Mechanisms of Vascular Injury: The Emerging Role of the Endothelium

Leopoldo Rajj

L. Rajj, Department of Medicine, Veterans Administration Medical Center, and University of Minnesota, Minneapolis, MN
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ABSTRACT
Evidence is increasing that vascular tone is highly dependent on the health of the endothelium and on the delicate balancing act between endothelium-derived relaxing and endothelium-derived contracting factors. Moreover, there is also evidence supporting the notion that the same factors which affect vascular tone also regulate, either in an autocrine or paracrine fashion, changes in vascular architecture. Synthesis and release of both endothelium-derived relaxing and contracting factors are affected by a number of physiologic and therapeutic agents as well as by other factors, among them vascular injury in disease states such as atherosclerosis, hypertension, diabetes, and acute renal failure. A number of trials indicate that therapeutic intervention may be capable of modulating the synthesis and release of these substances and the balance between the two as well as influencing the processes which control vascular remodeling.

Key Words: Endothelium, hypertension, atherosclerosis, acute renal failure, diabetes

Several functions of the vascular endothelium are well known, among them the selective "filtering" of substances, control of hemostasis, interaction with cellular and noncellular components of circulating blood, synthesis of angiotensin-converting enzyme (ACE), and modulation of vasoactive agents such as catecholamines, bradykinin, and angiotensin II by means of metabolic actions (1,2). Another function, direct action on vascular tone accomplished through production and release of both vasodilatory and vasoconstrictive substances, is the subject of this review. This latter function is emerging as a complex and crucial role of the endothelium and one that has important ramifications in a number of disease states as well as exciting implications for therapeutic intervention in the future.

THE VASODILATING ABILITY OF THE ENDOTHELIUM
Prostacyclin, a vasodilatory prostaglandin and an inhibitor of platelet aggregation (3), was one of the earliest of the endothelial-derived vasoactive substances to be described. Other such factors have been discovered more recently, perhaps the most important of which was identified in 1980 by Furchgott and Zawadski (4). These researchers found that relaxation of vascular smooth muscle (VSM) in isolated arteries with administration of the muscarinic agent acetylcholine occurs only if the endothelium is intact. They named the labile, diffusible substance whose release controls this relaxation "endothelium-derived relaxing factor" (EDRF).

EDRF is, at least for the most part, nitric oxide (NO) synthesized in the endothelial cell from a specific substrate, L-arginine. Generation of NO from L-arginine is inhibited by L-Nω-monomethyl arginine (L-NMMA), an L-arginine analog (5). The NO may be part of a nitrosothiol released by the endothelium and then taken up by or released at the cell membrane of VSM (6). Relaxation of the VSM may also be accomplished by release of a hyperpolarizing factor that activates the sodium-potassium pump, but understanding of this mechanism awaits further study (3).

Superoxide radicals, but not other oxygen-reactive species (e.g., hydrogen peroxide, hydroxyl radical), inactivate EDRF (7), as does hemoglobin, the latter probably by chemically binding it (8,9). EDRF thus probably acts in close proximity to its site of release and mainly on the abluminal side of the endothelium.

EDRF/NO has other effects, namely, inhibition of platelet aggregation and inhibition of platelet adhesion to collagen (10). The interaction between the effect of EDRF on vascular tone and its effects on blood coagulation itself has interesting implications. During platelet aggregation, thrombin, serotonin, and ADP are released and bind to specific receptors. Release of EDRF with resulting vasorelaxation occurs, providing the endothelium is intact (2,3). When the endothelium is damaged, the relaxation is overridden as these agonists stimulate vascular contraction (2). This would explain the vasospasm that oc-
curs in vascular beds with endothelial lesions, because blood coagulation is activated in areas of endothelial injury. Moreover, in vessels with abnormal endothelium, only agents such as organic nitrates and calcium channel blockers which induce direct relaxation of VSM cells are effective vasodilators.

VSM proliferation is inhibited by NO (11), as is proliferation of mesangial cells stimulated with platelet-derived growth factor or thrombin (12,13). Thus, EDRF may play a role in modulating growth-related events in vessels and in glomeruli. Although the evidence is inconclusive, several researchers have associated these effects with NO-induced stimulation of soluble guanylate cyclase and the elevated level of intracellular cGMP that results (10,11,14).

