Bimodal Effect of Stimulation on Light Fluctuation Transients Monitoring Spontaneous Sarcoplasmic Reticulum Calcium Release in Rat Cardiac Muscle

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Microscopic, myofilament motion caused by spontaneous oscillatory Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) of unstimulated rat papillary muscles produces scattered light intensity fluctuations (SLIF) in a laser beam scattered by the tissue. SLIF frequency increases with Ca\(^{2+}\) loading of resting muscle. We used novel time-gated SLIF measurements to determine how electrical stimulation (which per se both induces SR Ca\(^{2+}\) release and modulates total cellular Ca\(^{2+}\) loading) affects SLIF. Stimulation of thin rat, right ventricular muscles at 1 Hz in bathing [Ca\(^{2+}\)] (Ca\(_b\)) of 1.5 mM at 29° C abolished SLIF for 5-7 seconds; SLIF then reappeared and monotonically increased for 10-15 seconds to reach the steady resting level. Resting force transients paralleled those of SLIF. The magnitude of depression and time course of recovery of both resting force and SLIF at this Ca\(_b\) vary inversely with the rate of prior stimulation and the number of stimuli given. An increase in Ca\(_b\), or disablement of the Na-K pump increased both resting SLIF and force; transient stimulation under these conditions (i.e., in a 2.5-5.5-second “diastolic window” after cessation of stimulation) augmented SLIF and force above the resting level. Isoproterenol caused a modest reduction of resting SLIF, but it transiently increased SLIF after stimulation up to 10-fold above the resting level. Nifedipine did not affect resting SLIF but transiently depressed SLIF after stimulation. Ryanodine abolished SLIF both after stimulation and at rest. These results permit the inference that SLIF transients noninvasively monitor transient changes in the average extent of SR Ca\(^{2+}\) loading without necessitating test depolarizations and suggest that, depending on conditions, stimulation of rat muscle can either transiently increase or decrease the extent of SR Ca\(^{2+}\) loading. (Circulation Research 1988;63:960-968)

Spontaneous Ca\(^{2+}\) release can have marked effects on cardiac muscle function. First, the localized Ca\(^{2+}\)-myofilament interaction that results from spontaneous Ca\(^{2+}\) release alters sarcomere and cell length, and thereby alters tissue compliance. This imparts a Ca\(^{2+}\)-dependent “tone” to the tissue.1-3 Second, the resultant Ca\(^{2+}\) modulation of the nonspecific cation channel or Na-Ca carrier ion flux produces a depolarization8-10 that could initiate the spontaneous action potentials8-9 that underlie certain cardiac arrhythmias. Third, as discussed in the following paper,10 spontaneous Ca\(^{2+}\) release can interfere with the action potential-mediated twitch tension.11-15

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The subtle mechanical manifestation of the localized spontaneous Ca\(^{2+}\) release (i.e., the Ca\(^{2+}\)-induced, microscopic myofilament motion) has been observed in both rat myocardial tissue and single cardiac myocytes in the absence of experimental Ca\(^{2+}\) overload.1,2,4,9,11,16 When observed directly, this microscopic motion appears as a propagating contractile band (or “wave,” Figure 1) in both the unstimulated isolated myocyte16 and bulk muscle preparations.4,11 The frequency of the periodic mechanical oscillations that result from spontaneous oscillatory Ca\(^{2+}\) release in both unstimulated rat cardiac muscles and myocytes bathed in physiological [Ca\(^{2+}\)] (Ca\(_b\)) is as low as 0.1 Hz,16 and when cellular Ca\(^{2+}\) loading is increased, this frequency increases up to several hertz (see Lakatta et al17 for review).

Without the use of microscopy, the subtle mechanical motion caused by spontaneous Ca\(^{2+}\) release in bulk tissue can be easily and noninvasively mea-
Stimulation Alters Heart Calcium Oscillations

Fluxes (a-e) must occur in a "resting" myocardium of unstimulated tissue exists in different states. In a process,1 the present study used novel time-gated time-averaged SLIF could not resolve this restitution. The scheme implies that Ca²⁺ fluxes (a-e) must occur in a "resting" myocardium exhibiting spontaneous Ca²⁺ release and that the myoplasm of unstimulated tissue exists in different states. In one, the cytosolic [Ca²⁺] (Caᵢ) is low (i.e., at true "resting levels" [region 1]), in a second, Caᵢ is high as the result of spontaneous SR Ca²⁺ release (i.e., within the propagating contractile bands [region 2]), and probably in a third at intermediate levels as recovery occurs subsequent to wave passage (region 3).

