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Increased cell membrane capacitance is the dominant mechanism of stretch-dependent conduction slowing in the rabbit heart: a computational study

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Abstract

Volume loading of the cardiac ventricles is known to slow electrical conduction in the rabbit heart, but the mechanisms remain unclear. Previous experimental and modeling studies have investigated some of these mechanisms, including stretch-activated membrane currents, reduced gap junctional conductance, and altered cell membrane capacitance. In order to quantify the relative contributions of these mechanisms, we combined a monomain model of rabbit ventricular electrophysiology with a hyperelastic model of passive ventricular mechanics. First, a simplified geometric model with prescribed homogeneous deformation was used to fit model parameters and characterize individual MEF mechanisms, and showed good qualitative agreement with experimentally measured strain-CV relations. A 3D model of the rabbit left and right ventricles was then compared with experimental measurements from optical electrical mapping studies in the isolated rabbit heart. The model was inflated to an end-diastolic pressure of 30 mmHg, resulting in epicardial strains comparable to those measured in the anterior left ventricular free wall. While the effects of stretch activated channels did alter epicardial conduction velocity, an increase in cellular capacitance was required to explain previously reported experimental results. The new results suggest that for large strains, various mechanisms can combine and produce a biphasic relationship between strain and conduction velocity. However, at the moderate strains generated by high end-diastolic pressure, a stretch-induced increase in myocyte membrane capacitance is the dominant driver of conduction slowing during ventricular volume loading.

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Conflicts of interest

Bernardo L. de Oliveira, Emily R. Pfeiffer, Joakim Sundnes, Samuel T. Wall, and Andrew D. McCulloch declare that they have no conflicts of interest.

Ethical standards

No human studies or animal studies were carried out by the authors for this article. Studies were conducted on murine myocytes, which were isolated and cultured according to institutional, national, and international guidelines, and approved by the UCSD Animal Subjects Committee.

Key terms

Mechano-electric feedback; stretch-activated currents; cell membrane; bidomain model; tissue conductivity; multiscale model; pressure loaded heart

1 Introduction

Clinical conditions associated with cardiac arrhythmic risk are often linked with altered mechanical loading of the myocardium [30]. It is well known that mechanical factors may be arrhythmogenic through processes collectively referred to as mechano-electric feedback (MEF). However, the role of MEF in arrhythmogenesis is not fully understood, and may include factors such as altered action potential morphology [3], triggered depolarizations [6], and altered electrical conduction velocity (CV) [14].

Mechanical strains are known to influence CV, although the underlying biophysical mechanisms remain unknown. Several experimental and computational studies have investigated this phenomenon [14], though different studies have reported increasing [31, 21], decreasing [29, 10], constant [25, 36] or biphasic [22, 14] relationships between myocardial strain and CV. While this variety of observations can be explained by variations in species, tissue type, preparation and loading conditions, a consensus of studies in the isolated rabbit heart loaded at moderately high diastolic filling pressures show significant and reproducible conduction slowing by about 20% compared with unloaded preparations [15, 29]. A similar CV reduction was recently seen in isolated mouse hearts and cultured myocytes [18].

Cardiac CV is affected by distinct electrophysiological properties of the myocardium including the rate of membrane depolarization due to fast inward sodium current during phase zero of the action potential, intra- and intercellular electrical resistance, and cell membrane capacitance. The MEF mechanism most commonly studied is stretch activated channels (SACs), in particular stretch activated cation non-selective channels (nsSACs), which partially depolarize the membrane at rest [6]. This tends to increase cell excitability and hence CV, but it also inactivates fast sodium channels thereby reducing upstroke velocity and CV. The combination of these interactions may result in a biphasic response to stretch. However, experiments presented in [15] showed no effect of Gd^{3+} , a known blocker of nsSACs, on the observed CV reduction.

