Enhanced Contractile Mechanisms in Vasospasm

Is Endothelial Dysfunction the Whole Story?

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The importance of the endothelium in regulating vascular tone and reactivity is well recognized. The endothelium produces a variety of substances including nitric oxide (NO), prostaglandins, endothelin-1, and an unidentified hyperpolarization factor that control vascular tone, thrombus formation, platelet and leukocyte adhesion and aggregation, and cellular proliferation. Alterations in the synthesis or activity of these substances appear to play a major role in the pathogenesis of a variety of diseases. Emphasis has been placed on alterations in the generation and function of NO as an underlying mechanism for abnormal vascular reactivity in atherosclerosis and other vascular diseases. Studies in both animal models and humans demonstrate that NO-mediated responses are decreased in atherosclerosis, diabetes, and a wide variety of vascular diseases. But measurements of NO in atherosclerosis have produced variable results with decreases in the levels of NO reported. Inactivation of NO by reactive oxygen species may account for the decrease in NO-mediated responses.

Although the absence of vasodilator mechanisms may impair the ability of the coronary circulation to respond to increases in myocardial oxygen consumption or vasoactive substances, for vasospasm to occur, an increase in contractile mechanisms must also occur. Previous studies from the laboratory of Akira Takeshita and coworkers in animal models of coronary vasospasm have suggested that increased vascular contractile responses to serotonin were primarily related to smooth muscle hyperreactivity. Takeshita and coworkers compared the relative contributions of abnormal endothelium-dependent responses versus smooth muscle function to enhanced vascular muscle contractions in a model of vasospasm to determine whether vasospasm is due to altered NO-mediated responses, enhanced vasoconstrictor responses, or both. Endothelium-dependent responses were examined in two ways: measurements of serotonin-induced constriction and substance P–induced dilation both before and after inhibition of nitric oxide synthase (NOS) in normal and vasospastic arteries. Serotonin is a unique vasoactive substance because its action involves two opposing effects on vascular tissue: dilation via release of NO from endothelium and constriction via direct activation of serotonin receptors on smooth muscle. Inhibition of NOS augmented serotonin-induced constriction in a normal artery but not to the degree observed in the vasospastic artery. Inhibition of NOS did not increase serotonin-induced constriction further in the vasospastic artery. Dilation to substance P was attenuated by inhibition of NOS in the normal artery, but again there was no effect in the vasospastic artery. Thus, NO-mediated dilation by two different agonists was absent at the vasospastic site. Despite the lack of NO, constriction to serotonin in the normal artery was not as great as constriction in the vasospastic artery. This suggests that vascular muscle at the vasospastic site was hyperresponsive to serotonin. Inhibition of nitric oxide alone was not enough to produce coronary vasospasm.

What intracellular mechanism(s) could account for the enhanced contractile function of vascular muscle? The contractile status of vascular muscle regulates vessel tone and vascular resistance. Phosphorylation of regulatory subunits of myosin light chain is the primary determinant of cross-bridging and attachment of the actin–myosin complex and cycling during contraction and relaxation of smooth muscle. Phosphorylation of the regulatory subunits of myosin light chain (rMLC) is controlled by a balance between the Ca2+/calmodulin-dependent myosin light chain kinase (MLCK) and the myosin light chain phosphatase (MLCP). Increases in the concentration of intracellular calcium initiates contractions by activating MLCK and phosphorylation of rMLC. Contractions of smooth muscle are not dependent on the concentration of intracellular calcium alone but also on the sensitivity of the contractile apparatus to calcium. Thus, increases in vascular tone can result without a change in the concentration of intracellular calcium.

What regulates sensitivity to calcium? Two potential mechanisms may modulate calcium sensitivity: alterations in the regulation of phosphorylation of rMLC by kinase or phosphatase cascades or the calcium affinity of regulatory proteins such as caldesmon or calponin. Alterations in the balance of phosphorylation and dephosphorylation of rMLC is recognized as the major site of regulation of contractile function. Agonist-induced G protein–coupled receptor activation produces contractions by increasing cytosolic calcium concentration and/or the sensitivity of the contractile apparatus for calcium. Increases in the sensitivity of the contractile proteins for calcium can occur despite a constant calcium concentration. Selective agonists can increase sensitivity to cytosolic calcium by activation of a small GTPase, RhoA, which subsequently activates rho-kinase. Activation of rho-kinase may play a prominent role in sustained constriction to

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agonists like serotonin that stimulate G protein–coupled receptors. Rho GTPases, including RhoA, Rac, and Cdc42, are members of the Ras superfamily of small GTP-binding proteins that, similar to other G proteins, cycle between an inactive cytoplasmic GDP-bound state and an active membrane-associated GTP-bound state. Activation of the rho-kinase pathway regulates vascular contractility by inhibiting the phosphatase, preventing dephosphorylation of MLC, and increasing phosphorylation of MLC and vasoconstriction.

RhoA is activated by growth factors, cytokines, hormones, and G protein–coupled receptor ligands involved in cell motility, proliferation, regulation of cell shape, and alterations in sensitivity of vascular muscle to calcium. Recently several studies using animal models have provided strong evidence that alterations in the RhoA/rho-kinase pathway mediate hypercontractility to vasoactive substances. Rho-kinase–induced increases in calcium sensitivity mediate constriction of normal arteries to serotonin and thromboxane mimetic, U46619. Upregulation of RhoA and rho-kinase may also mediate the enhanced vasconstriction and vessel tone in atherosclerosis, hypertension, angiotensin II–induced vascular hypertrophy, and cerebral and coronary vasospasm. Using selective inhibitors of rho-kinase or RhoA, enhanced constriction in each of the models of heightened vascular tone was reversed to normal. These studies in animal models suggest that a large component of the altered responses in many models of vascular disease is due to altered contractile mechanisms in addition to or instead of alterations in endothelial function.

The study by Masumoto and coworkers in this issue of Circulation is the first study to demonstrate that an increased activity of rho-kinase may play a major role in vivo in the enhanced contractile responses in patients with coronary vasospasm. Fasudil, a rho-kinase inhibitor, was effective in selectively preventing acetylcholine-induced constriction at vasospastic sites (>75% decrease in vessel diameter) while not altering constriction to acetylcholine at other sites. Other indices of ischemia (chest pain and ECG changes) were prevented by fasudil as well. Although the selectivity of fasudil for acetylcholine-induced vasospasm in comparison to other vasoactive substances was not tested, these findings suggest a novel therapeutic approach for the treatment of coronary vasospasm. Instead of administration of vasodilators to reverse vasospasm, the target is to reduce the calcium sensitivity of the hypercontractile portion of the artery and prevent vasospasm. However, the long-term effects and the selectivity of this approach are not known.

Although decreases in NO-mediated function may not directly determine the hypercontractile status of blood vessels, it is possible that alterations in NO may indirectly alter the contractile apparatus. NO released by specific agonists increases cGMP and activates cGMP-dependent protein kinase (cGK). Activation of cGK decreases the concentration of cytosolic calcium and/or the sensitivity of the contractile proteins to calcium. The mechanism of cGMP/cGK-induced dilation is related to both decreases in cytosolic calcium concentration through activation of calcium- lowering pathways and calcium desensitization by stimulating MLCP. Although cGK can bind directly to MLCP and phosphorylate the regulatory subunit of MLC phosphatase, this mechanism may not account for the effects of cGMP modulation of contractile proteins. RhoA-dependent changes in calcium sensitivity are inhibited by cGMP through cGK-mediated phosphorylation of RhoA that results in translocation of the membrane-bound activated form of RhoA to the inactive cytosolic form. This inhibition of RhoA–induced calcium sensitivity of the contractile apparatus by cGMP/cGK suggests a novel signaling pathway for NO released from the overlying endothelium.

Impairment in the ability of the endothelium to produce NO or a reduction in NO bioavailability is one of the early indices of a variety of pathophysiological conditions, including hypertension, atherosclerosis, diabetes, aging, and cerebral and coronary vascular disease. A reduction in the generation and/or bioavailability of NO could potentially decrease the negative impact of NO on control of RhoA and rho-kinase and shift the balance in favor of RhoA and rho-kinase activation and enhanced vascular contractility. Potential interactions between release of vasoactive substances from endothelium and the regulation of the contractile apparatus in underlying vascular muscle needs further investigation to understand mechanisms of altered vascular responses in cardiovascular disease.

References


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