FIGURE 1. Spontaneous localized sarcoplasmic reticulum (SR) Ca²⁺ release in mammalian cardiac cells causes a localized myofilament interaction that propagates as a contractile band in a wavelike manner.4 In unstimulated, intact muscle illuminated by a laser, the myofilament motion caused by this spontaneous oscillatory Ca²⁺ release produces an optical path length variation reflected as scattered light intensity fluctuations (SLIF). Thus, the quantity of time-space averaged SR Ca²⁺ release that determines the contractile wave density within the tissue is monitored by SLIF. The scheme implies that Ca²⁺ fluxes (a-e) must occur in a "resting" myocardium exhibiting spontaneous Ca²⁺ release and that the myoplasm of unstimulated tissue exists in different states. In one, the cytosolic [Ca²⁺] (Caᵢ) is low (i.e., at true "resting levels" [region 1]), in a second, Caᵢ is high as the result of spontaneous SR Ca²⁺ release (i.e., within the propagating contractile bands [region 2]), and probably in a third at intermediate levels as recovery occurs subsequent to wave passage (region 3).

Materials and Methods

Thin (0.2 mm² or less) rat papillary muscles were mounted isometrically in series with a Statham UC-2 force gauge (Gould, Cleveland, Ohio) in a 3-ml chamber with glass sides as previously described.2 The preparation was superfused with a solution containing 140 mM Na⁺, 4.2 mM K⁺, 1.5 mM Ca²⁺, and 3.0 mM PIPES (piperazine-N⁴-N⁴-bis[2-ethanesulfonic acid]) buffered to a pH of 7.4, at a temperature of 29°C. A minimum equilibration period of 3 hours9,11 was allowed before the experimental protocols were implemented. The basic protocol for quantitation of SLIF has been described previously.2,11 In brief, muscles were illuminated by a He-Ne laser beam (with intensity adjusted by a series of rotating and fixed polarizing filters) collimated by a 1-mm pinhole. Light scattered from the muscle at a 30° angle was collected through two pinholes and a convex lens system designed to project the effective image of a constant-sized region of the illuminated muscle onto the photomultiplier. The resulting signal was analyzed by a digital autocorrelator (model K7025, Malvern Instruments, Southborough, Massachusetts) for which averaging times, prescale levels, and time-gating sequence protocols (see below) were automatically determined by a computer (model 9825A, Hewlett-Packard, Palo Alto, California). SLIF measurements are presented as \( f_{1/2} \) (Hz):

\[
f_{1/2} = 1/2 \pi t_{1/2}
\]

where \( t_{1/2} \) is the time (msec) for the normalized autocorrelation function to decay to 50% of its baseline.2

In time-gating protocols,24 the Hewlett-Packard Model 9825A computer served as a central control unit that synchronously coordinated the remainder of the system. A Grass Instruments SD9 stimulator (Quincy, Massachusetts) was triggered by the Hewlett-Packard to deliver a train of stimuli (at 60/min, unless otherwise noted) containing a sufficient number of beats, so that twitch force achieved steady state. The autocorrelation function of the scattered light was continuously calculated by the Malvern Model K7025 but accumulated in the output bins only during the discrete time windows selected by the Hewlett-Packard.

Figure 2 illustrates the two different time-gating protocols that were used to measure SLIF. In Panel A a portion of a sequence of a representative protocol in which SLIF were measured during four time windows (a–d) is shown. The trace begins during a 6-second window (a; designated as such because it extends from 4.5 to 7.5 seconds after the last electrical stimulus). This is followed by measurements in subsequent windows at 10, 14, and 18 seconds (b, c, d). In the middle region of Panel A (during interval c), the time base has been expanded...
FIGURE 2. A, Top Panel: Actual chart recording illustrates a series of stimulation trains and pauses (windows) used for time-gated scattered light intensity fluctuation (SLIF) measurements. Note the paper speed change in center of figure. Lower Panel: The time-gated windows in which SLIF measurements were being made. See text for further details. B: Actual chart recording from a truncated series of the time-gating protocols in which SLIF were measured in a diastolic window (DW) after stimulation or at rest (R). Note paper speed change in center of figure. See text for details.

