Novartis Award for Hypertension Research

Endothelium-Dependent Contractions in Hypertension
When Prostacyclin Becomes Ugly

Paul M. Vanhoutte

Most isolated arteries respond to shear stress and several vasodilator substances, as demonstrated first for acetylcholine, by releasing endothelium-derived relaxing factor (or nitric oxide [NO]), and various endothelium-derived hyperpolarizing signals. However, in certain blood vessels, when exposed to stretch, agonists such as thrombin, acetylcholine, and adenosine nucleotides (adenosine diphosphate [ADP] and adenosine triphosphate [ATP]) or calcium ionophores, the endothelium produces diffusible cyclooxygenase (COX)-derived vasoconstrictor prostanoids (endothelium-derived contracting factors [EDCF])6–11 Endothelial cells also produce vasoconstrictor peptides, in particular, endothelin-1.12 The attribution of a role to endothelin-1 in instantaneous changes in vascular tone has been made difficult by the almost insurmountable nature of the vasoconstriction caused by the peptide that can only be tempered by NO or calcitonin gene-related peptide.13,14 Likewise, the evidence linking acute release of endothelin-1 to constriction of arteries is still limited.15 Therefore, the present article focuses on the mechanisms leading to the production of endothelial COX-derived vasoconstrictors, in particular, in the rat aorta, which has been the standard preparation used by the author and his collaborators for the study of EDCF-mediated responses However, the occurrence of such EDCF-mediated responses can vary widely, depending on the species and the blood vessel studied. For example, they are prominent in canine veins but not arteries. In the mouse, endothelium-dependent contractions are more pronounced in the carotid artery than in the aorta. Further, in any given blood vessel, the production of endothelium-derived vasoconstrictor prostanoids is exacerbated by aging and disease, in particular, hypertension. The 2 isoforms of COX, COX-1, and COX-2, can contribute to the generation of EDCF depending on the species, the blood vessel studied, and the health conditions of the donor. Nonselective COX inhibitors (eg, indomethacin) abrogate endothelium-dependent contractions. Preferential inhibitors of COX-1, but not those of COX-2, prevent endothelium-dependent contractions in the SHR aorta. Although in that preparation, COX-1 is expressed in both endothelial and vascular smooth muscle cells, the gene encoding for this isomorph is overexpressed only in the endothelial cells of the SHR, and only the activation of endothelial COX contributes to the generation of EDCF. Endothelium-dependent contractions are absent in the aorta.
Endothelium-dependent contractions to acetylcholine are reduced partially by inhibitors of thromboxane A2 synthase. They contribute to EDCF-mediated responses elicited by these agonists. Likewise, PGE2 and PGF2a contribute to EDCF-mediated responses in the hamster aorta or in arteries of aging and diabetic rats. This contribution results from enhanced oxidative stress and the augmented generation of peroxynitrite, which inhibits PGI2 synthase and diverts arachidonic acid toward PGE2 and PGF2a syntheses.

The Amplifiers: Reactive Oxygen Species

The exaggerated production of reactive oxygen species (ROS) causes oxidative stress and is a hallmark of atherosclerosis, diabetes, and hypertension. In the canine basilar artery, superoxide anions mediate endothelium-dependent contractions. Although hydrogen peroxide can act as a vasodilator, it contributes to the stimulation by ROS of COX in vascular smooth muscle cells and thus can act either directly as EDCF or potentiate their response to endothelium-derived prostanoids. Superoxide anions also indirectly can amplify EDCF-mediated responses by reducing the bioavailability of NO. The production of ROS in endothelial cells is augmented during endothelium-dependent contractions to acetylcholine or A23187. Further, antioxidants reduce endothelium-dependent contractions, suggesting that ROS augment or even mediate part of the response.

To judge from results obtained in the rat pulmonary artery, the ROS-induced contraction involves the activation of protein kinase C in the vascular smooth muscle. In the rat aorta, ROS cause calcium sensitization, which is mediated by the activation of Rho and an increase in Rho kinase activity, which plays a key role in the response of the vascular smooth muscle to EDCF. In addition, ROS directly depolarize vascular smooth muscle cells by inhibiting various potassium channels.

The Intercellular Links: Gap Junctions

Endothelium-dependent contractions to acetylcholine are smaller in layered bioassay ("sandwich") preparations than in
intact rings, illustrating that the contact between endothelial and vascular smooth muscle cells is important in the genesis of EDCF-mediated responses. In bioassay preparations, the endothelium-derived prostanoids diffuse freely across the intercellular gap between the donor (containing endothelial cells) and the effector (without endothelium, responsible for the contraction), and cell-impermeable antioxidants reduce the response to acetylcholine, whereas they do not in intact rings in which intracellular antioxidants inhibit EDCF-mediated responses. Thus, ROS exert their facilitatory effect by either acting in the endothelial cells or being transported from the latter to the vascular smooth muscle cells via preferential channels not accessible to cell-impermeable antioxidants. One possible channel for linking the endothelial and vascular smooth muscle cells are the myoendothelial gap junctions. This interpretation is prompted by the observation that gap junction inhibitors reduce endothelium-dependent contractions to acetylcholine and the calcium ionophore A23187.

