Differentiating the effects of β-adrenergic stimulation and stretch on calcium and force dynamics using a novel electromechanical cardiomyocyte model

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Am J Physiol Heart Circ Physiol 319: H519–H530, 2020. First published July 31, 2020; doi:10.1152/ajpheart.00275.2020.—Cardiac electrophysiology and mechanics are strongly interconnected. Calcium is crucial in this complex interplay through its role in cellular electrophysiology and sarcomere contraction. We aim to differentiate the effects of acute β-adrenergic stimulation (β-ARS) and cardiomyocyte stretch (increased sarcomere length) on calcium-transient dynamics and force generation, using a novel computational model of cardiac electromechanics. We implemented a bidirectional coupling between the O’Hara-Rudy model of human ventricular electrophysiology and the MechChem model of sarcomere mechanics through the buffering of calcium by troponin. The coupled model was validated using experimental data from large mammals or human samples. Calcium transient and force were simulated for various degrees of β-ARS and initial sarcomere lengths. The model reproduced force-frequency, quick-release, and isotonic contraction experiments, validating the bidirectional electromechanical interactions. An increase in β-ARS increased the amplitudes of force (augmented inotropy) and calcium transient, and shortened both force and calcium-transient duration (lusitropy). An increase in sarcomere length increased force amplitude even more, but decreased calcium-transient amplitude and increased both force and calcium-transient duration. Finally, a gradient in relaxation along the thin filament may explain the nonmonotonic decay in cytosolic calcium observed with high tension. Using a novel coupled human electromechanical model, we identified differential effects of β-ARS and stretch on calcium and force. Stretch mostly contributed to increased force amplitude and β-ARS to the reduction of calcium and force duration. We showed that their combination, rather than individual contributions, is key to ensure force generation, rapid relaxation, and low diastolic calcium levels.

INTRODUCTION

Cardiac electrics and mechanics are strongly interconnected in the heart. Cardiac electrical activation and myocyte contraction are, indeed, strongly coupled through cardiomyocyte calcium handling (“excitation-contraction coupling”) (4, 34). On one hand, during systole, the calcium entering the cell triggers calcium release from the sarcoplasmic reticulum (SR). Calcium ions can subsequently bind to troponin, initiating conformational changes in the thin filament of the sarcomere that initiate contraction by enabling the formation of cross-bridges. Relaxation occurs when cytosolic calcium levels decline and calcium unbinds from troponin, inducing conformational changes to thin filament conformation back to a blocked, non-force-generating state. On the other hand, altered cardiac mechanics also affect electrophysiological properties (“mechanoelectrical feedback”) by modifying ionic currents and changing calcium-binding properties (30). This complex dynamic interplay between cardiac electrophysiology and mechanics makes it difficult to evaluate their relative and combined inotropic and lusitropic effects, especially under dynamic physiological conditions, such as exercise, where both may be altered. Exercise, for example, profoundly affects both cardiac electrophysiology and mechanics by increasing sympathetic stimulation, which strongly regulates cardiac electrophysiology, calcium handling, and contractility (6). Although mean cavity volumes are not increased during continuous exercise in athletes (3, 20), it may increase in sedentary controls (3). Moreover, at the start of exercise, a sudden increase in ventricular end-diastolic sarcomere length (SL) may occur through the acute increase of cardiac preload. In such situations, these electromechanical interactions may even be proarrhythmic. The initial step to understand these potential arrhythmogenic effects is to improve our quantitative understanding of cardiac electromechanical coupling interactions. This is the aspect that...
will be addressed in this article. Untangling the effects of this complex electromechanical coupling remains a challenge for standard experimental techniques. For example, optical mapping of voltage or calcium concentration often requires the use of blebbistatin, a myosin II inhibitor, to prevent contraction and reduce motion artifacts during measurement (36). Dynamic situations, such as exercise, are also challenging to study in an ex vivo setup. Computational models, however, provide a controlled environment to evaluate the influence of single physiological properties on myocardial system dynamics, providing mechanistic insights where clinical or experimental approaches are limited. Several modeling studies have, therefore, focused on different aspects of the interactions between cellular electrophysiology and mechanics by combining cellular electrical and contraction models to investigate, e.g., how force and action potential alternans may interact in the context of heart failure (44), or the effect of stretch and fibrosis on electrophysiology using multiple combinations of existing cellular models (39). However, the effects of sympathetic stimulation and SL have not yet been investigated in these models.

