Endothelium-Derived Relaxing Factor and Coronary Vasospasm

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The vascular endothelium is the monolayer of squamous cells in direct contact with the circulating blood. Since the first report in 1980 by Furchgott and Zawadzki of the endothelium-dependency of the relaxation to acetylcholine in isolated arteries, an overwhelming number of findings has indicated that this most intimal layer of cells importantly modulates the degree of contraction of the underlying vascular smooth muscle. It does so by liberating vasoactive substances, such as prostacyclin, a nonprostanoid endothelium-derived relaxing factor (EDRF), a hyperpolarizing substance (endothelium-derived hyperpolarizing factor, EDHF), and two constrictor substances (endothelium-derived contracting factors, EDCFs) (Figure 1). More and more data are emerging suggesting that alterations in endothelium-dependent responsiveness of vascular smooth muscle are involved in the pathogenesis of several cardiovascular diseases (see Vanhoutte, for reviews).

Coronary vasospasm plays an important pathogenetic role, not only in variant angina but also in a wide spectrum of ischemic heart disease. Because coronary vasospasm can be provoked by several stimuli with different mechanisms of action that do not induce a pathologic response in normal subjects, a local nonspecific hypersensitivity of the coronary artery appears to be involved. This interpretation is in agreement with the clinical observations that coronary vasospasm almost invariably occurs at atherosclerotic portions, although the extent of the coronary artery narrowing varies widely. Among the possible vascular components contributing to the spasm, the endothelium may play an important role. This brief review describes the current status of experimental and clinical studies concerning the possible role of EDRFs under normal conditions and in the pathogenesis of coronary vasospasm.

Physiology of Endothelium-Derived Relaxing Factors

Nature of Endothelium-Derived Relaxing Factor

EDRF is a diffusible vasodilator with a very short half-life (only seconds) in oxygenated buffer solution. It is inactivated by superoxide anions, and its half-life can be prolonged by superoxide dismutase, a scavenger of the oxygen-derived free radical; this indicates that EDRF is an oxidized substance and that the oxidized state is essential for its biologic activity.

In 1986, during an international symposium on "Mechanisms of Vasodilatation," Furchgott and Ignarro et al proposed that nitric oxide (the final messenger in the dilator action of the nitrovasodilators) may be EDRF. Their proposals were based on the facts that biologic (e.g., half-life) and pharmacologic (e.g., modes of inhibition) properties of EDRF are identical to those of nitric oxide. Subsequently, the perfusate from cultured porcine aortic endothelial cells was shown to contain nitric oxide in amounts that account for the biologic activity of EDRF. The nitric oxide formed by cultured porcine aortic endothelial cells probably is synthesized from L-arginine. It is still uncertain whether nitric oxide is secreted as such (NO) by the endothelial cells or bound to a carrier molecule (R-NO) that dissociates at the cell membrane of the vascular smooth muscle.

Other investigators have reported that EDRF and nitric oxide exhibit different relaxing effects among different smooth muscles and that nitric oxide does not necessarily explain endothelium-dependent relaxations in all preparations. As a matter of fact, canine arteries and cultured porcine endothelial cells release at least one EDRF that is not nitric oxide.

Production and Action of Endothelium-Derived Relaxing Factor

Although the EDRF identified as nitric oxide clearly causes relaxation of the underlying smooth muscle by activation of soluble guanylate cyclase leading to cyclic GMP-dependent protein phosphorylation, little is known about the mechanisms of its production (synthesis and release) by the endothelium. The production of EDRF depends on extracellular calcium, although the activation of voltage-operated Ca++ channels is not involved. Hence, Ca++ antagonists do not prevent the release of EDRF but rather act synergistically with it to relax vascular smooth muscle. In porcine coro-
nary arteries, pertussis toxin, an inhibitor of several G proteins (mainly the G, protein), significantly inhibits endothelium-dependent relaxations to serotonin and UK 14304 (a selective α2-adrenergic agonist) but not those to ADP, bradykinin, or the calcium ionophore A23187.31 The toxin does not inhibit direct, endothelium-independent relaxations to nitric oxide or sodium nitroprusside (these agents activate soluble guanylate cyclase of vascular smooth muscle as does EDRF20). These results indicate that there are at least two cellular pathways leading to the production of EDRF; one of the pathways involves a pertussis toxin–sensitive G protein (probably G, protein).

