Endothelium and Control of Vascular Function
State of the Art Lecture
Paul M. Vanhoutte

The response of isolated blood vessels to a variety of vasoactive agonists is modulated by the presence of endothelial cells. Indeed, these cells can release both dilator and constrictor substances. The major endothelium-derived relaxing factor may be nitric oxide, which activates soluble guanylate cyclase in the smooth muscle, although the endothelial cells also secrete an unidentified hyperpolarizing factor. Among the natural stimuli for the release of endothelium-derived relaxing factors are circulating hormones, platelet products, thrombin, shear stress, and certain autacoids. Endothelium-derived relaxing factors may contribute to the regulation of the release of atrial natriuretic factor and renin. The endothelial cells can also release constricting factors; among the likely candidates are superoxide anions or the peptide endothelin. In hypertensive blood vessels, the ability to release endothelium-derived relaxing factors but not endothelium-derived contracting factors is blunted. (Hypertension 1989;13:658–667)

After the initial report by Furchgott and Zawadzki1 of the obligatory role of endothelial cells in relaxations of isolated arteries of the rabbit to acetylcholine, vascular physiologists and pharmacologists have been forced to admit that the most intimal layer of the blood vessel wall, in addition to its role in metabolism, coagulation, and transport processes, possesses the ability to profoundly modulate the degree of contraction of the smooth muscle that surrounds it. It does so primarily by the release of short-lived vasodilator and vasoconstrictor substances, termed endothelium-derived relaxing factor (EDRF) and endothelium-derived contracting factor (EDCF), respectively. The ability of the endothelium to control the underlying vascular smooth muscle appears to be an ancestral property of these cells, emerging early in evolution (see Reference 2). This brief review summarizes the author’s current thinking on the questions concerning endothelium-dependent modulation—the answers to which could be of particular relevance in hypertension and its treatment.2-4

How Ubiquitous Are Endothelium-Dependent Responses to Acetylcholine?

In large arteries of most mammals including humans, acetylcholine causes endothelium-dependent relaxations (e.g., Figure 1; see also References 2–7). However, there are several unexplained exceptions; for example, unlike most arteries of these species, the basilar artery of the dog and the coronary artery of the pig do not exhibit endothelium-dependent relaxations to the cholinergic transmitter.8,9 The information at hand suggests that endothelium-dependent relaxations to acetylcholine occur at the level of small arteries (Figure 2) and resistance vessels (e.g., see References 10 and 11). Hence, together with the prejunctional (presynaptic) inhibitory effect of acetylcholine on adrenergic nerve endings, the endothelium-dependent effects of the cholinergic transmitter provide the explanation for the powerful vasodilator response that it causes in the intact organism (Figure 3).

The endothelium-dependent responsiveness to acetylcholine (or to other endothelium-dependent vasoactive agents) is not fixed, as it can be modulated chronically by hemodynamic variables or hormonal status.12,13 Chronic modulation by hemodynamic variables may explain why, in animals and in humans, the endothelium-dependent responses to acetylcholine (and other endothelium-dependent dilators) are considerably blunted in peripheral veins compared with those in arteries.14,15

What Are the Physiological Triggers for Endothelium-Dependent Relaxations?

Acetylcholine

Acetylcholine does not circulate in the blood. Most tissues, particularly those innervated by cho-
linergic neurons, contain acetylcholinesterase, an enzyme that is remarkably effective in rapidly destroying the cholinergic transmitter. Hence, it is not surprising that, to date, in large and mediumsized arteries, no evidence has been obtained that acetylcholine released from cholinergic nerves can reach the endothelial cells in amounts sufficient to evoke endothelium-dependent relaxations. Although certain endothelial cells may be able to synthesize acetylcholine, it is uncertain whether endothelium-derived acetylcholine contributes to vascular regulation.

**Catecholamines**

Substances circulating in the blood would be potential candidates for the triggering of endothelium-dependent responses. Circulating catecholamines (epinephrine and norepinephrine) are no exception. Indeed, endothelial cells, at least in large blood vessels carrying oxygenated blood, contain $\alpha_2$-adrenergic receptors that, when activated, can evoke endothelium-dependent relaxations of the underlying smooth muscle (Figure 4). It is likely that $\alpha_2$-adrenergic endothelium-dependent effects of the catecholamines contribute to the vasodilator effects that they have in the coronary circulation or the splanchnic bed. Theoretically, such endothelium-dependent responses could contribute to the vasodilator and hence the hypotensive effect of $\alpha_2$-adrenergic agonists like clonidine (Figure 5). In addition, the endothelium-dependent relaxation to $\alpha_2$-adrenergic agonists can be potentiated by $\beta$-adrenergic blocker carteolol (Figure 6), which implies that part of the (unexplained) vasodilator effect of $\beta$-blockers may be due to facilitation of endothelium-dependent relaxation of arterial smooth muscle.
Other Hormones

