Editorial Commentary

Triggering Mitochondrial Radical Release
A New Function for NADPH Oxidases

Ralf P. Brandes

Ischemic preconditioning describes a scenario in which brief intermittent periods of ischemia provide protection against subsequent ischemic injury.\(^1\) A number of elements in the signal transduction cascade mediating ischemic preconditioning have been identified. The opening of mitochondrial K\(_{ATP}\) channels is central for this process, as well as the subsequent release of reactive oxygen species (ROS) from mitochondria, and finally the activation of p38 mitogen–activated protein kinase (p38 MAP kinase). In addition to intermittent ischemia, several agonists, such as acetylcholine, phenylephrine, bradykinin, and opioids have been demonstrated to elicit (pharmacological) preconditioning\(^2\) by a similar pathway.

In this issue of Hypertension, Kimura et al extend this list of preconditioning agents by including angiotensin II.\(^3\) In particular, they demonstrate that angiotensin II leads to the assembly of the NADPH oxidase in the rat myocardium and that inhibition of this assembly process using apocynin blocks angiotensin II–mediated preconditioning. Moreover, the authors observed that inhibition of mitochondrial K\(_{ATP}\) channels by 5-hydroxydecanoate (5-HD) blocked not only the preconditioning effect of angiotensin II but also prevented the angiotensin II–induced ROS formation from cardiac myocytes. 5-HD had no antioxidative properties and did not block the respiratory burst in leukocytes but prevented ROS formation of isolated mitochondrial preparations. Therefore, the authors conclude that the effects observed in response to angiotensin II in rat myocardium, such as lipid peroxidation, p38 MAP kinase activation, and preconditioning, are mediated by mitochondrial ROS (Figure).

Given that the NADPH oxidase is considered a strong source of ROS,\(^4\) the observations of this study are unexpected. Nevertheless, the data are compatible with the concept of ROS–triggered ROS formation, which has been demonstrated in cardiac myocytes and pathophysiological vascular models. In cardiac myocytes, it was observed that ROS can induce mitochondrial depolarization and subsequent mitochondrial ROS generation through mitochondrial permeability transition.\(^5\) It is assumed that K\(_{ATP}\) channels are involved in the process of mitochondrial depolarization because the mitochondrial K\(_{ATP}\) channel can be directly activated by superoxide anions\(^6\) and because 5-HD, which blocks opening of the channel, prevented mitochondrial depolarization and ROS formation. Moreover, exogenously applied ROS can induce pharmacological preconditioning, and this effect was also blocked by 5-HD.\(^7\)

The concept of ROS–triggered ROS formation can be expanded further because exposure of vascular smooth muscle cells and fibroblasts to ROS has been shown to activate an NADPH oxidase, also facilitating ROS–triggered ROS release from this enzyme.\(^8\) Finally, Landmesser et al demonstrated that activation of an NADPH oxidase in deoxycorticosterone acetate– and salt-induced hypertension leads to the release of ROS from endothelial NO synthase.\(^9\) Therefore, it appears that several enzymes contribute to ROS formation and that positive feedback loops exist that lead to an amplification of ROS production and ROS–dependent signaling.

However, what is remarkable in the study by Kimura et al is that the authors failed to detect any substantial contribution of the NADPH oxidase to the total cellular ROS production. Treatment of the cells with the K\(_{ATP}\) channel inhibitor 5-HD completely prevented the ROS–mediated lipid peroxidation, isoprostane release, and MAP kinase activation, whereas the compound did not interfere with the assembly of the NADPH oxidase or with the respiratory burst in leukocytes. This suggests that all biologically active ROS are produced by mitochondria. In a recent article, the authors also report that 5-HD prevents the angiotensin II–mediated signaling in smooth muscle cells, attributed previously to NADPH oxidase–dependent generation of ROS.\(^10\) Certainly, these observations will prove controversial, and the authors do not provide data showing that established inhibitors of the respiratory chain, which block mitochondrial ROS formation, have the same effect as 5-HD. Indeed, others have determined the effects of these inhibitors on ROS formation in vascular or cell culture preparations after preincubation with angiotensin II and have consistently reported that inhibition of the respiratory chain had no effect on the angiotensin II–induced ROS formation. However, because these experiments were performed in preparations exposed to angiotensin II for a prolonged period, an induction of the enzyme is likely, whereas in the work by Kimura et al, only the constitutively expressed NADPH oxidase level was of relevance. It is conceivable that under basal conditions, the expression of the NADPH oxidase, at least in cardiac myocytes, is low and just sufficient to initiate some localized signaling. This concept is compatible with the observation that redox signaling can occur at ROS concentrations too low to alter whole cell redox status.\(^11\)

An alternative explanation could be that 5-HD interferes with the myocardial NADPH oxidase in a way that does not affect the leukocyte enzyme. In fact, little is known about the NADPH oxidase in rat cardiac myocytes. Given the multicellular composition of the heart and the limitations of the antibodies

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Mechanism of angiotensin II–induced preconditioning. Stimulation of rat cardiac myocytes with angiotensin II leads via the action of the angiotensin II type 1 (AT1) receptor to the assembly and activation of an NADPH oxidase. The subsequently generated superoxide anions (O$_2^-$) react with and stimulate ATP-sensitive K$^+$ channels in the inner mitochondrial membrane, which results in mitochondrial depolarization ($\Psi^+$. It is thought that this mechanism allows the mitochondrial permeability transition pore (MPT) to open and facilitates the efflux of large amounts of O$_2^-$ into the cytoplasm. Most likely via its dismutation product, H$_2$O$_2$ and additional, as yet unidentified, signaling pathways, O$_2^-$ activates ASK1, which phosphorylates MAP kinase kinases (MEKs) upstream of p38 MAP kinase and JNK. In turn, p38 MAP kinase is a key player in the process of preconditioning.

Another important observation of the study of Kimura et al was that the angiotensin II–mediated activation of p38 MAP kinase and c-Jun N-terminal kinase (JNK); at least in cardiac myocytes correlates with activation of apoptosis signal-regulating kinase 1 (ASK1). This observation will further help the understanding of the well-documented dissociation between extracellular signal-regulated kinase 1/2, p38 MAP kinase, and JNK: Although all 3 enzymes become phosphorylated when cells are exposed to oxidative stress (application of H$_2$O$_2$), only the angiotensin II–induced activation of p38 MAP kinase and JNK involves NADPH oxidases and oxidative stress. Signaling to exogenous H$_2$O$_2$ involves inhibition of phosphatases and thus “overphosphorylation” of a large number of enzymes. In contrast, ASK1 appears to be a specific redox sensor, although the precise mechanism how the enzyme senses oxidative stress is controversial and may still require specific phosphatases.

Altogether, the study by Kimura et al reports a new function for NADPH oxidase, which may also be central in the signaling of other agents eliciting pharmacological preconditioning and therefore may have important implications. Nevertheless, a lot still needs to be done because it is unknown which NADPH oxidase is required for the preconditioning process and because the present study largely relies on a few certainly not completely specific pharmacological inhibitors.

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