to illustrate both the twitch transients that occur with regular stimulation trains and the subsequent time window of SLIF collection. When a series of stimulation trains and subsequent collection of SLIF represented a statistically valid autocorrelation function, the Hewlett-Packard interrupted the series, read the data from the correlator, and quantitated the autocorrelation function as described above. At that point, the Hewlett-Packard commenced a new series of stimulation trains involving a new time window as shown in the remainder of Figure 2A. Most actual protocols had more than four windows. The measurements were repeated for the entire series of windows two more times in sequences randomized so as to minimize the effect of any SLIF drift with time. The reported values represent the average of the three measurements for each window.

Figure 2B shows a truncated time-gating protocol of stimulation sequences during which SLIF was measured only during a 2.5–5.5-second interval poststimulation and at rest. The trace begins in the middle of a series of stimulation trains involved in measurement of the poststimulation SLIF. The last of this series (arrow) is followed by a pause (the 30-second duration which was experimentally determined to be sufficient for SLIF to return to equilibrium or resting level), and then a measurement of the resting SLIF is made during the next 40 seconds. A new series of stimulation trains then begins. The paper speed was increased in the center portion of Panel B to allow better visualization of this aspect of the protocol. After achievement of steady-state twitch tension, a pause of 2.5 seconds preceded a 3-second period of SLIF collection in a diastolic window (DW). This series of stimulation trains, pauses, and SLIF collection was repeated 10 times to produce a total of 30 seconds of SLIF collection before f_1/2 was calculated from the autocorrelation function.

**Results**

The solid line in Figure 3 shows that a representative rat papillary muscle bathed in physiological (i.e., 1.5 mM) Ca_o and bathing [K+] (K_o; 4.2 mM) exhibits SLIF at 15–20 seconds after prior stimulation (i.e., in the unstimulated or resting state). However, for a 5-second interval after electrical stimulation at 60/min, SLIF are transiently abolished and then only gradually increase to their resting level. Because the 5-second interval before the onset of SLIF recovery is longer than the interstimulus interval during prior stimulation of this muscle, SLIF did not occur during the diastolic period of that stimulation.

Figure 3 also shows that in reduced K_o (0.8 mM), resting SLIF are increased, and stimulation of the muscle during the Na-K inhibition transiently enhances rather than suppresses SLIF in the early poststimulation state. Specifically, SLIF are now not only present during the same poststimulation time window in which they were transiently abolished in normal K_o, but they are also augmented by 2.5-fold over the already increased level achieved in the rested state in the reduced K_o.
Figure 4A illustrates the effect of stimulation on SLIF restitution in a representative muscle bathed in varying $C_{a_0}$. Note the increase in resting SLIF with increasing $C_{a_0}$. Differences in the pattern and time course of SLIF recovery following stimulation in the different $C_{a_0}$ are also clearly defined. In the earliest time window employed, SLIF were abolished in $C_{a_0}$ of 2.0 mM but increased with progressive increases in $C_{a_0}$; in the two highest $C_{a_0}$ studied, SLIF were greater than that at rest, that is, a transient overshoot in SLIF occurred. At longer times following stimulation, SLIF proceeded to increase or decrease so as to equilibrate back to the resting level characteristic of that $C_{a_0}$. In Figure 4B, the SLIF transients in Panel A are normalized to the resting level in each $C_{a_0}$. Although the recovery time varied somewhat with $C_{a_0}$, it is evident that the most marked $C_{a_0}$ effect occurs during the initial time window examined (i.e., during the 2.5–5.5-second window following stimulation).

Figure 5 illustrates the average effect of an increase in $C_{a_0}$ on the SLIF in the measured 2.5–5.5-second interval after stimulation and at rest in all rat muscles. While, as observed previously, the resting SLIF frequency is approximately a linear function of $C_{a_0}$ over this range, SLIF frequency measured in the poststimulation interval exhibits a nonlinear, steeply increasing dependence on $C_{a_0}$. The ratio of SLIF measured during this poststimulation interval to that measured at rest increases monotonically over this range of $C_{a_0}$. The cross-over point of the curves of Figure 5 represents the $C_{a_0}$ where this ratio is 1, and thus stimulation under these conditions (at 60/min at 29°C) does not alter SLIF from its resting level.