Vasopressin and oxytocin cause endothelium-dependent relaxations of the canine basilar artery without doing so in peripheral arteries; this implies that activation of endothelial vasopressin receptors may contribute to the preferential perfusion of the brain during acute volume depletion (Figure 7).8,22

Platelets, Platelet-Derived Products, and Thrombin

When isolated blood vessels are exposed to aggregating platelets, they contract (constrict) vigorously, mainly because their smooth muscle reacts to the direct activating effect of 5-hydroxytryptamine (serotonin) and thromboxane A2 released from the platelets. However, if platelet aggregation is induced in the vicinity of an isolated coronary artery that contains endothelial cells, the ensuing contraction is very limited; if the aggregation is initiated when the vascular smooth muscle is already activated, it causes further contraction in arteries without endothelium but profound relaxations in arteries with endothelium (Figure 8).23-24 The platelet-induced endothelium-dependent relaxations result from the response of the endothelial cells to adenine nucleotides (adenosine 5'-diphosphate [ADP] and adenosine 5'-triphosphate [ATP]) and serotonin (Figure 9).23-25 In the dog, platelet-activating factor contributes little to the platelet-induced responses.26

The ability of the endothelium to respond to intraluminal platelet products and to relax the underlying smooth muscle27 is only part of the role it plays in trying to prevent obstruction of blood flow in normal blood vessels (see below). Its role may be reinforced whenever the coagulation cascade is activated, with the resulting formation of thrombin, a potent trigger for endothelium-dependent relaxation in most blood vessels (Figure 10).8,28-29

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**FIGURE 3.** Sketch showing summary of effects leading to vasodilatation evoked by acetylcholine (ACh) in intact organism. At prejunctional (presynaptic) level, cholinergic neurotransmitter acts on M2-muscarinic receptors, which inhibit release of norepinephrine (NE), thus leading to reduced sympathetic vasoconstriction. If present at level of endothelial cells (e.g., during intra-arterial administration), ACh will cause release of endothelium-derived hyperpolarizing factor (EDHF) and of endothelium-derived relaxing factor (EDRF) by acting on endothelial M1- and M2-muscarinic receptors, respectively. ATP, adenosine triphosphate; EJP, excitatory junction potentials generated by ATP7, NE?

**FIGURE 4.** Sketch showing formation of vasoactive factor(s) by vascular endothelium. Various substances may, by activation of specific receptors on endothelial cells, evoke release of relaxing factor(s) (EDRF; nitric oxide [NO]) that, in turn, causes relaxation of arterial vessels. ACh, acetylcholine; M, muscarinic receptors; H2, histaminergic receptors; AVP, arginine vasopressin; VP1, vasopressinergic receptors; P1, purinergic receptors; A, adrenaline (epinephrine); NA, noradrenaline (norepinephrine); a2, a2-adrenergic receptor; AA, arachidonic acid; ADP, adenosine diphosphate; MAO, monoamine oxidase; 5-HT, 5-hydroxytryptamine (serotonin); S1, serotonergic receptors; and T, thrombin receptors.
**Bradykinin**

Bradykinin causes endothelium-dependent relaxations in a variety of isolated arteries. It is not surprising that, in the presence of a concentration of the peptide that does not evoke measurable endothelium-dependent responses per se, the inhibitor of converting enzyme trandolaprilat causes marked endothelium-dependent relaxation (Figure 11). This then implies that the (unexplained) vasodilator effect of the inhibitors of converting enzyme may be related to the unmasking of endothelium-dependent dilatations to endogenous bradykinin.

**Shear Stress**

It has been known for decades that, when flow increases through large arteries, dilatation ensues ("flow-induced vasodilatation"). It is now established that this phenomenon is endothelium dependent and can be attributed to the release of EDRF (see below) caused by an increase in shear stress.

**Figure 5.** Sketch showing that α2-adrenergic agonists can reduce peripheral resistance by acting on α2-adrenergic receptors located in brainstem (resulting in reduced sympathetic outflow), on peripheral adrenergic nerve endings (resulting in reduced release of norepinephrine [NE]), or on endothelial cells (resulting in augmented release of endothelium-derived relaxing factor (EDRF). NE released from adrenergic nerve terminal activates α1- and α2-adrenergic receptors on smooth muscle cells. Reprinted from Vanhoutte and Miller with permission.

