EDITORIAL FOCUS

A two-faced cysteine residue modulates skeletal muscle contraction. Focus on “S-nitrosylation and S-glutathionylation of Cys134 on troponin I have opposing competitive actions on Ca\(^{2+}\) sensitivity in rat fast-twitch muscle fibers

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The contraction of skeletal muscle involves a cascade of events, starting in the central nervous system. Action potentials in motor neurons are transmitted at neuromuscular junctions to the plasma membranes of muscle fibers. The action potentials in plasma membranes propagate into so-called T-tubules, which initiate the release of calcium from the sarcoplasmic reticulum into the muscle fiber cytosol. This calcium binds to troponins on the sarcomeric thin filament, a binding that results in movement of tropomyosin on actin, exposing myosin-binding sites and enabling actomyosin interaction, i.e., cross-bridge formation. Once a cross-bridge is formed, a power stroke takes place, which moves the thin filament relative to the thick filament. This is the basis of muscle contraction. To induce muscle relaxation, calcium is pumped back into the sarcoplasmic reticulum by SERCA proteins. When calcium dissociates from the troponins, tropomyosin moves back to the blocked state and actomyosin interaction cannot take place anymore. Thus, troponin plays a central role in the contraction of muscle (3).

Troponins form a complex on the thin filament. A complex consists of three troponin (Tn) isoforms: troponin I (TnI), troponin T (TnT), and troponin C (TnC). TnC binds calcium, TnI is primarily responsible for maintaining tropomyosin in the blocked state, and TnT modulates the closed and open state of tropomyosin (8). When muscle is activated, the force generated by its muscle fibers depends on the amount of calcium released by the sarcoplasmic reticulum. With more calcium released, more Tn will bind calcium, hence more thin filaments are activated, more cross-bridges are formed and more force is produced. The number of Tn complexes that bind calcium does not depend solely on the cytosolic calcium concentration, but also on the affinity of Tn for calcium. If this affinity is high, the force generated by muscle fibers at a given calcium concentration is high, and, vice versa, this force will be low if the affinity of Tn for calcium is low. A well-established method to determine changes in Tn’s affinity for calcium is to expose demembranated, single muscle fibers to various concentrations of calcium and record the generated force (9). The affinity of Tn to calcium, and thus the relation between calcium and force—typically referred to as the calcium-sensitivity of force—is, among others, modulated by the redox status of the muscle fibers.

During the past decades, propelled by the observation that products of oxidative reactions in muscle increase during exercise (7), the roles of reactive oxygen and nitrogen species (ROS and RNS, respectively) in skeletal muscle function have gained increasing attention. Although at first assumed that this increase reflects damage to the proteins, in recent years it has been recognized that ROS and RNS modulate many cellular processes, including the functioning of contractile proteins [for details, see review by Cheng et al. (1)]. Indeed, reversible oxidation of cysteine residues is a means of redox regulation of muscle contraction, and the study of Dutka et al. (2), published in the current issue of American Journal of Physiology-Cell Physiology, for the first time shows that nitrosylation and oxidation of a specific cysteine residue on TnI modulates the calcium-sensitivity of force generation in fast-twitch muscle fibers.

Dutka et al. convincingly show that S-nitrosylation and S-glutathionylation exert opposing effects on the calcium-sensitivity of force in fast-twitch muscle fibers, and, importantly, that these effects are mediated by competitive actions on Cys134 on fast TnI. Thus, S-nitrosylation of Cys134 blocks subsequent S-glutathionylation of this residue, and vice versa, S-glutathionylation of Cys134 prevents its subsequent S-nitrosylation. This characteristic of Cys134 is important as S-nitrosylation of Cys134 decreases the calcium-sensitivity of force, whereas S-glutathionylation of Cys134 increases the calcium-sensitivity of force (Fig. 1 shows the force-calcium relation and the effects of glutathionylation and nitrosylation). An important question that should be addressed in future studies is how this mutually exclusive posttranslational modification affects exercising muscle. During exercise, hypoxia, and conditions associated with inflammation, such as sepsis, both the production of RNS and of ROS increases. Based on the findings by Dutka et al., the effect of these conditions on the calcium-sensitivity of force depends on which species reaches Cys134 first, thus, on whether ROS or RNS is generated first and in which quantities. Furthermore, it can be questioned what the long-term effects of glutathionylation and nitrosylation of Cys134 are. Previous work (5) suggested that S-glutathionylation initially increases the calcium-sensitivity of force (based on the current paper by Dutka et al. through effects on Cys134 on TnI), but that long-term glutathionylation reduces the calcium-sensitivity of force. Future studies should...
Fig. 1. Effect of S-glutathionylation and S-nitrosylation on the force-calcium relationship of demembranated single muscle fibers. Typical force-calcium relation of a demembranated single muscle fiber that is sequentially exposed to incremental Ca^{2+} concentrations (here expressed as pCa: −log of molar free Ca^{2+} concentration). The black line depicts the sigmoidal-shaped force-calcium curve in the absence of posttranslational modifications. S-glutathionylation of Cys134 increases the calcium-sensitivity of force, reflected by the leftward shift of the curve (gray line). S-nitrosylation of Cys134 decreases the calcium-sensitivity of force, reflected by the rightward shift of the curve (gray dashed line).

reveal whether these long-term effects are also modulated by Cys134.

Finally, in vivo RNS and ROS modulate not only contractile proteins, but also proteins that regulate the release of calcium from the sarcoplasmic reticulum and those that regulate vasoconstriction. Thus, as also acknowledged by the authors, the relevance of posttranslational modifications of Cys134 on TnI remains to be established.

The paper by Dutka et al. not only reveals important, novel insights in the mechanism underlying ROS- and RNS-mediated modulation of contractility, it is also a showcase of thorough, vigorous research. The authors present various layers of evidence for the notion that Cys134 on TnI modulates the effects of ROS and RNS on muscle fiber contractility. First, they show that S-nitrosylation and S-glutathionylation treatments affect fast-twitch fibers only in mammalian muscle and not in muscle of toad and chicken, which both lack Cys134 on TnI. Whereas they could have presented these findings as strong evidence for a central role for Cys134, the authors next established that S-nitrosylation and S-glutathionylation have competitive actions, suggesting a common site of action. Furthermore, using the blocking action of NEM in combination with a biotin-HPDP assay which labels TnI at Cys134 and with pretreatment with an NO donor, they showed that this common site is indeed Cys134. Finally, data from troponin exchange studies (i.e., studies in which endogenous slow-twitch TnI in soleus muscle was replaced by exogenous fast-twitch TnI) as well as data from mass-spectrometry assays provided additional layers of evidence in support of a central role of Cys134 on TnI in modulating contractility. The vigorous and multifaceted approach applied by Dutka et al. is overwhelming and deserves applause.

In summary, the data by Dutka et al. reveal a novel RNS/ROS-based mechanism that modulates the calcium-sensitivity of force generation in skeletal muscle. This finding might benefit the identification of therapeutic targets to combat muscle weakness caused by derailed ROS/RNS production, such as occurs in patients with sepsis or chronic inflammatory diseases but also in those with inherited myopathies (4, 6).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES