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Coexistence of potentiation and fatigue in skeletal muscle

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Abstract

Twitch potentiation and fatigue in skeletal muscle are two conditions in which force production is affected by the stimulation history. Twitch potentiation is the increase in the twitch active force observed after a tetanic contraction or during and following low-frequency stimulation. There is evidence that the mechanism responsible for potentiation is phosphorylation of the regulatory light chains of myosin, a Ca^{2+}-dependent process. Fatigue is the force decrease observed after a period of repeated muscle stimulation. Fatigue has also been associated with a Ca^{2+}-related mechanism: decreased peak Ca^{2+} concentration in the myoplasm is observed during fatigue. This decrease is probably due to an inhibition of Ca^{2+} release from the sarcoplasmic reticulum. Although potentiation and fatigue have opposing effects on force production in skeletal muscle, these two presumed mechanisms can coexist. When peak myoplasmic Ca^{2+} concentration is depressed, but myosin light chains are relatively phosphorylated, the force response can be attenuated, not different, or enhanced, relative to previous values. In circumstances where there is interaction between potentiation and fatigue, care must be taken in interpreting the contractile responses.

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- Myosin light chains

Introduction

The contractile response of a muscle depends to a great extent on the history of its activation. A brief period of repetitive stimulation results in enhanced contractile response (potentiation) while continued stimulation results in impaired or attenuated contractile response (fatigue). Considering that potentiation and fatigue both result from prior activation, it seems reasonable to assume that these two processes are initiated when contractile activity is started, and that they coexist during and for some time after repetitive stimulation, as suggested by Krarup (1). This coexistence of potentiation and fatigue would make it difficult to quantify either process independently.

The purpose of this review is to present evidence that the underlying cellular mechanisms for potentiation and fatigue can and do coexist. We will briefly review the known mechanisms of potentiation and fatigue, then consider the probable consequences of their interaction. Following this, we will review the evidence that potentiation and fatigue do coexist. It is our hope that this review will raise awareness among scientists dealing with

contractile responses of muscles that the underlying mechanisms of potentiation and fatigue can coexist, and that care must be taken in interpreting the contractile responses under such circumstances.

Definitions

There are several terms used in this review which should be defined. Staircase is the progressive increase in twitch active force during repetitive low-frequency stimulation. Posttetanic potentiation is the enhancement of twitch active force following a tetanic contraction. Activity-dependent potentiation is a term we use to refer collectively to an enhanced contractile response which can be attributed to prior activity. It has been demonstrated that this enhancement is evident not only with twitch contractions, but with incompletely fused tetanic contractions as well (MacIntosh BR and Willis JC, unpublished results).

Fatigue refers to the depression of contractile response which can be attributed to prior activity, and is generally evident as less active force than otherwise expected. Fatigue can be further subdivided into low-frequency fatigue and high-frequency fatigue. Low-frequency fatigue is evident when prior activity results in depression of active force at frequencies which elicit submaximal force while the maximal force (or force at the frequency which elicited maximal force prior to the fatiguing exercise) is unaltered. High-frequency fatigue is evident when prior activity results in depression of maximal force (or force at the frequency which elicited maximal force prior to the fatiguing exercise) without depression of force at frequencies which elicit submaximal force.

The expression "coexistence of potentiation and fatigue" is used in this review to refer to the situation where the underlying causes of activity-dependent potentiation and fatigue are simultaneously present. The result of this coexistence of potentiation and fatigue may be enhanced contraction amplitude, depressed contraction amplitude, or no apparent change from the control situation. To understand how coexistence of potentiation and fatigue can occur, it is necessary to be aware of the presumed mechanisms of potentiation and fatigue.

Mechanisms of potentiation

There is considerable evidence that activity-dependent potentiation results from phosphorylation of the regulatory light chains of myosin (RLC). Several studies have demonstrated a correlation between the magnitude of potentiation and the magnitude of phosphorylation of RLC (2-5). In addition, skinned fiber experiments have shown that the force of contraction at a given submaximal Ca^{2+} concentration is increased, while maximal force is not altered (6,7). Taken together, these two observations provide strong support for the theory that RLC phosphorylation is responsible for activity-dependent potentiation.

