of endothelium-dependent vasodilatation, which is mediated by K⁺ released from endothelium and involves endothelial KCa₂.3 and KCa₃.1, local interstitial Ca²⁺, Ca²⁺-sensing receptors co-localized with KCa₃.1 in caveolin-poor regions of endothelial cells, myo-endothelial gap junctions, and the Na/K pump and Kᵥ₂.1 of the vascular smooth muscle [11]. Experiments in dysgenic mice suggest that KCa₂.3 and KCa₃.1 have important but different contributions to endothelium-dependent vasodilatation and, thus, represent novel therapeutic targets for the treatment of hypertension [5, 66].

The Ca²⁺-dependent component of local vasodilatation obviously depends on Ca²⁺ influx into endothelial cells. One of the most attractive candidate influx pathways has been the store-operated Ca²⁺ entry (SOC), which could be mediated by transient receptor potential (TRP) channels [see 43 for a critical review]. SOC was indeed identified in endothelium [1, 13], but a direct relation to NO production and release is still under evaluation [4]. Non-store-operated channels seem to play a more important role in regulation of NO release [65]. The involvement of TRPV4-channels in flow-induced endothelium-dependent vasodilatation is now generally accepted [35, see also 44 for a review]; the mechanism requires an active CYP epoxygenase and channel translocation to the cell membrane, where it is associated with caveolin-1. Moreover, the expression of caveolin-1 is required for EDHF-related relaxation, by modulating the membrane location and activity of TRPV4 channels and connexins, which are both implicated at different steps in the EDHF-signaling pathway [53]. The TRPV4 channels of both endothelial and vascular smooth muscle cells are critically involved in endothelium-dependent vasodilatation of mesenteric arteries and in TRPV4-knockout mice the hypertension induced by NOS inhibition is greater than in wild-type animals [12].

### The endothelial saga: the future

Much remains to be learned about the precise regulation of NO release by endothelial cells and also about the consequences of its perturbation within the complex chain of events leading to the vascular dysfunction characteristic of hypertension, diabetes, and atherosclerosis [64]. We still do not completely understand the exact role of EDHF-mediated responses in physiology and pathology, as we are still unable to selectively interfere with them in vivo [7]. We still do not fully comprehend the importance of EDCFs in endothelial dysfunction [60]. Finally, we have to unravel the complex interactions between the different endothelium-derived signals. For example, in diabetic mice, hyperglycemia-induced changes in endothelial function are linked to COX2 and oxidative stress (enhanced NADPH oxidase and decreased SOD expression), uncoupling of eNOS, and changes in its expression and regulation, while EDHF-mediated vasodilatation can be maintained, but with a modified profile [10]. Whatever the future of endothelial research will yield, we should not forget that this extraordinary scientific saga started with the very simple pharmacological experiments of Robert Furchgott [18, 61], whose memory we honor in this special issue.

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### References

405 oxide and hydroxyl radical an inhibition by haemoglobin and
408 potential channels in endothelium: solving the calcium entry
409 puzzle? Endothelium 10:5–15
411 potential cation channels in disease. Physiol Rev 87:165–217
413 cells synthesize nitric oxide from L-arginine. Nature 333:664–666
415 enzyme implicated in the formation of nitric oxide by vascular
416 endothelial cells. Biochem Biophys Res Commun 158:348–352
418 oxide and cGMP in platelet adhesion to vascular endothelium.
419 Biochem Biophys Res Commun 148:1482–1489
421 inhibitor of nitric oxide formation from L-arginine attenuates
423 49. Rees DD, Palmer RMJ, Moncada S (1989) The role of
424 endothelium-derived nitric oxide in the regulation of blood
425 pressure. Proc Natl Acad Sci U S A 86:3375–3378
426 50. Rubanyi GM, Lorenz RR, Vanhouette PM (1985) Bioassay of
427 endothelium-derived relaxing factor(s). Inactivation by catechol-
430 release of endothelium-derived relaxing factor. Am J Physiol 250:
431 H1145–H1149
432 52. Rubanyi GM, Vanhouette PM (1986) Superoxide anions and
433 hyperoxia inactivate endothelium-derived relaxing factor(s). Am
434 J Physiol 250:H822–H827
436 Rodella LF, Vriens J, Nilius B, Feron O, Balligand JL, Despy C
437 (2008) Role of caveolar compartmentation in endothelium-derived
438 hyperpolarizing factor-mediated relaxation: Ca2+ signals and gap
439 junction function are regulated by caveolin in endothelial cells.
440 Circulation 117:1065–1074
441 54. Sankaranarayanan A, Raman G, Busch C, Schultz T, Zimin PI,
443 ylamine (SKA-31), a new activator of KCa2 and KCa3.1
444 potassium channels, potentiates the endothelium-derived hyper-
445 polarizing factor response and lowers blood pressure. Mol
446 Pharmacol 75:281–295
447 55. Scott-Burden T, Vanhouette PM (1993) The endothelium as a
448 regulator of vascular smooth muscle proliferation. Circulation 87:
449 V51–V55
451 Microcirculation 12:33–45
452 57. Tallini YN, Brekke JF, Shui B, Doran H, Hwang SM, Nakai J,
454 Ca2+ waves and arteriolar dilation in vivo: measurements in
455 Cx40BAC GCaMP2 transgenic mice. Circ Res 101:1300–1309
457 signaling in vascular cells—implications in cardiovascular
459 59. Vanhouette PM (1998) Endothelial dysfunction and inhibition of
460 converting enzyme. Eur Heart J 19:37–315
462 col Ther (in press)
463 61. Vanhouette PM (2009) How we learned to say NO. Arterioscler
464 Thromb Vasc Biol 29:1156–1160
466 277
467 63. Vanhouette PM, Shimokawa H, Tang EH, Feletou M (2009)
468 Endothelial dysfunction and vascular disease. Acta Physiol
469 196:193–222
470 64. Vanhouette PM, Tang EH (2008) Endothelium-dependent contrac-
471 tions: when a good guy turns bad! J Physiol 586:5295–5304
473 T, Merisseau C, Hammock BD, Fleming I, Busse R, Nilius B
474 (2005) Modulation of the Ca2+ permeable cation channel TRPV4
475 by cytochrome P450 epoxygenases in vascular endothelium. Circ
476 Res 97:908–915
478 Role of caveolae in endothelium-derived hyperpolarizing
479 factor-type dilations and conducted responses in the microcircu-
481 67. Wolin MS (2009) Reactive oxygen species and the control of
483 H549
484 68. Yu J, deMuinck ED, Zhuang Z, Drinane M, Kauser K, Rubanyi
485 GM, Qian HS, Murata T, Escalante B, Sessa WC (2005)
486 Endothelial nitric oxide synthase is critical for ischemic remod-
487 eling, mural cell recruitment, and blood flow reserve. Proc Natl
488 Acad Sci U S A 102:10999–11004
489
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