Electron microscopy studies have shown that ventricular wall stretch causes unfolding of membrane pleats and incorporation of subsarcolemmal caveolae into the myocyte membrane [9, 18]. This response may incorporate lipid to the membrane to protect against high membrane tension [23]. A recent study showed that modest acute stretch significantly increases myocyte membrane capacitance by over 50% and that this response was prevented with genetic or biochemical ablation of caveolae [18]. This study used physiological stretches and found that these alteration take place and are reversed within 1–5 minutes, but shorter time scales have not been investigated. These changes are therefore acute responses to stretch but are not necessarily occurring on the time-scale of a single cardiac beat. Increased capacitance and membrane surface to volume ratio both increase the charge

required to depolarize the membrane, thus reducing upstroke velocity and consequently slowing conduction. There are other potential mechanisms by which tissue resistivity and capacitance could be affected by stretch. For instance, acute physiological volume loading experiments in isolated rabbit hearts [15] caused a 20% decrease in conduction velocity. This change coincided with an increased time constant for decay of a sub-threshold stimulus corresponding to a 56% increase in effective membrane capacitance. However, this same experiment also reported a 20% increase in the electrical space constant, defined as the characteristic length of decay of passive conduction, see e.g. [20]. An increased space constant is expected to increase conduction velocity, contrary to the observation in [15]. The space constant is determined by intracellular and extracellular resistances and cell geometry. Based on measurements of wall strain and the effects of a gap junction uncoupler in their preparation, these authors concluded that the increase in electrical space constant in the loaded ventricular was probably due to a decrease in gap junctional resistance.

The objective of this study is to quantify the contributions of different MEF mechanisms on CV, and to compare their significance. We apply a computational model of a rabbit heart subjected to passive ventricular loading that includes multiple MEF mechanisms, and compare the results with experimental results from the literature. Using the model, we are able to support previous conclusions on the dominant mechanisms for CV reduction during loading, and present a more detailed comparison of individual MEF mechanisms than what is possible in experimental studies.

2 Methods

2.1 Model equations

The effect of strain on CV was studied with a mathematical model of cardiac electrophysiology and mechanics. The model included several MEF mechanisms, but neglected forward coupling from action potential to mechanical contraction. The latter simplification is justified by the fast time scale of heart electrophysiology and the considerable delay between electrical activation and contraction. Because of this delay, the electrical activation is normally completed before onset of contraction, and the CV is therefore not affected by contraction. For these same reasons, most studies on the effects of ventricular MEF mechanisms on conduction velocity have been performed in electromechanically decoupled experimental preparations [29].

With this simplification, our modeling approach is reduced to solving a passive mechanics problem for a specified load, and inserting the resulting strain fields into a detailed model of cardiac electrophysiology and MEF. Established models based on partial and ordinary differential equations (PDEs and ODEs) are employed for electrophysiology and mechanics.

To describe the mechanics of the heart we employ the standard equilibrium equation for a hyperelastic material,

$$\nabla \cdot (\mathbf{FS}) = 0, \quad (1)$$

$$\mathbf{S} = \frac{\partial \Psi}{\partial \mathbf{E}}. \quad (2)$$

Here $\mathbf{F}$ is the deformation gradient and $\mathbf{S}$ is the second Piola Kirchhoff stress tensor. This stress tensor is defined as the first derivative of the strain energy function Ψ with respect to the components of $\mathbf{E}$, the Green-Lagrange strain tensor. In this study, we have employed the transversely isotropic version of the exponential strain energy function from Usyk et al. [32]:

$$\Psi = \frac{1}{2}C(e^W - 1) + C_{compr}(J \ln J - J + 1) \quad (3)$$

$$W = b_{ff}E_{ff}^2 + b_{xx}(E_{ss}^2 + E_{nn}^2 + E_{sn}^2 + E_{ns}^2) + b_{fx}(E_{fn}^2 + E_{nf}^2 + E_{fs}^2 + E_{sf}^2) \quad (4)$$

Here C , C_{compr} , b_{ff} , b_{xx} and b_{fx} are material parameters. J is the determinant of $\mathbf{F}$, and E_{ij} are components of the Green-Lagrange strain tensor $\mathbf{E}$. The subscripts f , s and n correspond to the fiber, sheet and sheet-normal directions respectively. The material is modeled as almost incompressible, and the parameter C_{compr} is used to control the volume changes. Eq (1) was discretized with a standard Galerkin finite element method, and the resulting nonlinear equations were solved with Newton's method, see [28] for details.