Figure 6 shows that the rate and pattern of the prior stimulation have a substantial effect on the time course of SLIF restitution. In $C_{a_0}$ of 1.5 mM, a single, stimulated twitch is sufficient to abolish SLIF for 5 seconds, after which a gradual return to the resting level requires 15 seconds. Stimulation at 40/min also completely abolishes SLIF for 5 seconds, but the subsequent restitution of SLIF to its resting level now requires 35 seconds. This dependence of SLIF restitution on the prior stimulation pattern is abolished in high $C_{a_0}$ (Figure 7).

Previous studies in unstimulated preparations have indicated that changes in SLIF cause parallel changes in resting force. Figure 8A illustrates that poststimulation transients in resting force occur in $C_{a_0}$ of 1.5 mM and parallel the restitution curves for SLIF under conditions in Figures 3–7. In Panel A, stimulation under the conditions that transiently depress SLIF in Figure 7 cause a transient depression ("hyper-relaxation") of resting force. Furthermore, the time required for the monotonic return to the steady level of resting force varies in the same manner as SLIF: a progressively longer restitution time is required with increased frequencies of stimulation between a single depolarization and rates of
up to 30–60 min. Additionally, a progressive increase in the number of beats delivered in a train at 60/min (Figure 8B) also results in a progressive increase in the time required for restitution of the resting force transient.

Figures 3–8 are consistent with an interpretation that, as measured by both SLIF and resting tension, stimulation of rat muscle in "physiological" Ca0 suppresses spontaneous SR Ca2+ release, and that the time course of the recovery depends on the rate and pattern of this stimulation. The next series of experiments examined the effect on SLIF of drugs known to alter cellular and SR Ca2+ fluxes.

Figure 9 illustrates the dose response of SLIF to isoproterenol. SLIF measured in the 2.5–5.5-second interval poststimulation are markedly enhanced, but resting SLIF are not. That the stimulation-induced increase in SLIF in the presence of isoproterenol dissipates by 20–30 seconds poststimulation suggests β-adrenergic modulation causes a substantial net increase in cell Ca2+ loading only during depolarization (i.e., systole), which then dissipates with rest, in contrast to Na-K inhibition (cf. Figure 2) or an increase in Ca0 (cf. Figure 3), which cause persistent increases in Ca2+ loading at the resting level of membrane potential. The observed isolated increase in poststimulation SLIF with isoproterenol might be attributed exclusively to cyclic AMP modulation of the slow inward current. At the higher concentrations of isoproterenol (Figure 9), a depression of the resting SLIF occurs. This may be due to a reduction in resting
Ca, by the catecholamine as recently demonstrated in rat myocytes. 25

Given the result in Figure 9, in conjunction with those in Figures 3–7, a blockade of the Ca\(^{2+}\) channel might be expected to transiently decrease SLIF after stimulation but not affect SLIF at rest. Figure 10 shows that nifedipine indeed depresses poststimulation SLIF, which then recovers with time to a resting level not substantially different from control. This result suggests that when cell Ca\(^{2+}\) loading during stimulation is reduced by a blockade of the L-type Ca\(^{2+}\) channel, 26-27 rat myocardium regains Ca\(^{2+}\) with time following the cessation of stimulation.

From multiple previous studies in unstimulated tissue, it has been inferred that the spontaneous contractile waves that underlie SLIF (Figure 1) require the presence of normal SR function, 24 as do the higher frequency spontaneous Ca\(^{2+}\) oscillations measured during Ca\(^{2+}\) overload states. 3,28 This conclusion depended in part on the effects of drugs (e.g., caffeine or ryanodine) that interfere with SR Ca\(^{2+}\) cycling and thereby depress or abolish resting SLIF. 2-4,11,17 However, corresponding measurements of the effects of caffeine and ryanodine on SLIF measured in the poststimulation interval uncover some important differences between these two agents (Figure 11).

