Role of Subplasmalemmal Mitochondria in Angiotensin II–Mediated Contraction

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Nearly 2 decades after the seminal work by Sundaresan et al., which established the requirement of hydrogen peroxide ($H_2O_2$) during platelet-derived growth factor–initiated signal transduction in vascular smooth muscle cells (VSMCs), it is now well accepted that enzymatically generated reactive oxygen species (ROS) play pivotal roles as signaling molecules during both physiological and pathological conditions. In fact, virtually all cellular programs in VSMCs including differentiation, growth, contraction, and migration use ROS as part of their signaling machinery.

ROS are produced from a sequential 1- or 2-electron reduction of molecular oxygen. Mitochondria are classically linked to ROS generation, which are formed as a by-product of mitochondrial respiration. In addition, VSMCs contain a variety of other sources of ROS, including xanthine oxidase, lipoygenases, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. The NADPH oxidase multienzymatic complexes are major sources of superoxide ($O_2^-$) and $H_2O_2$, which in this cell type have been shown to participate in the signaling pathways of growth and differentiation and in the mediation of the effects of vasoactive peptides such as angiotensin II (AngII). In particular, the Nox1-based NADPH oxidase is activated downstream of the AngII type 1 receptor in VSMCs, where it is required for AngII-induced hypertension in animal models. Although other sources of AngII-induced ROS have been documented, including mitochondrial-produced ROS, their consequences during AngII-initiated signaling are still not well characterized.

In this issue of Circulation Research, Chaplin et al. demonstrate a clear role for NADPH oxidase-induced mitochondrial ROS in the signaling pathway that leads to VSMC contraction, which plays a central role in the regulation of blood pressure under normal conditions, and when aberrant, in the development of hypertension. Interestingly, Chaplin et al. found that after AngII stimulation, a small subset of mitochondria—located near the plasmalemma and closely associated with L-type calcium channels—induce calcium influx that is known to be required for VSMC contraction and endothelial dysfunction–induced hypertension in vivo.

This is in agreement with previous works, which demonstrated that AngII signaling is responsible for an increase in calcium influx. However, it was not until later that AngII was shown to activate transmembrane plasmalemma L-type calcium channels, which play a large role in calcium homeostasis. Presently, using a combination of mitochondrial ROS inducers, a mitochondrial-targeted $O_2^-$ scavenger, and pharmacological inhibitors of protein kinase C, Chaplin et al. propose that the missing link between AngII signaling and L-type calcium channel activation is the oxidative activation of protein kinase C by adjacent mitochondrial-derived ROS downstream AngII-induced NADPH oxidase (Figure).

The production of $H_2O_2$ after AngII stimulation has been linked to contraction in VSMCs although the exact source of the ROS that mediates this effect has been unclear. In VSMCs from large arteries, Nox1 seems to be the most important agonist-induced NADPH oxidase isoform, whereas Nox2 may be more important in small-resistance arteries in vivo. Using basilar and cerebral artery (a conductance vessel)–derived smooth muscle and the Nox1 inhibitor ML171, Chaplin et al. demonstrated that Nox1 NADPH oxidase activity is necessary for local regulation of L-type Ca$^{2+}$ channels by AngII-induced $H_2O_2$ microdomain signaling. Further investigation will be necessary to determine whether resistance arteries Nox2 from VSMCs replaces the role of its homologue in this mechanism.

In addition, the work by Chaplin et al. poses a possible amplification step of Nox-produced ROS-induced mitochondrial ROS. This is in agreement with earlier work about the cross talk between the NADPH oxidase system and the mitochondria, and its requirement for AngII signaling in VSMCs, which has been recently documented for other vascular cells as well. Furthermore, work by Dikalov et al. in endothelial cells has established a positive feedback loop in which mitochondrial ROS induce NADPH oxidase activity by oxidative-mediated activation of cytosolic phox subunits. More experiments are required to investigate whether a similar mechanism contributes to the redox-sensitive AngII signaling during contraction (Figure, dashed line).

Together, it is evident that the mitochondrion has an emerging role in vascular signaling beyond the fundamental view as a critical organelle for bioenergetics and cell death. Although mitochondrial $O_2^-$ may be mainly contained or scavenged within the organelle, $O_2^-$–derived membrane permeable hydrogen peroxide may easily diffuse to the cytosol to participate in cellular signaling by modification of the redox state of thiol-containing proteins, such as protein kinase C.
ROS are highly diffusible and short-lived molecules, thus it is understood that subcellular compartmentalization is a requirement for redox signaling. The work of Chaplin et al reveals an exquisite spatial regulation of cross talk between NADPH oxidase and the mitochondria, which is restricted to a subpopulation of mitochondria localized to the subplasmalemma area. Although these mitochondria represent only a small percentage of the total population of mitochondria within the cell, Chaplin et al provide compelling evidence of cross talk between this small population of mitochondria and L-type calcium channels through ROS signaling. Indeed, subplasmalemmal mitochondria correspond to only 2% of the total mitochondrial network and yet such as small population of organelles seem to be essential to proper cellular function. This finding highlights the necessity of single cell, image-based approaches to appreciate localized production of ROS and its outcomes.

This work helps to uncover a novel piece of a classical signaling pathway that may lead to the development of therapies for hypertension-associated arterial dysfunction by targeting and scavenging mitochondrial-generated ROS. Although more work is needed to elucidate whether this mechanism is conserved in other cell types, intervening at the ROS amplification loop subsequently turning down calcium influx may prove to be a valuable treatment for many pathologies, not just limited to cardiovascular tissues.

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References


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