The electrical activity of the heart was modeled using the monodomain model on the reference undeformed configuration:

$$\chi \left(C_m \frac{\partial v}{\partial t} + I_{ion}(v, s, \lambda) \right) = \nabla \cdot (\sigma \nabla v), \quad (5)$$

$$\frac{\partial \mathbf{s}}{\partial t} = f(v, \mathbf{s}, \lambda), \quad (6)$$

where χ is the cell membrane surface-to-volume ratio, v is the transmembrane voltage, σ is the conductivity tensor, and C_m is the specific membrane capacitance. The components of the monodomain conductivity σ are computed as harmonic means of the intracellular and extracellular conductivities, see e.g. [17]. I_{ion} is the transmembrane current density, which depends on the transmembrane voltage v , the fibre extension ratio λ and on a vector of cellular state variables s . The fiber stretch λ comes from the solution of the mechanics

problem, $\lambda = \sqrt{2E_{ff} + 1}$, while the vector of state variables is governed by a system of non-linear ODEs. In this work we have used the rabbit ventricular model by Mahajan et al. [13] to model membrane ionic currents.

Eqs. 5–6 were solved with a Godunov operator splitting scheme, a Galerkin finite element method, and a combination of backward Euler and a singly diagonally implicit Runge-Kutta method for time discretization. See [27] for details on the solution procedure.

2.2 MEF Mechanisms

Different MEF mechanisms have been formulated and incorporated into the cell model used. These mechanisms use the stretch ratio in the fiber direction, λ , as input and allow the mechanical deformation to affect the electrophysiology.

The total SAC current, I_{SAC} , was assumed to be zero when the myofiber is shortened ($\lambda < 1$) and to vary linearly when the cell is stretched ($\lambda > 1$) [8]. Moreover, two different types of SACs were incorporated into the model; non-selective SAC (nsSAC) represented by the current I_{ns} and potassium specific SAC (KSAC) represented by the current I_{K0} . We have

$$I_{SAC} = \begin{cases} (I_{ns} + I_{K0})(\lambda - 1), & \text{if } \lambda > 1, \\ 0, & \text{if } \lambda \leq 1. \end{cases} \quad (7)$$

The K^+ SAC current was implemented as an outward rectifying K^+ current, adopting the formulation proposed in [7],

$$I_{K0} = \frac{g_{K0}}{1 + \exp\left(\frac{(19.05 - v)}{29.98}\right)}, \quad (8)$$

where g_{K0} is the channel conductance.

The non-specific current I_{ns} was implemented as a linear, non-inactivating current,

$$I_{ns} = g_{ns}(v - E_{ns}), \quad (9)$$

where g_{ns} is the channel conductance. E_{ns} is the nsSAC reverse potential and was considered to be constant and equal to -10mV . Both g_{K0} and g_{ns} were varied between 0 and $100\text{S}F^{-1}$, which corresponds to the range of published values for these currents [8].

Both the cell membrane capacitance and the tissue space constants have been suggested to increase with tissue strain, which may substantially affect the CV. Experimental data on these effects are fairly sparse, but experiments on isolated mouse myocytes indicate up to 98% increase in capacitance for biaxial stretch of 14% in the longitudinal direction and 3.6% transverse to the long axis of the myocytes [18]. Furthermore, experiments on isolated rabbit hearts [15] suggest increases of 56% in capacitance and up to 20% in space constants when ventricular filling pressure was increased from zero to 30 mm Hg. To incorporate these effects into the monodomain simulations, we propose a new set of models for how the capacitance and tissue space constant vary with strain.

