Cellular Mechanisms for the Slow Phase of the Frank–Starling Response

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Abstract: Following a step increase in sarcomere length, isometric cardiac muscle tension increases instantaneously by the Frank-Starling mechanism. In isolated papillary muscle and myocytes, there is an additional significant rise in developed tension over the following 15 min due to an unknown mechanism. This slow change in tension could not be explained by mechanical heterogeneity of the muscle preparations or by an increase in myofilament sensitivity to Ca\textsuperscript{2+}. The slow change in tension was not dependent on sarcoplasmic reticulum Ca\textsuperscript{2+} loading assessed with rapid cooling contractures, and was not significantly altered by sarcoplasmic reticulum Ca\textsuperscript{2+} depletion (ryanodine) or inhibition of sarcoplasmic reticulum Ca\textsuperscript{2+} reuptake (cyclopiazonic acid). We used the Luo-Rudy ionic model of the ventricular myocyte together with a model of the length-dependent myofilament activation by Ca\textsuperscript{2+} to examine the effects of step changes in the parameters of sarcolemmal ion fluxes as possible mechanisms for the slow change in stress. The slow increase in tension was simulated by step changes in the Na\textsuperscript{+}-K\textsuperscript{+} pump or Na\textsuperscript{+} leak currents, suggesting that the slow change in stress may be caused by length induced changes in Na\textsuperscript{+} fluxes. The model also predicted a slow increase in the magnitude of the initial repolarization during phase 1 of the action potential. The combination of experimental and computational models used in this investigation represents a valuable technique in elucidating the cellular mechanisms of fundamental processes in cardiac excitation-contraction coupling.

Key words: heart, Frank-Starling mechanism, excitation-contraction coupling, Luo-Rudy model.

Active stress in cardiac muscle increases with muscle length. This fundamental principle has been known since the classic studies of Frank and Starling almost a century ago. Over the last two decades, it has become evident that this increase is primarily caused by length-dependent activation of the thin filament. The length–tension relationship has long been considered a fundamental property of isolated cardiac muscle preparations and has been the subject of numerous review articles (1–5). However, the contractile force of cardiac muscle does not depend on the instantaneous muscle length alone. Active and passive forces in cardiac muscle both depend on the time history of sarcomere length.

In 1973, Parmley and Chuck (6) first showed that the immediate increase in cardiac muscle tension
due to a step increase in length is followed by a slow rise in stress that continues for at least 10 min in cat papillary muscle. Since then, this phenomenon has been investigated in only about a dozen previous studies. Most experiments have been performed in isolated papillary muscles or trabeculae from various species including cat (3.6–9), rabbit (10), ferret (11), and rat (12), with step increases in muscle length.

Figure 1 demonstrates the slow changes in stress (SCS) as observed in a rabbit ventricular papillary muscle that was stretched from 85% to 95% of the muscle length at which active force development was maximal ($L_{\text{max}}$). The slow component of the increase in tension represents a significant proportion of the total Frank-Starling effect in these preparations. From about a quarter to as much as half of the total increase in active stress after a step increase in length of a papillary muscle occurs after the immediate increase in stress. The phenomenon is also not unique to isolated muscle preparations. Time-dependent shifts in ventricular end-systolic pressure volume relations have also been reported in intact dog (13–15) and rat hearts (16). A report of slow changes in stress in guinea pig ventricular myocytes (17) has also been published.

These studies demonstrate that the SCS are a particularly important and significant example of length history–dependent behavior of active cardiac muscle that is observed in several different preparations of cardiac muscle from a variety of different species. However, to date, the mechanisms of this fundamental property of cardiac muscle contraction have not been clearly established. In this article, we review experimental and modeling investigations into the potential mechanisms of this poorly understood phenomenon and suggest possible future studies and analyses.