In this article, we present a directly coupled model of cardiac cellular electrophysiology and sarcomere mechanics. We used the O’Hara-Rudy (ORd) model (28) of the ventricular action potential, an extensively validated model of human cellular electrophysiology. We coupled it with the MechChem sarcomere contraction model (11, 12), which simulates the mechanochemical interactions underlying sarcomere contraction and was validated on experimental data. Although several models have previously coupled cardiac electrics and mechanics (5, 8, 15, 24–26, 31–33, 39, 44), the uniqueness of this ORd-MechChem combination is that it captures the mechanochemical interactions leading to sarcomere contraction, and it provides a direct link between the mechanics of the thin filament, the chemistry of contraction and relaxation, and the electrophysiology through calcium dynamics. In this work, we aimed to differentiate the electrical and mechanical effects induced by exercise on calcium-transient (CaT) dynamics and force generation. Toward this end, we validated the integrated electromechanical model and simulated the individual and combined effects of acute β-adrenergic stimulation (β-ARS) and cardiomyocyte stretch (i.e., increased SL).

MATERIALS AND METHODS

Human ventricular electromechanical model. To investigate the effects of β-ARS and SL alterations on cardiac mechanics and ventricular electrophysiology, we implemented a bidirectional coupling between two previously validated models. The ORd model of the human ventricular action potential (28) was used to simulate cellular electrophysiology. The MechChem model of sarcomere mechanics (12), describing the cooperative nature of myocardial contraction through mechanochemical interactions, was used to simulate cell contraction and relaxation. The MechChem model is the first to integrate two specific cooperative mechanisms: the intrinsic chemical cooperativity of calcium binding to troponin and the newly proposed mechanochemical cooperativity where mechanical tension in the thin filament impedes the unbinding of calcium from troponin. The ORd and MechChem models were coupled through the buffering of calcium. On the one hand, the free (nonbuffered) intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) determined by the ORd model induced force generation in the MechChem model. On the other hand, the contractile properties depending on the tension in the sarcomere thin filament determined calcium buffering by troponin, as described in MechChem, and were fed back into the ORd to compute [Ca\(^{2+}\)]\(_i\) (Fig. 1).

In the original ORd model formulation, calcium buffering by troponin was combined with buffering by calmodulin in a single buffer equation. To enable the bidirectional coupling with MechChem, we split the original ORd buffering description into two parts, following the approach taken by Zile and Trayanova (44), including dynamic calcium buffering by troponin, while modeling the buffering by calmodulin with a single ordinary differential equation.

![Fig. 1. Bidirectional coupling of the electrophysiological O’Hara-Rudy model and the mechanical MechChem model based on calcium buffered by troponin [Ca\(^{2+}\)]\(_i\),Tn. Figures are taken from the original publications (12, 28).](H520 DIFFERENT EFFECTS OF β-ADRENERGIC STIMULATION AND STRETCH)
The equations below describe the changes made to the original ORd model to include the mechanical feedback from the MechChem model through the calcium-troponin relation. The original ORd formulation can be found in Supplemental Materials S1 (see https://doi.org/10.6084/m9.figshare.12571949; all supplemental materials may be found at this repository).

Equation 1 describes the change in calcium buffered by troponin with \( CaTn \), defined as the amount of intracellular calcium bound to troponin; \( \text{trp} \text{max} \), defined as the maximum amount of available troponin; \( P \), defined as the fraction of calcium bound to troponin over the total amount of troponin per segment \( i \) \((i = 1 : X)\), with \( i \) defined as the position along the single overlap region (defined as the section of the thick filament outside of the double overlap region that overlaps with the thin filament) starting closest to the midline and ending toward the Z-disk; and \( X \), defined as the end of the single overlap region.

\[
\frac{d\text{CaTn}}{dt} = \text{trp} \text{max} \sum_{i=1}^{X} \left( \frac{dP}{dt} \right) X
\]  

Equation 2 describes the change in calmodulin buffer with CaCMDN being the amount of intracellular calcium bound to calmodulin; \( k_{\text{anc}} \) and \( k_{\text{adc}} \) being the on-rate and off-rate constants describing the binding of calcium to calmodulin, respectively, chosen such that

\[
K_m = \frac{k_{\text{anc}}}{k_{\text{adc}}}
\]

with \( K_m \) being the binding constant defined in the original ORd model and CMDN\text{max}, being the maximum amount of available calmodulin.

\[
\frac{d\text{CaCMDN}}{dt} = k_{\text{anc}} \cdot \left[ \text{Ca}^{2+} \right] \cdot (\text{CMDN}_{\text{max}} - \text{CaCMDN}) - k_{\text{adc}} \cdot \text{CaCMDN}
\]