Given the sparsity of experimental data, and limited understanding of the underlying biophysical mechanisms, a natural approach would be to assume a linear relationship between strain and the relevant electrophysiological parameters. However, while such a simple model would be able to fit the experimental data in, e.g., [15], it would generate non-physiological values for larger strains. Furthermore, as noted above, there are studies that report a biphasic relation between strain and CV, where the CV will first increase for small

strains and then decrease, see, e.g., [14, 22]. Based on these observations, we chose to use Hill curves to describe the increase in capacitance and space constants. This choice allowed us to avoid non-physiological values by presenting asymptotic behavior for large strains, and would also allow us to reproduce the biphasic behavior by individually tuning the inflection points of the two curves. We set

$$C_m = C_{m0} + H_C(\lambda) \quad (10)$$

$$\Lambda = \Lambda_0 + H_\Lambda(\lambda), \quad (11)$$

where C_{m0} , Λ_0 are the baseline values of the capacitance and space constant, and $H_C(\lambda)$, $H_\Lambda(\lambda)$ are scaled Hill curves given by

$$H_i(\lambda) = \Delta_i \frac{(\lambda-1)^{n_i}}{K_i^{n_i} + (\lambda-1)^{n_i}}, \quad \text{if } \lambda > 1, \\ H_i(\lambda) = 0, \quad \text{if } \lambda \leq 1, \quad (12)$$

for $i = C, \Lambda$. We chose the model parameters as $C = 1$, $n_C = 6$, $K_C = 0.04$, and $\Lambda = 0.4$, $n_\Lambda = 1$, $K_\Lambda = 0.02$, which matched the values measured at 4% strain in [15]. The resulting curves are shown in Figure 1.

The space constant is not explicitly represented in our monodomain model, but can be calculated from the tissue conductivities or resistances. In one space dimension, the relation is

$$\Lambda = \sqrt{\frac{R_m}{(1/\sigma_i + 1/\sigma_e)\chi}} = \sqrt{\frac{R_m\sigma}{\chi}} \quad (13)$$

where R_m is the membrane resistance, σ_i is the intracellular conductivity, σ_e is the extracellular conductivity, and χ is the surface to volume ratio. We have defined the components of the monodomain conductivity σ as harmonic means of σ_i and σ_e , which gives the second equality in (13). As explained above, the space constant changes observed in [15] were assumed to be related to altered gap junctional resistance. However, motivated by the simple relation between Λ and the monodomain conductivity σ , given by (13), we incorporated the space constant changes by scaling σ in (5). These and other model choices are discussed in more detail below.

2.3 Simulation experiments

Two different experimental setups were modeled. Using a cuboid geometry, we investigated how MEF mechanisms affect the CV when strains are homogeneous. The second model used a realistic rabbit ventricular geometry and investigated how CV is affected by the heterogeneous strain fields generated by passive inflation of the left ventricle.

The first test case was inspired by [16], where a number of electrophysiology solvers were tested, and results compared in terms of CV. We adopt the same geometry and stimulus

conditions. The geometry is a rectangular box of dimensions $3 \times 7 \times 20 \text{ mm}^3$, with parallel fibers aligned in the long, 20mm, axis. A regular tetrahedral mesh with node spacing of 0.125mm was used. The longitudinal conductivity was set to 1.2 mS cm^{-1} , the transversal conductivity was 0.45 mS cm^{-1} and the surface to volume ratio was 1400 cm^{-1} . The stimulus was applied in a $1.5 \times 1.5 \times 1.5 \text{ mm}^3$ cube in one corner. CV was calculated from the difference in the activation times between the stimulus corner and the corner opposite to the stimulus. Suitable initial conditions were established by running single cell simulations with the corresponding λ and MEF parameters, with a cycle length of 360ms. These single cell simulations used the models default initial conditions as described in its original publication [13]. After reaching a steady state, the resting values from these single cell simulations were used as initial condition for the cuboid simulations. We varied λ between 1.0 and 1.25, and when SACs were present the conductances were set to 100 SF^{-1} , which corresponds to the largest published value for these types of currents[8]. Also, some of the simulations incorporated both of the proposed models for stretch-dependent capacitance and space constants.

In the second test case, a rabbit biventricular geometry was modeled to study how MEF affects CV with the heterogeneous strains caused by left ventricular inflation. This test case was designed to mimic the *ex vivo* experiments reported by Mills et al. [15] who used optical mapping to measure epicardial activation patterns in unloaded and loaded conditions. The left ventricles from sample hearts were inflated with pressures from 0 to 30 mmHg (4.0 kPa). Furthermore, in some experiments Gd^{3+} , a known blocker of the non-specific SACs, was added to the perfusate.

