Hyperlipidemic Arterial Dysfunction

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"We have, I think, evidence that sclerotic arteries are specially prone to spasm."* (William Osler)

About 10 years ago, Furchgott and Zawadzki made a remarkable observation that helped clarify variable effects of acetylcholine on arterial tone. They demonstrated that isolated arteries relaxed in response to acetylcholine and other endogenous vasoactive agents only in the presence of an intact endothelium and postulated that endothelial cells released a diffusible vasodilator principle, which they called endothelium-derived relaxing factor (EDRF). Current evidence indicates that EDRF is a nitric acid radical (NO) or a precursor thereof (S-nitrosothiol) apparently derived from the terminal guanidino atom(s) of L-arginine. Stimulation of endothelial muscarinic receptors produces a rise in cytosolic calcium, the intracellular signal evoking release of EDRF. Numerous endogenous agents, including bradykinin, vasopressin, norepinephrine, histamine, serotonin, ADP, ATP, and thrombin, are capable of releasing EDRF by binding to endothelial surface receptors. Calcium ionophores (A23187, ionomycin) also may promote release of EDRF, but they produce the intracellular signal bypassing receptor-dependent membrane regulation. EDRF and nitroglycerin appear to relax vascular smooth muscle by a common mechanism of action. NO derived from both agents stimulates soluble heme-containing guanylate cyclase in smooth muscle cells, an event leading to enhanced sarcemmal outward transport of calcium and consequent muscular relaxation. In addition to acting indirectly by stimulating the release of EDRF, acetylcholine acts directly on vascular smooth muscle. Stimulation of smooth muscle muscarinic receptors evokes a rise in cytosolic calcium that determines an increase in smooth muscle tone. Dose-response experiments can dissociate the two muscarinic mechanisms to some extent because endothelial stimulation requires lower acetylcholine concentrations than those required to contract smooth muscle. With high agonist concentrations, direct constrictor effects tend to supersede endothelium-dependent relaxation. Accordingly, muscarinic effects on vasomotor regulation cannot be adequately characterized using only one or two arbitrary doses of acetylcholine.

The balance between two opposing muscarinic effects, endothelium-mediated vasodilation and direct stimulation of smooth muscle, appears to provide a satisfactory explanation for the variable vasomotor effects of acetylcholine. There are still unanswered questions, however. First, there is evidence that neurogenic muscarinic vasodilation may occur as an endothelium-independent event. Second, it is difficult to envision how acetylcholine released in quantal amounts from adventitial nerves can reach endothelial cells. Perhaps transmural diffusion of neurotransmitter is operative mainly at the level of thin-walled arterioles. Alternatively, muscarinic agonists might originate from nonneural sources. Third, the regulation of muscarinic receptors is still poorly characterized. Five muscarinic receptors have been cloned already, and selective agonists and antagonists are not available to differentiate them in intact tissues. Fourth, endothelial cells release in addition to EDRF multiple vasoactive agents with both constrictor and dilator effects. Possible interactive regulatory mechanisms between EDRF and these agents remain to be delineated.

In 1984, we reported that arteries isolated from rabbits with diet-induced hypercholesterolemia exhibited a defective endothelium-dependent relaxation in response to acetylcholine. We showed in such rabbits that acetylcholine was ineffective in reducing hindlimb total vascular resistance. We were then able to demonstrate by video-microscopic visualization of skeletal muscle microvessels in situ that arterioles (20–30 μm in diameter) failed to dilate normally in response to either topical or intra-arterial acetylcholine. Because arterioles do not develop atheromas, we concluded that hyperlipidemic endothelial dysfunction could occur in the absence of underlying atheromatous lesions. Impairment of endothelium-dependent relaxation has been demonstrated in other species maintained on high-fat diets, notably pigs and monkeys. In cholesterol-fed pigs, defective endothelium-dependent relaxation is manifest before light and electron microscopic changes of atherosclerosis developed. Of considerable interest...
is the report of Harrison et al.\textsuperscript{16} showing that defective endothelium-dependent arterial relaxation in cynomolgus monkeys with diet-induced hypercholesterolemia can be reversed by returning the animals to a low-fat diet.

Attempting to extend our experiments to human arteries, we have collected coronary arteries from hearts excised at cardiac transplantation.\textsuperscript{18} Coronary arteries selected from patients with a plasma cholesterol of less than 200 mg/dl and exhibiting no atherosclerotic changes, including absence of fatty streaks, relaxed dose-dependently in response to acetylcholine and concomitantly accumulated cyclic GMP. These effects resembled those obtained in aortas from normocholesterolemic rabbits. In contrast, the vast majority of arteries obtained exhibited histologic signs of atherosclerosis and relaxed poorly in response to acetylcholine and other endothelium-dependent vasodilators such as histamine and substance P.\textsuperscript{18} The calcium ionophore A23187, an endothelium-dependent agent that bypasses surface receptor-mediated regulation, and nitroglycerin, a vasodilator acting directly on smooth muscle, exerted potent relaxing effects.\textsuperscript{18} Thus, endothelial cells of atherosclerotic human arteries appeared to have a suppressed responsiveness to agonists mediating their effects through membrane-dependent mechanisms, but their ability to generate EDRF in response to an appropriate intracellular calcium signal seemed preserved. The precise mechanism(s) responsible for the failure of endothelial cells to respond to surface stimuli with appropriate intracellular calcium signals remains to be determined.

