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A new mechanism for flow-mediated vasoprotection? Focus on "Lung endothelial cell proliferation with decreased shear stress is mediated by reactive oxygen species"

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FLOW-ORIGINATED UNIDIRECTIONAL laminar shear stress is an essential physiological protector of vascular integrity. It exerts beneficial effects via an array of cellular responses, such as upregulation of endothelial nitric oxide synthase (eNOS) gene expression and nitric oxide formation (23), upregulation of the antioxidant enzyme superoxide dismutase (6, 11), prevention of endothelial cell apoptosis (22, 24), and attenuation of inflammatory protein expression (25). Under physiological conditions, low ambient concentrations of reactive oxygen species are required for growth signaling (9). Flow-dependent physiological production of hydrogen peroxide from mitochondrion mediates coronary vasodilatation (19). On the other hand, overly produced reactive oxygen species from further activated NAD(P)H oxidases and secondary oxidase systems generally promote inflammation, apoptosis, and uncoupling of eNOS (2, 5, 8). These observations seem to suggest that the central functional core of shear stress is to prevent reactive oxygen species overproduction. Indeed, a recent study by Yamawaki et al. (25) demonstrated that shear stress inhibition of vascular inflammation is mediated by downregulation of endothelial thioredoxin-interacting protein, a protein that is negatively involved in thioredoxin-mediated protection against oxidant stress. Nevertheless, the entire molecular mechanisms underlying shear stress control of redox signaling and the identity of the potential mechanotranducers involved remain to be fully elucidated.

Milovanova et al. (Ref. 18, see p. C66 in the present issue) report that cessation of flow activates endothelial NAD(P)H oxidases by inactivating Kir6.2-containing ATP-sensitive K^+ (K_{ATP}) channels, resulting in enhanced reactive oxygen species production in pulmonary endothelial cells and subsequent cell proliferation. The authors found that flow cessation-induced cell proliferation is absent in endothelial cells isolated from Kir6.2 and gp91^{phox}-deficient mice. In addition, activation of NAD(P)H oxidase did not occur in Kir6.2-deficient endothelial cells, indicating an upstream role of the K_{ATP} channels. Although they used mouse pulmonary microvascular endothelial cells in their study, the same group recently demonstrated that blockade of K_{ATP} channels activates NAD(P)H oxidases in large vessel (bovine aortic) endothelial cells (16). These data indicate that K_{ATP} channels could function as mechanotransducers in both large and small vessel endothelial cells. In contrast to lack of flow, normal shear stress could thus prevent NAD(P)H oxidase-dependent overproduction of reactive oxygen species by maintaining normal endothelial K_{ATP} channel activity. Activation of vascular NAD(P)H oxidases underlies development of atherosclerotic vascular diseases (2, 3). Likewise, NAD(P)H oxidase-derived reactive oxygen species contribute to chronic pulmonary hypertension by augmenting vasoconstriction and vascular remodeling (1, 15). Thus the observation that K_{ATP} channels play an important role in modulating endothelial redox balance could represent a novel mechanism whereby flow-mediated shear stress is vasoprotective in both large and small vessels.

In addition, this K_{ATP} channel-dependent activation of endothelial NAD(P)H oxidases appears specific for loss of flow. The authors demonstrated that K_{ATP} antagonist glyburid mimicked flow cessation-induced activation of NAD(P)H oxidases, and that this response was absent in Kir6.2-deficient cells. Angiotensin II, however, had similar effects in activating endothelial NAD(P)H oxidases in both wild-type and Kir6.2-deficient cells, strongly suggesting a flow-specific role of K_{ATP} channels in activation of endothelial NAD(P)H oxidases. Thus these data characterized an agonist-specific modulation of gp91^{phox} (Nox2)-containing endothelial NAD(P)H oxidases. Whether similar differential modulations occur with other isoforms of NAD(P)H oxidases, i.e., Nox1, Nox4, and Nox5 (all exist in endothelial cells) (2, 7), remain to be examined. This is an interesting question to address because Nox proteins are activated in an agonist-specific fashion that seems linked to their intracellular localizations (2, 7). Activation of Nox1 promotes endothelial cell proliferation (12). Then, besides Nox2, is endothelial Nox1 activated by flow cessation as well? If so, does it require K_{ATP} channels similarly or differently from other agonists such as angiotensin II?