**Figure 6.** Bar graph of augmentation by carteolol of relaxation (%) (which is endothelium-dependent) of canine femoral arteries with endothelium evoked by selective α2-adrenergic agonist UK 14,304. The arteries were contracted with prostaglandin F2α. Data are from Janczewski et al. Reprinted from Vanhoutte and Miller with permission.

**Figure 7.** Sketch showing the selective endothelium-dependent relaxing effect of vasopressin on cerebral (and to a lesser extent coronary) blood vessels. Combined with constriction of systemic blood vessels that it causes, it could favor redistribution of blood flow to cerebral circulation. Reprinted from Vanhoutte et al with permission.
Whether this is due secondarily to the release of autacoids (acetylcholine, ATP, serotonin, or substance P) by certain endothelial cells affecting their immediate neighbors or to direct activation of all endothelial cells by shear stress is uncertain.

**How Do the Endothelial Cells Control the Underlying Vascular Smooth Muscle?**

**Nitric Oxide**

In view of current physiological knowledge, there are only two major possible mechanisms by which the endothelium could act as a modulator of the responsiveness of the medial smooth muscle. The first one would be cell-to-cell contacts with induction of electrical signals. Although this remains a distinct possibility at the microcirculatory level where the attachment of endothelial cells to vascular smooth muscle is particularly intimate, it is now established that, in large blood vessels, the message from the endothelium consists of a potent but short-lived substance (EDRF). Cultured endothelial cells maintain the ability to release the vasodilator mediator. It had been known for several years that relaxations by EDRF are accompanied by an accumulation of cyclic guanosine 5'-monophosphate in the vascular smooth muscle. This cellular action of EDRF is similar to that exerted by nitric oxide (NO), the final mediator of the relaxing response to the nitrosovasodilators. NO shares with EDRF the property to be exquisitely sensitive to destruction by superoxide anion. Thus, it has been proposed that EDRF may be nothing other than NO (Figure 12). This interpretation was confirmed by chemical measurements of the release of NO from cultured endothelial cells; in those...
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EDRF and EDCF 663

Control
Trandolapril, -log M
9 
8 
7 
6

In the presence of 10^-9 M bradykinin

PGF 2α
2x10^-6 M

FIGURE 11. Line plots of isometric tension (g) recordings in two rings of canine femoral arteries (with endothelium) contracted with prostaglandin F2α (PGF2α) in absence (upper trace) and presence (lower trace) of bradykinin (10^-9 M). Trandolapril (an inhibitor of angiotensin converting enzyme, -log M) caused relaxation only in presence of bradykinin. Experiments were performed in presence of indomethacin to exclude prostanooid formation (M.J. Vidal-Ragout and P.M. Vanhoutte, unpublished observations).

experiments, the biologic and physiochemical properties of NO and EDRF were indistinguishable. It seems probable that NO originates from the metabolites of L-arginine. Whether NO is released as such or is bound to a precursor molecule remains a matter of debate.

Is Nitric Oxide the Only Answer?

Early in the description of endothelium-dependent relaxations, it appeared plausible that the endothelium could release more than one relaxing factor. It is now established that, in addition to NO (which does not alter the membrane potential of smooth muscle cells), at least certain endothelial cells release a distinct factor causing hyperpolarization of the underlying vascular smooth muscle; this factor has been called endothelium-derived hyperpolarizing factor (EDHF) (Figure 12). Pharmacological analysis of the responses to acetylcholine has demonstrated that the muscarinic receptor mediating the release of EDHF differs from that responsible for the release of EDRF (Figure 3). Experiments in isolated canine blood vessels and in cultured porcine endothelial cells (Figure 13) (U. Hoeffner and P.M. Vanhoutte, unpublished observations) demonstrated also that endothelial cells can release at least two distinct EDRFs. Interestingly, ouabain inhibits the action of one factor on vascular smooth muscle and the release of the other (which most likely is NO) (Figure 13). These observations may have implications in the understanding of the vasoconstrictor effects of endogenous inhibitors of Na⁺,K⁺ exchanges if these play a role in the genesis of certain types of hypertension.

In addition, the endothelium can produce adenosine, ammonia, and prostacyclin, all of which can cause relaxation of vascular smooth muscle. However, although all these substances can be considered as EDRFs, none of them cause hyperpolarization of canine arterial smooth muscle (K. Komori and P.M. Vanhoutte, unpublished observations).

Does the Endothelium Release Contracting Factors?

