NADH, a New Player in the Cardiac Ryanodine Receptor?

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In this issue of Circulation Research, Cherednichenko et al describe an NADH oxidase activity that regulates the ryanodine receptor ion channel (RyR2) in cardiac muscle. Mammalian tissues express three closely related 560-kDa ryanodine receptors (RyRs) encoded by separate genes. RyR1 is the predominant isoform in skeletal muscle, and RyR2 predominates in heart. RyR3 is widely expressed at low levels. RyRs control diverse cellular functions by releasing Ca²⁺ ions from intracellular membrane-bound Ca²⁺ stores. In cardiac muscle, release of Ca²⁺ from the sarcoplasmic reticulum (SR) into the cytoplasm leads to muscle contraction. Released Ca²⁺ returns to SR by an ATP-dependent Ca²⁺ pump, resulting in muscle relaxation. The RyRs are regulated by myriad pathways through small diffusible molecules such as Ca²⁺, Mg²⁺, and ATP and by calmodulin, kinases, and phosphatases.

The RyRs are also targets for redox active molecules (Figure). Active muscle produces reactive oxygen and reactive nitrogen species that modulate RyR2. Changes in oxygen tension or the ratio of reduced to oxidized glutathione modulate RyR2 activity by reducing and oxidizing cysteine residues (J. Sun and G. Meissner, unpublished data). RyR2 is endogenously S-nitrosylated and an association of RyR2 with neuronal nitric oxide synthase has been described, suggesting NO and related molecules are physiological modulators of cardiac muscle excitation-contraction coupling. The study by Cherednichenko et al along with two recent reports provide evidence for an additional redox-sensing mechanism in cardiac muscle. An NADH oxidase is shown to modulate RyR2 through the cytosolic NADH/NAD⁺ redox potential in cardiac myocytes.

The present report builds on an earlier observation of a regulation of RyR2 by NADH. Zima et al compared the effects of NADH, NAD⁺, and NADPH on single RyR1 and RyR2 ion channels isolated from rabbit skeletal muscle and rat cardiac muscle, using the planar lipid bilayer method. RyR2 activity decreased in the presence of NADH and increased with NAD⁺. Inhibition by NADH was reversed by equimolar amounts of NAD⁺. NADPH was without effect. Regulation of RyR1 by NADH via the ATP-binding site was observed but ruled out for RyR2. A striking finding of the present report is that mitochondrial electron transport inhibitors (rotenone, pyridaben, and antimycin A) relieve inhibition of RyR2 activity by NADH. The data suggest that NADH exerts its effect via an NADH oxidase activity rather than a direct interaction with RyR2.

In support of an action via an RyR2-associated protein, NADH reduced RyR2 activity in the presence of MgATP, when cardiac SR vesicles, but not purified RyRs, were fused with lipid bilayers (L. Xu and G. Meissner, unpublished data). Photolabeling studies with [¹H](trifluoromethyl)diazirinyl-pyridaben revealed the presence of a mitochondrial PSST-like 23-kDa protein in membrane fractions enriched in RyR2. PSST acts as an intermediate in electron transfer in complex I from NADH to ubiquinone. In contrast, RyR2-associated NADH oxidase was not stimulated by an exogenously added ubiquinone analogue. Cardiac mitochondrial particles exhibited a low NADH oxidase activity that was activated by the exogenous electron acceptor, pointing to distinct differences in the pharmacology of RyR2-associated and mitochondrial NADH oxidases.

Cardiac myocytes have a microsomal NADH oxidase that is a major source of O₂⁻ production in cardiomyocytes. The enzyme is regulated by P O₂ and contains a diphenylene iodonium-inhibitable flavoprotein site. Cherednichenko et al confirmed the presence of a diphenylene iodonium-inhibitable NADH oxidase in their cardiac SR preparation but did not describe the effect of the inhibitor on NADH-mediated RyR2 activity. Reactive oxygen species such as O₂⁻ and H₂O₂ affect SR function by modulating RyR and SR Ca²⁺ pump activities. However, single-channel measurements showed that NADH inhibition of RyR2 was not affected by superoxide dismutase and thus was independent on O₂⁻ production. Rather, O₂⁻ had a slight stimulatory effect, consistent with observations that O₂⁻ activates RyR2.

In skeletal muscle, the major isoform RyR1 is also modulated by an NADH oxidase. Xia et al found the enzyme requires O₂ but otherwise differs from the oxidase acting on RyR2. Mitochondrial electron transport inhibitors did not inhibit modulation by NADH. In contrast to inhibition of RyR2, the skeletal NADH oxidase stimulated RyR1 and activation was inhibited by superoxide dismutase, suggesting that the enzyme that activated RyR1 produced O₂⁻. Baker et al identified an N-terminal oxidoreductase-like domain in RyR1. RyR1 bound NAD⁺ to sites other than the ATP-binding site, but it is unclear whether the oxidoreductase-like domain is enzymatically active. Contrary to the results of Xia et al, NADH had only minor effects on RyR1 activity.

The mechanism of NADH inhibition of the cardiac RyR2 ion channel activity is unclear. One possibility is that RyR2 senses changes in NADH oxidase conformation that are controlled by NADH/NAD⁺. Alternatively, the NADH oxidase may transfer reducing equivalents to RyR2. RyR ion channels contain regulatory thiol groups susceptible to redox-
High pO₂, GSSG, NAD⁺, low [ROS, RNS]

RyR2 (less active)

Low pO₂, GSH, NADH, high [ROS, RNS]

RyR2 (more active)

Modulation of RyR2 activity by redox active molecules.

based modifications, suggesting that thiols modulate RyR2 activity. Future studies will establish whether NADH and NAD⁺ affect RyR2 function via specific modulatory thiols.

The native cardiac muscle RyR2 has a large number of free cysteines (>25 per 560-kDa RyR subunit) in the presence of 5 mmol/L reduced glutathione (J. Sun and G. Meissner, unpublished data). Thus, many thiols are likely in a reduced state in normal functioning hearts due to the reducing environment created by thiol-reducing compounds such as glutathione. Cherednichenko et al. suggest that the NADH oxidase is part of a negative-feedback mechanism that couples SR Ca²⁺ release with mitochondrial Ca²⁺ and energy metabolism. The ratio of cytosolic NADH/NAD⁺ ratio in aerobically perfused working hearts is low (~0.1%) when calculated from the intracellular concentrations of lactate and pyruvate. Only reducing conditions with an NADH/NAD⁺ ratio >1 inhibited Ca²⁺ sparks in permeabilized cells and RyR2 activity in single-channel measurements. It therefore is doubtful that cytosolic NADH has a significant negative impact on SR Ca²⁺ release in normal functioning cardiomyocytes.

Perhaps a more important function of the NADH oxidase is to maintain RyR2 in an “oxidized,” more functional state since NAD⁺ counteracts the action of NADH. Even during the extreme anaerobic condition of sustained ischemia, the cytosolic NADH/NAD⁺ ratio was below that found to inhibit SR Ca²⁺ release and RyR2 activity. Future therapeutic approaches might benefit from a focus on the redox modulation of the cardiac muscle ryanodine receptor.

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References

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