Hypertension is a clinical situation associated with endothelial dysfunction and increased vascular reactive oxygen species (ROS) formation. The observations that antioxidants, such as Tempol or apocynin, lower the blood pressure in spontaneously hypertensive rats gave rise to the concept that ROS essentially contribute to hypertension. Recent work, however, has sharpened this view, and it is now clear that the underlying mechanism of ROS-induced hypertension may not be endothelial dysfunction but rather an ROS-dependent modulation of the sympathetic drive, the kidney, and potentially alterations in the immune system. Despite these findings, hypertension has been consistently linked to increased ROS formation in the vascular system. It is, therefore, plausible that ROS are not necessarily the cause but potentially the consequence of hypertension. This notion certainly does not exclude that ROS are involved in the pathogenesis of specific aspects of hypertension, such as the development of fibrosis.

In keeping with the concept of hypertension-induced ROS formation, it was noted previously that distension of a vessel with an oversized balloon acutely increases ROS formation. Moreover, acute elevation of pressure from 80 to 160 mm Hg enhances the ROS formation and induces an ROS-dependent attenuation of the endothelium-dependent relaxation in isolated rat femoral arteries. Moreover, even in chronic models of isolated hypertension, such as aortic banding, the ROS formation was consistently higher in the hypertensive rather than in the normotensive part.

There is, however, uncertainty regarding the enzymatic sources of ROS, and a contribution of mitochondria, endothelial NO synthase uncoupling, and NADPH oxidases has been suggested. Moreover, little is known about the signaling pathway linking hypertension with ROS formation.

In this issue of Hypertension, Vecchione et al. significantly add to our knowledge concerning the latter aspect. With the aid of a wire-myograph system and the isolated murine carotid artery, the authors demonstrate that an increase in vascular stretch, equivalent to a rise in blood pressure from 100 to 180 mm Hg, translocates the integrin-linked kinase 1 (ILK-1) to the plasma membrane, where it interacts with the multidomain adaptor protein paxillin. Small interfering RNA directed against ILK-1 and adenovirus coding for dominant-negative Rac-1 demonstrated that ILK-1 activates Rac-1, which subsequently promotes superoxide anion (O$_2^-$) formation and endothelial dysfunction. Because NO is a mediator of vascular compliance, it appears possible that endothelial dysfunction further promotes vascular stiffening in hypertension and, thus, stretch.

Rac-1 activation is mediated by guanine exchange factors (GEFs). Overexpression of a dominant-negative version of the GEF βPIX (p21-activated kinase-interacting exchange factor) prevented the stretch-induced increase in O$_2^-$ formation and endothelial dysfunction. That these effects might also be operative in vivo was suggested by the fact that an increased ILK-1 expression, Rac-1 activation, and O$_2^-$ formation was also detected in a hypertensive carotid artery of mice subjected to transverse aortic banding. Thus, this work not only documents the great importance of ILK-1 as part of a stretch-induced signaling cascade in the intact vessel, it also further establishes the link between ILK-1 and Rac-1 via the GEF βPIX. What makes this study unique is that the authors rigorously concentrate on the intact vessel, whereas previous studies were performed in cultured cells or even cell lines with the aid of stretch apparatus. A couple of limitations arise from this approach, which should be mentioned to avoid an overinterpretation of the results: the mechanisms that induce ILK-1 translocation are not necessarily identical to those that increase the protein level. Inactive ILK-1, that is, without the interaction with other partners of the IPP (ILK PINCH parvin) complex, however, is rapidly degraded by the proteasome. Thus, ILK-1 protein abundance can be a marker for ILK-1 signaling activity.

Moreover, the approach with dominant-negative proteins potentially blocks downstream target proteins for independent stimuli. Thus, at the current stage, it cannot completely be excluded that a different GEF, like Vav2 or pRx1, mediates Rac-1 activation in response to stretch. Because antibodies are not available for most GEFs to control small interfering RNA experiments, the current approach with dominant-negative proteins is the only feasible technique. So far, maybe with the exception of Rho-GEFs, the proteins that activate small GTPases in the vascular system have gained relatively little attention. However, because small GTPases themselves are so tremendously important for almost all cellular processes, a specified control system is required to render GTPase signaling site and stimulus specific. Indeed, >80 GEFs have been identified in human cells so far, but research on this topic is still in its infancy because of the high diversity of these proteins and their multifunctionality.

Sensing stretch is a complex task for cells. Although integrins are well-established mechanosensors, there are sev-
A role for protein kinase C in stretch-induced ROS production is possible, because src is known to be easily activated by ROS. The current concept is that stretch induces a contribution of ROS to cell signaling at an early point, a consequence of increased formation of ROS, which is also the consequence of a Rac-1-dependent activation of the NADPH oxidase.

In conclusion, with the present work, Vecchione et al. provide additional support for the concept that increased vascular ROS formation is a consequence of hypertension. They suggest that an activation of vascular mechanosensors and the subsequent stimulation of the ILK-1/βPix/Rac-1 pathway activate the NADPH oxidase and that O$_2^-$ generated by this class of enzymes reduces NO availability. The consequence of this process will be endothelial dysfunction and an increase in vascular stiffness (Figure).

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**References**


A New PIXel in the Puzzle: How Increased Vascular Pressure Induces Oxidative Stress
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