Role of Endothelium-Derived Relaxing Factor in Regulation of Vascular Tone and Remodeling

Update on Humoral Regulation of Vascular Tone

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In addition to preserving the permselectivity of the vascular wall and providing an antithrombogenic surface, the vascular endothelium contributes importantly to the regulation of vasomotor tone. Indeed, the endothelium participates in the conversion of angiotensin I to angiotensin II; the enzymatic inactivation of several plasma constituents such as bradykinin, norepinephrine, serotonin, and ADP; and the synthesis and release of vasodilator substances such as prostacyclin and the recently discovered endothelium-derived relaxing factor (EDRF). The diffusible EDRF released from the endothelium is nitric oxide or a substance closely related to it such as nitrosothiol. The endothelium also synthesizes and releases vasoconstrictive factors, including products derived from arachidonic acid metabolism and the recently discovered peptide endothelin. An increasing body of evidence from experimental and clinical studies indicates that EDRF and endothelium-derived contracting factors play an important role in vascular physiology and pathology. It has become apparent that the balance of these factors may be a major determinant of systemic and regional hemodynamics. Moreover, through generally opposite effects on growth-related vascular changes, contracting factors such as endothelin and relaxing factors such as EDRF also may be important determinants of the vascular response to injury in various disease states such as atherosclerosis and hypertension.

It is clear that the vascular endothelium is a complex and dynamic organ. Understanding endothelium function in normal physiology and disease states is of potential clinical importance and should be the focus of future investigation.

Historically, the vascular endothelium was considered to be a structurally and metabolically simple cellular lining to blood vessels that was important only insofar as it contributed to regulation of vascular permeability and hemostasis. More recently, it has become overwhelmingly apparent that the endothelium represents a complex, dynamic organ with diverse functions, including the synthesis and release of vasoactive agents that play a role in modulating vascular tone. Moncada et al first demonstrated that vascular endothelial cells produce prostacyclin, a vasodilator prostaglandin. In 1980, Furchgott and Zawadzki postulated the release by endothelial cells of a labile factor termed endothelium-derived relaxing factor (EDRF). Subsequent work in this area has made it clear that control of vascular function by the endothelium is quite complex and involves the synthesis and release not only of vasodilator substances such as prostacyclin and EDRF, but of vasoconstrictor substances such as endothelin and other less well-characterized endothelium-derived contracting factors (EDCFs) as well. The role of these substances in normal vascular physiology and disease states is the subject of this review.

Endothelium-Derived Relaxing Factor

EDRF first was described as a labile, diffusible substance released by the endothelium in response to acetylcholine. Since this initial study, a variety of agonists, including thrombin, bradykinin, serotonin, ADP, and the calcium ionophore A23187, have been shown to induce release of EDRF from the endothelium of various vascular beds and endothelial cells grown in culture. A large body of evidence,
including comparative pharmacological studies and direct measurements, supports the hypothesis that EDRF activity is due to release of nitric oxide (NO) by stimulated endothelial cells.\textsuperscript{8-10} Palmer et al\textsuperscript{11-13} demonstrated that the EDRF NO originates from the terminal guanidino nitrogen atom of the amino acid L-arginine. The characteristics of the NO synthase enzyme involved in this reaction in endothelial cells have been reviewed recently by Nathan and Stuehr.\textsuperscript{14} In endothelial cells, this enzyme is soluble, calcium- and NADPH-dependent, and can be inhibited by L-arginine derivatives such as N\textsuperscript{G}-monomethyl L-arginine (LNMMA) or N\textsuperscript{G}-nitro L-arginine.\textsuperscript{15} It is a constitutive enzyme, in that it requires no induction and synthesizes NO within seconds of agonist stimulation of the endothelial cell.\textsuperscript{14} The active product, NO, has a short half-life and decomposes rapidly to form mixtures of nitrate and nitrite in oxygenated solutions. The postulated metabolic pathway by which agonist stimulation of endothelial cells results in vascular smooth muscle cell relaxation is shown schematically in Figure 1. It should be kept in mind that other less well-characterized factors, such as endothelium-derived hyperpolarizing factor,\textsuperscript{15} may be released by agonist-stimulated endothelium and result in vasodilation as well. In this regard, Tare and coworkers\textsuperscript{16} have demonstrated recently that endothelium-derived NO can contribute to the hyperpolarization of vascular smooth muscle cells in response to acetylcholine. Thus, it is likely that NO is responsible for the majority of observed EDRF activity, and this substance will be the focus of the current review.