To replicate this setup, we developed a finite element mesh of the rabbit left ventricle based on the measurements of Vetter and McCulloch [33]. The surfaces from the original geometry were extracted and a tetrahedral mesh with quadratic basis functions and average node spacing of 0.3mm was generated for the domain. A fiber field was generated using the method described by Bayer et al. [1], which produced smooth fiber fields in good agreement with measurements from [33]. The left ventricle was inflated from 0 to 30 mmHg (4.0 kPa) over the time course of 100 ms, while the pressure on the right ventricle and epicardium was set to zero. Also, to avoid rigid body movements, a few nodes on the basal region of the ventricular septum were prescribed with a zero displacement boundary condition. To set the parameters in the material law (3)-(4), we started from a typical parameter set in the literature, and tuned the parameters to match the cavity volume reported in [15]. The strains on the anterior LV epicardium were then compared with the experimental values. The parameter set $C = 13 \text{ kPa}$, $C_{\text{compr}} = 50 \text{ kPa}$, $b_{ff} = 12$, $b_{xx} = 6$, and $b_{fx} = 6$ gave a good match with the observed strains, and are consistent with previous results in the literature.

During the inflation of the ventricle, we continuously updated λ and solved Eqs. 5–6, to allow the electrophysiology model to reach equilibrium before the heart was stimulated on the LV free wall approximately 5 mm from the apex. Several simulations were performed, with a wide range of values for the SACs conductances, from 0 to 100 SF^{-1} , to investigate the potential impact of these channels on CV. Stretch-dependent space constants given by (11) were included in all simulations, while the impact of membrane capacitance was studied by performing simulations both with constant capacitance and with the capacitance

given by (10). While most of the simulations applied an LV pressure of 30 mmHg, to match the experimental results in [15], we also investigated the strain-CV relation by varying the target LV pressure from 0 to 60 mmHg. Apparent epicardial CVs were calculated from the reciprocal gradient of the activation time field [2]. This field also represents the directions of fastest and slowest conduction.

2.4 Validation experiments

While most of the results we use for parameter fitting and model validation are taken from the literature, and in particular from [15], we also performed a qualitative model validation against new experimental data. Through stretch and optical mapping of micro-patterned neonatal murine ventricular myocyte cultures, we obtained a set of strain-CV relations to which our model results could be compared.

Polydimethylsiloxane (PDMS) tissue culture substrates (Sylgard 186 silicone elastomer, Dow Corning Corp., Midland, MI) were molded from silicon wafers, as described previously [4, 5] to achieve microgrooves 10 μm wide, 10 μm apart and 5 μm deep. Murine laminin (Sigma-Aldrich, St. Louis, MO) was adsorbed onto the micro-patterned membranes, which were mounted onto custom homogeneous biaxial stretching devices described previously [4, 11, 35]. The micropatterned substrates were mounted into the elliptical stretch devices so that the axis of maximum stretch was parallel to the longitudinal axis of the micropatterning.

Neonatal murine ventricular myocytes (1NMVMs) were isolated from P1-P2 CD-1 mouse pups (Charles River Labs) using protocols approved by the UCSD Animal Subjects Committee [26]. Hearts were excised and enzymatically digested. Fibroblasts were removed from the cell suspension using a 90-minute pre-plating incubation and discarded. Cardiomyocytes were plated in the stretch device culture chambers in 15% serum plating media, and maintained at 37°C and 5% CO₂ for 2–4 days with media changes every 1–3 days. Cultures were maintained in standard 6% serum media until 24 hours prior to optical mapping experiments, when the media was changed to antibiotic-free media.

