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Permalink
https://escholarship.org/uc/item/1046t3kp

Journal
Biochemistry, 57(27)

ISSN
0006-2960

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Publication Date
2018-07-10

DOI
10.1021/acs.biochem.8b00429

Peer reviewed
Exosomal NADPH Oxidase: Delivering Redox Signaling for Healing

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Reactive oxygen species (ROS) make up a class of transient, redox-active small molecules that have long been studied for their deleterious effects in aging and disease progression. On the other hand, emerging data have revealed a more sophisticated biology for ROS, where regulated bursts of specific ROS like hydrogen peroxide (H\(_2\)O\(_2\)) that are generated by the NADPH oxidase (NOX) family of metalloproteins can trigger oxidative post-translational modifications at cysteines and methionines on target proteins to elicit downstream physiological responses. This signal/stress dichotomy is exquisitely illustrated in the brain, which consumes up to 20% of the oxygen taken up despite being only 2% of body weight, with aging and neurodegenerative disorders exhibiting strong connections to oxidative stress.\(^1\)

A key feature of deciphering whether a particular ROS is a signal or stress agent in a given situation is the spatial and temporal nature of its production. In this context, a foundational physiological role for NOX-derived H\(_2\)O\(_2\) signaling in the brain, as shown by two independent studies, is to maintain proliferation and neurogenic potential of adult neural stem cells derived from the SVZ.\(^\text{2,3}\) Indeed, stem cells derived from the two main neurogenic niches of the adult brain, the subventricular zone (SVZ) and the subgranular zone of the hippocampus, rely on regulated H\(_2\)O\(_2\) production for proliferation and self-renewal. Adult hippocampal progenitors produce H\(_2\)O\(_2\) upon stimulation with growth factor via NOX2. Likewise, proliferating neural stem cells derived from the SVZ maintain a high level of ROS. Functional studies in NOX2 knockout and mutant mice show impaired proliferation and neurogenesis, establishing that redox-mediated regulation is critical for adult neurogenesis.\(^\text{2,3}\) Interestingly, these models share a common mechanism, in which the effects of H\(_2\)O\(_2\) as an intracellular signal are mediated by oxidative inactivation of the phosphatase PTEN, which promotes phosphorylation of Akt and leads to enhanced survival and growth of these stem cells.\(^\text{2,3}\)

An exciting study by Hervera et al. has expanded the scope of this biology to transcellular H\(_2\)O\(_2\) signaling, reporting an essential role for H\(_2\)O\(_2\) in axonal regeneration after an acute injury.\(^4\) A novel aspect of this system is that the injured neurons do not appear to generate H\(_2\)O\(_2\) locally instead relying on recruited macrophages to deliver NOX2 and redox signaling through exosomes, which are then transported to the proper location to promote axonal growth (Figure 1). This finding is in line with previous observations that H\(_2\)O\(_2\) can enhance axonal growth of sensory neurons after a skin injury in zebrafish models.\(^5\)

Unlike the peripheral nervous system (PNS), central nervous system (CNS) neurons have limited abilities for regeneration after injury. One interesting mechanism by which CNS neurons can regenerate is described by the “conditioning lesion paradigm”. According to this concept, a prior lesion to the peripheral system can enhance central axon growth from a CNS injury that transpires at a later time point. The molecular players that mediate this recovery are insufficiently characterized but remain an attractive therapeutic avenue for stimulating regeneration after CNS injuries. Given that H\(_2\)O\(_2\)...
accumulates at injury sites, the authors sought to investigate whether levels of H$_2$O$_2$ are increased locally at peripheral and central lesions. Using a ROS-responsive dye, the authors showed that sensory neurons produce ROS at the sites of injury of both peripheral and central lesions. However, only the conditioning peripheral lesion increased the level of ROS at distant cell bodies in the dorsal root ganglion (DRG) region. Indeed, this oxidative burst is required for axonal growth and regeneration, as shown by treatment with antioxidants or NOX inhibitors. Moreover, exogenous H$_2$O$_2$ mimicked the effects of the preconditioning lesion by promoting axonal growth.

To elucidate the major molecular players in the observed oxidation-mediated regeneration, the authors subjected mice to H$_2$O$_2$ treatment or injuries with or without antioxidants and isolated DRGs for transcriptional profiling. Gene expression analysis implied a key role for NOX2, which was corroborated by the increased presence of the NOX2 subunit, p47phox, in both the axons and distant DRGs after injury. Indeed, mice with nonfunctional NOX2 exhibited lower ROS levels in the DRG and diminished axonal growth after CNS injury. Interestingly, the level of NOX2 mRNA was not elevated in the injured neurons themselves, suggesting that H$_2$O$_2$ bursts were derived from an exogenous source of NOX2. Indeed, immunostaining revealed the presence of p47phox positive macrophages near the site of injury, suggesting that these cells could serve as reservoirs of NOX2. Co-culture of DRG neurons with bone marrow-derived macrophages (BMDMs) from wild-type or NOX2 deficient mice established that it was indeed the macrophage-derived NOX2 that mediated the post-injury neurite outgrowth.

Finally, the authors addressed the question of how NOX2 is transferred from the macrophage to the injured DRG and site of injury by identifying extracellular vesicles called exosomes as the carriers of NOX2. These structures, which are secreted by many cell types, are emerging as important units for intercellular transmission as they can fuse with specific recipient cells and transfer their content, including proteins, nucleic acids, lipids, and ions, to elicit downstream responses. In the case presented here, macrophage-derived exosomes containing NOX2 travel to the DRGs and appear to be specifically transported to the site of CNS injury to promote axonal growth and regeneration at the typically nonregenerative injury site. Like the neural stem cells, oxidative inactivation of PTEN is central to these regenerative effects of H$_2$O$_2$.

In summary, Hervera et al. identify a new physiological role for H$_2$O$_2$ in the brain in which it acts as a transcellular signal and establish exosome-mediated NOX transfer as a mechanism for this pathway. They propose a model in which a conditional injury at a distant site promotes the recruitment of positive inflammatory signals, such as metalloprotein-derived H$_2$O$_2$, that are specifically targeted to the nonregenerative injury site. This study highlights the underlying complexities of CNS axonal regeneration and sheds light on the delicate oxidant/antioxidant balance motivating further investigation aimed at improving recovery after debilitating spinal cord and other CNS injuries. From a more fundamental perspective, studies to expand the signaling roles of metals and the transient redox species they produce will continue to provide fresh insights into biochemistry in the body.

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**Funding**

This work was supported by funding from National Institutes of Health Grant R01-GM79465 (to C.J.C.). C.J.C. is an investigator with the Howard Hughes Medical Institute and a CIFAR senior fellow.

**Notes**

The authors declare no competing financial interest.

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**Author’s note**

This research was supported by funding from National Institutes of Health Grant R01-GM79465 (to C.J.C.). C.J.C. is an investigator with the Howard Hughes Medical Institute and a CIFAR senior fellow.