Role of Calcium Sensitivity Modulation in Skeletal Muscle Performance

Brian R. MacIntosh
Faculty of Kinesiology and Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 2N4, Canada

A common mechanism affecting Ca$^{2+}$ sensitivity in skeletal muscle is the proximity of myosin heads with actin filaments, a function of myofilament lattice spacing and myosin head mobility with respect to the myosin filament. This is an important mechanism of pCa$^{2+}_{50}$ modulation by length, pH, regulatory light-chain phosphorylation, and temperature.

Skeletal muscle contraction is initiated by an increase in cytoplasmic Ca$^{2+}$ concentration ([Ca$^{2+}$]). This Ca$^{2+}$ binds to troponin C, which permits actin-myosin interaction. Myosin heads will remain detached as long as tropomyosin impedes actin-myosin interaction but will undergo cycles of binding and exerting force when the binding of Ca$^{2+}$ to troponin has resulted in the movement of tropomyosin to a position where it cannot impede actin-myosin interaction. The number of cross-bridges engaged, and hence the magnitude of contractile force, will be primarily dependent on the relative occupation of troponin C by Ca$^{2+}$. Clearly, [Ca$^{2+}$] is a major determinant of the number of myosin heads participating in cyclic attachment, force generation, and detachment. However, there are several factors that can modify the force exerted by a muscle fiber at a given [Ca$^{2+}$].

Ca$^{2+}$ sensitivity is the term that is used to express the fact that force at a given [Ca$^{2+}$] can vary. Modulation of the force response of skeletal muscle by changes in Ca$^{2+}$ sensitivity occurs in a variety of natural circumstances including prior activity, as in activity-dependent potentiation, with length changes, and possibly in fatigue. In addition, there are several factors that can modify the force exerted by a muscle fiber at a given [Ca$^{2+}$].

Measurement of Ca$^{2+}$ sensitivity

Ca$^{2+}$ sensitivity is most easily and most often measured in skinned fiber preparations. A skinned fiber is a muscle cell that has had the membrane compromised mechanically or chemically, often by Triton (18) or glycerol exposure (5, 20). Once the membrane has been disrupted, force can be measured under a variety of conditions that yield different [Ca$^{2+}$], and the relation between free [Ca$^{2+}$] and force can be determined (Fig. 1). Ca$^{2+}$ sensitivity can be quantified by determination of the [Ca$^{2+}$] (usually expressed as the negative log or pCa$^{2+}$) at which the force is half of the maximal value (pCa$^{2+}_{50}$). The slope of the force-pCa$^{2+}$ relation, which indicates the degree of cooperativity, can also be investigated, but for the purposes of this review consideration will only be given to pCa$^{2+}_{50}$. When pCa$^{2+}_{50}$ increases or shifts to the left, Ca$^{2+}$ sensitivity is greater; a shift to the right indicates a decrease in Ca$^{2+}$ sensitivity.

An advantage of the skinned fiber preparation for the study of skeletal muscle properties is that the investigator has control over the chemical composition of the intracellular environment. This could also be considered a disadvantage, because the chemical composition and temperature selected by the investigator may have very little physiological relevance. Care must be taken when interpreting the results of these studies in the context of physiological circumstances. One of these considerations is the level of activation. It can be seen in Fig. 1 that a change in Ca$^{2+}$ sensitivity alone does not alter the magnitude of contraction when activation is maximal. The steep part of the force-pCa$^{2+}$ relation relates to submaximal activation. In terms of motor unit recruitment, this would correspond with incompletely fused tetanic contractions or submaximal effort.

General mechanisms of modulation of the pCa$^{2+}_{50}$

Conceptually, there are two possible mechanisms by which the sensitivity to Ca$^{2+}$ can be modified: altered binding of Ca$^{2+}$ to troponin C and modified kinetics of cross-bridge cycling. Binding of Ca$^{2+}$ to troponin has been investigated by either the double-isotope method (18) or by labeling troponin C with a probe that changes fluorescence when Ca$^{2+}$ is bound (6). No evidence has yet been provided that Ca$^{2+}$ sensitivity in skeletal muscle is altered by changes in Ca$^{2+}$ binding to troponin C. Cross-bridge kinetics can be evaluated by consideration of the rate of force redevelopment after a fast shorten/relengthen protocol, by the ratio of stiffness to force, and by measurement of ATPase activity for a given developed force (16). Altered cross-bridge kinetics appears to be a primary mechanism by which Ca$^{2+}$ sensitivity is modulated in skeletal muscle.