Finally, Eq. 3 describes the change in \( \left[ \text{Ca}^{2+} \right] \), with \( I_{\text{CaC}} \), defined as the sarcolemmal calcium pump current; \( I_{\text{Cabs}} \), defined as the calcium background current; \( I_{\text{NaCa}} \), defined as the myoplasmic component of \( \text{Na}^+ / \text{Ca}^{2+} \) exchange current; \( A_{\text{cap}} \), defined as the cell capacitive area; \( F \), defined as the Faraday constant; \( \text{vmyo} \), defined as the myoplasmic volume; \( J_{\text{as}} \), defined as total \( \text{Ca}^{2+} \) uptake, via SERCA pump, from myoplasm to nsr; \( \text{vnsr} \), defined as the nsr volume; \( J_{\text{diff}} \), defined as the total diffusion flux; and \( \text{vsub} \), defined as the volume of the subspace compartment, as described in the original ORd (28). The terms \( \frac{d\text{CaTn}}{dt} \) and \( \frac{d\text{CaCMDN}}{dt} \) were added as described above. The factor 3 is included to account for the intrinsic cooperativity coefficient of calcium-troponin buffering from the MechChem model.

\[
\frac{d\left[ \text{Ca}^{2+} \right]}{dt} = -(I_{\text{pCa}} + I_{\text{Ca}} - 2 \cdot I_{\text{NaCa}}) \cdot A_{\text{cap}} \cdot \left( \frac{F}{2} \cdot \text{vmyo} \right) - J_{\text{as}} \cdot \text{vnsr} + J_{\text{diff}} \cdot \frac{\text{vsub}}{\text{vmyo}} - 3 \cdot \frac{d\text{CaTn}}{dt} \cdot \frac{d\text{CaCMDN}}{dt}
\]

Because the original MechChem model was developed on the basis of experimental data from rat ventricular cardiomyocytes and the prescribed CaT from Rice et al. (31), we reoptimized its parameters to reproduce experimental data in the presence of the human ventricular CaT from the ORd model. In particular, we adjusted the original mechanical parameters: \( C_{\text{ad}} \), representing the rate of \( \text{Ca}^{2+} \) binding to troponin; \( C_{\text{a}} \), representing the rate of \( \text{Ca}^{2+} \) unbinding from troponin; and \( K_{\text{pCaTn}} \), representing the equilibrium constant of calcium-troponin buffering in the absence of tension in the thin filament, by minimizing the error between the CaTs of the coupled model and the original ORd under baseline conditions (cycle length of 1,000 ms and SL of 2.1 \( \mu \)m). A one-factor-at-a-time sensitivity analysis was also performed to evaluate the influence of these individual mechanical parameters (\( C_{\text{ad}}, C_{\text{a}}, \text{ and } K_{\text{pCaTn}} \)) have on tension and calcium properties. The methods and results of the MechChem model parameterization and sensitivity analysis have been included in Supplemental Material S1 together with the values of the parameter describing the coupling of the mechanical with electrophysiological model. The code of the final coupled model is also available in Supplemental Material S1.

Validation on experimental data. The behavior of the coupled ORd-MechChem model was validated over a range of experimental data from large mammals and human ventricles. We focused on two experimental protocols (force-frequency and quick-release experiments) that illustrate the bidirectional interactions between intracellular calcium and myofiber force, allowing validation of the coupling of the model. Force-frequency simulations were used to evaluate the peak of generated tension for different stimulation frequencies. They were compared with isometric contractions of ventricular trabeculae at 37°C measured at varying frequencies from Janssen and Periasamy (16) (human data). Quick-release simulations were performed in the coupled model by initiating a sudden drop in afterload with SL held constant upon reaching 92% of its original value. Simulated tension was compared with ferret papillary muscle data, as measured by Kurihara and Komukai (19) and cat papillary muscles by Allen and Kurihara (2). In the model, the reduction of the afterload was set to \( t = 300 \) ms during the repolarization, to account for species differences in AP duration and better match the calcium transient morphology between experiments and simulations. Together, these experiments showed the effect of calcium concentration changes on force and vice-versa. They validated the bidirectionality of the coupled model by illustrating the effect of mechanical alterations, such as stress release, on calcium dynamics, as well as the effect of calcium concentration changes, due to varying stimulation frequency, on force generation. Isotonic contractions were also simulated, evaluating CaT, force, and sarcomere length changes for a range of afterloads and compared with experimental data from Vahl et al. (42) from left ventricular myocardium of patients with dilated cardiomyopathy.