An important area for future research is delineation of exactly which part of the enzymatic process, through which EDRF is synthesized from L-arginine, is damaged in the various settings where endothelium-dependent relaxations are impaired (e.g., atherosclerosis, presence of toxins). Once this is known, treatments can be developed to intervene effectively.

THE VASOCONSTRICTING ABILITY OF THE ENDOTHELIUM

The endothelium can release vasoconstricting substances as well as the vasodilatory one just described. These are termed endothelial-derived contracting factors (EDCF) and include superoxide radicals (15), which inactivate NO, as well as certain eicosanoids and endothelin, a 21-amino-acid peptide with potent vasoconstrictive properties that has recently been isolated from endothelial cell-conditioned medium (16). It appears that endothelin, unlike EDRF, may circulate in the bloodstream, so it can cause vasoconstriction in a location far from the one from which it was released.

Endothelin has binding sites in the blood vessels and the kidneys (17). The substance exerts a vasodilatory effect initially, followed by vasoconstriction. Blood pressure increases and renal blood flow decreases in its presence, but coronary blood flow is unaffected (18,19). Renal vessels are more sensitive to vasoconstriction by endothelin than coronary, femoral, or bronchial vessels by a factor of 10 (20). Patients with hypertension, cardiogenic shock, pulmonary hypertension, and uremia, especially those undergoing hemodialysis, show elevated levels of the substance (21,22).

Endothelin has a more powerful effect when given intra-arterially than when given i.v. (23), which may mean that it is inactivated during passage through the pulmonary circulation. The kidney may play a role in clearance of the substance, as evidenced by the finding in animal studies (24) that bilateral nephrectomy substantially delays plasma clearance of i.v. administered endothelin, with resulting elevations in blood pressure.

Endothelin has been found to be released in vitro by intact porcine vessels under basal conditions. Thrombin has been found to stimulate endothelin synthesis and release (25), and this action is enhanced when L-NMMA is present (a state in which, as already mentioned, NO synthesis is inhibited [5]). This suggests that under normal conditions (i.e., absence of L-NMMA), NO inhibit the synthesis of endothelin (24,25).

VASODILATION AND VASOCONSTRICTION: A DELICATE BALANCE

Because the endothelium is capable of releasing vasoconstricting factors as well as vasorelaxing ones, vasospasm is not necessarily caused by impaired vasorelaxation but may be caused by overproduction or unopposed release of vasoconstrictive agonists. Perhaps under normal conditions, contracting factors are released but in quantities that are counterbalanced by relaxing ones. Alternatively, vasoconstrictive substances might be released only when vessels are damaged.

Thrombin, A23187, and other agonists stimulate release of both EDRF and endothelin (3), which supports the possibility that under normal conditions these substances counterbalance one another to maintain vascular tone. Also, as noted above, EDRF may modulate the synthesis and release of endothelin (24,25). Endothelin can probably stimulate EDRF and prostacyclin release as well (23), another possibility that would indicate a circular relationship between the vasoconstrictors and the vasodilators.

The control of renovascular tone and renal function may be affected significantly by vasodilator-vasoconstrictor balance, particularly glomerular function, because EDRF and endothelin exert a number of opposing effects on the glomerulus and mesangial cells.

THE ATHEROSCLEROSIS-ENDOTHELIUM CONNECTION

Injury to the endothelium and the physiologic response to this injury has been postulated by many as an important factor in the formation of atherosclerotic plaque (26). In addition, exposure of endothelial cells to low-density lipoprotein but not to high-density lipoprotein has been shown experimentally to lead to impairment of prostacyclin and EDRF release, thus directly inhibiting endothelium-dependent relaxations (27). Monocytes migrate into the intima early during development of atherosclerotic plaque and then become macrophages laden with lipids (foam cells). Macrophages can release oxygen rad-
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cals (27,28), which, as already mentioned, inactivate EDRF. The resulting vasospasm in atherosclerosis may be further accentuated by release of vasoconstrictive products such as thromboxane A₂ (28).

Atherosclerotic vessels have been shown to have exaggerated vasoconstrictive responses in a number of animal experiments (29–31). Monkeys with diet-induced atherosclerosis show impairment in endothelium-dependent relaxations to acetylcholine and thrombin (32). Acetylcholine, which in human coronary arteries normally depresses relaxations \textit{in vitro} but causes dilation \textit{in vivo} (33), induces vasoconstriction in atherosclerotic coronary vessels. Infusion of organic nitrates results in vasodilation. These findings further suggest that endothelium-dependent, but not endothelium-independent, relaxations are impaired in atherosclerosis (33–35). The mechanism involved in the impairment are unclear but may include abnormal production or release of EDRF or altered diffusion or damage in transit through an injured vessel wall. Patients with high cholesterol levels show impaired endothelium-dependent relaxations in forearm resistance vessels even in the absence of clinical atherosclerosis (36).