Specific modulators of pCa$^{2+}_{50}$

There are several known factors that modulate the contractile response at a given level of free Ca$^{2+}$ in the sarcoplasm or cause a change in pCa$^{2+}_{50}$. These include, but are not limited to, regulatory light-chain phosphorylation, sarcomere length, temperature, and pH. The mechanism of action of each of these is briefly presented below.

Regulatory light-chain phosphorylation. Activity-dependent
potentiation of submaximal contractions is thought to occur primarily due to phosphorylation of the regulatory light chains of myosin. Repeated Ca^{2+} transients will activate myosin light-chain kinase, and this enzyme phosphorylates the regulatory light chains. Dephosphorylation is achieved by light-chain phosphatase but occurs at a relatively slow rate, so light-chain phosphorylation persists for a few minutes after a tetanic contraction or a maximal voluntary contraction. It has been shown that light-chain phosphorylation results in increased Ca^{2+} sensitivity (11) and that the mechanism of this change is via an effect on cross-bridge kinetics. The constant proportionality between myosin ATPase activity and force and between force and stiffness (16) provides evidence that the increased force at a given [Ca^{2+}] is due to an increase in the number of cross-bridges in the strong-binding state with no change in the time each cross-bridge remains in the strong-binding state. It has been shown with electron microscopy and optical diffraction that light-chain phosphorylation also results in loss of the ordered (helical) pattern of myosin heads adjacent to the thick filaments (3), suggesting that phosphorylation causes greater mobility of the myosin heads. This increased mobility would mean that the actin-binding region of the myosin head would spend more time close to the actin filament, resulting in an increased probability of myosin binding to actin. In a two-state model of actin-myosin interaction, this increased probability of myosin binding to actin is expected to lead to an increased rate of cross-bridge formation. Assuming no change in the rate of dissociation, this should result in more myosin heads in a strong-binding or force-generating state during the contraction. This change in cross-bridge kinetics would result in more force at a given submaximal [Ca^{2+}].

**Sarcomere length.** It has been known for quite a long time that Ca^{2+} sensitivity is greater at long sarcomere length than at short length (1). The mechanism of this increased sensitivity has been shown to be an effect on cross-bridge kinetics, related to the proximity of the actin and myosin filaments. As a muscle fiber is stretched, the diameter decreases, and the myofilaments become closer to each other. When length decreases, the myofilament lattice spreads out, increasing the gap between myosin and actin (see Fig. 2). That this is the primary mechanism of length-dependent modulation of Ca^{2+} sensitivity in skeletal muscle has been demonstrated by osmotic compression of the myofilament lattice at different lengths. By keeping the lattice at the same size across different lengths, the length dependence of Ca^{2+} sensitivity can be eliminated (17). This mechanism is apparently analogous to the mechanism for increased pCa_{50} with regulatory light-chain phosphorylation. Whether the proximity of actin and myosin results from increased mobility of the S1 head of myosin or compression of the myofilament lattice, the end result is the same: more cross-bridges are formed at a given submaximal [Ca^{2+}].

**Temperature.** Skeletal muscle has been studied at temperatures ranging from 0 to 40°C. There are conflicting reports on the temperature dependence of Ca^{2+} sensitivity, but in general pCa_{50} in skeletal muscle is high at low temperatures and decreases as temperature increases (15). The mechanism of this effect is not well known. However, it has been demonstrated that at low temperatures (near 0°C), there is less organization of the myosin heads with respect to the thick filament backbone (4). This observation has been supported by Xu et al. (19), who report that there appears to be a gradual change in dispersion of the myosin heads with change in temperature. This structural change is like the loss of organization that was described for regulatory light-chain phosphorylation (see above). On the basis of this observation, it can be speculated that decreasing the temperature of the bathing solution will increase the probability for cross-bridge formation. Considering that mammalian muscle functions primarily between 34 and 40°C, the temperature dependence of pCa_{50} may have limited physiological application. However, awareness of this property and its mechanism will help with understanding some of the interactions described later in this paper.