Stretch devices were mounted into an optical assembly for voltage mapping experiments [35]. Changes in cell membrane potential were detected using voltage-sensitive transmembrane fluorescent probe di-8-ANEPPS (Life Technologies, Grand Island, NY). 470-nm wavelength excitation light from an LED source was directed to the cell culture with a dichroic mirror, and emitted light passed through a 610 nm long-pass filter and was detected at 500 frames per second by a CMOS camera (MiCAM Ultima-L, SciMedia, Costa Mesa, CA). Depolarization was assessed according to time of maximum rate of fluorescence change at each pixel. The velocity of propagation of 5–6 consecutive, stimulated, and smoothly propagating action potentials per condition was measured along the longitudinal and transverse axes of the cell culture.

The imaging chamber was maintained at 37°C and cells were electrically stimulated at 300 ms intervals for the duration of the experiment. Following recordings in the unstretched state, stretch was applied to a maximum biaxial strain of 14% parallel to the myocyte longitudinal axis and 3.6% perpendicular and maintained for 5 minutes prior to imaging in

each culture. Conduction velocity in the fiber direction was measured in the stretched state and normalized to the unstretched control in each culture.

3 Results

3.1 Cuboid Geometry

Several simulations were performed for this test case, using all possible combinations of MEF mechanisms implemented. All simulations used homogeneous strain fields consisting of stretch in the fiber direction. As this simple mechanical problem has an analytical solution, no numerical simulations were necessary for the mechanics, and MEF could be simulated by simply inserting constant values for λ into the models.

Figures 2 and 3 show the normalized CVs obtained with these simulations superimposed with the experimental data from the stretch and optical mapping experiments described above. Several different combinations of MEF mechanisms were tested, and presented a variety of different strain-CV relationships.

In simulations only including the nsSACs, we see a biphasic relationship, which is related to the increase in resting potential caused by the stretch. A slightly depolarized resting membrane increases cell excitability and CV, while larger increases tend to inactivate sodium channels and lead to decreased upstroke velocity and thereby CV. Even though a biphasic relationship is observed, reduced CV only occurs for very large strain values. In the simulations including only KSAC, a moderate decrease in CV is observed as a consequence of decreased resting potentials. Combining nsSACs and KSACs, a monotonic increase is observed on the CV. The elevation in resting potential induced by nsSAC is stronger than the reduction induced by KSAC. It is interesting to note that the two SACs counterbalance each other and the resting potential does not reach an elevation high enough to affect the sodium dynamics.

In the simulations where we include the stretch-dependent capacitance and space constants, we observe an increase in the CV for small strains followed by a steep slowdown for moderate strains. The same behaviour is observed in all the curves even with the inclusion of the SACs, indicating that the capacitance and space constants have a dominant impact on CV compared to SACs. We see that the inclusion of the strain-dependent capacitance and conductivity is needed to obtain a qualitative match with the experimental data. While figures 2 and 3 only show a selection of the more interesting curves, we have performed numerous experiments with either stretch-dependent capacitance or stretch-dependent conductivity. While certain combinations of SACs would give a biphasic strain-CV relation for large strains, as illustrated by the solid curve in Figure 2, no reasonable parameter choices would come close to reproduce the biphasic strain-CV relationship for small to moderate strains, as seen in the experiments. These results indicate that the mechanisms underlying observed CV changes are a combination of space constant and capacitance changes.

3.2 Rabbit Ventricular Geometry

Initially a control case was simulated by stimulating and calculating the CV without inflation. A CV value of 33.1 cm s^{-1} was obtained. This value is in agreement with the experimental values of $36.0 \pm 3.3\text{ cm s}^{-1}$ [15]. All the following simulations used the same strain field obtained by inflating the LV to 30mmHg (4.0 kPa). Figure 4 shows the strain distributions on the LV free wall of our model after inflation. The obtained results are in good agreement with the reported values of average 4% strain in fiber direction and 3% strain in cross-fiber direction [15, 33].

Figure 5 shows the normalized CVs obtained with three representative sets of simulations, for LV pressure set to 30 mmHg. Including only SACs has a very limited effect on CV, even for exaggerated conductance values up to 100 SF^{-1} . All changes in CV resulting from SACs were less than 3%. In the simulation that includes the stretch-dependent space constant, but a constant capacitance and no SACs, i.e. SAC conductances equal to zero, we observe an increase in the CV of 25%. A similar increase (23%) is observed in simulations with homogeneous space constants that are scaled with the experimental values measured in [15].

