Redox Signaling of the Arteriolar Myogenic Response

P.T. Nowicki, S. Flavahan, H. Hassanain, S. Mitra, S. Holland, P.J. Goldschmidt-Clermont, N.A. Flavahan

Arteriolar vascular smooth muscle cells (VSMCs) are mechanosensitive, constricting to elevations in transmural pressure (P_TM). The goal of the present study was to determine whether oxidant signaling regulates this myogenic response. In response to P_TM elevation, VSMCs of arterioles but not arteries generated constriction and increased reactive oxygen species (ROS) activity (using the H_2O_2-sensitive probe dichlorodihydrofluorescein). Arterioles had increased expression of NADPH oxidase components compared with arteries. Inhibition of NADPH oxidase, using mice with targeted impairment of enzyme components (p47^{phox} or rac1) or diphenyleneiodonium, prevented the pressure-induced generation of ROS. When ROS activity was inhibited, either by inhibiting NADPH oxidase or with N-acetylcysteine, the myogenic constriction was abolished. The myogenic constriction was also inhibited by catalase, which inactivates H_2O_2 but was unaffected by a cell-permeant mimic of superoxide dismutase (MnTMPyP). α_1-Adrenergic constriction was also inhibited by catalase, which inactivates H_2O_2, but was unaffected by a cell-permeant mimic of superoxide dismutase (MnTMPyP). Therefore, the myogenic constriction of arterioles was mediated by NADPH oxidase and ROS, contributing to the myogenic response of arteriolar VSMCs.

Vascular smooth muscle cells (VSMCs) of arterioles, but not arteries, are mechanosensitive, constricting to elevations in transmural pressure (P_TM). This myogenic response contributes to blood flow autoregulation and the establishment of basal vascular tone. The response is an inherent property of arteriolar VSMCs involving calcium-dependent actin/myosin interaction, but the more proximal signaling components have not been clearly defined. In cultured cells, mechanical stress initiates integrin-dependent activation of rho GTPases (rho, rac1, and CDC42), leading to reorganization of the cytoskeleton. Cytoskeleton reorganization by rac1 is mediated by NADPH oxidase and generation of reactive oxygen species (ROS). This enzyme complex, comprising Nox1, p47^{phox}, p67^{phox}, p22^{phox}, and rac1, is a key signaling system in cultured, noncontractile VSMCs. The aim of the present study was to determine whether the rac1/NADPH oxidase/ROS signaling pathway regulates the myogenic response of arteriolar VSMCs.

Materials and Methods

Vasomotor Responses

Mouse-tail arterioles and arteries were cannulated in a microperfusion chamber (Living Systems) and studied in the absence of flow as described. Unless stated otherwise, arterioles with intact endothelium were analyzed. Involvement of animals in the study was approved by the Ohio State University Animal Care and Use Committee.

ROS Determination

Endothelium-denuded vessels were incubated with the H_2O_2-sensitive probe 5-(and 6)-chloromethyl-2,7'-dichlorodihydrofluorescein diacetate (DCF), 5 μg/mL, for 30 minutes (37°C, P_TM of 10 mm Hg). Because activation of DCF fluorescence is reversible, fluorescent images (Zeiss, LSM 410) were captured at a P_TM of 10 mm Hg before and after P_TM had been increased (90 mm Hg, 1 minute). Maintaining vessels at 10 mm Hg did not change DCF fluorescence. Images were quantified using MetaMod software.

Mice

Transgenic mice expressing a dominant-negative mutant of human rac1 (rac-DN, cDNA; gift of Alan Hall, London, England), with threonine17 to asparagine substitution, were generated in FVB/N mice using the smooth muscle α-actin promoter (gift of Art Strauch, Ohio State University, Columbus, Ohio). The genome of the mice incorporated the cDNA of rac-DN, including its polyadenylation site. Founder mice were selected on the basis of Southern blot analysis, and one confirmed to have the highest number of human rac-DN gene copies was used to establish a stable transgenic line by breeding it with nontransgenic FVB/N mates. Reverse transcriptase–polymerase chain reaction analysis confirmed expression of rac-DN in smooth muscle, including blood vessels and intestine, whereas the transcript was not detected in control mice nor in the brain, heart, liver, skeletal muscle, and testis of transgenic mice. Responses in rac-DN mice were compared with nontransgenic littermates. All other experiments were performed on C57Bl6 mice; p47^{phox/-} mice were congenic to C57Bl6.