Phosphorylation of the RLC occurs when myosin light chain kinase (MLCK) is activated (8,9). Activation of MLCK occurs when Ca^{2+} concentration rises and the Ca^{2+}-calmodulin complex binds to MLCK (9). Therefore, when a muscle is activated, Ca^{2+} concentration rises resulting in the activation of MLCK and increased RLC phosphorylation. Presumably the increased RLC phosphorylation causes increased sensitivity of the contractile proteins to Ca^{2+}, thereby enhancing the submaximal contractile response. The enzyme myosin light chain phosphatase is responsible for removing the phosphate group from the RLC. This enzyme proceeds at a relatively slow rate, approaching control levels of phosporylation after 4-5 min in mammalian muscle at 37°C.

It has been demonstrated that the enhanced submaximal force during high levels of RLC phosphorylation results from an increased rate of attachment of cross-bridges

or a greater rate of transition from weak-binding to strong-binding which results in a greater number of force-generating cross-bridges during a contraction. Evidence supporting this conclusion includes the following: muscle stiffness increases in proportion to the increase in active force; myosin ATPase activity increases in proportion to the increase in active force, and the rate of relaxation does not decrease (10). The proportional increase in stiffness indicates that the enhanced force is associated with an increased number of attached cross-bridges, and the proportional increase in ATPase activity suggests that the time-dependent cross-bridge turnover is not affected by RLC phosphorylation. The steady state number of cross-bridges engaged is proportional to the sum of the rate of attachment and the rate of detachment of cross-bridges. The evidence presented above suggests that the increase in attached cross-bridges results from an increase in the rate of attachment. Evidence that the rate of detachment is not affected includes no increase in relaxation time (11) as well as no change in the economy of force production (12).

The collective evidence presented above confirms that force enhancement associated with RLC phosphorylation results from an increase in the rate of attachment of cross-bridges, with no change in the rate of detachment and this change in cross-bridge kinetics results in an increased Ca^{2+} sensitivity. Much of the evidence for this mechanism is based on skinned fiber experiments. Our knowledge of the mechanisms of activity-dependent potentiation cannot rule out the possibility of a contribution by other factors. In fact it has recently been demonstrated that staircase can occur without corresponding RLC phosphorylation (13). In spite of this, for the purposes of this review, increased Ca^{2+} sensitivity in association with RLC phosphorylation will be considered to be the primary mechanism of activity-dependent potentiation.

Mechanisms of fatigue

Care must be taken when discussing the potential mechanisms of peripheral muscle fatigue. The pattern of stimulation which induces the fatigue may be an important determinant of the mechanism. Considering that there are several steps in the sequence of activation of a muscle, failure at any of these could be the mechanism of fatigue. Ultimately, however, depression of active force, as a consequence of prior activity, results from either decreased peak (or average) myoplasmic free Ca^{2+} concentration (14-16), or decreased sensitivity to Ca^{2+} (17). The decreased sensitivity to Ca^{2+} may be due to a decreased Ca^{2+}/troponin affinity, or due to a decreased force produced by each cross-bridge during contractions. For the purposes of this review, these two factors will be considered together, and will be referred to as Ca^{2+} sensitivity.

For many years, indirect evidence has suggested that failure of excitation-contraction coupling is the primary mechanism of fatigue, particularly low-frequency fatigue. Eberstein and Sandow (18) reported that force depression during fatigue could be overcome by treatment with caffeine. This has been confirmed by others (11). Furthermore, dantrolene, which inhibits Ca^{2+} release in skeletal muscle, mimics several aspects of the contractile response of skeletal muscle fatigue (19,20).

Recently, it has been reported that there is a reduced peak free Ca^{2+} concentration in the myoplasm (16,21,22) in fatigued muscle. In addition, it has been suggested (17,23,24) that several factors associated with fatigue could result in decreased Ca^{2+} sensitivity (as illustrated in Figure 1A). These factors include mainly decreased pH and increased inorganic phosphate concentration. Skinned fiber experiments conducted at room temperature (or colder) have been done to confirm this effect (25-27). However, the impact of acidosis on the contractile response

of skinned mammalian muscle fibers is less at 30°C than at 10°C (28). Also, it has recently been demonstrated that the effect of acidosis on the contractile response of intact single mammalian muscle fibers is much less at 32°C than at room temperature (29). These observations place some doubt on the role of changes in Ca^{2+} sensitivity in fatigue at physiological temperature.

