Mitochondrial Reactive Oxygen Species and Vascular Function
Less Is More

Julie K. Freed, David D. Gutterman

Reactive oxygen species (ROS), conventionally known for causing cellular damage, are now accepted as important signaling molecules both physiologically and in the pathogenesis of cardiovascular disease. Elevated levels of ROS have been linked to the development of disorders, such as diabetes mellitus, hypertension, hypercholesterolemia, obesity, and atherosclerosis.1–4 Conversely, oxidative stress has been shown to stimulate and regulate multiple cell functions, including cell growth and proliferation, apoptosis, host defense, genomic stability, and vascular tone.5–8 An emerging concept is evolving of an optimal physiological range of ROS required to maintain cell homeostasis. Redox balance in the endothelium is a model example where ROS critically regulate vascular smooth muscle tone and tissue perfusion.

The various sources of oxidative stress within the vascular endothelial cell include xanthine oxidase, NAD(P)H oxidase, uncoupled endothelial nitric oxide synthase, and the mitochondrial electron transport chain (mETC), whereas antioxidant defense enzymes are located in peroxisomes (catalase), extracellularly (superoxide dismutase), within organelles (glutathione peroxidase, peroxiredoxins), or within the cytosol (glutathione peroxidase, superoxide dismutase, amino acids, peroxiredoxins).12 These varied distributions support the potential for regionally selective ROS production, an important characteristic of signaling molecules. Of the mentioned ROS-producing enzymes, the mETC is a significant source of ROS. Complexes I, II, and III within the mETC are all capable of producing $O_2^-$, which is rapidly converted to $H_2O_2$, but only complex III can generate ROS within the intermembrane space, which facilitates signaling to the cytosol.13 14 Mitochondrial-derived ROS carry the stigma of being linked to pathological conditions, for example, diabetes mellitus, alcoholic liver disease, Alzheimer, and ischemic heart disease, among others.15–18 However, more recent insights suggest that more constrained generation of ROS by the mitochondria is integral in physiological regulation as well.

The tight regulation of mitochondrial redox balance under normal conditions is supported by a number of observations. For example, NO protects against mitochondrial ROS production through a variety of mechanisms, including direct quenching of superoxide and direct inhibition of mETC components.19 In contrast, elevations in intracellular Ca$^{2+}$ in the presence of partial mETC inhibition or inner membrane depolarization promote ROS formation.20 Other known regulators and inducers of mitochondrial ROS include the inner membrane potential (A$\psi$) regulated by potassium channels, including ATP-dependent K+ (mito K$\text{ATP}$) channels and endogenous uncoupling proteins (UCP1, UCP2), sphingolipids, and isoflavones.21–24

Katakam et al provides new evidence for mitochondrial membrane potential as a critical vascular signaling mechanism by demonstrating mitochondrial-dependent stimulation of endothelial nitric oxide synthase in response to mitochondrial membrane depolarization. Interestingly, there was a ROS-dependent and ROS-independent component to this response, providing evidence of a novel redox negative feedback loop, as NO inhibits mitochondrial ROS production. The current study also implies that, whereas mitochondrial ROS in high concentrations or strategic cellular locations may quench and eliminate NO, under some circumstances (eg, treatment with mito K$\text{ATP}$ openers), mitochondrial ROS can stimulate endothelial production of NO from nitric oxide synthase. Wortmannin reduced dilation to 2 distinct mito K$\text{ATP}$ openers, Bristol Meyers Squibb-191095 (BMS) and diazoxide, showing commonality of the phosphoinositide-3 kinase/endothelial nitric oxide synthase pathway in dilator signaling for both agents. Interestingly, mitochondrial ROS generation was necessary only for the dilation to diazoxide, but not BMS. The authors explain that both agents act on mito K$\text{ATP}$ channels to initiate this depolarization, based on their previous work.
However, other explanations may be relevant. As the response to each was different, it is difficult to elucidate which response, if either, is most physiologically relevant. A major obstacle is the fact that the molecular identity of mito \( K_{ATP} \) remains unknown, precluding selective genetic manipulation of channel expression, which might answer these questions. Additional investigation is needed to reveal the non-ROS–mediated pathway of nitric oxide synthase activation by mito \( K_{ATP} \) channel openers. Perhaps, BMS has nonspecific pleotropic effects beyond mitochondrial membrane depolarization.

Numerous studies have shown that hyperpolarization of the mitochondria (elevation of \( \Delta \psi \)) triggers release of \( O_2^- \), predominantly at complex III.\(^1\) Combined with evidence that mitochondrial uncoupling proteins can inhibit formation of ROS, it is plausible that depolarization of the mitochondria would prevent ROS release.\(^2\) However, more recent studies show that the situation is more complex. Depending on the respiratory state of the mitochondria and redox state of the cell, either depolarization or hyperpolarization can stimulate oxidative stress within the mitochondria.\(^3\) It has been proposed that there is a U-shaped curve representing the relationship between mitochondrial \( \Delta \psi \) and ROS formation, with physiological levels of ambient oxidant production spanning the nadir of the curve.\(^4\)

The role of intracellular \( Ca^{2+} \) on mitochondrial ROS formation is similarly complex. Mitochondria respond to elevations in cytosolic \( Ca^{2+} \) by acting as a high-capacity, low-affinity buffering system. Based on previous studies, increases in mitochondrial \( Ca^{2+} \) can increase ROS production through stimulation of the mETC, as well as promoting release of cytochrome C.\(^5\) Katakam et al reported increases in intracellular \( Ca^{2+} \) levels from both diazoxide and BMS, both attributed to mito \( K_{ATP} \) channel activation. However, a necessary role for mitochondrial membrane depolarization in cytosolic calcium elevation was not proved in the current study. Furthermore, it is possible that increased \( Ca^{2+} \) levels are buffered by the mitochondria to effect ROS production.\(^6\) Of note, there was a greater increase in intracellular \( Ca^{2+} \) after diazoxide versus BMS treatment, consistent with the greater rise in mitochondrial superoxide (MitoSOX; mito-hydroethidine fluorescence) seen with diazoxide treatment. Future studies can evaluate whether diazoxide-induced increases in \( Ca^{2+} \) are buffered by the mitochondria, resulting in an increase in mitochondrial \( Ca^{2+} \)-stimulated ROS production. BMS appeared to have a greater effect on mitochondrial membrane depolarization, cytosolic calcium elevation, and mitochondrial ROS generation.

In summary, the report by Katakam et al suggests that mitochondrial membrane depolarization, through mitochondrial ROS-dependent and -independent mechanisms, generates endothelium-derived relaxing factors, including NO, that promote tissue perfusion. This study supports the role of mitochondrial ROS as signaling molecules that regulate endothelial cell function, and ultimately cerebral vascular tone. Although the connection involving the mito \( K_{ATP} \) channel and mitochondrial-derived ROS has proven to be key in limiting ischemia/reperfusion damage in myocardium, the current study supports prior work\(^7\) that sets the stage to explore these beneficial mechanisms in the cerebral vasculature as well, contributing to the concept of targeting mitochondria as a novel therapeutic target in the treatment of stroke.

Disclosures

None.

References


Key Words: superoxide • mitochondria • nitric oxide • oxidative stress • vasodilation • cerebral circulation • potassium channels
Mitochondrial Reactive Oxygen Species and Vascular Function: Less Is More
Julie K. Freed and David D. Gutterman

doi: 10.1161/ATVBAHA.13.301039
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/33/4/673

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/