It recently has become apparent that there is another subtype of NO synthase enzyme, designated as type I by Nathan and Stuehr.\textsuperscript{14} This enzyme has been found in macrophages,\textsuperscript{17} hepatocytes,\textsuperscript{18} and tumor cells\textsuperscript{19} and must be induced by cytokines such as interleukin-1, interferon gamma, and tumor necrosis factor or by microbial products, such as endotoxin. A similar enzyme also may be present in endothelial cells\textsuperscript{20}; however, the positive identification of an inducible NO synthase enzyme in endothelium remains to be demonstrated. Induction of this enzyme, and subsequent generation of NO, requires 4-18 hours of exposure to the immunostimulator and is dependent on protein synthesis. The amount of NO generated by these cell types is much larger than that generated by the NO synthase involved in EDRF responses and appears to mediate tumor cell cytostasis and macrophage-mediated bacterial killing.\textsuperscript{21} The NO generated by this system also may have vasodilator effects, because both endotoxin and interleukin-1 have been shown to inhibit contraction of and increase cyclic GMP (cGMP) levels in rat aortic rings in vitro\textsuperscript{22} and LNMMA has been shown to inhibit the hypotension associated with intravenous infusion of tumor necrosis factor in dogs.\textsuperscript{23} It is likely that this system may be present within the glomerulus as well, because a preliminary report suggested that rat mesangial cells also can generate NO and demonstrate increased intracellular cGMP levels after exposure to endotoxin or interferon gamma.\textsuperscript{24} Both the NO generation and cGMP changes were prevented by LNMMA. Thus, it is possible that during glomerular inflammation or endotoxemia, NO may participate in the modulation of glomerular function.

Murad et al\textsuperscript{25} reported that in vascular smooth muscle there is a positive correlation between an increase in cGMP levels and vascular relaxation. Subsequent work has shown that NO, as well as other nitrovasodilators, stimulates increases in cGMP levels in smooth muscle by activating soluble guanylate cyclase\textsuperscript{25,26} and that vascular relaxation is associated with an increase in tissue cGMP levels.\textsuperscript{27} Thus, it is apparent that NO released by stimulated vascular endothelium activates soluble guanylate cyclase in adjacent vascular smooth muscle cells, inducing a rise in intracellular cGMP levels and subsequent vascular relaxation.

**Figure 1.** Schematic of postulated metabolic pathway by which agonist stimulation of endothelial cells results in vascular smooth muscle cell relaxation. L-NMMA, N\textsuperscript{G}-monomethyl L-arginine; NO, nitric oxide; cGMP, cyclic GMP.
relaxation (Figure 1). The exact chemical form in which the endothelium releases NO remains controversial; more recent studies have suggested that endothelium-derived NO may be incorporated into a nitrosothiol. Uptake or degradation of this nitrosothiol carrier molecule at the vascular smooth muscle cell membrane would release NO and lead to subsequent stimulation of guanylate cyclase. Resolution of this issue awaits further study.

The role of EDRF/NO in normal physiology has begun to be explored only recently. Investigation in this area has been facilitated by the development of EDRF/NO synthesis inhibitors that can be administered in vivo, such as LNMMA. In the rat and the rabbit, systemic bolus administration of LNMMA results in a marked and long-lasting increase in blood pressure, and this hypertensive effect is reversed by administration of L-arginine, the endogenous precursor to NO. This suggested that there is a basal rate of EDRF/NO synthesis by vascular endothelial cells that may be important in the modulation of systemic blood pressure. Tolins et al administered LNMMA to rats by continuous intravenous infusion and reported that arterial blood pressure and renal vascular resistance were increased and that the normal hypotensive and renal vasodilator responses to acetylcholine were prevented. Furthermore, they demonstrated that the vasodilator effects of acetylcholine were associated with increases in urinary cGMP excretion, a result not observed with endothelium-independent vasodilators. They concluded that in vivo responses to endothelium-dependent vasodilators are due to stimulation of endogenous EDRF/NO release by vascular endothelium and that these responses are most likely mediated by changes in cGMP levels in vascular tissue. These same investigators infused LNMMA into the renal artery of the rat and demonstrated a decrease in glomerular filtration rate, marked renal vasoconstriction, and a rise in filtration fraction, suggesting an important role for EDRF/NO synthesis in the regulation of basal renal hemodynamics. The importance of EDRF/NO in the regulation of renal hemodynamic responses in vivo has been confirmed recently in the isolated perfused rat kidney and in the dog.

