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COMMENTARY

Move quickly to detach: Strain rate–dependent myosin detachment and cardiac relaxation

Charles S. Chung

The myosin ATPase mediates myosin-actin crossbridges that generate force and/or shorten striated muscles, including cardiomyocytes. The ATPase rate modifies the rates of contraction, shortening, and—importantly—relaxation, which leads to a tight coupling between contraction and relaxation in intact, isometrically twitching muscle (Janssen, 2010). That is to say, that when the myocardium cannot shorten or lengthen (i.e., cannot strain), contraction and relaxation are directly related to each other. In physiology, however, muscles are almost never isometric (Chung, 2019). What happens to myosin–actin crossbridges that are mechanically perturbed by a lengthening strain? A study by Palmer et al. in this issue of the *Journal of General Physiology* sought to answer this question by developing a novel model of crossbridge interactions. In doing so, they reveal how the force response to stretch is linear, how strain might modify the myosin ATPase cycle, and, excitingly, how myosin detachment in an integrative muscle is dependent on strain rate rather than strain, per se.

Strain (rate) and cardiac physiology

While many studies on strain and strain rate evaluate the physiological implication of shortening of muscles (Hanft and McDonald, 2019), lengthening strains are the basis for several important physiological and biophysical muscle processes: from eccentric contraction of skeletal muscles to the effects of strains on the forces measured from cells and tissues. For one, passive stiffness—or the force response of a resting (calcium-free) muscle to strain—has been used extensively to determine the physiological and biophysical properties of proteins like titin and the collagen-dominant extracellular matrix (Granzier and Irving, 1995; Koser et al., 2019). In contrast, the viscoelastic behavior of muscles stretched at various strain rates results from protein–protein interactions. Such viscoelastic and movement-dependent (thixotropic) responses underlie an acute resistance to strain and can be attributed to titin, myosin-binding protein C, and, importantly, the stretch of myosin heads that have formed crossbridges (Lakie and Campbell, 2019).

The force response to a step-stretch in constant calcium-activated muscle has also been studied in cells and tissues. For example, a maneuver to determine the rate of force redevelopment (k_{tr}) quickly shortens and then stretches an activated muscle to detach all of the bound crossbridges (Brenner, 1986). The subsequent force regeneration is governed by the rates of crossbridge attachment (f) and detachment (g), which are typically defined as constants. Similarly, studies have sought to characterize the four-phase force response of an activated muscle suddenly strained from an isometric state. The fourth phase is notable because it plateaus at a greater force than a muscle would achieve by activating it after stretching it to the same strain, a result known as residual force enhancement (Herzog, 2014). The enhanced force has been attributed to thin filament activation, titin–actin interactions, and crossbridge modifications (Herzog, 2014; Palmer et al., 2020).

The above methods typically focus on muscles with constant calcium conditions. In contrast, we have begun to use intact, paced cardiac trabeculae to evaluate active relaxation where force, strain, and calcium are all dynamic. While isometric relaxation is coupled to contraction (Janssen, 2010), strain can accelerate relaxation. In the 1970s, Brutsaert and others described how "relaxation loading"—a stretch of the muscle before relaxation—is necessary for afterload to control relaxation (Brutsaert et al., 1984). Recently, we reexamined Butsaert's seminal works and showed that stretch is not only necessary, but is sufficient to accelerate the relaxation rate, independent of afterload. Importantly, we also described that the relaxation rate was actually sensitive to the lengthening strain rate, not strain, before relaxation—a relationship that we termed "mechanical control of relaxation" (Chung et al., 2017).

As we recently reviewed (Chung, 2019), the myocardium continuously undergoes dynamic strains that may have critical physiological implications. A normal healthy heart stretches just before isovolumic relaxation (Rosen et al., 2004), and hearts of patients with hypertension and hypertrophy lose this lengthening strain (Saito et al., 2011). Recent studies report that cardiac global longitudinal strain is impaired in patients suffering from heart failure with preserved ejection fraction (Tschöpe and Senni, 2020). Integrating these clinical data, the results of Brutsaert et al. (1984), and our computational modeling (using a

Department of Physiology, Wayne State University, Detroit, MI.