Japanese workers have been pioneers in the pharmacologic exploration of coronary arterial function in humans. Their studies were prompted by the consideration that vasospastic (variant) angina was the expression of autonomic dysregulation. In 1974, Yasue and coworkers\textsuperscript{19} reported on studies in which patients with variant angina were challenged with parasympathetic (methacholine) and sympathetic (epinephrine) agonists. They suggested that enhanced activity of the parasympathetic nervous system was involved in initiating attacks of variant angina. Subsequently, they injected 10–80 \( \mu \)g acetylcholine into coronary arteries of patients with variant angina and concluded that this maneuver was useful for the diagnostic provocation of coronary spasm.\textsuperscript{20} They then extended their studies to patients with coronary disease not suffering from variant angina and found that acetylcholine (30–100 \( \mu \)g i.c.) induced coronary constriction in many patients with “normal or almost normal” coronary arteries, although other patients with similar angiographic anatomy responded with diffuse dilation.\textsuperscript{21} Furthermore, they emphasized that vasomotor responses along the segments of single coronary arteries were nonuniform. They proposed that angiographically normal–appearing arteries constrict in response to acetylcholine because of a dysfunctional endothelium in arterial segments without appreciable angiographic changes.\textsuperscript{21} In the present issue of \textit{Circulation},\textsuperscript{22} Yasue and collaborators have extended their observations on muscarinic coronary reactivity. They performed selective coronary arteriography in 74 patients, 49 of whom were diagnosed as having “normal smooth coronary arteries.” These patients were subdivided into subjects younger and older than 30 years, but one does not know how the authors arrived at this arbitrary age limit. In the younger group, acetylcholine (50 \( \mu \)g i.c.) evoked coronary dilation that was most prominently expressed in distal coronary segments, but in the older group the preponderant response was constriction. In 29 patients exhibiting coronary luminal irregularities and stenoses, acetylcholine elicited only constrictor responses. Nitroglycerin was an efficacious dilator in all groups. Coronary risk factors, including a total plasma cholesterol of more than 240 mg/dl, arterial pressure of more than 160/90 mm Hg, and tobacco smoking, were less prevalent in young patients and most prevalent in the group with coronary disease. The authors made no attempt to analyze possible associations between coronary reactivity and individual risk factors. Also, data on the dose-dependency of the muscarinic effects were not presented. Yasue and collaborators conclude that patients more than 30 years old who have angiographically normal coronary arteries tend to have endothelial dysfunction or atherosclerosis.

In another article in this issue of \textit{Circulation}, Vita et al.\textsuperscript{23} have attempted to determine possible relations between coronary responses to intracoronary acetylcholine and coronary risk factors. During a 52-month period, they studied the response of intracoronary acetylcholine in 106 patients. It is not explained how the patients were selected for these studies. Thirty-four patients were singled out for further evaluation because their angiograms revealed “entirely smooth coronary arteries, without stenoses or even minimal luminal irregularities.” Graded infusions of acetylcholine into the left anterior descending coronary artery were estimated to have produced final “intracoronary concentrations” of 0.01, 0.1, and 1.0 \( \mu \)M, but eight patients received only two of the three infusions. Computer-assisted quantitative arteriography was used to determine average percent changes in arterial diameter for the different acetylcholine doses were calculated. Data points for the different acetylcholine concentrations were used to determine slopes of the dose-response effects, positive and negative slopes indicating dilation and constriction, respectively. A multiple stepwise regression analysis was interpreted as showing independent associations between acetylcholine responses and coronary risk factors including total serum cholesterol, family history, and gender. The authors conclude that the development of muscarinic constrictor responses are likely to be an abnormality of endothelial function and a precursor of angiographically detectable coronary disease.

In conclusion, the two studies are in apparent agreement with experimental data indicating that
hypercholesterolemia may be associated with impaired endothelial function. One persisting question is what we should consider to represent biologically optimal cholesterol levels in humans. Great apes, animals that closely resemble humans with respect to digestive physiology and plasma lipoprotein metabolism, are almost exclusively herbivorous and have plasma total cholesterol levels of 100 mg/dl or below.16,24–26 In baboons simply doubling cholesterol levels (200 mg/dl) for 1 year may suffice to produce early atheromatous lesions.25 Vegetarians and Chinese populations who are less than Yasue et al’s 30-year-old age limit typically exhibit primate-like cholesterol levels of 130 mg/dl or less,27 but in young US children, levels unfortunately may already average 170 mg/dl.28,29 Starry’s studies suggest that as many as 50% of US teenagers may have early atheromatous lesions that resemble those of hypercholesterolemic animals with impaired endothelium-dependent relaxation. Most of these children would probably exhibit “entirely smooth coronary arteries” on arteriography, but what will their angiograms look like 30 years later?

References


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