**Experimental Investigations**

We have conducted a number of experimental studies to investigate possible mechanisms of this slow increase in active stress. Multicellular preparations of cardiac muscle undergo heterogeneous contractions with shortening in the central regions at the expense of lengthening at the muscle ends (18–21). The central shortening in a muscle length isometric contraction leads to a significant reduction in peak active stress compared with sarcomere...
length isometric contractions (22). Because of the viscoelastic properties of cardiac muscle (23,24), there may also be a slow decrease in the resting length of central segments after a step increase in muscle length. This would lead to a slow decrease in stress, which would subtract from SCS.

We explored how a heterogeneous preparation might affect measurements of SCS (25). Nonhomogeneous strain distributions along the length of rabbit papillary muscles measured during isometric contraction showed shortening in the central region at the expense of lengthening at the ends (Fig. 2). Measurements of resting lengths over 20 min after a step increase in muscle length showed slow shortening of central segments at the expense of slow lengthening at the ends, implying that the decrease in central segment length may have attenuated SCS. Further supportive evidence was provided by correlating peak active stress and SCS with the total muscle length at $L_{max}$. The peak active stress at $L_{max}$ increased significantly with the muscle length, and, as Figure 2 indicates, SCS also increased significantly with muscle length. Because nonhomogeneity associated with damaged muscle ends is more pronounced in shorter muscles, this result suggests that the SCS is not attributable to heterogeneity of the papillary muscle preparation; indeed the effects of mechanical heterogeneity tend to oppose SCS.

We next examined whether the SCS of cardiac muscle are an inherent property of isolated cardiac myocytes. The specific methods and physiologic characteristics of our isolated myocyte preparation have been previously established (26). Myocytes were attached to two poly-L-lysine-coated glass coverslips and force was measured with a capacitive force transducer. The stress response to step changes in length was studied by increasing cell length from slack to about 5% to 15% of the cell length (25). As shown in Figure 3, the immediate increase in stress was followed by an additional increase over 10 min. The relative SCS was 28 ± 23% of the total increase in stress after 10 min. The results of this study confirmed that SCS occurred in isolated cells with a magnitude comparable to that of papillary muscles, suggesting that the mechanisms of SCS do not necessitate the presence of the extracellular matrix but are an intrinsic property of the cardiac myocyte.

Having observed that the SCS are apparently not caused by a slow increase in sarcomere length due to changes in mechanical heterogeneity or a slow transduction of a stretch from the extracellular matrix, we investigated whether SCS may be a property of cardiac excitation-contraction coupling. Allen and Kurihara (12) found that the slow increase in active stress after a step increase in muscle length is accompanied by a corresponding slow increase in the magnitude of the intracellular calcium transient. This suggests that the slow increase in stress may be caused by an increase in the amount of calcium delivered to the myofilaments. During a cardiac muscle contraction, extracellular
calcium entering the cell triggers a further release of calcium from the sarcoplasmic reticulum (SR) (27). This SR calcium contributes the majority of activating calcium in most mammalian species, including the rabbit (28).

Because of the dominant role of SR in cardiac excitation–contraction coupling, we studied the contribution of the SR to the SCS in rabbit right ventricular papillary muscles after increasing muscle length from 85% to 95% of $L_{\text{max}}$ (29). In summary, the SCS amounted to $32.5 \pm 12.2\%$ (mean $\pm$ SD) of the total increase in active stress. This was associated with a $13.2 \pm 8.7\%$ increase in calcium content of the SR as estimated with rapid cooling contractures. However, SCS were not dependent on SR calcium content. There was no significant attenuation in SCS after SR calcium depletion with ryanodine, SR Ca-ATPase inhibition with cyclopiazonic acid, or combined treatment with ryanodine and cyclopiazonic acid. We thus concluded that in the rabbit, SR calcium content increases slowly following a step increase in cardiac muscle length, but that the slow changes in active stress are not dependent on the sarcoplasmic reticulum.