Western Blot Analysis

Endothelium-denuded vessels from 20 mice were processed as described. Antibodies were β-actin (Sigma), p47^{phox}, p67^{phox} (Transduction Laboratories), and rac-1 (Upstate Biotechnology).

Data Analysis

Data are expressed as mean±SEM for n number of animals. Vasomotor responses were expressed as percentage change in the basal diameter at 10 mm Hg. Statistical evaluation was by Student’s t test, except for multiple comparisons, when ANOVA followed by Scheffe’s analysis was used.

Results

At a P_TM of 10 mm Hg, the internal diameters of arterioles and arteries were 88.5±5.4 μm (n=18) and 184.4±13.4 μm, respectively (n=8). An increase in P_TM (10 to 90 mm Hg) caused arterioles to dilate, then constrict and initiate vasomotion, maintaining a diameter similar to that at a P_TM of 10 mm Hg (Figure 1A). The dilation represented passive
The basal level of ROS was reduced and vasodilators (papaverine 100 μmol/L plus nitroprusside 10 μmol/L) abolished the myogenic response. In arteries, elevated \( P_{\text{TM}} \) caused passive dilation that was unaffected by vasodilators. B, Increased \( P_{\text{TM}} \) (10 to 90 mm Hg) increased ROS activity in arteriolar VSMCs, assessed by LSM analysis of DCF fluorescence. Inhibition of ROS (catalase 3000 U/mL) (Figures 2A and 2B) caused dilatation that was unaffected by vasodilators. Similar results were obtained by inhibition of NADPH oxidase (DPI; p47\textsuperscript{phox} or rac-DN mice) or by impairment of NADPH oxidase using arterioles from p47\textsuperscript{phox} or rac-DN mice or using the nonselective inhibitor diphenyleneiodonium (DPI) (1 μmol/L; p47\textsuperscript{phox}−/− or rac-DN arterioles) reduced basal ROS activity and prevented the increase to elevated \( P_{\text{TM}} \). Arteries had reduced ROS activity, which did not increase after \( P_{\text{TM}} \) elevation. Bar=50 μm.

Opening was dramatically increased by vasodilators (Figure 1A). Increasing \( P_{\text{TM}} \) in arteries caused only passive dilation, and vasodilators had no effect (Figure 1A).

Elevating \( P_{\text{TM}} \) in arterioles increased ROS activity in VSMCs (2.53±0.30-fold increase in DCF fluorescence, \( n=13 \)) (Figure 1B). The basal level of ROS was reduced and the pressure-induced elevation was abolished by the antioxidant N-acetylcysteine (NAC) (20 μmol/L, data not shown) or by impairment of NADPH oxidase using arterioles from p47\textsuperscript{phox}−/− or rac-DN mice or using the nonselective inhibitor diphenyleneiodonium (DPI) (1 μmol/L) (Figure 1B). In contrast, arteries had low levels of ROS activity, which did not increase after \( P_{\text{TM}} \) elevation (Figure 1B). Arteries had decreased expression of the NADPH oxidase components rac1, p47\textsuperscript{phox}, and p67\textsuperscript{phox} compared with arterioles (Figure 2D).