EDRF and prostacyclin can interact synergistically to cause relaxation because there are different mechanisms of action underlying the vasodilatation that they cause; elevation of cyclic GMP evoked by EDRF inhibits Ca2+ release from intracellular storage sites and Ca2+ influx through receptor-operated channels,32 whereas the elevation of cyclic AMP induced by prostacyclin stimulates the phosphorylation of myosin light chain kinase.33 In addition, in the porcine coronary artery, prostacyclin causes the release of, or protects, the released EDRF.34 Hence, although both EDRF and prostacyclin have a rather short half-life, their interactions and mutual amplification must augment their relaxing effect on vascular smooth muscle (Figure 2).

**Physiologic Role of Endothelium-Derived Relaxing Factor**

EDRF is released under basal conditions and upon stimulation.2-4,35 Among the many physiologic stimuli that can elicit the release of EDRF are platelet products, thrombin, hormones, neurotrans-
mittens, local autacoids, changes in flow (shear stress), and changes in oxygen tension (Figure 1). In addition, EDRF itself can inhibit adhesion and aggregation of platelets. Local release of EDRF must contribute to local responses evoked by histamine (inflammatory response), bradykinin (hyperemia of exocrine glands), and substance P (vasodilatation accompanying axon reflexes) and to the vasodilator properties of catecholamines and vasoressin in certain vascular beds. The release of EDRF in response to variations in shear stress also plays a pivotal role in the changes in diameter of large arteries in response to increases in blood flow (flow-induced vasodilatation); at the microcirculatory level, it may help to control the distribution of blood flow among arteriolar branches.

However, the inhibitory effect of EDRF on platelet-induced contractions and platelet aggregation are particularly important in the large coronary arteries.

**Inhibition of Platelet-Induced Contractions.**
Endothelium-dependent relaxations to aggregating platelets have been observed in isolated coronary arteries in dogs, pigs, and recently in humans. Endothelium-dependent responses to aggregating platelets are the global expression of the divergent reaction (relaxations and contractions) to several released platelet products and their interactions (Figure 2). There are species and regional differences in the mechanisms of the platelet-induced responses. For instance, platelet-induced release of EDRF is mediated by both serotoninergic and purinergic (ADP) receptors on the endothelium in porcine coronary and peripheral arteries but mainly by endothelial purinergic receptors in porcine basilar arteries and canine coronary arteries. In isolated human coronary arteries, it remains to be determined whether or not platelet-derived serotonin contributes to the endothelium-dependent relaxation. Platelet-induced contractions of vascular smooth muscle are mediated by serotoninergic receptors in porcine coronary and peripheral arteries and by serotoninergic receptors in porcine basilar and canine coronary arteries. The contribution of platelet-derived thromboxanes to platelet-induced contractions is minimal, at least in porcine arteries. Interestingly, in porcine pulmonary arteries, the platelet-induced contractions are mediated mainly by histamine, which acts on H₁-histaminergic recep-

**Figure 2.** Illustration of several effects of endothelium-derived relaxing factor (EDRF) during platelet aggregation; inhibition of platelet adhesion and aggregation and of platelet-induced contraction. Endothelium-dependent relaxations to aggregating platelets are achieved mainly by adenine nucleotides (ADP and ATP) and serotonin (5-HT), whereas direct, endothelium-independent contractions are achieved mainly by serotonin (5-HT) and thromboxanes (TBA₂), depending on the species and vascular beds tested. Under normal conditions in the presence of intact endothelium, those relaxations and contractions may be well balanced. EDRF and prostacyclin could act synergistically (arrowheads) against platelet aggregation and platelet-induced contractions. +, facilitation; −, inhibition. MAO, monoamine oxidase; PGI₂, prostacyclin.
tors of the vascular smooth muscle, with little contribution of serotonin or thromboxanes.\textsuperscript{51} These results indicate that not only between different, but even in the same species, different receptor mechanisms underlie platelet-induced responses in different vascular beds. Among the porcine arteries, endothelium-dependent relaxations to aggregating platelets are most pronounced in the coronary artery\textsuperscript{45}; therefore, in this blood vessel, the platelet-induced contractions are prevented most effectively by the presence of endothelium.\textsuperscript{44}