In atherosclerosis, proliferation of VSM is likely stimulated by release of growth factors from endothelial cells, monocytes/macrophages, and platelets (26). Proliferation may also be promoted by the vascular renin-angiotensin system and by locally released endothelin. The latter would more likely be operative if production of EDRF and prostacyclin, which have antiproliferative action, has been depressed because of injury to the endothelium (13,37).

Atherosclerosis-damaged endothelium may release EDCF (38,39), and thus vasospasm may result not only from impaired vasorelaxation but also from overproduction of vasoconstrictive agonists. Morphologic features of atherosclerosis in large vessels resemble those of glomerulosclerosis (40). This suggests that impaired endothelium-dependent mesangial relaxation and unopposed or enhanced endothelium-dependent mesangial contraction may contribute to glomerular dysfunction.

Exaggerated responses of vascular beds and of isolated blood vessels to vasoconstrictor agonists are typical in hypertension (44), as are decreased relaxations in response to such vasodilators as sodium nitroprusside and isoproterenol (45–47). Structural changes, altered transmembrane calcium exchanges in VSM, and modified receptor density or affinity for the substances probably explain the changes in response (44).

Isolated aortas of Dahl salt-sensitive and salt-resistant rats with hypertension show significant impairment in vascular relaxations when exposed to EDRF agonists (46). Endothelium-independent relaxations to sodium nitroprusside show abnormalities but are not as impaired (46). Comparable endothelium-dependent responses have been found in other models of hypertension. For example, spontaneously hypertensive rats show depressed endothelium-dependent relaxations in the aorta and mesenteric vessels. (In addition, these rats show endothelium-dependent arterial contractions to acetylcholine which can be inhibited by indomethacin, however, suggesting mediation by products of cyclooxygenase [48].) The preglomerular vessels, but not the aortas, of spontaneously hypertensive rats have shown abnormal endothelium-dependent responses even before the onset of hypertension (49). This may indicate that the abnormality is primary and not a result of hypertension-caused changes to the endothelium.

Studies have shown abnormal endothelium-dependent relaxations in hypertensive humans as well (50). Further study is needed to determine whether the abnormal responses are a cause or a result of the hypertension.

As for endothelin, it may also be a factor in hypertension. Elevations in immunoreactive endothelin levels in the plasma of severely hypertensive patients have been found, especially when glomerular filtration rate is also reduced (22). Further study is needed here as well to determine whether the elevated levels were a cause or a result of the hypertension.

The vascular changes seen in hypertension and atherosclerosis are thought to be important as well in the pathogenesis of stroke, aortic aneurysm, and renovascular disease. Also, evidence exists that the endothelial injury after ischemia and reperfusion results in impaired endothelium-dependent responses (51), as does injury from atherosclerosis and from hypertension.

**THE HYPERTENSION-ENDOTHELIUM CONNECTION**

After long-standing hypertension, vascular endothelial cells change shape and size and they show cytoplasmic changes (41) and increased cell replication (42). Changes also appear in the structure and biochemistry of the arterial intima, and VSM shows hypertrophy.

Under normal conditions, circulating platelets and monocytes do not adhere to the endothelium but adhesions of monocytes increase in number when hypertension is present and the cells migrate to the subendothelium (41,43).

**THE DIABETES-ENDOTHELIUM CONNECTION**

Because vascular disease goes hand in hand with diabetes, it is not surprising that vascular responses to agonists of endothelium-dependent relaxations have been shown to be impaired in animal studies of diabetes (52,53).
Hyperinsulinemia and peripheral insulin resistance have been found in populations of hypertensive humans as well as in noninsulin-dependent diabetics (54,55). Insulin is a growth-promoting factor (56,57), so it may be that the hyperinsulinemia in these patients contributes to structural changes in their vessels.

Hypertension and accelerated atherosclerosis are known complications of diabetes (58). In view of all of these findings, it appears that in diabetes, endothelial injury may be an important factor in a continuous cycle of changes in vascular tone and vascular architecture.