The myogenic constriction to elevated \( P_{\text{TM}} \) was inhibited by impairment of NADPH oxidase (rac-DN or p47\textsuperscript{phox}−/− arterioles; DPI, 1 μmol/L), and only passive dilation was observed (Figures 2A and 2B). Similar results were obtained by inhibiting ROS with NAC (20 μmol/L) (Figures 3A and 3B). Inhibition of NADPH oxidase and decreased ROS activity, in particular superoxide, might depress constriction indirectly by augmenting endothelium-derived nitric oxide (NO). However, inhibition of NADPH oxidase (DPI; p47\textsuperscript{phox}−/− or rac-DN arterioles) or ROS activity (NAC) also inhibited the myogenic response in endothelium-denuded arterioles or in the presence of \( \Delta^{\#} \)-nitro-L-arginine methyl ester (L-NAME) (100 μmol/L), an NO synthase inhibitor (Figure 2E and data not shown). Furthermore, the myogenic constrictor response was not affected by a cell-permeant mimic of superoxide dismutase (SOD), MnTMPyP, which catalyzes the dismutation of superoxide to H\textsubscript{2}O\textsubscript{2}, but was abolished by catalase, which inactivates H\textsubscript{2}O\textsubscript{2} (Figures 3A and 3B). The inhibitory effect of catalase on myogenic constriction and DCF fluorescence (Figures 1B, 3A, and 3B) was maximal after 4 hours, consistent with intracellular accumulation in VSMCs. Exogenous H\textsubscript{2}O\textsubscript{2} constricted endothelium-denuded arterioles but not arteries (Figure 3D).

Constriction to the \( \alpha \)-adrenergic agonist phenylephrine was not associated with increased ROS activity (data not shown) and was not affected by inhibition of NADPH oxidase (DPI; rac-DN or p47\textsuperscript{phox}−/− arterioles) or of ROS (NAC, catalase) (Figures 2C and 3C).

**Discussion**

Elevation in \( P_{\text{TM}} \) increased ROS activity in arteriolar VSMCs. The source of ROS was likely to be NADPH oxidase based on the reduced activity in p47\textsuperscript{phox}−/− or rac-DN arterioles and the inhibitory effect of DPI. When the increase in ROS was inhibited, either by the antioxidant NAC or by inhibition of NADPH oxidase, the myogenic constriction to elevated \( P_{\text{TM}} \) was abolished. Therefore, oxidant signaling by the rac/NADPH oxidase/ROS pathway is essential for the myogenic response of arterioles. Because \( \alpha \)-adrenergic constriction was not associated with nor affected by changes in ROS activity, the role of this pathway may be restricted to mechanotransduction and myogenic constriction.
In cultured, noncontractile VSMCs, oxidant regulation of cell growth is mediated by H$_2$O$_2$. H$_2$O$_2$ also seems to be the predominant species involved in myogenic constriction, because (1) the myogenic response was associated with increased activity of H$_2$O$_2$, as detected by DCF; (2) the responses were inhibited by catalase but unaffected by a SOD mimic; and (3) exogenous H$_2$O$_2$ was a potent constrictor of arteriolar VSMCs. Studies in large arteries have suggested that oxidant signaling is associated predominantly with diseased phenotypes of VSMCs, compatible with oxidant regulation of VSMC growth. Our observations on small arteries are consistent with this proposal, and suggest that under physiological conditions, oxidant signaling may be more important in arteriolar compared with arterial VSMCs.

Mouse-tail arterioles represent a novel model of the microcirculation. They demonstrated robust myogenic responses and remarkable spontaneous vasomotion, suggesting that they are a useful and somewhat unique model of the terminal arteriolar vasculature. Several mechanisms have been proposed for the arteriolar myogenic response, and there is heterogeneous regulation between different vascular beds. The applicability of the present results to other vascular beds is not known. Indeed, although H$_2$O$_2$ can cause constriction through multiple mechanisms, it can also act as a vasodilator. This suggests that ROS contribution to myogenic constriction may not be universal or indicates a complex regulation of vasomotor responses by ROS.

In conclusion, elevation in P$_{TM}$ causes a NADPH oxidase–dependent generation of ROS in VSMCs of tail arterioles, and these ROS, in particular H$_2$O$_2$, initiate myogenic constriction. Proximal arteries do not participate in this response because of decreased expression of NADPH oxidase, reduced production of H$_2$O$_2$, and decreased ability to constrict to H$_2$O$_2$. Altered regulation of this mechanism may contribute to heightened constriction and oxidant stress in hypertension.

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References


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