Simulations of \( \beta \)-adrenergic stimulation and SL change. We investigated how acute changes of \( \beta \)-ARS and SL affect force and CaT dynamics. First, the effect of varying degrees of acute \( \beta \)-ARS was implemented by modifying electrophysiological parameters accounting for protein kinase A phosphorylation, as previously described (14, 23, 27). The following changes have been experimentally observed as typical effects of acute \( \beta \)-ARS and were incorporated in the coupled ORd-MechChem model:

- \( \text{L-type } \text{Ca}^{2+} \text{ current (}I_{\text{CaL}}\text{)}\): a three-fold increase in peak \( I_{\text{CaL}} \), a 10–15-mV leftward shift in the activation \( I–V \) relationship, and a 10-mV leftward shift in the inactivation \( I–V \) relationship.
- \( \text{Slow delayed rectifier } K^+ \text{ current (}I_{\text{KS}}\text{)}\): a three-fold increase in current amplitude, leftward shift in tail current amplitude-current voltage curve, increased activation kinetics, and decreased deactivation kinetics.
- \( \text{Plateau } K^+ \text{ current (}I_{\text{KP}}\text{)}\): a 2.5-fold increase in \( I_{\text{KP}} \).
- \( \text{Fast } Na^+ \text{ current (}I_{\text{Na}}\text{)}\): 25% increase in maximal conductance and a 5-mV hyperpolarizing shift of the activation and inactivation of \( I–V \) relationships.
- \( \text{Calcium uptake via the SERCA2a pump (}J_{\text{pap}}\text{): affinity for cytosolic calcium was increased by 50%}\).
- \( \text{Troponin I (}TnI\text{): decreased calcium binding affinity through a 50% increase in }K_m\text{, affecting both electrical and mechanical factors of the bidirectional model}\).
- \( \text{Na}^+/K^+ \text{ ATPase current (}I_{\text{NaK}}\text{): 30% increase in }Na^+ \text{ affinity, resulting in a 17–33\% increase in pump current}\).

Thereafter, changes in cardiomyocyte end-diastolic SL (preload) were modeled with various isometric (i.e., no change in SL) SLS ranging from 1.7 \( \mu \)m to 2.3 \( \mu \)m. Different initial SLSs were simulated to evaluate the individual effect of SL on CaT and force dynamics.
To investigate the effect of stimulation frequency, [Ca\textsuperscript{2+}] \textit{i} and tension were simulated under all different conditions, i.e., 21 combinations of \( \beta \)-ARS and SL, at a baseline cycle length of 1,000 ms (1 Hz) and at a reduced cycle length of 500 ms (2 Hz). Amplitude and duration (measured as 70% of relaxation) of simulated action potentials, CaT, and force were computed at steady state.

RESULTS

Validation of the novel electromechanical model. Both force-frequency and quick-release simulations showed qualitative agreement with the experimental data (Fig. 2). In both the experimental data and the simulations, peak force linearly increased with stimulation frequency from ~40% of peak force at 0.5–1.0 Hz to 100% of peak force at 3 Hz (Fig. 2, A and B). With the quick release protocol, the sudden drop in afterload led to an abrupt drop in tension at 138 ms, which resulted in a surge of intracellular calcium, both in the experiments (Fig. 2C) and the model simulations (Fig. 2D). These validation results establish that the ORd-MechChem model can be used to study the consequences of both electrical and mechanical alterations during exercise. Species differences may contribute to quantitative differences between quick release experiments and simulations and are addressed in the DISCUSSION.

Isotonic contractions with shortening and lengthening of the sarcomere were also simulated to validate the model in these situations. The model was able to reproduce isotonic contractions for various afterloads (Fig. 3B, bottom), showing reduction of the sarcomere length during the twitch (Fig. 3B, middle). This led to changes in the morphology of the CaT (Fig. 3B, top), exhibiting a surge in [Ca\textsuperscript{2+}] \textit{i}. This was in agreement with experimental recordings from patients (Fig. 3A).

Effects of \( \beta \)-ARS and increased SL on calcium and force dynamics. Figure 4 shows the combination of acute \( \beta \)-ARS and increased sarcomere length (2.3 \( \mu \)m) in the simulations, representing conditions that would be expected to occur during exercise (Fig. 4, orange lines), resulting in a 1.5-fold increase in peak [Ca\textsuperscript{2+}] \textit{i} and faster relaxation than at rest (Fig. 4, blue lines). The changes in ionic currents and calcium fluxes with acute \( \beta \)-ARS and increased SL were consistent with the observed alterations described in the METHODS section (14).

\( \beta \)-ARS and increased preload have synergic positive inotropic effects and differential lusitropic effects on calcium and force dynamics. When comparing the individual calcium and tension traces (Fig. 5A), increasing \( \beta \)-ARS led to a large increase in CaT amplitude and a steeper rise of tension at all SLs (Fig. 5A, right). In contrast, for a given \( \beta \)-ARS level, varying SL did not affect the upslope of tension. \( \beta \)-ARS also led to faster relaxation for both CaT and force. An increase of SL led to higher diastolic tension levels (5 mN at SL = 2.1 \( \mu \)m vs. 12.5 mN at SL = 2.3 \( \mu \)m).