The Effectors: The TP Receptors

TP receptor antagonists abrogate endothelium-dependent contractions in mouse, rabbit, and rat arteries. In SHR aorta, the expression of the gene encoding for and the protein presence of TP receptors are comparable in the aortae of Wistar-Kyoto rats and SHR, but the contractions evoked by endoperoxides are larger in the latter, suggesting that this hyper-responsiveness contributes to the prominence of endothelium-dependent contractions in the hypertensive strain. Because the hyper-responsiveness is present already in young SHR, it thus is not a consequence of the chronic exposure of the endothelium to the high arterial blood pressure and constitutes a genetic platform leading to endothelial dysfunction. In addition, vascular smooth muscle cells of older Wistar-Kyoto rats (in which endothelium-dependent contractions appear progressively with aging) and of SHR no longer relax when exposed to PGI₂, despite an unchanged expression of IP receptors. It is unknown whether or not this lack of responsiveness of IP receptors initiates a positive feedback on the endothelial cells, leading to the abundant overexpression of PGI₂ synthase and the predominant release of PGI₂ by endothelial cells stimulated by acetylcholine or A23187. However, the large amounts of PGI₂ become important enough to bind with TP receptors (Figure 2). The contraction of the latter on TP receptor activation is attributable to the combination of an increased entry of Ca²⁺ resulting from the opening of both receptor-operated and voltage-gated Ca²⁺ channels and Rho kinase–mediated sensitization of the myofilaments. In turn, the binding of endoperoxides and PGI₂ to these receptors activates the downstream Rho kinase pathway, leading to the increased contractile activity of the vascular smooth muscle.

The Gatekeeper: NO

NO inhibits endothelium-dependent contractions, and thus inhibitors of NO synthases cause an immediate potentiation of EDCF-mediated responses. Further, previous exposure to endothelium-derived or exogenous NO results in long-term inhibition of endothelium-dependent contractions (Figure 1). Thus, one can predict that EDCF-mediated responses will become prominent, in particular, when the release of endothelium-derived NO is curtailed by aging or disease.

Hallmark of Vascular Disease

Endothelium-dependent contractions are exacerbated by aging and vascular disease. Thus, the blunted vasodilation to acetylcholine observed in the forearm of essential hypertensive patients is nearly normalized by indomethacin, indicating that COX-derived vasoconstrictor prostanoids contribute importantly to the abnormal endothelial response. The indomethacin-sensitive impairment of the response to muscarinic agonists is accentuated by aging.

Figure 2. Endothelium-dependent effects of acetylcholine in rat aorta. Left, Endothelium-dependent relaxations in normotensive rats. Right, COX-dependent, endothelium-dependent contractions to acetylcholine in SHR aorta. R indicates receptor; IP, PGI₂ receptor; TP, TP receptor; AA, arachidonic acid; S-18886 (terutroban), antagonist of TP receptors; M, muscarinic receptor; PGIS, prostacyclin synthase; PGH₂, endoperoxides; sGC, soluble guanylyl cyclase; AC, adenylyl cyclase; SR, sarcoplasmic reticulum; +, activation; -, inhibition; ?, unknown site of formation. (Modified from Vanhoutte et al, 2009.)
disease, this isoform of the enzyme can contribute in part to endothelium-dependent contractions. This is illustrated in the human by the observations that in patients with endothelial dysfunction, an improvement was observed with COX-2 inhibitors. The prominence of endothelium-dependent contractions observed in arteries of aging animals and humans, in particular, in subjects with essential/spontaneous hypertension, results from the progressive inability of the endothelial cells to generate NO with, as consequence, a facilitated release of EDCF.

Summary
Endothelial cells release not only NO and other relaxing factors but can generate COX-derived vasoconstrictor prostanooids and ROS, termed EDCF. The sequence of events (Figure 1) leading to endothelium-dependent contractions first requires an increase in endothelial Ca\(^{2+}\) concentration. This activates calcium-dependent \(\text{PLA}_2\), which provides the substrate for COX to yield the vasoconstrictor prostanooids involved in EDCF-mediated contractions. These include primarily endoperoxides and \(\text{PGI}_2\) and, to a lesser extent, thromboxane \(\text{A}_2\) and other prostaglandins. EDCF activates TP receptors of the vascular smooth muscle cells, which initiate the contractile process (Figure 1). When IP receptor signaling is impaired, \(\text{PGI}_2\) no longer causes dilatation but becomes a prominent endothelium-derived vasoconstrictor activating TP receptors (Figure 2). EDCF-mediated responses are exacerbated in aging normotensive, hypertensive, and diabetic animals. In hypertensive patients, EDCF contributes importantly to the endothelial dysfunction that accompanies aging, atherosclerosis, myocardial infarction, and essential hypertension.

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None.

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