Comment to the Editor

Muscle Thixotropy: More than Just Cross-Bridges? Response to Comment by Campbell and Lakie

ABSTRACT Although Campbell and Lakie in a Comment to the Editor in this issue of *Biophysical Journal* suggested that exclusive cross-bridge action is behind muscle thixotropy, recent findings and our preliminary observations suggest that additional mechanisms could also be involved.

In their Comment to the Editor (1), Campbell and Lakie argue that although contradictory reports abound, the thixotropic behavior of muscle is exclusively explained by a sole cross-bridge mechanism.

Thixotropy, as broadly defined, is the history-dependent change in fluid viscosity: the longer the applied shear stress, the lower the viscosity. In recent years the term muscle thixotropy has become reserved for the history-dependent changes in the so-called short-range elastic component (SREC) (2), even though there remains controversy about the appropriateness of confining the terminology (3). The key supporting findings for a cross-bridge mechanism behind muscle thixotropy are the $[Ca^{2+}]$ dependence (3,4) and 2,3 butanedione 2-monoxime sensitivity (5) of SREC. The evidence seems compelling. However, before a final conclusion is drawn prematurely, some important aspects are worth considering. First, individual titin molecules display pronounced history-dependent viscoelastic behavior (6,7), and they are therefore thixotropic by the true definition of the term. It is highly unlikely that titin's thixotropy remains completely hidden in sarcomere mechanics. Second, $[Ca^{2+}]$ dependence in titin mechanics (8) and titin-actin interactions (9) have been reported, which could contribute to calcium-dependent thixotropic behavior of muscle. Third, the absolute specificity and the full spectrum of 2,3 butanedione 2-monoxime effect remain controversial (10). Finally, the number of newly discovered sarcomeric structural and associated proteins and interactions is on the rise (11); they may change our perception of the fine details of muscle mechanics.

We suggest resolving the debate about muscle thixotropy experimentally. For example, by using small recombinant PEVK fragments to specifically compete with in situ interactions, the role of titin and its domains in muscle thixotropy can be directly tested. In fact, in preliminary experiments, soaking rabbit psoas muscle fibers in relaxing solution containing a polyE motif-rich titin PEVK-domain fragment resulted in concentration-dependent, reversible reduction in SREC stiffness and maximum tension of the SREC response (12). The observation suggests that sarcomeric interactions of the PEVK domain may contribute significantly to muscle thixotropy. Ultimately, single-molecule experiments on pure systems will be required to resolve the muscle thixotropy controversy, that is, to determine whether a single mechanism is responsible for this phenomenon. After all, does it matter if more mechanisms are involved? A dynamic interplay between concurrent active and passive sarcomeric components may provide a more finely tuned control of muscle function.

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