An increase in myofilament sensitivity to calcium is another possible explanation for the slow rise in active tension. Kentish and coworkers (10) showed that SCS were reversed by large concentrations (0.1 $\mu$M to 1 $\mu$M) of the inotropic agent isoproterenol. Although isoproterenol has multiple effects, they suggested that SCS were attenuated through a cyclic AMP sensitive sarcolemmal process rather than a saturation of calcium binding to the myofilaments. We examined the effects of various inotropic agents on SCS using the same papillary muscle preparation which we used in our SR studies (25). Our intent was to determine the role of myofilament sensitivity to calcium in the SCS response. If SCS were caused by a time-dependent shift in myofilament sensitivity to calcium, SCS should be attenuated by agents that increase myofilament sensitivity and augmented by agents that decrease it. The inotropic interventions which we applied included agents which decrease myofilament sensitivity (isoproterenol, forskolin), interventions which increase myofilament sensitivity (phenylephrine, increases in muscle length), and interventions that have no effects on myofilament sensitivity (Bay K 8644, changes in extracellular calcium).

We found that SCS were attenuated or reversed by all positive inotropic interventions, regardless of their disparate effects on the myofilaments. These results are therefore inconsistent with the hypothesis that SCS might be caused by a slow increase in myofilament sensitivity to calcium and most consistent with the hypothesis that SCS are caused by changes in sarcolemmal ion currents.

We then speculated that the mechanisms of mechanotransduction of an increase in muscle length into a change of sarcolemmal ion currents might be governed by stretch-activated channels. Stretch-activated channels have been reported in many cell types, including cardiac myocytes (30,31), and it was suggested that they may be important in the Frank–Starling mechanism (30). The blocking of several types of stretch-activated channels has been reported with gadolinium at
Gd concentration [µM]  

Fig. 4. Effects of gadolinium on SCS in papillary muscle. Average (+ standard deviation) SCS for step sizes from 80% to 100% of L_max and from 90% to 100% of L_max in the presence of various concentrations of gadolinium. Gadolinium did not significantly alter SCS.

concentrations of 10 to 50 µM (30,32). To test our hypothesis, papillary muscles were studied for step increases from 80% to 100% of L_max and from 90% to 100% of L_max in the presence of 0 to 100 µM gadolinium. As Figure 4 indicates, block of the stretch-activated channels by gadolinium did not appear to alter the SCS. However, gadolinium is highly nonspecific, precipitates physiological anions, and has even been shown to block L-type Ca^{2+} channels in ventricular myocytes (33). Thus, the insensitivity of SCS to gadolinium does not completely rule out the involvement of stretch activated sarcolemmal channels.

Ionic Model of Length History–Dependent Tension

The foregoing experimental investigations focused our attention on altered sarcolemmal ion fluxes as a possible mechanism for the slow increase in active stress. Much of the extensive experimental data on the ionic kinetics of the cardiac myocyte are summarized within various mathematical models of the cardiac action potential that have included an increasing number of sarcolemmal ion channels (34–37). The dynamics of excitation–contraction coupling in general and intracellular Ca^{2+} transients in particular have also been investigated in detail. Luo and Rudy (38) published a refined version of their earlier action potential model that includes the SR with Ca^{2+} uptake and release, and several intracellular Ca^{2+} buffers. Sarcolemmal Ca^{2+} currents are more accurately described than by previous models, making it possible to account for changes in intracellular Ca^{2+} ion concentration. This model therefore allows computational experiments as a powerful alternative to experimental investigations in cells and tissues. Since myocardial force development is directly related to intracellular Ca^{2+}, we used the Luo–Rudy model (38) to investigate potential contributions of sarcolemmal ion fluxes to SCS. By analyzing the effects of a step change in each parameter of individual ion fluxes on the subsequent force development, we used the model to test whether a stepwise change in a sarcolemmal ion current accompanying stretch could provide a potential mechanism for SCS.