In the simulation including stretch-dependent capacitance and space constant, but with zero SAC conductance, we observe a slowdown of 12.1% with respect to the control case. This is in agreement with the experiments that reported $12 \pm 3\%$ slowdown after inflation [15]. Including SACs shows the similar behavior as for the cuboid test case. Increasing nsSAC conductance causes a CV increase, and increasing KSAC conductance gives a decrease, but these effects are very small compared with the effects of the space constants and capacitance. The only simulations that presented CV reductions comparable to the experimentally measured values, were the ones that included the model of increased capacitance. This again indicates that changes in the space constants and mainly in the capacitance have a dominant effect on the CV.

Figure 6 shows the normalized CV as a function of LV filling pressure, combining the models for SDC and SDSC. The SAC models were not included in this simulation because of their very small impact on CV shown in Figure 5. We observe that the pressure-CV relation is very similar to the stretch-CV relation observed for the cuboid geometry, with a marked increase in CV for lower loading values followed by a steep decrease towards an asymptotic value.

4 Discussion

We used computational models of ventricular mechanoelectric feedback to quantify the relative contribution of multiple reported mechanisms by which myocardial strain can alter ventricular conduction velocity. Using a simple cuboid geometry it was possible to study how homogeneous strains affect CV, and using a realistic rabbit geometry, the effect of heterogeneous strain fields on CV was studied.

In the cuboid test case, different relations between strain and CV were observed, including biphasic, increasing and decreasing. The two different formulations of stretch-activated currents did affect CV, but the changes were small for low to moderate strain, and a reduced

CV was only seen for large strain values. Hence, although the simulations including nsSACs presented a biphasic stretch-CV relationship, the large strain required to reduce the CV was not consistent with the presented experimental data.

The curves including the proposed stretch-dependent capacitance and space constant models showed a more accentuated effect on the CV. The inclusion of SACs in addition to these models showed little effect for small strains, and only contributed significantly to the CV for very large strains, where the effect of membrane depolarization and consequent sodium channel inactivation becomes significant. Although not quantitatively accurate, the results with the stretch-dependent capacitance and space constants showed a good qualitative agreement with experimental results from murine ventricular myocyte cultures, presenting a considerable speed up for small strains followed by a steep slowdown in the moderate strain region. The quantitative mismatch between our results and the experimental data may be due to a number of sources. Most significantly, all model parameters were fitted to reproduce the experimental results from [15], and were not adjusted to match the data shown in Figure 3. Since these experiments are done using different species, and very different experimental preparations, a close quantitative match should not be expected.

Simulations on a realistic volume-loaded isolated rabbit ventricular geometry showed largely the same findings as for the simplified cuboid case. The parameters of the monodomain model, mechanical model, and proposed space constant and capacitance model were adjusted to match the control case as well as the measured space constant and capacitance changes in [15]. No parameters were adjusted to fit the observed CV changes, but the model still provided an excellent match with the experimental values. Including only the space constant changes resulted in a 25% increase in CV, while the inclusion of SACs showed a very weak effect on CV. Several simulations were performed, using multiple combinations of SACs and parameters that spanned a wide range of experimentally measured conductance values. Both the non-specific and potassium SACs had a minor effect on CV, and no simulations showed variations in the CV larger than 3%. This indicated that the CV has a low sensitivity to SACs in this test case, with the heterogeneous and moderately large strain fields obtained by ventricular loading. While our results indicate that SACs have a minor effect on CV, previous studies have shown the significance of SACs in regulating action potential morphology, see e.g. [12, 19], and model results in [34] indicate that the presence of SACs tends to reduce APD dispersion in healthy myocardium.