Correspondence to Charles S Chung: cchung@med.wayne.edu.

simple two-state crossbridge model), we previously suggested that there is a strain rate–dependent detachment of crossbridges, resetting myosin's ATPase cycle (Chung et al., 2017). We concluded that a muscle must "move quickly to relax," but several mechanistic questions remained unanswered.

Focusing in on myosin

In the current study, Palmer et al. developed a model-based approach to assess how strain might impact diastolic function (Palmer et al., 2020). The model included an explicit strain dependence to the two-state crossbridge model; i.e., that crossbridge attachment and detachment become functions of strain (f(x), g(x)) instead of simple constants. The study generated several notable results, of which we highlight three.

First is that a viscoelastic response following a power-law relaxation relationship may explain the force levels of a muscle returning to steady-state after stretch. As noted, stretching a muscle while it is activated results in an enhanced force compared with activating the muscle after stretching it to the same strain. In contrast to some residual force enhancement studies, the reattachment of crossbridges that had detached due to strain is sufficient to explain the enhanced force, without any increased thin filament activation.

Second is that the stretch causes a transient detachment of myosin heads that is dependent on strain rate. This is a subtle difference from a myosin head simply detaching and returning to a state of myosin with ATP (M•ATP) or myosin with hydrolyzed ATP (M•ADP•Pi) just because myosin has been pulled past its lever arm length. Palmer et al.'s experiments varying Pi concentration suggest that this transiently detached state of myosin with ADP, which is not attached to actin (M•ADP), but could return to the actin-bound M•ADP (A•M•ADP) state.

Thirdly, biophysical single-molecule assays provide clear evidence for strain-dependent detachment rates. However, when integrated in a sarcomere or muscle system, strain rate, rather than strain, seems to be the key mechanical modifier—the myosin head must be moved quickly relative to actin to detach.

Does myosin really underlie mechanical control of relaxation?

Palmer et al.'s model suggests that (1) strain rate is the critical parameter in controlling myosin detachment in an integrated muscle system (in contrast to the single-molecule response) and (2) strain causes myosin detachment from actin in the post-power stroke, preADP release state (Palmer et al., 2020). These suppositions remain to be fully experimentally validated in both steady-state and dynamic systems.

Besides trabeculae studies, there is limited data associated with strain rate, but integrating previous studies involving strain do, in general, support the strain rate hypothesis. In a study of myofibrils, Tesi et al. induced step-stretches on myofibrils just after calcium was removed from an activated myofibril, during the slow, linear phase of the relaxation process (Tesi et al., 2002). They reported that higher amplitude strains were associated with more rapid transitions to fast relaxation. Higher strain rates might be inferred for higher strains since the stretch durations were similar, but strain rate–specific results were not reported in this study.

A key biophysical unknown is how exactly the myosin ATPase cycle is modified by strain. Previously, our two-state model could only suggest that myosin would enter a traditional detached state (such as M•ATP or M•ADP•Pi) when detached by a strain (Chung et al., 2017). Palmer et al. suggest that strain enhances myosin detachment from actin before MgADP release, resulting in a transient M•ADP state (Palmer et al., 2020). Previously, Smith and Geeves presented a model that explicitly included a rotated or rigor-like state of myosin heads (Smith and Geeves, 1995). Interestingly, this earlier study also predicted a high probability of the M•ADP state during strain. In this journal in 2014, Campbell evaluated a six-state model using a new modeling interface called MyoSim (Campbell, 2014). Expanding on work by Lombardi and Piazzesi (1990), the six-state scheme also included a detached state of M•ADP that could return to the A•M•ADP state. The model resulted in excellent fits to step-stretch responses probing force redevelopment (k_{tr}), supporting the physiological relevance of this transiently detached M•ADP state.