While investigating the responses of arteries and veins to endothelium-dependent relaxing agents, it became apparent that in certain blood vessels,
certain of these agents caused endothelium-dependent contractions rather than relaxations.14 Two types of endothelium-dependent contractions have been identified. In peripheral veins and cerebral arteries, contractions evoked by arachidonic acid, the Ca\(^{2+}\) ionophore A23187, and even acetylcholine appear to require the presence of the endothelium and the activity of cyclooxygenase.50,51 A likely candidate as mediator of this type of endothelium-dependent contraction is superoxide anion (Figure 14).52 In a number of isolated arteries and veins, endothelium-dependent contractions can be evoked by anoxia14,53-55; the contractions can be inhibited by Ca\(^{2+}\)-antagonists, presumably because of their action on vascular smooth muscle rather than on the endothelial cells.55 Although the mediator of the endothelium-dependent hypoxic contraction is unknown, the possibility exists that the hypoxic endothelial cells may release a vasoconstrictor peptide like endothelin (Figure 14).52,56,57

Does the Endothelium Influence Cells Other Than Vascular Smooth Muscle?

EDRF and NO inhibit the adhesion and the aggregation of platelets; their antiplatelet activity is considerably augmented in the presence of prostacyclin and vice versa.58-62 The synergistic action of prostacyclin and EDRF at the interface between the plasma and the blood vessel wall probably contributes substantially to the antiaggregating properties of the endothelium (Figure 9).

The endothelial cells, which possess many hormonal receptors with unknown function, also may constitute a link in the feedback loops controlling hormonal secretion. This is suggested by the observations that inhibitors of endothelium-dependent relaxations augment the basal release of atrial natriuretic peptide from the rat atrium (C. Sanchez-Ferrer and P.M. Vanhoutte, unpublished observations). EDRF markedly inhibits the basal release of renin (Figure 15), suggesting that the renal endothelium

![Figure 14](https://hyper.ahajournals.org/)

**Figure 14.** Sketch showing release of two endothelium-derived contracting factors (EDCF) by different stimuli. One factor may be endothelin and the other superoxide anions (\(\cdot\)O\(_2\)\(^{-}\)). A23187, calcium ionophore. Reprinted from Vanhoutte and Katusic\(^{52}\) with permission.

![Figure 15](https://hyper.ahajournals.org/)

**Figure 15.** Bar graph showing that when release of renin (ng/ml/hr) by canine cortical slices is measured during superfusion with physiological salt solution, acetylcholine (ACh) has no direct effect (left panel). However, if superfusate flows through a carotid artery with endothelium and if endothelial cells are exposed to acetylcholine (right panel), resulting release of endothelium-derived relaxing factor causes marked inhibition of renin release. *\(p<0.05\). Reprinted from Vidal-Ragout et al\(^{63}\) with permission.
may be a regulator of the release of the hormone and possibly constitutes the intrarenal baroreceptor that links increases in shear stress in the afferent arterioles with the deactivation of the secretory cells of the juxtaglomerular apparatus.63

What Is the Relevance for Hypertension?

Most authors agree that, in a variety of experimental models of hypertension, the responsiveness of isolated large arteries to acetylcholine and other endothelium-dependent relaxing agents is reduced (Figure 16; e.g., References 64–68). A similar conclusion has been reached for small arteries and resistance vessels.69 In salt-induced hypertension, the reduced endothelium-dependent relaxations are due to the combination of a diminished release of EDRF and a lesser sensitivity of the vascular smooth muscle to the relaxing effect of the factor (Figure 17).68 In that type of hypertension, a normal endothelium-dependent responsiveness can be restored by appropriate antihypertensive treatment or by dietary adjustments in potassium intake.70,71

In the spontaneously hypertensive rat, the reduction in endothelium-dependent relaxation cannot be due to a reduced release of EDRF but to the liberation of a constricting factor by the endothelial cells (Figure 18).67 The release of the factor involved requires the activation of cyclooxygenase (Figure 17).67 Higher

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**FIGURE 16.** Plot showing relaxations (%) to acetylcholine (−log M) are blunted in aorta of salt-sensitive Dahl rat. Reprinted from Lüscher et al68 with permission.

**FIGURE 17.** Sketch summarizing changes in endothelium-dependent responsiveness occurring in hypertension. 5-HT, 5-hydroxytryptamine (serotonin); ADP, adenosine diphosphate; ACh, acetylcholine; NE, norepinephrine; M, muscarinic receptors; P1, purinergic receptors; S1, serotonergic receptors; T, thrombin receptors; α2, α2-adrenergic receptors; EDRF, endothelium-derived relaxing factor; EDCF, endothelium-derived contracting factor; cGMP, cyclic guanosine monophosphate.
concentrations of serotonin also cause endothelial contractions in the aorta of the spontaneously hypertensive rat. Thus, in hypertension and in other cardiovascular diseases, the ability of the endothelium to release EDRFs is blunted, while, if anything, its ability to release EDCFs is augmented (Figure 17). It is tempting to assume that this imbalance may contribute to the increase in peripheral resistance characteristic of the disease.

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