Fig. 2. Validation of the electromechanical model based on experimental data. A and B: force-frequency protocol in human experiments redrawn from Janssen and Periasamy (16) (A) compared with simulations using the ORd-MechChem model (B). C and D: quick release experiments from Kurihara and Komukai (19) and Allen and Kurihara (2) (C) compared with simulations (D). Red arrows indicate the “bump” in CaT observed during quick-release experiments.

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We made use of the perfect control offered by computational modeling to vary β-ARS and SL independently, allowing us to differentiate their effects on CaT and force dynamics (Fig. 5, B–E). Increasing β-ARS led to an increased force amplitude (Fig. 5C), reflecting positive inotropy. β-ARS also increased CaT amplitude (Fig. 5B). An increase in β-ARS decreased CaT and force duration, leading to faster relaxation (Fig. 5, D and E). Increasing SL also led to an increased force amplitude (Fig. 5C), but a reduction of CaT amplitude (Fig. 5B) due to increased Ca\(^{2+}\) buffering by troponin. A longer SL resulted in an increased CaT and force duration (Fig. 5, D and E), therefore, slowing down relaxation. SR calcium load was primarily dependent on β-ARS with increased β-ARS leading to increased SR load. SL changes had almost no effect on SR load (Fig. 5F).

β-ARS and SL, therefore, had similar positive inotropic effects, increasing force amplitude, although the influence of β-ARS was smaller than that of SL. β-ARS and SL had different lusitropic effects. In addition, at high β-ARS, CaT and force duration were mostly SL-dependent, and at SL < 1.9 μm, β-ARS had little effect on force duration, indicating a preload-dependent β-ARS response. β-ARS-dependent lusitropic effects enhance calcium and force development at higher frequency. Figure 6 compares the effects of a reduction in cycle length from 1,000 ms to 500 ms for various combinations of β-ARS and SLs. At a higher frequency, amplitudes of both CaT and force increased at all SL and with and without β-ARS (Fig. 6, A and B), and duration decreased at all SL and β-ARS = 1. (Fig. 6, D and E). Decreasing the cycle length from 1,000 ms to 500 ms in the absence of β-ARS at high SL led to a large increase in diastolic calcium levels (from 0.08 to 0.25 M at SL = 2.3 m, Fig. 6C). Increasing the extent of β-ARS reduced the influence of SL on diastolic calcium levels (to 0.07 M at SL = 2.3 μm) and led to faster relaxation (shorter duration of force and CaT) (Fig. 6D). The presence of β-ARS allowed a larger amplitude of force and a faster relaxation at higher frequency.

Gradient in unbinding of Ca\(^{2+}\) from troponin modulates CaT morphology. In the presence of combined high β-ARS and high stretch (SL = 2.3 μm), or at higher frequencies (CL = 500 ms), the descending limb of the CaT became less steep as soon as the tension started decreasing, and a slight nonmonotonic delay (‘bump’) in the CaT decay could be observed (Fig. 5, left and Fig. 3). This bump coincided with the
decrease in tension during relaxation and was observed in the situation in which the highest twitch tension was reached (high β-ARS and high SL). Using the coupled model, we could attribute this phenomenon to the late unbinding of Ca<sup>2+</sup> from troponin in areas of the sarcomere thin filaments with the highest tension. Indeed, our model includes a spatial representation of cross-bridges, as proposed by the MechChem model, with a larger density of cross-bridges in the areas of the single overlap region under high tension, located further away from the midline toward the Z-disk (12). During high β-ARS and high stretch, the areas of the sarcomere thin filaments under high tension relaxed considerably later than the areas under low tension (212 ms at the start of the single overlap region closest to the midline vs. 278 ms at 600 nm to reach 0.09 M of Ca<sup>2+</sup> bound by troponin) (Fig. 7, right) and at a faster rate, which led to a sudden increase in Ca<sup>2+</sup> in the cytosol with the unbinding of Ca<sup>2+</sup> from troponin from these regions. In contrast, at baseline, relaxation along the thin filament was homogeneous, and little delay was observed between regions (Fig. 7, left), which led to a smooth CaT decay without bump.

DISCUSSION

Altered β-ARS and increased preload may occur in dynamic situations such as exercise. In this study, we differentiated the effects of β-ARS and increased preload on CaT and force generation, using a newly developed, bidirectionally coupled electromechanical model of the human ventricular cardiomyocyte. First, we validated the bidirectional behavior of the coupled model with quick-release, force-frequency, and isotonic contraction experimental data from human samples and large mammals. Second, we identified synergistic positive inotropic effects and differential lusitropic effects of β-ARS and stretch on CaT and force dynamics, together enabling the development of more force in less time at higher heart rates. Finally, our simulations showed that heterogeneity in CaT decay during high β-ARS and SL may be related to the mechanically controlled gradient of calcium unbinding from troponin along the thin filament affects. These results may have implications for better understanding of cardiac function, various forms of heart failure, and arrhythmias.

