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RESEARCH NEWS

A new stress test for ryanodine receptors

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JGP study (Steinz et al. https://doi.org/10.1085/jgp.202313515) reveals that oxidative stress can induce stable posttranslational modifications of RyR1 that increase the channel's open probability and could therefore disrupt muscle contractility.

The ryanodine receptor type 1 (RyR1) is a homotetrameric Ca^{2+} channel that mediates the release of Ca^{2+} from the sarcoplasmic reticulum (SR) of skeletal muscle cells to facilitate muscle contraction. It is regulated by a variety of factors, including Ca^{2+}, ATP, and auxiliary proteins such as FKBP12 (1). Channel activity is also sensitive to S-nitrosylation, a reversible posttranslational modification induced by reactive oxygen species (ROS), and this may contribute to muscle dysfunction in a variety of conditions associated with prolonged oxidative stress, including heat stroke, cancer, chronic inflammation, as well as normal aging. In this issue of *JGP*, Steinz et al. reveal that ROS also induce stable, irreversible modifications of RyR1 that, by increasing channel open probability, could further contribute to muscle dysfunction (2).

ROS can induce several irreversible protein modifications, including malondialdehyde adducts (MDA) and 3-nitrotyrosine (3-NT). Johanna Lanner and colleagues at the Karolinska Institute recently discovered that these bulky modifications are elevated on the skeletal muscle actin of patients and mice with rheumatoid arthritis, impairing polymerization and force production (3). "We thought that oxidative stress would probably induce these stable modifications on RyR1 as well but, to the best of our knowledge, no one has ever looked at this," Lanner says.

Lanner and colleagues, including first author Maarten Steinz, first examined whether stable oxidative modifications could alter RyR1 activity. The researchers treated purified SR membranes with SIN-1, a compound that promotes the formation of 3-NT and MDA modifications by generating the ROS peroxynitrite, and then recorded the activity of single RyR1 channels (2). "We saw that there was a dose-dependent increase in the levels of modification and in channel open probability," Lanner says. "Over a prolonged time, that could be detrimental in vivo, as there would be increased Ca^{2+} leakage from the SR, leading to muscle weakness."

To investigate which residues of RyR1 are irreversibly modified in the presence of peroxynitrite, Steinz et al. performed mass spectrometry on SIN-1–treated SR membranes. The researchers identified 30 modified residues out of the 5,035 amino acids in each RyR1 monomer. Although 3-NT and MDA modifications are added by non-enzymatic mechanisms, these modified residues were clustered in regions of the protein important for channel activation and regulation.

In particular, Lanner and colleagues identified several modified residues in the cleft region that binds to the auxiliary protein FKBP12. Though none of these modified residues are thought to mediate FKBP12 binding, Steinz et al. found that SIN-1 treatment reduced the association of FKBP12 with RyR1, suggesting that 3-NT and MDA modifications either sterically impede the RyR1–FKBP12 interaction or else trigger a conformational change that favors FKBP12 dissociation.

The absence of FKBP12 has previously been shown to increase RyR1 open probability (4), and Lanner and colleagues found that pretreating channels with rapamycin to induce FKBP12 dissociation blunted SIN-1's effects on RyR1 activity. Thus, at least in part, the stable, oxidative modifications induced by SIN-1 promote RyR1 opening by inhibiting the interaction with FKBP12, though they may also have other effects, such as interfering with the binding of other regulatory factors or directly changing channel gating properties.

Maarten Steinz and Johanna Lanner.

"We now want to look at the levels of these irreversible modifications in mouse models of arthritis and other diseases to see how they might contribute to the muscle weakness associated with these disorders," Lanner says.

References

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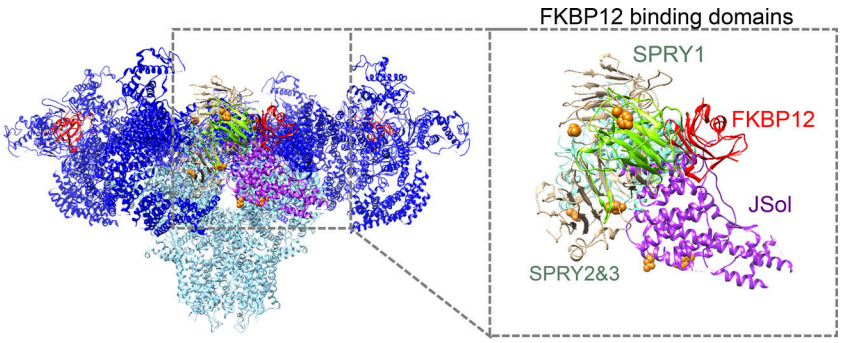

Steinz et al. reveal that stable posttranslational modifications induced by ROS increase the open probability of RyR1, potentially contributing to muscle weakness in diseases associated with chronic oxidative stress. The increase in RyR1 activity is caused, at least in part, by 3-NT and MDA modifications (orange) in the FKBP12 binding cleft, promoting FKBP12 dissociation.