\textbf{Inhibition of platelet aggregation.} EDRF inhibits platelet aggregation\textsuperscript{36–39} and platelet adhesion in vitro.\textsuperscript{52} Both EDRF and nitrovasodilators (e.g., sodium nitroprusside and nitroglycerin) have an antiaggregatory effect by activating soluble guanylate cyclase, elevating the levels of cyclic GMP in the platelets, and thus reducing cytosolic free Ca\textsuperscript{2+}. EDRF and prostacyclin interact synergistically to inhibit platelet aggregation\textsuperscript{53} (Figure 2). Thus, subthreshold concentrations of EDRF and prostacyclin markedly inhibit platelet aggregation when given together.\textsuperscript{53} This interaction must contribute in a major way to the antiaggregatory properties of the endothelial surface.

\textit{Cod-liver oil}. Dietary fish oil (cod-liver oil) markedly augments the production of EDRF in porcine coronary arteries, thus augmenting endothelium-dependent relaxations\textsuperscript{54} and probably the antiaggregatory properties of this blood vessel. Eicosapentaenoid acid contained in the fish oil probably plays the major role in the augmented production of EDRF.\textsuperscript{55} This augmentation is also observed at the coronary microvessel level.\textsuperscript{56} Dietary fish oil also improves the production of EDRF in atherosclerotic porcine coronary arteries together with suppression of atherosclerosis, which must contribute to the antiatherogenic effects of fish oils.\textsuperscript{57}

\textbf{Endothelium-Derived Hyperpolarizing Factor}

The endothelium of certain blood vessels produces a diffusible factor (EDHF), which transiently hyperpolarizes the cell membrane of the vascular smooth muscle\textsuperscript{5,58,59} (Figure 1). EDHF may contribute in part to relaxation, especially in the initial phase of the acetylcholine-induced endothelium-dependent relaxation, although most of the relaxation is achieved by EDRF.\textsuperscript{60}

\textbf{Coronary Vasospasm and Endothelium-Dependent Relaxing Factors}

\textit{Acute Endothelial Injury In Vivo}

Acute endothelial injury by a balloon-tipped catheter causes enhanced vasoconstriction in response to serotonin in canine coronary arteries.\textsuperscript{61,62} This enhanced responsiveness can be explained by loss of the inhibitory effects of the endothelium and the resultant expression of the direct activation of the smooth muscle by the monoamine. There is a positive correlation between the extent of platelet deposition and the extent of vasoconstriction shortly after balloon angioplasty in porcine carotid arteries.\textsuperscript{63} In the isolated rabbit heart, addition of activated platelets to the perfusion solution causes constriction of epicardial coronary arteries only after the endothelium has been removed.\textsuperscript{64} These data indicate that the endothelium exerts its inhibitory actions on platelet aggregation and platelet-induced contraction under normal conditions but that endothelial injury results in platelet aggregation and contraction by platelet products.

\textbf{Importance of Atherosclerotic Changes}

Augmented vasoconstrictor responses to serotonin and other vasoactive substances have been reported in atherosclerotic blood vessels of animals and humans.\textsuperscript{65–67} Endothelium-dependent relaxations to acetylcholine are impaired in a variety of atherosclerotic blood vessels,\textsuperscript{68–70} including the human coronary artery.\textsuperscript{68} This impairment is due to a reduced production of EDRF by the atherosclerotic endothelium (endothelial dysfunction),\textsuperscript{71,72} whereas the ability of the vascular smooth muscle to relax to nitrovasodilators (e.g., sodium nitroprusside) is minimally affected. The thickened intima with lipid deposition (atheroma) may not play a major role as a distance barrier against the diffusion of EDRF because endothelium-dependent relaxations are restored after regression of the atherosclerosis despite the persistence of significant intimal thickening.\textsuperscript{73} The reduced production of EDRF at atherosclerotic sites probably is associated with adhesion and aggregation of platelets and adhesion and penetration of white blood cells, initiating the vicious cycle of the atherosclerotic process.\textsuperscript{74}

Although endothelial injury or dysfunction could play a major role in the pathogenesis of coronary vasospasm, other pathogenetic factors should be considered, including hypersensitivity of the smooth muscle (e.g., increased receptor density), increased density of vasa vasorum, formation of vasoconstrictor mitogens by platelets and inflammatory cells, generation of free radicals by inflammatory cells, and local dysfunction of the autonomic nerves.\textsuperscript{75}