Necessary basic and integrative studies of strain rate dependent myosin detachment

The modeling data discussed above suggest the need to confirm that myosin heads detach and reattach to the thin filament. Designing new experiments with guidance from published studies might help refine such models. For example, Amemiya et al. performed time-resolved x-ray diffraction on activated muscles during a steady stretch (Amemiya et al., 1988). They report a reduced $I_{1,1}$ intensity during the stretch, suggesting that myosin was moving away from the thin filament. Interestingly, the thick filament-associated $I_{1,0}$ position was not repopulated. Upon the end of the stretch, the $I_{1,1}$ intensity began to recover, but $I_{1,0}$ didn't change until relaxation began. Additional studies are needed to clarify if this change in intensity is truly due to the detached M•ADP state and whether the result is strain rate dependent. Similarly, while there is evidence that thin filament activation is not modified by stretch (Tesi et al., 2002; see also references within Palmer et al., 2020), additional studies may be required to conclusively determine if it does contribute during physiological stretches imposed upon muscle.

An area of broad physiological interest is the myosin isoform dependence of the strain rate response. Palmer et al. primarily used α-myosin heavy chain dominant mouse myocardium at 17°C to mimic the overall kinetics of the slower β-myosin heavy chain human myocardium at 37°C. However, ADP release rates differ between cardiac myosin isoforms (Wang et al., 2013; and, indeed, skeletal myosin isoforms as well; Weiss et al., 2001). One could question, for example, whether the strain rate–dependent detachment of myosin would be more important in β-myosin heavy chain dominant muscles because the myosin heads might stay in the A•M•ADP state for a longer duration. While this is an exciting possibility, especially for clinical translation, our preliminary data using intact trabeculae actually suggested that the relaxation rate of human trabeculae had reduced sensitivity to strain rate than that of mouse and rat trabeculae (Chung et al., 2017). Given the limited sample size in these studies, further investigation of myosin isoform dependence is required.

Our current knowledge regarding the role of strain rate on physiological relaxation is limited, partially due to the protocols and models used. For example, Palmer et al. showed that the force response to strain at various rates is linear in a constantly

activated (or passive) system (Palmer et al., 2020). However, the calcium activation of an intact muscle is dynamic. In our studies of intact trabeculae (Chung et al., 2017), we did show a strain rate dependence of relaxation, but could only speculate on crossbridge detachment processes via computational modeling. Additional studies, including variable strain rate conditions, physiological calcium dynamics, and measurement of myosin head position or orientation within sarcomeres and muscles, are needed to better characterize the mechanisms and physiological implications of these strain rate–dependent processes.

Perspective

A closer examination of how strain rate causes myosin to detach from actin could provide greater understanding and insight on how to integrate seemingly disparate physiological and biophysical measurements. Both clinically and mechanically, strain magnitude has been at the forefront of muscle physiology, but integrating studies using lengthening strain rate might provide improved physiological insights. As an example, based on bench-side experiments, we were previously befuddled by the difference in the relaxation rates of loaded and unloaded muscles. An isometrically relaxing trabeculae or cardiomyocyte will reach its final diastolic force very slowly. In contrast, an unloaded myocyte can relengthen more rapidly than it contracts. Integrating the concept of strain rate–dependent relaxation, we can now speculate that the isometric relaxation is really a biochemically limited process, whereas the unloaded myocyte is regulated by a feedback loop of strain rate–dependent detachment of myosin causing more and more rapid relaxation.

Integrating the concepts of strain rate–dependent myosin detachment could have significant implications in cardiac physiology, especially in revealing new treatments for diastolic relaxation (Chung, 2019). It may also provide new insights in the physiology of myosin-related proteins with similar ATPase cycles. While integrative physiology is complex, studies such as the one by Palmer et al. (2020) just might help explain many dynamic, physiological, and strain rate–dependent processes such as mechanical control of relaxation.

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