From Isolated Vessels to the Catheterization Laboratory

Studies of Endothelial Function in the Coronary Circulation of Humans

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In 1980, Furchgott and Zawadski first showed that an intact endothelium must be present for acetylcholine to relax isolated vessels. This was subsequently found to be true for many other vasoactive substances. As a result of work from several laboratories, this response has been shown to occur because the endothelium releases a short-lived nonprostanoid compound or family of compounds collectively termed the "endothelium-derived relaxing factor" (EDRF). These substances relax vascular smooth muscle by activating guanylate cyclase and by producing transient smooth muscle hyperpolarization. The role of EDRF in regulating vascular tone was first implicated in large vessels but has now been extended to the microcirculation.

Identity of Endothelium-Derived Relaxing Factor

The chemical identity of EDRF has been the subject of intense investigations. Many of EDRF's biologic activities are shared by nitric oxide. These include the ability to activate guanylate cyclase, inhibition of platelet aggregation, short biologic half-life, and inactivation by hemoglobin. In 1987, Palmer et al reported that nitric oxide is synthesized and released by cultured endothelial cells and that EDRF may be nitric oxide. The evidence for nitric oxide being the sole EDRF is far from conclusive, however. The amount of nitric oxide released from cultured endothelial cells is insufficient to account for all of the vasodilator activity of EDRF simultaneously released from the same source. Moreover, recent studies have shown that EDRF may not be authentic nitric oxide but a nitrosyl compound (such as a nitrosothiol) that is substantially more potent than nitric oxide. Further, nitric oxide cannot account for some of EDRF's effects such as hyperpolarization of vascular smooth muscle. Hopefully, future research will provide further insight into the chemical identity of these substances.

Abnormalities of Endothelium-Dependent Vascular Relaxation in Atherosclerosis

Shortly after the original observation by Furchgott and Zawadski, several groups began to examine endothelium-dependent vascular relaxation in vessels removed from experimental animals with diet-induced atherosclerosis. The rationale underlying these studies was that many of the substances that elicit the release of EDRF (serotonin, norepinephrine, acetylcholine, vasopressin, and others) also exert a direct vasoconstrictor effect on vascular smooth muscle. The release of EDRF normally modulates the constrictor action of these compounds. If the endothelium were dysfunctional (and thus did not release EDRF) in the setting of atherosclerosis, these neurohumoral agents could conceivably produce exaggerated vascular constriction because of their unopposed effect on the smooth muscle. The unopposed vasoconstrictor actions of these agents may account for abnormal vasomotor phenomenon encountered in individuals with coronary atherosclerosis. In 1986 and 1987, several groups reported that endothelium-dependent relaxations of vessels removed from animals with diet-induced atherosclerosis were markedly impaired, whereas responses to agents that directly relax smooth muscle were either normal or only minimally impaired. Subsequent studies have shown that this defect of vascular function in atherosclerosis is not due to decreased responsiveness of vascular smooth muscle to EDRF but related to either decreased release of EDRF from the endothelium or the release of a defective EDRF.
Recently, this defect in endothelial function was shown not to be limited to larger vessels but that it also extends to endothelial function in the microcirculation. In vivo studies of the rabbit cremaster muscle have shown that short-term cholesterol feeding markedly impairs acetylcholine-induced vasodilation of vessels as small as 25-μm diameter.23 Preliminary in vitro studies in our laboratory on coronary resistance vessels from monkeys with diet-induced atherosclerosis suggest that endothelium-dependent responses are strikingly abnormal in these vessels, whereas responses to endothelium-independent vasodilators are unchanged. These findings show that endothelial dysfunction can occur in vessels that do not develop overt atherosclerosis and indicate that abnormal vasomotor control in atherosclerosis can involve microvessels as well as large vessels.

Close on the heels of the demonstration that endothelium-dependent vascular relaxation is abnormal in atherosclerotic animals, several groups began to report similar abnormalities of endothelium-dependent vascular relaxation in atherosclerotic human coronary arteries. Two groups have studied human coronary arteries removed from hearts of transplant recipients in vitro.24,25 In these experiments, endothelium-dependent relaxations were depressed in rings of vessels with atherosclerosis compared with relaxations in nonatherosclerotic vessels. Subsequently, several groups have examined the vasodilatation produced by acetylcholine (presumed to be dependent on an intact endothelium) in the cardiac catheterization laboratory.26–29 In general, most of these studies have shown that vessels with even minimal atherosclerosis do not dilate to acetylcholine, whereas normal vessels do.