Endothelial cells cultured from bovine glomeruli have been reported to be capable of producing EDRF. Within the glomerulus, the endothelial and mesangial cells are in close proximity, and it is possible that EDRF is an important mediator of endothelial-mesangial cell communication and thus of renal function. EDRF released from cultured endothelial cells, as well as exogenously applied NO, increases cGMP levels in cultured rat glomerular mesangial cells. NO also attenuates the mesangial cell contractile response to angiotensin II. Others have shown that EDRF can inhibit renin release from juxtaglomerular cells and therefore can limit the generation of angiotensin II. Because the state of mesangial contraction could affect the ultrafiltration coefficient (Kf) by altering the surface area available for glomerular filtration, this may represent another mechanism, distinct from direct hemodynamic effects, by which EDRF could modulate glomerular function.

Recent studies have suggested that EDRF plays a role in the regulation of vascular tone in the pulmonary circulation. Brashers et al demonstrated that NO, bradykinin, and acetylcholine cause vasodilation in isolated, perfused lungs during hypoxic pulmonary vasoconstriction and conversely, that hypoxic vasoconstriction is enhanced by nonspecific inhibitors of EDRF action. Archer and coworkers reported that inhibition of EDRF/NO synthesis with LNMMA enhanced hypoxic vasoconstriction in the isolated perfused rat lung as well as in isolated pulmonary artery rings. In this same study, L-arginine rapidly reversed hypoxic vasoconstriction, especially when EDRF synthesis had been inhibited previously with LNMMA. They concluded that EDRF/NO synthesis is enhanced during hypoxia and acts as a balancing factor, limiting the constrictor response. Similarly, recent observations by Crawford et al suggest that vasorestriction of human pulmonary arteries is attenuated by NO release.

Further evidence has accumulated that EDRF/NO contributes to the control of resting tone in diverse vascular beds. Gardiner et al infused LNMMA intravenously into rats and noted a dose-dependent, sustained vasoconstriction in the carotid, mesenteric, renal, and hindquarters vascular beds. Vallance et al infused LNMMA into the brachial artery of human volunteers and noted a 50% fall in basal blood flow rate and inhibition of the vasodilator response to acetylcholine, suggesting that EDRF/NO contributes to control of basal peripheral arterial blood flow in humans. EDRF/NO also has been reported to be important in modulating hemodynamic responses in the cerebral and coronary circulations.

In addition to effects on vascular tone, EDRF/NO influences components of the blood coagulation system and cellular proliferative responses. In vessels with normal endothelium, thrombin, as well as serotonin and ADP released by aggregating platelets, binds to specific receptors and induces EDRF release. EDRA/NO inhibits platelet aggregation. The adhesion of platelets to cultured endothelial cells in vitro is decreased by the EDRF agonist bradykinin or exogenous NO. Furthermore, platelets themselves contain an NO synthase that can generate NO and increase platelet cGMP levels in response to activating stimuli. Therefore, acting synergistically with prostacyclin, NO may participate in the local regulation of coagulation responses. NO also has been shown to inhibit vascular smooth muscle and mesangial cell proliferation induced by serum- or platelet-derived growth factor in vitro and so could act as a limiting factor in vascular and glomerular responses to injury. Whether this antiproliferative effect is mediated by increases in cGMP is currently unclear.
Endothelium-Derived Contracting Factors

Since the discovery of EDRF, it has become clear that endothelial cells also release several substances that mediate contraction of vascular smooth muscle. Possible candidates for EDCFs include the cyclooxygenase products thromboxane A2 and prostaglandin H2 and superoxide anion. Vascular tissue also may have a locally active renin-angiotensin system that is capable of generating the vasoconstrictor angiotensin II. Furthermore, the vascular endothelium recently has been demonstrated to release endothelin, a 21-amino acid peptide with potent vasoconstrictive properties. Freshly isolated porcine arteries with intact endothelium release endothelin under basal conditions, and thrombin significantly enhances this release. Binding sites for endothelin have been described in blood vessels and in renal tissues. Recently, a radioimmunoassay for measurement of endothelin in plasma has been developed that should greatly facilitate further investigation into the role of this novel peptide in vivo. Preliminary reports using this radioimmunoassay have found detectable circulating levels of endothelin in normal humans, with increased levels noted in patients with essential hypertension and patients with renal insufficiency. In nephrectomized rats, the plasma clearance of endothelin is prolonged, and exogenously administered endothelin induces exaggerated responses, indicating that the kidney may play a role in the elimination of endothelin. It appears that, unlike EDRF, endothelin can circulate in the bloodstream and therefore may be released from one vascular bed and have vasoconstrictive effects in distant parts of the circulation.