In cases in which there is clear evidence of high-frequency fatigue, the likely mechanism is depressed conduction of the action potential along the sarcolemma and/or into the transverse tubules (17). Caution must be exercised, however, to be certain that apparent high-frequency fatigue is not a result of coexistence of general fatigue and activity-dependent potentiation. General fatigue would depress active force at all frequencies of stimulation, whereas activity-dependent potentiation would enhance the force only at low frequencies. The net result of their coexistence would be little or no change in active force at low frequencies and depression of active force at high frequencies. This possibility is described further below.

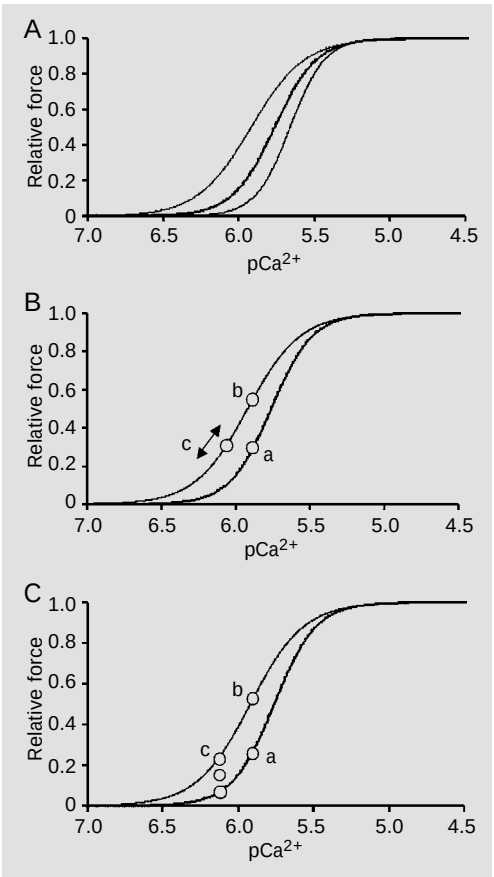

Figure 1 - Hypothetical force-calcium relationships which demonstrate various combinations of potentiation and fatigue. *A*, The thick line in this figure represents the control condition, where Ca^{2+} sensitivity is neither increased nor decreased. The thinner lines on either side represent enhanced (to the left) or decreased (to the right) sensitivity of the contractile proteins to Ca^{2+}. *B*, This figure shows the transition from a control situation (a) to an enhanced condition (b) which could represent myosin light chain phosphorylation. When fatigue is superimposed on potentiation, the force of contraction could be at c, which is indicated to be mobile. That is, the position of c could represent the same active force as a, or something above or below that. The point at b could also represent high-frequency stimulation (depressed relative to what it would be). *C*, Illustrates the transition from a control situation (a) to an enhanced condition (b), and the combined effects of fatigue due to decreased Ca^{2+} concentration (c), and decreased Ca^{2+} sensitivity, superimposed on myosin light chain phosphorylation.

Interactions of potentiation and fatigue

A steady-state contractile response can be conveniently related to the force-pCa^{2+} relationship (Figure 1). Three lines are drawn in Figure 1A to illustrate potential factors which may enhance or depress the steady-state contractile response. The center heavy line represents a control condition. Force can be increased or decreased by changing the myoplasmic free Ca^{2+} concentration. The line which is on the left represents increased sensitivity and the line on the right represents decreased sensitivity to Ca^{2+}. Increased Ca^{2+} sensitivity will result in greater force at any given Ca^{2+} concentration (except at saturating levels of Ca^{2+}), while decreased Ca^{2+} sensitivity has the opposite effect. Maximal active force is not affected in either case.

Interactions of potentiation and fatigue can be illustrated using the relationship shown in Figure 1. Let us assume that potentiation results from enhanced calcium sensitivity, and fatigue results primarily from depressed Ca^{2+} release per activating pulse. A further assumption is needed to relate the force-frequency relationship with the force-pCa^{2+} relationship. It is assumed that as frequency of stimulation increases for a given brief period of repetitive stimulation, the average free Ca^{2+} concentration increases. This assumption is consistent with published reports of the free Ca^{2+}-frequency relationship for mammalian muscle (30). With these assumptions, it can be seen that combinations of fatigue and potentiation will result in a shift to the left in the force-pCa^{2+} relationship, and any given stimulation will result in a lower peak (or average) free Ca^{2+} concentration. This is illustrated in Figure 1B. If the

control condition results in a free Ca^{2+} concentration and active force corresponding with point a, a shift to the left would give active force indicated by b at the same Ca^{2+} concentration, but point c at a lower Ca^{2+} concentration.