Novel electromechanical model. Several studies have focused on the coupling of electrophysiological and contraction models to investigate feedback relations between calcium and force (5, 8, 15, 24–26, 31–33, 39, 41, 44). Among them, the Ekaterinburg-Oxford model was used to study the effects of myocardial mechanical heterogeneity on calcium handling and action potential properties (33). The Niederer and Smith rat ventricular cardiomyocyte model (26) was used to study the effects of stretch-dependent mechanisms on the slow force response by coupling rat electrophysiology and contraction models. By combining the Rice contraction model (31) with a model of rabbit ventricular cardiomyocyte electrophysiology

Fig. 4. Simulation results of action potential (\(V_m\)), intracellular calcium concentration ([Ca<sup>2+</sup>]i), L-Type calcium current (\(I_{Ca,L}\)), fast Na<sup>+</sup> current (\(I_{Na}\)), slow delayed rectifier potassium current (\(I_{Ks}\)), plateau K<sup>+</sup> current (\(I_{Kp}\)), calcium uptake via SERCA2a (\(J_{up}\)), Na<sup>+</sup>/K<sup>+</sup> ATPase current (\(I_{NaK}\)), [Ca<sup>2+</sup>]i buffered by troponin (CaTn) and tension, in the presence of acute β-ARS and increased SL, as may happen in exercise (orange) and in the control situation (resting condition without β-ARS and resting SL, blue).
and extending it to canine data, Campbell et al. (5) proposed an electromechanical model to investigate transmural coupling variations in canine left ventricular cardiomyocytes. Recently, Zile and Trayanova (44) investigated how force, calcium, and action potential voltage alternans may be related and altered in the context of heart failure. However, none of these models used a physiology- and physics-based contraction model describing the mechanochemical interactions underlying the cooperative behavior of cardiac sarcomeres. The bidirectionally coupled human ventricular cardiomyocyte model presented in

Fig. 5. A: intracellular calcium concentration ([Ca^{2+}]_i) and tension for various combinations of β-ARS and SLs. Calcium transient amplitude (B), force amplitude (C), calcium transient duration (D), force duration (E), and maximum SR Ca^{2+} load (F) for various degrees of β-ARS and varying SL. Colored diamonds correspond to the traces in A.
this article is the first coupling of an electrophysiological ventricular action potential model with the physiology- and physics-based MechChem contraction model. Its novelty resides in the hypotheses underlying the mechanical model, integrating two cooperative mechanisms: the intrinsic cooperativity of calcium bound by troponin and the proposed mechanochemical cooperativity where mechanical tension in the thin filament impedes the unbinding of calcium from troponin (11, 12). This approach enabled a link between mechanical and electrophysiological properties, enabling further mechanistic understanding of the physiological and chemical properties of force generation and how these mechanisms respond and influence calcium handling and electrophysiology.

The bidiirectionality of the electromechanical feedback in our model was verified by replicating experimental protocols with various sets of data from human or large mammals. Recently, a novel human ventricular cardiomyocyte model (based on the ORd model) was developed by Tomek et al. (40) (ToR-ORd). However, the ORd remains the most widely used electrophysiological model currently, and changes made in the ToR-ORd are not expected to affect the conclusions drawn in this work.

Validation based on experimental data. The ORd-MechChem coupled model does not exactly reproduce the CaT generated by the published ORd model (Supplemental Material S1). This was expected, given the different boundary conditions as a result of the electromechanical coupling. However, our ORd-MechChem model qualitatively reproduced the experimental protocols tested. In the force-frequency situation, the amount of force generated showed a linear increase with increased frequency, matching the experimentally measured forces.

Fig. 6. Calcium transient amplitude (A), force amplitude (B), diastolic calcium levels (C), calcium transient duration (D), and force duration (E) for different combinations of sarcomere length (SL) of 1.7, 2.1, and 2.3 μm, and the presence or absence of β-ARS at frequencies of 1 Hz (blue) and 2 Hz (green). F: traces of CaT and tension at 2 Hz and SL = 1.7 μm, in the presence (orange) and absence (blue) of β-ARS.
data from Janssen and Periasamy (16) for human ventricular trabeculae and validating the estimation of the parameters from the mechanical model for human data. In the case of the quick release experiment, the model simulation showed the same qualitative behavior, with the drop of afterload resulting in a surge of intracellular calcium. The shape of the tension curve differs between the model and the experiments, with a slower upslope in the experimental data compared with the simulations, which may be due to a slower CaT upslope in ferret (19) compared with human cardiomyocytes (7). To account for interspecies differences in action potential duration, we simulated the afterload drop at the same stage of repolarization as in the experiments, rather than reproducing the exact timing. Differences in experimental conditions may also play a role and account for the differences observed between the two sets of experimental data. Finally, our model could simulate isotonic contractions, reproducing the morphology of the CaT observed in human experimental data (42). The amount of afterload required to reproduce the experimentally observed isotonic contractions was smaller in the model than in the experimental data (18). This was due to the differences in force measured, observable between the experiments themselves [4 mN (42) vs. 90 mN (18)] and with the model. This can be explained by species differences [human (42) vs. rat (18)], as well as normalization of the reported tension to muscle length of the preparation.

