Rho-Kinase

A Potential Link Between Hypercholesterolemia and Abnormal Vascular Smooth Muscle Contraction

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Although elevated serum cholesterol levels and hypertension are independent risk factors for cardiovascular disease, far too often they are manifested in the same individual. Whether this is a coincidental finding between 2 highly prevalent risk factors or perhaps because of some mechanistic link between these 2 processes is unclear. Regardless, the concomitant treatment of hypercholesterolemia and hypertension has yielded additive and possibly synergistic reductions in cardiovascular events.

Previous studies attempting to link hypercholesterolemia with abnormal vascular smooth muscle (SMC) contractions have focused on the endothelium. Hypercholesterolemia and atherosclerosis impair the production and/or availability of endothelium-derived NO, leading to endothelial dysfunction and abnormal vascular reactivity. Indeed, acute lowering of serum cholesterol with low-density lipoprotein-apheresis or with statin therapy improves endothelium-dependent relaxations.

In the present study, Morikage et al extend the effects of cholesterol on vascular reactivity to changes in SMC contractions. They showed that elevated serum cholesterol levels were associated with increased calcium sensitization of SMC through the sphingosylphosphorylcholine (SPC)/Rho-kinase (ROCK)-mediated pathway in humans and rabbits. This enhanced SMC contraction was observed only with ROCK activator SPC and not with phenylephrine or potassium; was accompanied by minimal changes in intracellular calcium concentration; and was unaffected by addition of the eNOS inhibitor, N\textsuperscript{\text{-}}\text{G}-monomethyl-L-arginine, or removal of the endothelium. However, the SMC contraction was abolished by addition of the ROCK inhibitor, Y27632, or by applying a dominant-negative mutant of ROCK (dn-ROK) to the cytosol of permeabilized SMC. Interestingly, Y27632 and dn-ROK had no effect on calcium-dependent SMC contraction, suggesting calcium sensitization of SMC rather than calcium-induced SMC contraction by cholesterol. The authors conclude that cholesterol enhances calcium sensitivity of SMC to SPC via ROCK and suggest that this may be a mechanism by which hypercholesterolemia could precipitate vasospasms or perhaps increase the risk for hypertension.

ROCK plays a central role in diverse cellular functions such as SMC contraction, stress fiber formation, and cell migration and proliferation. Presently, 2 ROCK isoforms have been identified, ROCK1 and ROCK2, which share an overall 65% homology in amino-acid sequence and 92% homology in their kinase domains. Thus, pharmacological inhibitors of ROCK such as Y-27632, fasudil (HA1077), and hydroxyfasudil, which target their ATP-dependent kinase domains, can inhibit both ROCK1 and ROCK2. Furthermore, at higher concentrations, Y-27632 can also inhibit protein kinase C–related kinase-2, protein kinase N, and citron kinase, whereas fasudil can inhibit protein kinase A and protein kinase C. Physiologically, ROCKs can be activated by serum lysophospholipids such as lysophosphatidic acid, sphingosine-1 phosphate, and SPC, which stimulate RhoGEF and lead to the formation of active GTP-bound Rho GTPases. ROCKs are serine-threonine protein kinases and immediate downstream targets of Rho GTPases. They control the assembly of the actin cytoskeleton, increase calcium sensitization, and enhance cell contractility by phosphorylating a variety of proteins, such as myosin light chain phosphatase, LIM kinases, adducin, and ezrin-radixin-moesin proteins. Indeed, inhibition of ROCK prevents cerebral ischemia, coronary vasospasm, and hypertension and atherosclerosis. Thus, it is likely that SPC-induced calcium sensitization and the subsequent enhanced contractions of SMC, as demonstrated in the present study by Morikage et al, is mediated by ROCK.

Immunolocalization and cell-fractionation studies of ROCKs, especially ROCK2, have shown that this protein is distributed mainly in the cytoplasm. Activated ROCK2 partly translocates from the cytoplasm to membranes. For example, a small amount of ROCK2 has been found in the membrane fraction, and some immunostaining is detectable at the cell periphery or membranes of growing cells. In the present study, SPC-induced translocation of cytosolic ROCK to the cell membrane and SMC contractions were blocked by depletion of cellular cholesterol with β-cycloextrin and the subsequent loss of cholesterol-enriched lipid rafts. These findings suggest that translocation of ROCK to lipid rafts is essential for its function and that cholesterol enhances this...
process. Thus, cholesterol primes SMC in terms of enhancing ROCK-mediated calcium sensitivity by facilitating ROCK translocation to cholesterol-enriched lipid rafts. Clinically, calcium sensitization of SMC by ROCK is observed in patients with hypertension, and as shown in the present study, in patients with hypercholesterolemia.

Although cholesterol may be necessary for ROCK-mediated effects, several questions remain. Because most studies have used ROCK inhibitors, which cannot discriminate between ROCK1 and ROCK2, it is unknown what the relative importance of ROCK isoforms is in calcium sensitization of SMC. Perhaps future studies with isoform-specific ROCK inhibitors or ROCK-deficient mice will shed light on this matter. Furthermore, it is not known whether patients who have lower serum cholesterol levels or who received lipid-lowering therapy have decreased membrane ROCK and lipid rafts, and if so, whether this is associated with decreased blood pressure. Although blood pressure was not monitored as frequently in previous lipid-lowering trials with statins as with hypertensive trials, none of lipid-lowering trials have ever shown a clear reduction in blood pressure with statin therapy. Thus, further studies are required to determine whether the priming effect of cholesterol on ROCK-mediated calcium sensitivity of SMC contributes clinically to higher blood pressure. Unless these questions are addressed, it remains to be determined whether ROCK is an important therapeutic target for hypertension in patients with and without hypercholesterolemia.

Sources of Funding
Supported by National Institutes of Health grant HL052233.

Disclosures
None.

References


**Key Words:** cholesterol, Rho kinase, calcium, smooth muscle contraction.
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Circ Res. 2006;99:238-239
doi: 10.1161/01.RES.0000236798.01988.5d

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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