Recent experimental studies have focused on the systemic and renal hemodynamic effects of exogenously administered endothelin. When administered to "experimental animals" in pharmacological doses, endothelin induces a transient vasodilation followed by a marked and prolonged vasoconstriction, resulting in elevation of blood pressure and reduction in renal blood flow. The renal vascular bed appears to be 10-fold more sensitive to vasoconstriction by endothelin than are the coronary, femoral, or bronchial vascular beds. Endothelin also has been shown to stimulate atrial natriuretic peptide release and to increase plasma renin activity and aldosterone levels.

The effect of endothelin on the glomerular microcirculation has been investigated with the use of micropuncture techniques. In this setting, endothelin has been shown to reduce single-nephron glomerular filtration rate and glomerular plasma flow by 30–50%. Both afferent and efferent arteriolar resistances increase with endothelin and \( \varepsilon \) has been reported to be reduced or unchanged. Several studies have investigated the effects of endothelin on glomerular mesangial cells in culture. In contrast to EDRF, endothelin stimulates mesangial cell proliferation especially when added in the presence of low concentrations of serum. Endothelin stimulates rapid increases in mesangial cytosolic calcium, phosphoinositide turnover, and intracellular alkalinization similar to many other vasoconstrictors and growth factors. In this regard, endothelin would fit the apparent pattern observed in studies of mesangial cells in which vasodilators (EDRF/NO, prostacyclin) are antiproliferative, whereas vasoconstrictors (angiotensin II, platelet-derived growth factor, endothelin) are pro-proliferative. Whether glomerular endothelial cells can synthesize endothelin remains to be determined, but because endothelin circulates within the bloodstream, the glomerulus could be exposed to this agent from various sites within the circulation. Thus, it appears that EDRF and endothelin exert a number of opposite effects on the glomerulus and mesangial cells, suggesting that they may have counterbalancing effects on glomerular function.

Role of the Endothelium in Disease States

From the above discussion it is apparent that the control of vascular tone by the endothelium has proved to be much more complex than previously suspected and would appear to reflect a balance between locally acting mediators such as EDRF/NO and the various EDCFs, as well as circulating endothelin-derived factors such as endothelin. Furthermore, the interaction of EDRF and endothelin is of potential importance in both normal physiology and disease states. Several agonists, such as thrombin and A23187, stimulate simultaneous release of EDRF/NO and endothelin. In addition, recent studies have demonstrated that thrombin-induced release of endothelin is potentiated when NO synthesis is inhibited by LNMMA, suggesting that NO may exert an inhibitory effect on endothelin synthesis. On the other hand, endothelin may stimulate the release of EDRF and prostacyclin. Thus, it can be seen that in disease states in which the endothelium is injured, the delicate balance between endothelium-mediated vasodilation and vasoconstriction can be disrupted, leading to unopposed vasoconstriction. This imbalance may play a role in the pathophysiology of diseases such as hypertension and atherosclerosis.