Details and results of the model have been previously published (39). Briefly, the Luo–Rudy model (38) is based on a numerical reconstruction of the action potential using the following equation:

\[
\frac{dV}{dt} = -\frac{1}{C_m} \cdot (I_i + I_{st})
\]

where \( V \) is the membrane potential, \( C_m \) is the membrane capacitance, \( I_{st} \) is a stimulus current, and \( I_i \) is the sum of 13 ionic currents through sarcolemmal ion channels, pumps, and exchangers. The ionic currents are determined by voltage-dependent ion gates that are obtained as the solution to a system of coupled differential equations. The model accounts for changes in the intracellular concentrations of Ca^{2+}, K^{+}, and Na^{+} ions. Force was computed as an output variable of the model from a sigmoidal function of the free intracellular calcium concentration \([Ca^{2+}]_i\) with a Hill coefficient of 2.5, representing the binding of calcium to troponin C. The Hill coefficient chosen was similar to published values (40,41). Force generation at 85% or 95% of L_max was simulated using low (\( \text{Cas}_0 = 2 \) µM) or high (\( \text{Cas}_0 = 1 \) µM) myofilament sensitivities to calcium, where \( \text{Cas}_0 \) is the calcium concentration for half-maximum force generation. With these values for the Hill coefficient and the myofilament sensitivity, simulated step changes from 85% to 95% of L_max produced force changes similar to those observed experimentally (29). Muscle contractions were simulated to occur at 5-sec intervals.
Model Results

Model-simulated muscle contractions were stable over long periods of time with the parameters given. A step in myofilament sensitivity from 2 μM to 1 μM generated a step increase in force from 14.3% to 48.5% of maximum generated force. The peak value of the Ca\(^{2+}\) transient was 0.976 μM and did not change with the step. In the 15 min after the step change, force and the Ca\(^{2+}\) transient changed by less than 0.1%, with this change most likely being related to small remaining imbalances in intracellular Na\(^+\) and K\(^+\) homeostasis. Therefore, the model showed no slow change in force with a step change in myofilament sensitivity to calcium alone.

Superimposing a step change in myofilament sensitivity with a step change in any one of the parameters governing sarcolemmal ion fluxes enabled the potential role of a single ion flux in SCS to be examined. We examined step changes in 20 parameters of 13 ionic fluxes included in the model. Step changes in 12 parameters of 7 ionic currents produced changes in force of more than 10% following the steps. However, for all but 4 of these parameters, the time courses of the increases in force did not match the experimental results. Step changes in the amplitude of \(I_{Na,b}\), the sodium background current, and three parameters describing \(I_{Na,K}\), the Na\(^+-\)K\(^+\) pump did produce force changes which closely resembled the experimental SCS. Of these parameters, \(I_{Na,K}\), the maximum current through the Na\(^+-\)K\(^+\) pump, needed to be changed the least to yield a slow change of force comparable in magnitude to the experimentally observed SCS, and this parameter was therefore selected for further analysis. \(I_{Na,K}\) was decreased by 20% from 1.7 μA/μF to 1.36 μA/μF. The immediate increase in force from 14.3% to 48.3% was followed by a further increase to 65.8%, amounting to a SCS of 34% with a half-time of 2.6 min, where the value of SCS expresses the slow increase in stress as a percentage of the total increase in stress following the step length change. This is comparable to our experimental studies in which we observed the magnitude of SCS to be 38%, with a half-time of 4.3 min (29). The model also predicted a slow increase in the magnitude of the initial repolarization during phase 1 of the action potential.

The mechanisms for this simulated SCS are illustrated in Figure 5. The decrease in \(I_{Na,K}\) from 1.7 μA/μF to 1.36 μA/μF resulted in a decrease in \(I_{Na,K}\). Peak systolic pump activity dropped from 0.599 μA/μF to 0.479 μA/μF, and diastolic activity decreased from 0.315 μA/μF to 0.252 μA/μF. As a result of the diminished Na\(^+\) extrusion from the myoplasm, \([Na\(^+\)]\) rose from 10 mM before and immediately after the step to 12.5 mM after 15 min. The rise in \([Na\(^+\)]\), in turn led to a recovery of the Na\(^+-\)K\(^+\) pump, to a systolic pump activity of 0.599 μA/μF and a diastolic pump activity of 0.294 μA/μF.
was no immediate increase in force, because the returning to the original values at the time of the period starting 1 sec after each stimulus, and in Caso and INa,K only during a 4-sec diastolic
tions and to return it to the original shorter increased to 0.64 μM, and force increased from Ca 2+ entry caused an increase in the Ca 2+ transient from 0.976 μM to 1.299 μM, and therefore the increase in force.