THE ACUTE RENAL FAILURE-ENDOTHELIUM CONNECTION

Renal vasoconstriction, with resulting increases in renal vascular resistance, has been shown to be important in acute renal failure. Thus, as more is learned about the importance of the endothelium in regulating vascular tone, interest is growing in its potential role in the pathogenesis of acute renal failure. Specifically, ischemic acute renal failure has been associated with vascular damage and abnormal vascular responses in animal studies. The abnormalities were hypothesized to be caused by endothelial damage and impaired release of EDRF (59). Intrarenal infusion of acetylcholine and bradykinin failed to stimulate a vasodilating response, adding more weight to this hypothesis. On the other hand, calcium channel blockers were effective in restoring vasodilation (59). Thus, endothelial damage and endothelium-dependent vasodilation may be important determinants in ischemic renal injury.

Further evidence of the importance of intact endothelium to renal health comes from studies of the immunosuppressive agent cyclosporin A. Use of this agent has been limited by frequent development of nephrotoxicity, which in experimental models has been found to be associated with renal vasoconstriction and to increase in severity when renal resistance increases (60). Researchers have now found that cyclosporin A induces endothelial cell injury (61). Such injury, with the resulting impairment of EDRF release and renal vasoconstriction, may be an important factor in the nephrotoxicity of the agent.

Endothelin, presumably released from damaged endothelium, may also play a role in postschematic acute renal failure, as evidenced by studies with antiendothelin antibody infusions into branches of the renal artery as well as micropuncture techniques (62,63).

Renal function is thus affected by the health of the endothelium and its vasodilatory and vasoconstrictive actions, and acute renal failure may be one potential result of damaged endothelial function. In the glomerulus, endothelial damage may be due to direct injury by toxins or immune reactants or to hemodynamic factors. This may then result in abnormalities in glomerular microcirculation, such as altered traffic of macromolecules in the mesangium (64).

CAN THERAPEUTIC INTERVENTION NORMALIZE ENDOTHELIUM FUNCTION?

Abnormal endothelium-dependent responses have been reversed in a number of ways in experimental models of several diseases. In atherosclerosis, the reversal has occurred with cessation of atherogenic diets (33) and supplementation of diets with fish oil (65). Furthermore, a calcium channel blocker has been shown to prevent development of atherosclerosis and abnormal endothelium-dependent relaxations in cholesterol-fed rabbits (66). An ACE inhibitor has been shown to slow the development of atherosclerotic changes in hypercholesterolemic rabbits (67). ACE inhibitors have also been found to prevent myointimal thickening in response to balloon injury of the carotid artery (68).

Dahl rats with salt-dependent genetic hypertension have shown a reversal of hypertension with administration of antihypertensive drugs and restoration of abnormal endothelium-dependent relaxations (69,70). Future study is needed to determine whether the beneficial effect was exclusively due to the lowered blood pressure achieved through use of the antihypertensive agents or to some direct effect of the agents themselves.

Dietary supplementation with potassium has resulted in improved endothelium-dependent vascular relaxations in hypertensive rats (71), independent of blood pressure level. The fact that human populations with a high potassium intake have a low incidence of cerebrovascular accidents would follow from this finding (72). In view of the beneficial effect of potassium, it may be that potassium depletion, common with administration of diuretic treatment, has harmful effects on vascular tone and architecture.

The specific mechanisms at work in the therapeutic interventions mentioned here remain unclear, but the results of experimental trials to date point to the value that might be afforded by future research in this area.

SUMMARY

The endothelium is an important link in the maintenance of vascular tone by way of its ability to synthesize and release both substances that dilate and substances that constrict the vessels. The most recently discovered of the vasodilating substances, EDRF, has direct action on vascular tone as well as
effects on the vascular architecture. The substance inhibits VSM and mesangial cell proliferation. The EDCF, which oppose the actions of EDRF, also have direct action on vascular tone and probably in vascular remodeling as well. Vasoconstriction may be caused by an impairment in vasorelaxation or by overproduction or unopposed release of vasoconstrictive agonists. Endothelial health may be affected in a number of disease states, including atherosclerosis, hypertension, diabetes, and acute renal failure. Alternatively, endothelial abnormalities may play a role in the pathogenesis of the diseases. This relationship is clearly an important area for future research. Reversal of disease in experimental models has been found with various interventions, such as dietary manipulation and treatment with antihypertensive agents. This, too, is a promising area for further investigation.

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