**pH.** Maximum force and pCa_{50} both decrease when pH decreases, the myofilament lattice spreads out, increasing the gap between myosin and actin (see Fig. 2). That this is the primary mechanism of length-dependent modulation of Ca^{2+} sensitivity in skeletal muscle has been demonstrated by osmotic compression of the myofilament lattice at different lengths. By keeping the lattice at the same size across different lengths, the length dependence of Ca^{2+} sensitivity can be eliminated (17). This mechanism is apparently analogous to the mechanism for increased pCa_{50} with regulatory light-chain phosphorylation. Whether the proximity of actin and myosin results from increased mobility of the S1 head of myosin or compression of the myofilament lattice, the end result is the same: more cross-bridges are formed at a given submaximal [Ca^{2+}].
decreased. These effects of acidosis have been considered to be a primary mechanism of fatigue for quite some time. Maximum force is probably decreased due to reduced force per cross-bridge (7). The mechanism of the pH-dependent decrease in pCa$_{50}$ is less clear. Martyn and Gordon (5) have shown that decreasing pH of the bathing solution causes fiber diameter to decrease. This probably occurs as a result of changes in the fixed charge of the myofilaments (5). Remarkably, this change in fiber diameter should be expected to increase Ca$^{2+}$ sensitivity, according to the effects described above. However, there are other effects of H$^+$. Podlubnaya et al. (12) have reported that increased pH results in a disordered pattern of myosin head position relative to the thick filament, similar to the changes observed when the regulatory light chains are phosphorylated. This could mean that the mechanism of increased pCa$_{50}$ with alkalosis is similar to the mechanism described above for regulatory light-chain phosphorylation. Acidosis would have the opposite effect.

**Interactions among modulators of Ca$^{2+}$ sensitivity**

It can be imagined that when procedures that operate by different mechanisms are combined, their effect on Ca$^{2+}$ sensitivity should be additive, whereas interventions leading to increased sensitivity by similar mechanisms may not be additive. In skeletal muscle, the primary mechanism for altered Ca$^{2+}$ sensitivity for the effects described herein is a change in cross-bridge kinetics. Not only is altered cross-bridge kinetics the primary mechanism, but changes in the probability of cross-bridge formation due to proximity of actin with the actin-binding site on S1 appears to be the primary mode of action for these modulators of Ca$^{2+}$ sensitivity. It is important to note that there appears to be an optimal level of proximity of the myofilaments. Compression of the myofilament lattice beyond the optimal level will decrease Ca$^{2+}$ sensitivity (17).

Considering the shared fundamental mechanism associated with these modulators of Ca$^{2+}$ sensitivity, it would seem unlikely that they would be additive. In fact, it could be surmised that an increase in Ca$^{2+}$ sensitivity by any of these mechanisms would be most evident when the other effectors were decreasing pCa$_{50}$. An increase in Ca$^{2+}$ sensitivity due to any one of these factors would not be evident in circumstances in which Ca$^{2+}$ sensitivity had already been increased maximally by another of these factors. In the following section, the results of a few studies that considered interactions of these factors that alter Ca$^{2+}$ sensitivity will be presented.

Figure 3 illustrates potential interactions of mechanisms of modulation of Ca$^{2+}$ sensitivity. Several assumptions have been made in formulating this model. It has been assumed that the S1 segment is 16.5 nm in length and that it is typically oriented at 45$^\circ$ in the gap between the actin and myosin filament. The S2 segment is assumed to be 40 nm long and is mobile at the distal end. This assumption was necessary because the estimates of intermyofilament distance yielded values $>$16.5 nm for sarcomere lengths $<$1.95 $\mu$m (see Fig. 2). If the S2 segment was not mobile, then cross-bridge interaction and force development would be impossible for sarcomere lengths $<$1.95 $\mu$m.

**FIGURE 3.** Hypothetical conditions affecting pCa$_{50}$. A: conditions that give a high Ca$^{2+}$ sensitivity due to proximity of the actin and myosin filaments: long sarcomere length (2.81 $\mu$m) or compression of the myofilament lattice. B: conditions that give a low Ca$^{2+}$ sensitivity due to increased distance between the actin and myosin filaments. C: conditions that give a high Ca$^{2+}$ sensitivity due to enhanced mobility of the myosin head: regulatory light-chain phosphorylation, decreased temperature, or alkalosis, when myofilament spacing is increased due to short length or skinning the fiber without osmotic compression. A ribbon diagram used to represent the S1 segment is used with permission from Annual Review of Biochemistry (Volume 68 ©1999 by Annual Reviews www.annualreviews.org).

In Fig. 3A, the myosin filament is close to the actin filament. This represents a long sarcomere length, and sensitivity to Ca$^{2+}$ would be high. Under these conditions, increased regulatory light-chain phosphorylation, decreased temperature, or increased pH would have minimal impact on Ca$^{2+}$ sensitivity. This has been shown to be the case for regulatory light-chain phosphorylation (14). In fact, at room temperature, posttetanic potentiation is reversed (posttetanic depression) at sarcomere lengths (14), possibly due to mobility of the myosin heads moving the heads past the actin filament. This would be analogous to osmotic compression of the myofilament lattice past the optimal position (17) for actin-myosin interaction. Furthermore, acidosis decreases Ca$^{2+}$ sensitivity more when the myofilaments are osmotically compressed or when the muscle fiber is at long lengths (5).