We used the slow force response to the step changes in Ca50 and INa,K as a control which was subjected to simulations of several experimental interventions. We modeled SCS dependence on the SR. SR calcium uptake was inhibited by setting I_{up} = 0. This led to SR calcium depletion, a decline of the peak Ca 2+ transient to 0.52 μM, and a decline of peak force to 3.3% of maximum force generation. The same step changes in Ca50 and INa,K that were used to simulate the SCS were then imposed. The peak Ca 2+ transient increased to 0.64 μM, and force increased from 16.2% immediately after the step to 24.4% after 15 min, amounting to a 39% SCS. Therefore, the simulated SCS did not depend on the SR, similar to our experimental observations.

Nichols (9) and Allen et al. (11) showed that for SCS to occur, the muscle never had to contract at an increased muscle length, but that it was sufficient to stretch the muscle only between contractions and to return it to the original shorter length prior to the next stimulus. We simulated this length protocol by imposing the step changes in Ca50 and INa,K only during a 4-sec diastolic period starting 1 sec after each stimulus, and returning to the original values at the time of the next stimulus. At the onset of this protocol, there was no immediate increase in force, because the contractions always occurred at the same myofilament sensitivity of 2 μM. However, there was a slow increase in force from 14.3% to 21.7% with a half-time of 2.9 min. The peak Ca 2+ transient increased from 0.976 μM to 1.198 μM. This increase of 0.222 μM was 68% of the 0.323-μM increase in peak Ca 2+ transient with a sustained change in Ca50 and INa,K. We also ran a simulation in which the changes in Ca50 and INa,K were made only during a systolic 1-sec period immediately following each stimuli. The immediate increase in force from 14.3% to 43.8% was followed by an increase to 51.4% after 15 min. This amounted to an 8% SCS with a half-time of 2.1 min. The peak Ca 2+ transient increased from 0.976 μM to 1.023 μM, and the increase was 15% of the increase with maintained changes in Ca50 and INa,K.

Discussion

We approached the investigation of the slow increases in stress following a step change in cardiac muscle through a combination of experimental and computational models. Our experimental results indirectly implicated alterations in the sarcolemmal ion fluxes as a possible mechanism for SCS. However, experimental approaches for directly screening individual sarcolemmal ion currents and their contributions to SCS are technically difficult as such studies involve a large number of individual ionic channels, exchangers, and pumps. Instead, we used a theoretical ionic model to investigate whether SCS could be caused by length-induced step changes in individual sarcolemmal ion currents. Step changes in the model parameters of sarcolemmal Ca 2+ or K + fluxes did not yield force changes that could resemble the experimentally observed SCS. SCS, however, could be reproduced by step changes in the parameters of the Na +–K + pump or the Na + background current. The increase in intracellular Na + concentration concurred with an increase in systolic Ca 2+ entry through the Na +–Ca 2+ exchanger, which caused an increase in Ca 2+ transients and thus force. This simulated SCS compared well with experimental observations in its response to several simulated interventions. Therefore, the model suggests changes in sarcolemmal Na + fluxes as a possible mechanism.