At low caffeine concentrations, while suppression of the resting SLIF was minimal, poststimulation SLIF were markedly enhanced. This mimicked the effect of isoproterenol (Figure 9) and may be due to the well-known property of methylxanthines to inhibit phosphodiesterase activity 29 leading to an increase in intracellular cyclic AMP, which could lead to an increase in the Ca\(^{2+}\) current. As expected from previous results, 2 higher concentrations of caffeine markedly suppressed resting SLIF. Figure 11 shows that poststimulation SLIF are abolished as well. This effect of high caffeine concentrations can be attributed to either the marked decrease in the contractile wave amplitude, which became less than the laser wave length and therefore difficult to detect, or to an actual cessation of the waves. 2-4,30 The decrease in amplitude or abolition of the spontaneous Ca\(^{2+}\) release by caffeine can be interpreted to result from a depletion of SR (and eventually cellular) Ca\(^{2+}\).

Figure 12 illustrates that, unlike a low concentration of caffeine, ryanodine does not increase poststimulation SLIF; rather, SLIF measured in both the poststimulation interval and at rest exhibit a monotonic decrease in response to increasing exposure to ryanodine. Since ryanodine neither affects myofilament Ca\(^{2+}\) sensitivity 31 nor increases cyclic AMP, this parallel decrease in SLIF measured at both times can be interpreted to result directly from a decrease in the magnitude of SR Ca\(^{2+}\) release. This could be due to a ryanodine depletion of the SR Ca\(^{2+}\) load as demonstrated in recent studies in which quin 2 was used in rat cardiac myocyte suspensions 32 or in which Ca\(^{2+}\)-selective microelectrodes and rapid cooling contractions were used in rabbit muscle. 33

Discussion

Suppression of Spontaneous Ca\(^{2+}\) Release by Electrical Stimulation in Physiological Ca\(_o\), in the Absence of Drugs

The present results demonstrate that electrical stimulation alters SLIF and thus alters the density of spontaneous contractile waves present within the
tissue. In Ca$_2^+$ of 1.5 to 2.0 mM (i.e., that which has been conventionally employed in in vitro studies of cardiac muscle) and in which SLIF are present in the unstimulated, resting states in rat cardiac muscle, electrical stimulation abolishes SLIF for up to several seconds (Figures 3, 4, 6, and 7). The bulk mechanical counterpart of the transient abolition of SLIF after the twitch is the hyper-relaxation of resting tension (Figure 8). Thus, under the conditions of physiological Ca$_{o}$, spontaneous SR Ca$_2^+$ cycling does not occur in the rat muscle in the diastolic period between regular stimulation despite its occurrence in the unstimulated state. Rather, in the absence of intended experimental Ca$_2^+$ loading, this tissue is protected from the potential adverse effects of spontaneous diastolic Ca$_2^+$ release during a "delay interval" of a few seconds following prior contractions. This result is strikingly similar to direct observations in the isolated Ca$_2^+$ tolerant rat myocyte in physiological Ca$_{o}$, in which a delay interval on the order of seconds elapses before the occurrences of a spontaneous contractile wave following an electrically driven twitch. The present results show that the delay interval for SLIF to recur after stimulation in muscles bathed in physiological Ca$_{o}$ at 29°C is approximately 5 seconds (Figures 3, 4, and 6). If the interstimulus interval exceeds this delay interval, spontaneous Ca$_2^+$ release occurs between twitches. In single cardiac myocytes, it has been directly observed that reduction of the interstimulus interval to times less than the delay interval prevents the occurrence of spontaneous waves. Although the experimental observations in the present and prior studies have indicated that SLIF varies with "cell Ca$_2^+$ loading," the specific experiments to differentiate the loading of which of the various intracellular sites (i.e., the various possible SR compartments, the myoplasmic space or other cell Ca$_2^+$ sinks) is critical to spontaneous SR Ca$_2^+$ release, and thus SLIF production, are lacking. The transient abolition of SLIF by stimulation could be interpreted as arising from 1) a generalized Ca$_2^+$ depletion of some or all cellular compartments; 2) Ca$_2^+$ depletion or delayed replenishment of the SR Ca$_2^+$ release site without a net Ca$_2^+$ loss from the cell; or 3) inhibition of SR Ca$_2^+$ release mechanisms. Regarding the first possibility above, evidence for a substantial Ca$_2^+$ efflux during stimulation has recently been provided from measurements of interstitial [Ca$_{2+}$] with Ca$_2^+$ sensitive dyes or microelectrodes, even during a given twitch in cardiac muscle of some species. If the recovery of SLIF with rest following stimulation of rat muscle is due to a slow "diastolic" reloading of cell Ca$_2^+$, it may relate to the higher Na$_{e}$ measured in rat preparations in some$^{35-36}$ but not in all$^{37}$ studies. Suspensions of rat cardiac myocytes have a higher Ca$_{i}$ than cat myocytes. It may be possible that in rat myocardium the Na-Ca exchanger operates closer to equilibrium than in other species.$^{39}$ A transient removal of Ca$_2^+$ from the SR "releasable pool" but not from the cell is a second mechanism that might explain the transient suppression of SLIF. The idea of a transient inaccessibility of SR Ca$_2^+$ for release has traditionally been modeled as a two compartment SR with pumping occurring into a nonreleasable compartment followed by subsequent transfer to the release compartment. Some anatomical evidence for this has been provided from autoradiographic and electron microscope probe studies that suggest a time-dependent recycling of Ca$_2^+$ after contractile activity from the longitudinal SR to the terminal cisternae in various muscle types. Alternatively, a delay in the restitution of Ca$_2^+$ loading of the SR release site could occur if Ca$_2^+$ released by an action potential were taken up by or bound to non-SR cell buffers (i.e., myofilaments, mitochondria, calmodulin, or phospholipids within the sarcolemma) from which it was only slowly released or if complete SR Ca$_2^+$ filling involved a relatively slow SR pumping rate at the relatively low Ca$_{i}$ subsequent to the contraction itself. Ca$_2^+$ inhibition of SR Ca$_2^+$ release not related to SR Ca$_2^+$ loading and resulting from the increase in
Ca, elicited by a prior action potential is a third possible explanation for the transient depression of SLIF after stimulation. However, at the moment, this hypothesis has no direct evidence from intact preparations in its favor. In fragments of single cells, the time constant for removal of the hypothesized Ca\(^{2+}\) inactivation of SR Ca\(^{2+}\) release mechanisms\(^4\) is very short compared to the prolonged suppression of SLIF by stimulation in the present study.