Arterial hypertension results in the exposure of the vascular endothelium to abnormal physical forces such as shear stress and stretch. As a result, endothelial cells from hypertensive animals and humans develop morphological and functional abnormalities. Abnormal vascular responses have been noted in experimental models of hypertension. Responses of vascular beds and isolated blood vessels to vasoconstrictor agonists generally are exaggerated, whereas relaxation after exposure to vasodilators such as nitroprusside and isoproterenol is decreased. It is apparent that abnormal endothelial function, with an imbalance of endothelium-dependent relaxations and contractions, could contribute to these abnormal vascular responses and the increased peripheral vascular resistance that is the central hemodynamic abnormality in hypertension.
Studies in hypertensive Dahl salt-sensitive rats have shown that relaxations to agonists of EDRF are significantly impaired when compared with responses of normotensive Dahl salt-sensitive and salt-resistant rats. In this same model, endothelium-independent relaxations to sodium nitroprusside also were impaired, but to a lesser degree. Spontaneously hypertensive rats also have abnormalities in endothelium-dependent responses. In fact, vessels from spontaneously hypertensive rats also develop endothelium-dependent contractions in response to acetylcholine. Indomethacin normalized the reduced endothelium-dependent relaxations and eliminated endothelium-dependent contractions in the spontaneously hypertensive rat, suggesting that the primary defect in this form of hypertension is not defective release of EDRF/NO but simultaneous increased release of cyclooxygenase-dependent contracting factors. A recent study by Panza and coworkers demonstrated that endothelium-dependent relaxations, determined by noninvasive techniques, are abnormal in patients with hypertension. In this study, forearm vascular relaxation was normal in response to nitroprusside but reduced with acetylcholine. Whether these abnormalities are secondary to hypertensive changes or are part of the pathogenesis of hypertension, or both, is an important question that will need to be addressed by future studies.

It has become apparent that injury to the endothelium and the response of vascular tissue to endothelial injury are important in the pathogenesis of atherosclerosis. At an early stage in the development of the atherosclerotic plaque, monocytes migrate into the intima. These cell types are capable of releasing oxygen radicals, which could decrease the half-life and bioactivity of EDRF/NO, thus removing a vasodilator influence on vessel tone. In addition, oxidized low density lipoproteins, known to be important in atherosclerosis, have been reported to directly inhibit endothelium-dependent relaxations further contributing to a vasospastic tendency.

The endothelium can sense changes or abnormalities in blood flows and pressures and can participate in vascular responses not only by rapid generation of vasoactive mediators, such as NO or prostacyclin, but also by modulating long-term structural responses of the smooth muscle in the vascular wall. The role of the endothelium in vascular remodeling has been reviewed recently by Gibbons and Dzau. Growth factors released by endothelial cells, monocytes, and platelets most likely stimulate vascular smooth muscle cell proliferation as part of the development of atherosclerotic lesions or the response to hypertension. Because EDRF/NO and prostacyclin have been demonstrated to have antiproliferative effects on vascular smooth muscle cells, decreased production of these agents due to endothelial injury and dysfunction could result in enhanced proliferation and accelerated atherogenesis. Thus, endothelium-derived substances may regulate vascular remodeling and play a role in the development of vascular disease.

Functionally, vasoconstrictor responses of atherosclerotic vessels from experimental animals tend to be exaggerated, although this varies depending on the agonist used and the animal species studied. Aortic rings from hypercholesterolemic rabbits with atherosclerosis have depressed responses to agonists of endothelium-dependent vasodilation, such as acetylcholine and ADP. Monkeys with diet-induced atherosclerosis have impaired endothelium-dependent relaxations to acetylcholine and thrombin. Atherosclerotic human coronary arteries have depressed endothelium-dependent relaxations when studied in vitro, whereas normal human coronary arteries in vivo dilate in response to acetylcholine. In coronary vessels with atherosclerosis, acetylcholine actually induces vasoconstriction despite the fact that organic nitrates still cause vasodilation. This suggests that endothelium-dependent but not endothelium-independent relaxations are impaired. In patients with hypercholesterolemia, impaired endothelium-dependent and endothelium-independent relaxations can be demonstrated in forearm resistance vessels that are not, themselves, injured by atherosclerosis. Furthermore, injury to the endothelium from atherosclerosis may stimulate release of EDCF. Thus, in certain vascular beds, vasospasm associated with atherosclerosis may not be due only to deficient vasorelaxation but also to an unbalanced production of vasoconstrictive agonists.

Conclusions

An increasing body of evidence from experimental and clinical studies indicates that EDRFs and EDCFss play an important role in vascular physiology. It has become apparent that the balance of these factors may be a major determinant of systemic and regional hemodynamics. Through generally opposite effects on hemostatic mechanisms and cellular proliferation, contracting factors such as endothelin and relaxing factors such as NO also may be important determinants of the vascular response to injury in various disease states such as atherosclerosis and hypertension. It is clear that the vascular endothelium is a complex and dynamic organ. Understanding endothelial function in normal physiology and disease states is of potential clinical significance and should be the focus of future investigation.

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