Moreover, differences in the CaT relaxation and tension morphology may be observed between experimental and simulated data, which may be attributed to the absence of clear description of the experimental protocol. The two sets of experimental data reported in Fig. 3 stress the variability present in signal morphologies, probably related to experimental circumstances.

In this article, we study the influence of changes in acute β-ARS, SL, and frequency on CaT and force properties. These changes can reflect variations occurring during exercise. Although left ventricular end-diastolic volume was reported to remain rather constant during exercise (20), SL is likely to increase considerably in the very acute phase (due to an acute increase of cardiac preload). Indeed, significant beat-to-beat changes have been reported in SL (from 1.6 to 1.8 to 1.6 to 1.95 μm) with acute preload changes in rat sarcomeres (as well as acute changes in stimulation frequency) (29). Investigating the effect of SL changes, therefore, allows the simulation of acute beat-to-beat preload changes as may occur in acute exercise. This may also be relevant in other pathologies. Indeed, in dyssynchronous situations such as left bundle branch block, myocardial tissue may exhibit local heterogeneities in tissue properties, including local variations in SL, affecting global pump function (10).

Synergistic and opposing effects of mechanics and electrics and bidirectional interactions. This in silico study shows that β-ARS and SL both have positive inotropic effects, but exhibit different lusitropic effects, with β-ARS enhancing relaxation rate, while increasing SL slowed relaxation. These results, therefore, suggest that electrical effects (acute β-ARS) and mechanical changes (increased preload) may have both synergistic and opposing effects on CaT and force properties. It is the combination of both β-ARS and SL that enables an increase of force development in less time and the maintenance of low diastolic calcium concentration, for example, during exercise when more force needs to be generated at a faster pace. The coupled model also helps untangle the bidirectional interactions between electrics and mechanics in the presence of combined acute β-ARS, increased SL, and increased stimulation frequency, resembling exercise-like conditions. First, the presence of β-ARS increases the initial upslope of the tension trace, leading to a faster contraction, while length-dependent changes have little influence on the speed of the tension response. This strongly suggests that the β-ARS-induced electrical consequences of exercise rather than the stretch-induced mechanical ones promote increased speed of contraction. On the other hand, greater tension is generated at larger SLs, and the decay in force at the beginning of relaxation leads to a bump in the intracellular calcium concentration, explained by the unbinding of calcium from troponin. In this case, exercise-induced increased SL, a purely mechanical alteration, modulates the electrophysiological state via CaT dynamics. Our results also show that SR calcium load is primarily determined

Fig. 7. Amount of Ca\(^{2+}\) bound by troponin over time for 50 distinct points along the thin filament from the start of the single overlap region closest to the midline (distance x from 0 to 600 nm, from the beginning of the single overlap region to the Z-disk), in the situation with no β-ARS and SL = 1.7 μm (A), and high β-ARS and SL = 2.3 μm (B).
by β-ARS, with an increase in β-ARS leading to an increase in SR load, as has been shown in previous experimental studies (9). Our simulations also suggest that SL has little to no influence on SR Ca load. The model can, therefore, highlight the electrical and mechanical interactions and explain how they may have different effects on electrophysiology and tension generation. Finally, our results showed preload-dependent changes in force generation, with a preload-dependent response to β-ARS, as indicated by a minor effect of β-ARS on force generation at low preloads (low SL). This was also mentioned in previous experimental studies (1, 22). Therefore, our results highlight the importance of the preload-dependency of contractility observed at the cellular and whole organ level (Frank-Starling law).