Coexistence of fatigue and potentiation could be detected by measurement of the force-pCa^{2+} relationship. For a given submaximal stimulation, the combined effects of potentiation and fatigue could result in an increase, no change or a decrease in active force, depending on the relative change in the two parameters (increased sensitivity and decreased Ca^{2+} concentration). This situation would be further complicated if fatigue was partly due to a decrease in Ca^{2+} sensitivity, as is clearly the case at room temperature *in vitro*. It would no longer be sufficient to measure the force-pCa^{2+} relationship to identify coexistence of fatigue and potentiation. The net effect of the corresponding increased Ca^{2+} sensitivity associated with potentiation and the decreased sensitivity associated with fatigue could cancel each other out (see Figure 1C). This difficulty may explain why assessment of coexistence of potentiation and fatigue has not been studied in this way.

How then, do we know when potentiation and fatigue coexist? There are several phenomena that can be observed which indicate coexistence of fatigue and potentiation. However, there are also situations where potentiation or fatigue appear to be exclusively evident, but the opposing factor may be present, decreasing the apparent magnitude of the other. The primary observations which present evidence for coexistence of fatigue and potentiation include the following: depressed high-frequency response while the twitch is enhanced; a time-dependent decrease in twitch amplitude following repeated activation to active force levels below control; discrepancies in the relationship between potentiation and RLC phosphorylation, and altered time-course of the twitch (slowing of contraction and relaxation times) while the twitch active force is enhanced.

Evidence for coexistence of potentiation and fatigue

Discrepancies in frequency response

One way of observing the coexistence of potentiation and fatigue is looking at the discrepancies in the force response during different frequencies of stimulation. In circumstances where high-frequency force is depressed by fatigue, yet RLC are phosphorylated (see Figure 1B), there could be an enhanced twitch with depressed high-frequency contractile response (b in Figure 1B).

Rankin et al. (31) measured the twitch and tetanic (100 Hz) contractile response of individual motor units of soleus and extensor digitorum longus muscles following 6 min of intermittent (1 Hz) tetanic contractions (40 Hz for 330 ms). In a substantial proportion of the motor units of extensor digitorum longus muscles the twitch response was enhanced relative to the prefatigue twitch while the tetanic contraction was depressed. This is a clear example of coexistence of potentiation and fatigue. Only 15% of the soleus motor units presented this pattern of response.

Another example of coexistence of potentiation and fatigue is presented by Jami et al. (32) who investigated force enhancement and a delayed force decrease in different motor units of the cat peroneus tertius muscle in association with the Burke fatigue protocol (successive trains of 13 pulses at 40 Hz, each train lasting 330 ms and being repeated every 1 s). Immediately following the Burke protocol, twitch force was enhanced while response to 40-Hz and 200-Hz stimulation was enhanced, depressed or unchanged. However, after a period of "recovery" depression of active force for twitch and tetanic contractions was evident. This observation reveals another important aspect of

the interaction of fatigue and potentiation. These two properties of muscle have different time-courses of recovery. Activity-dependent potentiation dissipates within minutes (5,11), whereas fatigue, particularly low-frequency fatigue, persists for hours (33).

The two studies cited above, both of which were done at physiological temperatures, show the specific cases in which coexistence of potentiation and fatigue is apparent. At room temperature (or colder) the interactions of potentiation and fatigue are less clear, for two reasons: 1) a given level of RLC phosphorylation results in less potentiation, and 2) there is evidence that fatigue can result from decreased Ca^{2+} sensitivity. In spite of these complications, similar observations have been reported at temperatures colder than room temperature.

Vergara et al. (34) used single fibers from the frog semitendinosus muscle to study the coexistence of potentiation and fatigue at 15°C. The authors employed 200-ms stimuli (20 Hz) to evoke near maximal mechanical responses, and twitches to measure posttetanic potentiation (PTP) before and after the fiber was stimulated tetanically at 20 Hz for 5-200 s (fatiguing contraction). Tetanic contractions maintained for 50 s or longer produced substantial fatigue, evidenced by the decline in the 200-ms 20-Hz contraction observed after the tetanus and through a period of ~90 min. The twitch response was enhanced after the tetanic contraction, and after 10-40 min, the twitch active force declined. PTP was greater after the long tetanus when fatigue was present than it was after a short tetanus.