\textbf{The Porcine Model of Coronary Vasospasm}

Based on the clinical and experimental findings of the intimate relation between coronary vasospasm and atherosclerosis, a porcine model of coronary atherosclerosis was developed.\textsuperscript{76} In this model with miniature pigs, the combination of balloon removal of the endothelium and a high-cholesterol diet induced selective coronary atherosclerotic lesions; coronary vasospasm associated with regional myocardial ischemia could be repeatedly provoked with histamine or serotonin.\textsuperscript{76,77} Ergonovine also caused hyperconstriction of the spastic segment, but vasoconstrictor responses to phenylephrine (a selective \(\alpha\)-adrenergic agonist) were not enhanced.\textsuperscript{77} In similar experiments in the dog, hyperconstriction to ergonovine was noted, but the vasoconstriction was
not enough to induce myocardial ischemia. They observed a close correlation between the localization of the spastic sites and the histologic demonstration of atherosclerotic lesions, although the lesions could not be detected angiographically. The atheromatous lesions were of the eccentric type and were covered by regenerated endothelium. Thus, the coronary vasospasm observed in this animal model has many similarities with that occurring in humans.

In this porcine model of coronary vasospasm, vasoconstrictor responses to STA\textsubscript{2} (a thromboxane A\textsubscript{2} analogue) were not enhanced in the spastic sites, and prostacyclin infusion had no preventative effect on the spasm, suggesting that an imbalance between thromboxane A\textsubscript{2} and prostacyclin alone cannot explain the occurrence of the spasm. The spasm could also be provoked 3 months after balloon removal of the endothelium without high-cholesterol feeding, indicating that endothelial injury and subsequent tissue repair (including endothelial regeneration) may be more important than hypercholesterolemia alone. Identical coronary artery spasm observed in vivo can be provoked in an intact isolated heart perfused with Krebs-Ringer solution, showing the importance of local hypersensitivity and justifying the in vitro approach to study the pathogenesis of the spasm. In this model, endothelium-dependent relaxations to serotonin were impaired, whereas the responsiveness of the smooth muscle was slightly but significantly enhanced to histamine but not to serotonin or potassium chloride.

The endothelium probably plays a pivotal role in the abnormal responses of the spastic sites. Indeed, 4 weeks after denudation of the endothelium and despite complete endothelial regeneration, the endothelium-dependent relaxations to serotonin, the \( \alpha_2 \)-adrenergic agonist UK 14304, and aggregating platelets are impaired while those to ADP, bradykinin, and the calcium ionophore A23187 are unaltered. At this stage, serotonin and aggregating platelets caused coronary vasospasm with myocardial ischemia and coronary hyperconstriction without myocardial ischemia, respectively, in vivo. Thus, the regenerated endothelium is dysfunctional and may play an important role in the pathogenesis of coronary vasospasm. Endothelium-dependent relaxations to serotonin, UK 14304, and aggregating platelets remained impaired up to 6 months after endothelium removal; relaxations to ADP and bradykinin but not to A23187 were also impaired at later stages, indicating that inhibitory effects of the endothelium progressively worsen after regeneration rather than recover with time and that the endothelium-dependent relaxation to aggregating platelets is impaired importantly at the early stage of atherosclerosis. The inhibitory effects of pertussis toxin are reduced significantly in porcine coronary arteries with regenerated endothelium. Thus, a dysfunction of a pertussis toxin-sensitive \( G \) protein (possibly the \( G \) protein) may contribute to the dysfunction of regenerated endothelium, particularly in the early stage of atherosclerosis. These findings in the pig seem to be clinically relevant because endothelial turnover is accelerated markedly in hypertension, hyperlipidemia, and diabetes mellitus because of continuous endothelial injury; the vasoconstrictor responses to serotonin are enhanced under those pathologic conditions.