**Augmentation of Spontaneous Ca\(^{2+}\) Release by Stimulation in High Ca\(_o\), or in the Presence of Inotropic Drugs**

A second major finding of the present study is that the stimulation-induced suppression of SLIF in physiological Ca\(_o\) in the absence of drugs could be reversed by perturbations that increase cell Ca\(^{2+}\) loading by either increasing cAMP or by increasing Ca\(_o\) or reducing K\(_s\). In many of these instances stimulation could accelerate the SLIF measured just after stimulation to levels higher than the resting level. In other studies of intact muscle, this same phenomenon is manifested as the de novo occurrence of, or an exaggeration of, the spontaneous oscillations in aequorin luminescence during diastole.\(^{13,14}\) These are accompanied by after-contractions,\(^{10,46,47}\) that is, an oscillatory recovery of resting tension rather than the monotonic increase after a hyper-relaxation (Figures 8A or 8B). The exaggeration of SLIF by stimulation of rat muscle in the presence of these perturbations can be attributed to a resultant transient increase in net cell and SR Ca\(^{2+}\) loading.\(^6\)

A role for the Ca\(^{2+}\) channel in modulation of cellular Ca\(^{2+}\) in rat cardiac muscle during stimulation is particularly evident in the contrasting effects of isoproterenol and nifedipine (Figure 9 and 10) on poststimulation SLIF. Specifically, in the absence of an effect of either of these drugs on resting SLIF, SLIF measured in the 2.5-5.5-second interval following stimulation was markedly altered. The change in SLIF in the 2.5-5.5-second interval following stimulation in the presence of nifedipine or isoproterenol thus appears to reflect a change in the net cellular Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channels.\(^{26,27,48}\) This suggests that the level of excitation-contraction coupling in the rat ventricle beating in the steady state, as in cardiac muscle of other species, is critically dependent on this current to replenish cells with Ca\(^{2+}\) lost via other mechanisms either during or between twitches.

Action potential mediated SR Ca\(^{2+}\) release in rat cardiac muscle and the resulting twitch tension, like SLIF, exhibit marked variations with time following a prior twitch.\(^{49,50}\) In the following paper\(^6\) we have examined 1) the transient state restitution following a prior twitch of spontaneous SR Ca\(^{2+}\) release measured as SLIF and twitch tension, and 2) the steady state relation of SLIF and twitch tension both in rat muscle, which in physiological Ca\(_o\) displays SLIF in the unstimulated state, and in rabbit muscle, which does not.

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