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RESEARCH NEWS

Cold temperatures put a freeze on myosin activation

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***JGP* study reveals that low temperatures reduce force production in mammalian skeletal muscle by trapping myosin motors in a refractory state unable to bind actin.**

Contraction of striated muscle is triggered by an influx of calcium into muscle fibers that induces structural changes in the thin filament proteins troponin and tropomyosin, making actin available to the myosin motors present in the thick filaments. In recent years, however, it has become clear that structural changes in the thick filament are also crucial for contraction; in relaxed muscle, myosin is organized into helical tracks on the filament surface that render the motors incapable of binding to actin and hydrolyzing ATP. In this issue of *JGP*, Caremani et al. reveal that low temperatures convert this ordered OFF state into a disordered state that is nevertheless unavailable to bind actin (1). This refractory state explains why mammalian muscles generate less force at lower temperatures and may help hibernating animals conserve energy.

The ordered OFF state of myosin in relaxed muscle was originally defined by electron microscopy (2). In 2015, Vincenzo Lombardi and colleagues from the University of Florence and from King's College London used the x-ray diffraction patterns of frog muscle cells (fibers) to show that this organization is disrupted upon muscle stimulation by stress in the thick filament, increasing the number of myosin molecules in a disordered ON state capable of attaching to actin when the muscle works against a high load (3).

In frog muscles, at least, this mechanoregulation of thick filaments is not affected by temperature; the number of actin-attached motors recruited during an isometric contraction is identical at both 0°C and 20°C (4). The reduced amount of force generated by frog muscles at lower temperatures is explained by the reduced structural change responsible for force generation in the motor domain of individual myosin molecules (5). "However, temperature has a much greater effect on mouse muscles," Lombardi says. "The amount of force generated is threefold less at 10°C than it is at physiological temperatures."

Left to right: Marco Caremani, Massimo Reconditi, Vincenzo Lombardi, Elisabetta Brunello, and colleagues discovered that cold temperatures convert myosin motors in mouse skeletal muscle at rest to a disordered refractory state unable to bind actin upon muscle activation. This refractory population is revealed by x-ray diffraction measurements showing that, in resting muscle, low temperatures reduce the number of myosin motors in the ordered OFF state (top graph) whereas, in contracting muscle, low temperatures cause a parallel reduction in the number of actin-attached motors (bottom graph).

Lombardi and colleagues, including Marco Caremani, Elisabetta Brunello, and Massimo Reconditi, therefore suspected that low temperatures might have a more dramatic effect on the control of myosin in mouse thick filaments. To investigate this possibility, the researchers collected x-ray diffraction patterns from intact mouse skeletal muscle at temperatures ranging from 10–35°C.

The x-ray signals revealed that lowering the temperature progressively disrupts the helical arrangement of myosin in the muscle at rest, such that the number of motors in the OFF state at 10°C is half of that at 35°C. But these disordered motors appear to have lost the ability to be switched into an ON state capable of binding actin; in response to stimulation, the number of actin-attached motors at 10°C is also half of that at 35°C. This suggests that cold temperatures push myosin motors into a disordered state that is refractory to activation, and only those motors that remain in the ordered OFF state are capable of being activated and attaching to actin upon muscle stimulation.

"The challenge now is to define this refractory population either biochemically or pharmacologically," Lombardi says. The researchers propose that cold temperatures may disrupt the intra- and intermolecular interactions that stabilize the helical arrangement of myosin motors on the surface of thick filaments, while maintaining the interactions that inhibit the motors' actin-binding and ATPase activity.

Lombardi and colleagues further speculate that this cold-induced refractory state may be beneficial to hibernating animals when their body temperatures fall to ambient temperatures. "Muscles account for ∼40% of body mass, so reducing the fraction of muscle that can be switched on by residual neuronal activity would greatly reduce metabolic demand," Lombardi suggests.

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