This study is unique because the experimental strategy offers an oxygenated "ischemic" condition. Ischemia is often accompanied with hypoxia. The latter is also associated with modulation of redox signaling (20). A "pure" ischemic condition would be more effective in revealing the signaling events activated solely by the reduction in blood flow. In this study, the authors used a system to simulate ischemia by rerouting the flow from the luminal (endothelial cells cultured on capillary fibers to form circulation) to the abluminal compartment. This eliminates shear stress to endothelium while preserving oxygen and nutrient accessibility. Using this system, the authors clearly demonstrated that endothelial NAD(P)H oxidases are the sole dominant source for reactive oxygen species in response to ischemia/loss of flow. The gp91^{phox}-deficient endothelial cells completely failed to produce reactive oxygen species in response to flow cessation. Kir6.2 knockout partially but significantly reduced reactive oxygen species production, indicating that Kir6.2-K_{ATP} is required for NAD(P)H oxidase activation. On the other hand, the partial effectiveness seems to suggest that other flow-insensitive endothelial potassium chan-

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nels also contribute to activation of NAD(P)H oxidases. This hypothesis, however, requires further investigation.

In contrast to the vasoprotective unidirectional flow, disturbed or oscillatory flow at bifurcation and branching points of the vascular bed is atherogenic (13). Molecular studies further demonstrated that oscillatory shear stress provokes a marked and sustained increase in NAD(P)H oxidase-dependent reactive oxygen species production (6) in the endothelium and reactive oxygen species-dependent monocyte adhesion to endothelium (10). Importantly, NAD(P)H oxidase-derived hydrogen peroxide causes xanthine oxidase cleavage and activation in response to oscillatory shear (17). Oscillatory shear may also uncouple eNOS via hydrogen peroxide-dependent mechanisms (5), and under this condition, previously observed upregulation of eNOS (4) could be detrimental. Thus activation of NAD(P)H oxidases by oscillatory shear is a key step in activating secondary oxidase systems to sustain reactive oxygen species production. It would be very interesting to understand whether the same K_{ATP} channels involved in unidirectional shear attenuation of endothelial NAD(P)H oxidases are inhibited by oscillatory shear to allow activation of endothelial NAD(P)H oxidases. If true, one K_{ATP} channel agonist could augment beneficial effects of unidirectional shear at the mean time of diminishing pathological signaling initiated by oscillatory shear.

Finally, based on the observation of K_{ATP} channels as modulators of endothelial NAD(P)H oxidases, many questions remain as to how intracellular K^+ ions mediate downstream signaling that ultimately affect NAD(P)H oxidase activity. Using angiotensin II as a potent agonist, Seshiah et al. (21) characterized a NAD(P)H oxidase-activating signaling cascade in vascular smooth muscle that involves sequential activation of PKC, Src, EGF receptor, phosphatidylinositol 3-kinase, and phosphatidylinositol 3,4,5-trisphosphate-dependent Rac-1 membrane translocation. Activation of endothelial NAD(P)H oxidases by angiotensin II requires $p47^{phox}$ phosphorylation and membrane translocation (14). The authors found the roles of K_{ATP} channels in activating endothelial NAD(P)H oxidases are specific for flow cessation. What are the signaling effectors downstream of intracellular K^+ accumulation? Are none of the above-described signaling events involved in flow cessation-induced activation of NAD(P)H oxidases? Or if some are involved, at which levels do the signals diverge to allow agonist specificity? These signaling mechanisms are important as they would predict efficacy of K^+ channel agonists in counterbalancing NAD(P)H oxidase activation by pathological stimuli other than loss of flow.

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REFERENCES

1. **Brennan LA, Steinhorn RH, Wedgwood S, Mata-Greenwood E, Roark EA, Russell JA, and Black SM.** Increased superoxide generation is associated with pulmonary hypertension in fetal lambs: a role for NADPH oxidase. *Circ Res* 92: 683–691, 2003.
2. **Cai H.** NAD(P)H oxidase-dependent self-propagation of hydrogen peroxide and vascular disease. *Circ Res* 96: 818–822, 2005.
3. **Cai H, Griendling KK, and Harrison DG.** The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. *Trends Pharmacol Sci* 24: 471–478, 2003.
4. **Cai H, McNally JS, Weber M, and Harrison DG.** Oscillatory shear stress upregulation of endothelial nitric oxide synthase requires intracellular hydrogen peroxide and CaMKII. *J Mol Cell Cardiol* 37: 121–125, 2004.
5. **Chalupsky K and Cai H.** Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of eNOS. *Proc Natl Acad Sci USA* 102: 9056–9061, 2005.
6. **De Keulenaer GW, Chappell DC, Ishizaka N, Nerem RM, Alexander RW, and Griendling KK.** Oscillatory and steady laminar shear stress differentially affect human endothelial redox state: role of a superoxide-producing NADH oxidase. *Circ Res* 82: 1094–1101, 1998.
7. **Griendling KK.** Novel NAD(P)H oxidases in the cardiovascular system. *Heart* 90: 491–493, 2004.
8. **Griendling KK and FitzGerald GA.** Oxidative stress and cardiovascular injury. Part I: basic mechanisms and in vivo monitoring of ROS. *Circulation* 108: 1912–1916, 2003.
9. **Griendling KK and Harrison DG.** Dual role of reactive oxygen species in vascular growth. *Circ Res* 85: 562–563, 1999.
10. **Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, Dikalov S, Giddens DP, Griendling KK, Harrison DG, and Jo H.** Oscillatory shear stress stimulates endothelial production of O_2^- from $p47^{phox}$-dependent NAD(P)H oxidases, leading to monocyte adhesion. *J Biol Chem* 278: 47291–47298, 2003.
11. **Inoue N, Ramasamy S, Fukai T, Nerem RM, and Harrison DG.** Shear stress modulates expression of Cu/Zn superoxide dismutase in human aortic endothelial cells. *Circ Res* 79: 32–37, 1996.
12. **Kobayashi S, Nojima Y, Shibuya M, and Maru Y.** Nox1 regulates apoptosis and potentially stimulates branching morphogenesis in sinusoidal endothelial cells. *Exp Cell Res* 300: 455–462, 2004.
13. **Ku DN, Giddens DP, Zarins CK, and Glagov S.** Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. *Arteriosclerosis* 5: 293–302, 1985.
14. **Li JM and Shah AM.** Mechanism of endothelial cell NADPH oxidase activation by angiotensin II: role of the $p47^{phox}$ subunit. *J Biol Chem* 278: 12094–12100, 2003.
15. **Liu JQ, Zelko IN, Erbynn EM, Sham JSK, and Folz RJ.** Hypoxic pulmonary hypertension: role of superoxide and NADPH oxidase ($gp91^{phox}$). *Am J Physiol Lung Cell Mol Physiol* (In Press). First published August 5, 2005; doi:10.1152/ajplung.00135.2005.
16. **Matsuzaki I, Chatterjee S, Debolt K, Manevich Y, Zhang Q, and Fisher AB.** Membrane depolarization and NADPH oxidase activation in aortic endothelium during ischemia reflect altered mechanotransduction. *Am J Physiol Heart Circ Physiol* 288: H336–H343, 2005.
17. **McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H, and Harrison DG.** Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol Heart Circ Physiol* 285: H2290–H2297, 2003.
18. **Milovanova T, Chatterjee S, Manevich Y, Kotelnikova I, DeBolt K, Muniswamy M, Moore JS, and Fisher AB.** Lung endothelial cell proliferation with decreased shear stress is mediated by reactive oxygen species. *Am J Physiol Cell Physiol* 290: C66–C76, 2006.
19. **Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, and Gutterman DD.** Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. *Circ Res* 92: 31–40, 2003.
20. **Schumacker PT.** Hypoxia, anoxia, and O_2 sensing: the search continues. *Am J Physiol Lung Cell Mol Physiol* 283: L918–L921, 2002.
21. **Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, and Griendling KK.** Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res* 91: 406–413, 2002.
22. **Surapisitchat J, Hoefen RJ, Pi X, Yoshizumi M, Yan C, and Berk BC.** Fluid shear stress inhibits TNF-α activation of JNK but not ERK1/2 or p38 in human umbilical vein endothelial cells: Inhibitory crosstalk among MAPK family members. *Proc Natl Acad Sci USA* 98: 6476–6481, 2001.
23. **Uematsu M, Ohara Y, Navas JP, Nishida K, Murphy TJ, Alexander RW, Nerem RM, and Harrison DG.** Regulation of endothelial cell nitric oxide synthase mRNA expression by shear stress. *Am J Physiol Cell Physiol* 269: C1371–C1378, 1995.
24. **Urbich C, Walter DH, Zeiher AM, and Dimmeler S.** Laminar shear stress upregulates integrin expression: role in endothelial cell adhesion and apoptosis. *Circ Res* 87: 683–689, 2000.
25. **Yamawaki H, Pan S, Lee RT, and Berk BC.** Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. *J Clin Invest* 115: 733–738, 2005.