In porcine coronary arteries, endothelium-dependent relaxations to serotonin and ADP and hence those to aggregating platelets are impaired by chronic hypercholesterolemia; they deteriorate further in atherosclerosis (Figure 3). Eventually, aggregating platelets cause large contractions even in the presence of the atherosclerotic endothelium (Figure 4). The impairment of the relaxations and
the enhancement of the contractions are mainly due to the reduced production of EDRF (both under basal conditions and upon stimulation) because the ability of the vascular smooth muscle to relax or contract is not significantly altered (Figure 5). The inhibitory effect of pertussis toxin on endothelium-dependent relaxations to serotoninergic and α²-adrenergic activation is significantly reduced in hypercholesterolemic and is nearly abolished in atherosclerotic coronary arteries, suggesting that the function of a pertussis toxin-sensitive G protein is significantly impaired in hypercholesterolemia (probably due to increased endothelial turnover) and almost absent in atherosclerosis. This then explains in part why vasoconstrictor responses to agents with combined direct-constricting and endothelium-dependent relaxing effects are enhanced in atherosclerotic coronary arteries.

Ergonovine causes endothelium-dependent relaxations in the rabbit aorta and in the porcine coronary artery. In the latter, the relaxations were mediated by endothelial 5-HT₁-serotonergic and α₂-adrenergic receptors and were sensitive to pertussis toxin. In healthy blood vessels, the ergonovine-induced contractions were markedly suppressed by the presence of endothelium; this inhibition again was blunted significantly in the regener-erated endothelium possibly because of the dysfunction of a pertussis toxin–sensitive G protein. These findings imply that endothelial dysfunction leads to the occurrence of ergonovine-induced spasm. Those results obtained in isolated arteries are consistent with the previous in vivo observation that ergonovine-induced constriction is significantly enhanced in coronary arteries with endothelial dysfunction. In porcine coronary arteries, regenerated and atherosclerotic endothelium may also produce vasoconstrictor substances (EDCFs) in response to higher concentrations of serotonin.

Thus, the porcine model of coronary vasospasm has proved to be a useful tool for the understanding of the pathogenesis of the disease. It has shown that endothelium-dependent relaxations to aggregating platelets and related vasoactive substances are severely impaired at sites of endothelial regeneration leading to atherosclerotic lesions to the point that coronary vasospasm can be repeatedly provoked in vivo (Figure 5).

**Clinical Findings**

The elusive link between EDRF and coronary vasospasm in humans remains to be determined. The occurrence of the spasm related to coronary...
angioplasty\(^9\) suggests the important inhibitory effect of the intact endothelium on vascular smooth muscle constriction. More direct evidence for the involvement of endothelial dysfunction in the occurrence of human coronary vasospasm comes from the finding in human subjects that intracoronary injection of acetylcholine causes vasoconstriction in atherosclerotic coronary arteries, whereas it causes relaxation in normal coronary arteries.\(^{10}\) In addition to other stimuli, acetylcholine can provoke coronary vasospasm in patients with variant angina.\(^{11}\)

Clinical and experimental findings indicate that a local, nonspecific supersensitivity of the coronary artery related to atherosclerosis is responsible for spasm. Injury or dysfunction of the endothelium appear to be involved in the supersensitivity, but other possible alterations of vascular wall properties associated with atherosclerosis should also be considered. In addition, it has to be explained why some people but not others suffer from spasm-related ischemic heart disease, even though most of them have coronary atherosclerotic lesions. The progression of coronary atherosclerosis may vary from one person to another, from one time to another for a given person, or from one lesion to another.\(^{12}\) Conceivably, coronary vasospasm may be associated with some unknown events of atherosclerosis; under those circumstances, endothelial injury or dysfunction may be a *sine qua non* condition. Those spasmodenic situations may vary, resulting in spontaneous remission in some and sudden death immediately after accidental discontinuation of drugs in others.\(^{13},^{14}\) The atherosclerotic lesions in the porcine model of coronary vasospasm may represent a rapidly progressing process (including a rapid worsening of endothelial function) rather than the more slowly developing lesions observed in healthy subjects; in that regard, it may closely mimic the accelerated atherosclerosis observed in certain patients with cardiac transplantation.

**Summary**

The endothelium releases the powerful vasodilator and antiaggregatory substance, EDRF, both under basal conditions and upon stimulation by a wide variety of agents. Endothelial injury or dysfunction may play an important role in the spasmodenicity of the coronary artery, although other possible alterations related to atherosclerosis should also be considered. Among the possible stimuli, aggregating platelets are important as a source of vasoconstrictor substances. The endothelium may also produce the vasoactive substances EDHF and EDCF(s). Their pathophysiologic significance remains to be determined.

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