In Fig. 3A, the myosin filament is close to the actin filament. This represents a long sarcomere length, and sensitivity to Ca$^{2+}$ would be high. Under these conditions, increased regulatory light-chain phosphorylation, decreased temperature, or increased pH would have minimal impact on Ca$^{2+}$ sensitivity. This has been shown to be the case for regulatory light-chain phosphorylation (14). In fact, at room temperature, posttetanic potentiation is reversed (posttetanic depression) at sarcomere lengths (14), possibly due to mobility of the myosin heads moving the heads past the actin filament. This would be analogous to osmotic compression of the myofilament lattice past the optimal position (17) for actin-myosin interaction. Furthermore, acidosis decreases Ca$^{2+}$ sensitivity more when the myofilaments are osmotically compressed or when the muscle fiber is at long lengths (5).

Figure 3B illustrates the condition in which the S1 segment is a long distance from the actin. This represents a short sarcomere length, low levels of regulatory light-chain phosphorylation, and low pH and/or high (physiological) temperature. In this case, sensitivity to Ca$^{2+}$ would be low. An increase in regulatory light-chain phosphorylation would be expected to cause a greater change in Ca$^{2+}$ sensitivity in this condition than in the condition shown in Fig. 3A. This is indeed the case (9).
It has also been observed that addition of myosin light-chain kinase to skinned fibers at room temperature results in considerable increases in Ca\(^{2+}\) sensitivity (11). This observation would appear to be in contradiction to the effect of light-chain phosphorylation with a cool temperature (already a high Ca\(^{2+}\) sensitivity). However, skimming the muscle fiber causes expansion of the myofilament lattice, increasing the space between actin and myosin. Osmotic compression of the myofilament lattice prevents this increase in pCa\(^{2+}\) with addition of myosin light-chain kinase (20). The impact of light-chain phosphorylation on Ca\(^{2+}\) sensitivity when pH is low has not been directly evaluated, but the length dependence of twitch potentiation is abolished in acidosis (13). This would be the expected result if acidosis restores staircase potentiation at long length and/or suppresses potentiation at short length. This appears to be the result if repetitive stimulation is applied when the muscle has been made acidic by exposure to high levels of CO\(_2\) (13).

Figure 3C represents the condition in which the myofilament lattice is expanded, or sarcomere length is short and regulatory light-chain phosphorylation is high, or temperature is low, or pH is high. Under these conditions, acidosis would be expected to have the effect of decreasing Ca\(^{2+}\) sensitivity. Similarly, warming the preparation would decrease Ca\(^{2+}\) sensitivity. If this conformation was obtained by a very low temperature, then phosphorylation of the regulatory light chains would not be as effective at increasing the Ca\(^{2+}\) sensitivity as it would in the situation shown in Fig. 3B.

**Physiological implications for Ca\(^{2+}\) sensitivity**

Clearly, the conditions that can be obtained with a skinned fiber preparation are not necessarily of physiological relevance. However, the interactions that have been described here have important implications for contractile responses in physiological circumstances. For example, these interactions explain why acidosis causes decreased pCa\(^{2+}\) in frog skeletal muscle at ambient temperatures, but at physiological temperatures for mammals, acidosis will not decrease Ca\(^{2+}\) sensitivity (10). The warm temperature has already decreased pCa\(^{2+}\) (50).

Furthermore, the impact of light-chain phosphorylation on pCa\(^{2+}\) is more evident when muscle temperatures are in the physiological range rather than at room temperature (9, 14) and are also more evident on the ascending limb of the force-length relation than on the descending limb. Awareness of the interactions of these factors that modulate skeletal muscle sensitivity to Ca\(^{2+}\) will help us understand why these modulators are effective in some circumstances but not in others.

**Conclusions**

Several processes can affect Ca\(^{2+}\) sensitivity in skeletal muscle by altering the proximity of the S1 segment of myosin with the actin filament in skeletal muscle. These processes include temperature, pH, sarcomere length, and regulatory light-chain phosphorylation. These processes interact with each other such that the increased Ca\(^{2+}\) sensitivity that can be expected by any of these mechanisms is greatest when Ca\(^{2+}\) sensitivity has already been diminished by the other processes. The increase in Ca\(^{2+}\) sensitivity by any of these processes can be expected to be minimal if Ca\(^{2+}\) sensitivity is already enhanced by another of these modulators of Ca\(^{2+}\) sensitivity.

I thank Dilson Rassier, Krista Svedahl, and Bruno Stuyvers for their critical comments on preliminary drafts of the manuscript.

Research in my lab is supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

**References**


News Physiol Sci • Vol. 18 • December 2003 • www.nips.org