**Release of Endothelium-Derived Relaxing Factor by Mechanical Stimuli**

In addition to the large number of neurohumoral agents that can stimulate the release of EDRF, mechanical stimuli such as changes in shear stress or pulsatile flow also modulate EDRF release.30,31 Teleologically, this would appear important in maintaining vascular shear stress at a constant level during periods of increased blood flow by providing a mechanism for local dilatation of large vessels in response to changes in blood flow that are mediated by downstream vessels. Two recent studies have shown that flow-mediated vasodilatation occurs in the brachial artery of humans.32,33

In this issue of *Circulation*, Cox et al34 and Drexler et al35 show that this important regulatory function of the endothelium exists in the human coronary circulation. Furthermore, unequivocal evidence is provided that flow-induced, endothelium-dependent vasodilatation is abnormal in humans with coronary atherosclerosis.35 These studies were made possible first by initial observations in animals and second by the application of important new technology to the study of the coronary circulation in humans. Studies such as these would be impossible without a reasonably accurate method of measuring blood flow velocity and quantitative approaches to the measurement of arterial dimensions from the coronary angiogram. These observations represent an extension from experimental studies in animals (in vivo and in vitro) to clinical practice. Efforts such as these are important because occasional findings in experimental animals have not been applicable to clinical situations.

The implications of abnormal large vessel dilatation in response to increased flow in atherosclerosis are several. In addition to the effect of exposing the vessel wall to increased shear stress, the potential exists for an increase in pressure losses during high flow states across the epicardial coronary artery. Under normal conditions, there is minimal pressure gradient (<5 mm Hg) from the coronary ostium to the most distal areas of the large epicardial coronary arteries. Under conditions of increased flow (such as may exist during pharmacologic- or exercise-induced vasodilatation), this gradient may substantially increase and exceed 20 mm Hg. This may, in a small but important way, affect the driving pressure for perfusion in the coronary microcirculation. In addition, the pressure gradient that develops in the proximal coronary arteries during pharmacologic vasodilatation may decrease the pressure at the origin of coronary collateral arteries, effectively reducing coronary collateral blood flow. This phenomenon, which has been termed the "collateral steal phenomenon," would be exacerbated when the proximal coronary arteries could not dilate in response to increased vascular shear.

**Future Directions**

Numerous questions remain to be answered regarding endothelial regulation of vascular smooth muscle and how this regulation is altered by several disease states including atherosclerosis. Several questions remain to be answered regarding the biochemical identity of EDRF and how its biosynthesis is altered by disease. There are a myriad of other functions of the endothelium that may be abnormal in atherosclerosis.

The advent of new technology has substantially enhanced the capability of obtaining accurate information regarding coronary vasoactive reactivity in the cardiac catheterization laboratory so that many additional studies will likely be forthcoming. One inhibitor of EDRF, methylene blue, has recently been used in humans, unmasking the constrictor effect of acetylcholine.36 Such an approach, used cautiously, may provide an additional tool to examine dual opposing vascular effects of various neurohumoral substances. Abnormal endothelium-dependent vascular relaxation can be reversed after successful dietary treatment of atherosclerosis in experimental animals.37 Whether or not this can occur in humans has yet to be established. Newer therapies for correction of hypercholesterolemia
should allow such studies to be forthcoming in the near future. For example, Shimokawa and Vanhoutte have shown that dietary supplementation with fish oil can enhance endothelial-dependent vascular relaxation and in part correct abnormal responses observed in experimental atherosclerosis. Because endothelium-dependent relaxations are abnormal in the coronary resistance circulation of cholesterol-fed animals, such an abnormality probably exists in humans with atherosclerosis. Studies of blood flow with the Doppler catheter should allow the study of resistance vessel responses in normal and hypercholesterolemic subjects. Atherosclerosis develops in the setting of a wide range of plasma cholesterol levels, including low plasma cholesterol levels. It will be interesting to determine whether or not the plasma cholesterol level has any direct bearing on abnormalities of endothelial function in humans. Last, other than atherosclerosis, a number of pathologic conditions including ischemia with reperfusion, hypertension, and diabetes are associated with abnormal endothelium-dependent vascular relaxation in experimental animals. Whether or not these commonly encountered disorders alter coronary vascular reactivity in humans is unclear. Studies in patients with these diseases similar to those presented in this issue of Circulation will undoubtedly be forthcoming and should provide important additional information regarding abnormalities of the coronary circulation in other disease states.

References


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