The results of Vergara et al. (34) were supported in later studies from the same group (35,36). An important aspect of these observations is that these studies show levels of potentiation that were much smaller than those found in the studies performed by Rankin et al. (31) and Jami et al. (32). In general, reports of activity-dependent potentiation studied at room temperature or colder demonstrate considerably less potentiation than when muscle is studied at 35-37°C (37). In fact, Moore et al. (38) observed that there is less RLC phosphorylation despite a greater relative staircase potentiation at 35°C than at 30° and 25°C in fast muscles from the mouse stimulated at 5 Hz for 20 s. Since potentiation is related to an increased Ca^{2+} sensitivity, it is probably being partially masked by a decrease in Ca^{2+} sensitivity caused by fatigue at cooler temperatures.

Together, the studies cited above have presented evidence for coexistence of potentiation and fatigue. In all of these studies, the muscle was not able to produce maximal (control) force during moderate to high-frequency stimulation, but the force during low-frequency (twitch) stimulation was enhanced for several minutes after the fatiguing stimulation. Coexistence of fatigue and potentiation would be less evident when maximal force is not decreased as a result of fatigue, but force response at low-frequency stimulation is decreased (low-frequency fatigue). In this case, potentiation could be masked, since twitch force enhancement and depression would be present at the same time. Under these circumstances, it would be necessary to follow twitch active force for some time to see if twitch active force decreases to below the control level during a period of relative inactivity.

Altered relationship between RLC phosphorylation and potentiation

In all cases presented above, we have considered the consequences of a period of stimulation on enhancing and diminishing the subsequent active force. Another form of coexistence of potentiation and fatigue exists when a muscle which has been fatigued is subsequently activated in a manner which elicits potentiation.

MacIntosh and colleagues (11,39-41) have done a series of experiments looking at the effects of low-frequency fatigue on the

subsequent enhancement of active force during 10-Hz stimulation or after a tetanic contraction in the whole gastrocnemius muscle of the rat. These experiments confirmed that fatigued muscle can undergo staircase and PTP, and that activity-dependent potentiation is proportional to RLC phosphorylation. However, the relationship between RLC phosphorylation and potentiation is altered in fatigue (see Figure 2) and this alteration is different for staircase (39) compared to PTP (40). The relative increase in active force during staircase (10 Hz for 10 s) and PTP (500 ms or 2 s at 200 Hz) was similar in fatigued and rested muscle, but the increase in RLC phosphorylation was considerably less during staircase in fatigued than rested muscle. This was not the case for PTP. This difference between staircase and PTP is represented in Figure 2 by the greater apparent slope of the staircase response in fatigued muscle.

Staircase in the fatigued muscle resulted in greater enhancement of twitch active force for a given change in RLC phosphorylation. This is similar to results of Palmer and Moore (42) who observed greater potentiation for a given change in RLC phosphorylation in muscle treated with dantrolene, a drug which inhibits Ca^{2+} release. This observation can possibly be explained by the greater relative enhancement of force which would be expected for a given shift (to the left) in the force-pCa^{2+} relationship at low Ca^{2+} concentrations (see Figure 1). However, this is not the only possible explanation for this discrepancy in the relationship between RLC phosphorylation and potentiation in fatigued muscle. This mechanism cannot explain why PTP is different from staircase in fatigued muscle. This difference suggests that at least in fatigued muscle the mechanism for enhancement may be different for staircase and PTP. This conclusion is consistent with the observation that staircase can occur in the absence of RLC phosphorylation at low levels of Ca^{2+} release (13). One explanation for this discrepancy is that there could be another mechanism contributing to staircase under these circumstances.