The model chosen for this study (38) was primarily designed as a model of the mammalian ventricular action potential. However, the model is also able to account for dynamic changes in intracellular ion concentrations, which distinguishes it from several previous action potential models (34–37). Accurate intracellular Ca 2+ transients, which are clearly important for the model to be applied to study myocyte contraction, were obtained by including an SR with Ca 2+ uptake and release as well as several intracellular Ca 2+ buffers. The description of sarcolemmal K + and Na + fluxes proved sufficient to maintain intracellular K + and Na + homeostasis, since [K +] i and [Na +] i remained stable over more than 15 min. This is important because changes in [K +] i and [Na +] i lead to secondary changes in sarcolemmal Ca 2+ fluxes, and hence in myocardial contraction. Based on more recent experimental work, the delayed rectifier current has been replaced by two distinct components (42), and recently, an ATP-sensitive potassium current was also introduced into the model (43). Although these changes present important refinements of the version used in our simulations (38), they should not affect the present results.
The model showed that a step change in myofilament sensitivity alone produced only an immediate step in active force, but no additional slow component. By design, the model does not include time-dependent changes in myofilament sensitivity. However, the model does include calcium buffering by troponin, calmodulin, and calsequestrin. The lack of SCS following a step change in myofilament sensitivity therefore suggests that calcium buffering is not changed in such a way as to contribute to the SCS. The model results do not rule out the possibility that SCS might be related to length-induced slow changes in myofilament sensitivity. However, our experimental investigations using inotropic agents argue against this hypothesis. Recently, Hongo and coworkers (44) also dismissed a change in the $\text{Ca}^{2+}$ sensitivity of the myofilaments as a possible mechanism for SCS because they did not observe a change in the time course of contraction during the slow phase.

The Na$^+$–K$^+$ pump plays an important role in the regulation of myocardial contraction in the heart. Several processes alter force development through changes in the activity of the Na$^+$–K$^+$ pump, which is the major mechanism for the inotropic action of cardiac glycosides (45). The Na$^+$ pump lag hypothesis for the force–frequency relationship of cardiac muscle states that the greater Na$^+$ entry due to more frequent stimulation is balanced by increased Na$^+$ pump activity but only at the cost of elevated [Na$^+$]. and hence increased Ca$^{2+}$ entry. Mechanical restitution may be mediated in part through changes in [Na$^+$]. Wilde and Klöber (46) observed in guinea pig ventricular trabeculae that a transition from regular stimulation to quiescence was followed by a decline in intracellular Na$^+$ activity with a time course of about 1.5 min. In sheep cardiac Purkinje fibers, Eisner et. al. (47) found that Na$^+$–K$^+$ pump inhibition with strophanthidin resulted in parallel increases in tension and intracellular Na$^+$ activity with half-times of approximately 5 min.

Conversely, Hongo and coworkers (44) found that in isolated rat ventricular myocytes, neither [Na$^+$]. nor diastolic [Ca$^{2+}$]. changed significantly during the slow increase in contraction following a stretch. They suggest changes in sarcolemmal Ca$^{2+}$ influxes as a possible mechanism which still remains to be examined. These results indicate that further experimental studies are needed to determine the validity of our computational model hypothesis that sarcolemmal Na$^+$ fluxes may play a role in SCS. Our model predicts a slow increase in the initial repolarization phase of the action potential over the time course of the slow increase in tension. Thus, a future experiment could involve the measurement of the action potential to determine if this slow change in the action potential is experimentally observed. Due to the highly non-specific nature of gadolinium, our experimental results using this stretch activated channel blocker cannot conclusively dismiss mechanosensitive channels as a mechanism for SCS. Further studies involving other stretch-activated channel blockers, such as Grammastola spatulata spider venom (48), may provide further insight.

In summary, step changes in the parameters of sarcolemmal ion currents were analyzed with a theoretical ionic model. The results suggested that SCS may be caused by length-induced step changes in sarcolemmal sodium fluxes, leading to an increase in intracellular sodium concentration, and a concurrent increase in systolic calcium entry through the Na$^+$–Ca$^{2+}$ exchanger. Although future studies are needed to determine whether this proposed mechanism is indeed responsible for SCS, the present results demonstrate the great value of detailed ionic models as powerful tools for conducting computational experiments into the cellular mechanisms of fundamental processes in cardiac excitation–contraction coupling.

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