A gradient in cross-bridge detachment at high β-ARS and SL explains the nonmonotonic [Ca\(^{2+}\)]i, decay. At high β-ARS and SL, the CaT showed a nonmonotonic decay, observed as a bump on the CaT traces (Fig. 5, left). This phenomenon was also observed during the simulation of isotonic twitches (Fig. 3). This late slowing in the decay of the CaT signal has previously been reported during isometric twitches of rat ventricular trabeculae (17). In their article, Jiang et al. (17) propose that the observed bump in [Ca\(^{2+}\)]i is due to the release of Ca\(^{2+}\) from troponin C during relaxation that is strongly cooperative with cross-bridge detachment. They also observed an increase in the bump amplitude with increased tension amplitude and faster relaxation rate, which is consistent with our observations. Here, we propose a mechanism to explain this surge in cytosolic Ca\(^{2+}\) during relaxation from contractions that generated high tension. Analyses of the amount of Ca\(^{2+}\) bound by troponin in different regions of the thin filament showed that in the situation of high β-ARS and SL, the regions of high tension (located toward the Z-disk) contain a relatively high density of crossbridges, and relax later and at a faster rate compared with the regions of low tension. The bump observed in [Ca\(^{2+}\)]i, can, therefore, be explained by the unbinding of calcium from troponin in the high tension regions of the sarcomere, leading to a sudden increase in cytosolic Ca\(^{2+}\). Interestingly, this phenomenon has been presented as a possible mechanism for the initiation of Ca\(^{2+}\) waves in nonuniformly contracting hearts that may lead to arrhythmia (38, 43). Therefore, this might be a potential mechanism for arrhythmogenicity triggered by physical exercise.

Importance of β-ARS at higher frequencies and role of β-ARS for diastolic function. In vivo, β-ARS has pronounced positive chronotropic effects. Here, we showed that β-ARS plays a key role in the development of force and the regulation of the CaT at higher stimulation frequencies. The elevation of the diastolic calcium concentrations reached at high SL is reduced by the presence of β-ARS, and this allows a faster relaxation and, hence, a larger range of contraction frequencies that can be reached. β-ARS allows CaT to reach higher amplitudes, while returning earlier to lower diastolic values. In the absence of β-ARS at high frequencies, the presence of stretch leads to high diastolic calcium, reduced force generation, and slower relaxation. We showed that β-ARS speeds up relaxation by reducing the CaT duration and speeding up force generation. With a sole increase in SL, relaxation is much slower, so tension takes a longer time to reach diastolic levels. This suggests that the presence of β-ARS in exercise-like situations is key to ensuring proper diastolic function by speeding up relaxation at the sarcomere level and highlights the potential detrimental effects of tachycardia and increased SL in the absence of β-ARS. We show here that it is the combination of β-ARS and SL, rather than their individual contributions, that is key to ensure generation of more force, while maintaining sufficient time for diastolic filling and low diastolic calcium levels during exercise.

Potential limitations and future directions. The current model proposes a bidirectional coupling between cardiac electrophysiology and mechanics incorporating mechanical feedback through the binding of calcium to troponin, which could be validated against multiple experimental protocols. Mechanical feedback may also be mediated by other mechanisms, such as stretch-activated ion channels (30), which are activated by membrane stretch and produce an ion current that modulates the cardiomyocyte membrane potential. In addition, several of the traditional voltage-gated ion channels included in the electrophysiological model are known to be modulated by changes in cell volume and/or stretch (30). Future work should, therefore, include stretch-activated ion channels, as well as the addition of a spatial modeling component to account for (heterogeneous) calcium diffusion across the cytoplasm (35).

With future developments, this model has the potential to provide mechanistic understanding in various unresolved topics in literature. For example, the extended model can improve our understanding of the contribution of stretch-activated channels to calcium transient and tension during stretch. It can provide insight on the independent contributions of stretch-activated channels and changes to the contractile machinery (buffering to troponin) to calcium transient or tension development and relaxation during stretch, but also how these two mechanisms interact. This adds mechanistic value to the effects of stretch on calcium transient observed experimentally, as reported in Tavi et al. (37). The model may also help in the context of cardiomyopathies, where mutations affect both calcium handling and sarcemeric function, leading to complex interconnected electromechanical effects (21). The model may help reconcile the role of these electromechanical alterations on calcium transient, action potential, and tension generation. Finally, future work is needed to integrate this coupled electromechanical cellular model in a model of cardiac mechanics and whole-system hemodynamics. This work will then have the potential to untangle the effects of electrical and mechanical substrates on arrhythmia and cardiac pump function during exercise at a larger hemodynamic scale, and, thus, investigate their arrhythmogenicity at organ scale.

Conclusion. We identified the contribution of electrical and mechanical alterations to regulation of CaT and force under exercise-like conditions using a novel human electromechanical model, integrating ventricular electrophysiology and sarcemere mechanics. Our results 1) identify different effects of acute β-ARS and changes in SL, including synergistic positive inotropic effects and differential lusitropic effects, 2) suggest that the relaxation-induced surge in cytosolic calcium observed with high tension is due to a gradient in relaxation along the thin filament, with the unbinding of Ca\(^{2+}\) from troponin in areas of high tension occurring later and at a fast rate, and 3) it is the combination of β-ARS and increased preload that enables force amplitude to increase and relax fast enough, despite the reduction of twitch duration.
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