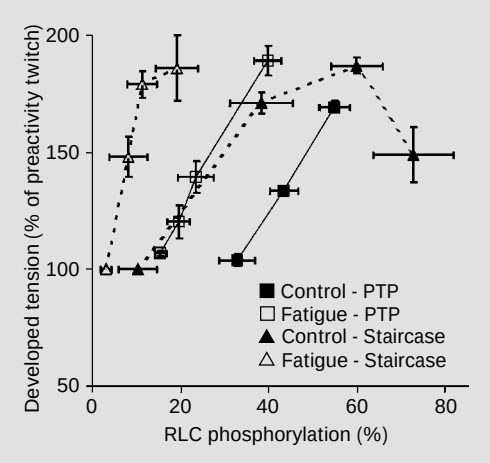

Figure 2 - Potentiation and myosin light chain phosphorylation of the rat gastrocnemius muscle for staircase (39) and posttetanic potentiation (PTP) (40). The results for control and fatigued muscles refer to the situation in which potentiation was elicited before or after the fatigue protocol, respectively. In both studies, fatigue was induced by stimulation at 10 Hz for 5 min, followed by 20 min of test contractions at 0.1 Hz. In both cases, the line representing the relationship between potentiation and light chain phosphorylation is shifted to the left by fatigue. Note that the slope of the line representing staircase in the fatigued muscle is steeper than the other lines. This may represent an additional mechanism of potentiation during staircase in fatigued muscle.

Time-dependent twitch characteristics

Twitch contractions can be characterized by measures of the time-course: contraction time (C_t), and half-relaxation time ($½R_t$). Fatigue is known to increase both C_t and $½R_t$ (43). Therefore, detection of these changes during activity-dependent potentiation may be evidence of coexistence of fatigue and potentiation.

It is known that activity-dependent potentiation can occur without an increase in C_t or $½R_t$ (11,44), yet there are several reports of prolongation of C_t and/or $½R_t$ during PTP (31,32,34,36,45). In general, studies which have observed a prolongation of C_t with PTP have utilized fairly long tetanic contractions (usually several seconds) to elicit PTP. Many authors have argued that the prolongation of the twitch is a characteristic which limits the impact of fatigue on the active force (32,36). Prolongation of C_t and $½R_t$ can result in enhanced summation and a decrease in the fusion frequency. This prolongation of the twitch is independent of RLC phosphorylation, and may represent an independent (additional) mechanism of activity-dependent potentiation.

Caution must be exercised when dealing with measurements of active force of a twitch contraction when C_t and/or $½R_t$ is/are prolonged. There is very good reason to believe that there is coexistence of factors which enhance and diminish the active force. This makes it impossible to accurately quantify either potentiation or fatigue.

Physiological consequences

The twitch contraction is the smallest contractile event which can be evaluated in an intact skeletal muscle preparation. Measurement of the active force and time-course of the twitch can be very revealing with respect to the contractile state of the muscle. However, voluntary activation of a muscle results in incompletely fused tetanic contraction. It is appropriate to consider the impact of coexistence of fatigue and potentiation on such contractions. However, very little research has been done which considers activity-dependent potentiation for incompletely fused tetanic contractions. MacIntosh BR and Willis JC (unpublished results) have demonstrated that activity-dependent potentiation is evident during repeated brief incompletely fused tetanic contractions at stimulation frequencies up to 70 Hz. Therefore there is reason to believe that intermittent voluntary activation of a muscle would result in activity-dependent potentiation.

Assuming that the force-frequency curve can be interpreted in terms of the force-Ca^{2+} relationship, it seems reasonable to anticipate that activity-dependent potentiation would result in greater isometric force for a given pattern of (submaximal) stimulation, and that fatigue would result in less isometric force for a given pattern of stimulation. In order to obtain a target force for repeated contractions, voluntary recruitment of motor units would have to be modulated in such a way that enhancement and reduction were accounted for. This is an aspect of the study of activity-dependent potentiation which has been largely ignored, but which may provide a fruitful avenue of research in the future.

During repetitive stimulation there are two opposing processes happening at the same time inside the muscle cells: one that enhances muscle performance and one that decreases muscle performance. This phenomenon results in a coexistence of activity-dependent potentiation and fatigue, for which the underlying mechanisms are not yet totally understood. In this review, it was assumed that low Ca^{2+} concentration caused fatigue and increased RLC phosphorylation was the mechanism of activity-dependent potentiation. Clearly these two opposing mechanisms can coexist.

Most investigators have not taken into account the possible coexistence of potentiation and fatigue. Ignoring this coexistence may cause problems in the interpretation of studies done with skeletal muscle. Specifically, when using results of force measurements made during or just after repetitive muscle stimulation, fatiguing factors may be present yet not observable due to potentiation. In order to better understand these phenomena, it seems appropriate to evaluate force transients over a long period of time, to be sure that the potentiating factor(s) do not influence active force when fatigue is assessed.

One of the objectives of this paper was to raise awareness of the coexistence of potentiation and fatigue. From the studies cited in this review, it seems clear that this coexistence occurs and is an important component to be taken into account when investigators analyze force production during and following repetitive muscle stimulation.

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