In this experiment, the only way we could obtain a significant reduction in CV was to include the stretch-dependent capacitance. In the simulations including this model, a considerable CV reduction was observed, even in the presence of increased space constants. The simulations including stretch-dependence both in capacitance and space constants showed good agreement with experimental data in [15]. This result supports the conclusion from [15], that the main cause of conduction slowing due to ventricular volume loading is a change in capacitance and not SACs. Furthermore, the good match with experimental data provides some support for our models of the stretch-dependent capacitance and space constants. Finally, although there is no experimental data to support this result, the relation between CV and LV loading pressure shows a similar biphasic behavior as for the simple cuboid model. Since the same MEF mechanisms are in place, the similarity of the curves is

not unexpected. However, the closely matching curves indicate that a biphasic strain-CV behavior is present also in the moderately loaded intact heart, which is a result of potential importance. More experimental data is needed to validate the computational model and provide further support for this result.

There are several limitations to our models, most of which are related to the sparsity of experimental data to choose or constrain parameters. The most significant ones are the uncertainties in the functions for stretch-dependent capacitance and space constants, as these are seen to substantially affect CV. Our choice of Hill equations for these functions was not based on the underlying biophysics, but was motivated by previous experimental results. The need to reproduce the measured values in [15] for moderate strains, without reaching non-physiological values for large strains, motivated functions with an asymptotic behavior for large strains. Also, unfolding of membrane pleats and consequently increased membrane area has been proposed as the main mechanism for capacitance increase. This increase in area should have a physical limit, also seen experimentally [24], which justifies the asymptotic behavior for the capacitance model. Furthermore, following our own results that capacitance and space constants are the key factors in CV changes, the biphasic small strain CV behavior seen in the literature motivated the use of functions with different inflection points, as seen in Figure 1. In fact, this choice was the only way we could obtain a qualitative match with experimental data, without including unknown additional effects in the model, using non-monotonic functions for capacitance or space constants, or choosing the SAC parameters far outside the realistic range. However, experimental data measuring these phenomena are still sparse, and no experimental evidence exists to support the exact shape, functions and parameterization of the models adopted here. The function shapes adopted here could therefore be interpreted as a motivation and a reasonable starting point for experimental studies into how capacitance and space constants are altered with strains. The goal of such studies would be to develop more precise and validated functional relationships, as well as to uncover the underlying biophysical mechanisms.

In our model equations, all MEF mechanisms are assumed to be instantaneous functions of stretch, ignoring a potential time delay. This assumption may be a good approximation for SACs, but the proposed mechanisms of capacitance and space constant changes are likely to work on slightly longer time scales. In particular, we suggested above that membrane unfolding may be responsible for increased capacitance, which probably has a non-negligible time delay. Results in [18] indicate that the time scale of capacitance changes is fairly short, at least not longer than 1–5 minutes, but no experimental data exists to characterize the changes on shorter scales. In light of these limitations, our model results are to be interpreted as steady state results, recorded after any transient period of stretch dependent CV changes, approximately 1–5 minutes after onset of stretch. With further experimental data available to characterize the initial response to stretch, refined computational models will be valuable tools for further investigation of the temporal dynamics of these processes.

It is also important to note that, similar to other MEF mechanisms, the variations of capacitance and space constants with strains may be species-dependent. Although our models are primarily fitted to rabbit data, they contain a number of default parameters and

formulations that are based on other species, and part of the validation is based on experimental data from mice. Once again, more experimental data would be necessary to assess if the mechanisms we include are also present in other species, and to assess the potential species-dependence of the relative significance of different MEF mechanisms.

5 Conclusions

The presented model studies support previous conclusions that while several mechanisms may affect CV under stretch, the dominating factors are increased tissue space constants and increased cell membrane capacitance. Furthermore, we have proposed novel models for how capacitance and space constants may vary with stretch, that are consistent with experimental data but have limited biophysical justification. Using different model parameters and strain values, we are able to reproduce multiple stretch-CV relations reported in the literature, including increasing, decreasing, biphasic and approximately constant CV. Given the differences in species and experimental protocols used to obtain these previously reported results, the large variety in observations are compatible with our findings. Furthermore, although the effect of SACs was present and visible in most of the experiments, no justifiable choice of SAC model parameters was sufficient to reproduce the conduction slow-down observed in loaded rabbit ventricles in [15], or the biphasic relation observed in our own experiments on murine myocyte cultures. However, a simple model of stretch-dependent capacitance and space constants, with parameters consistent with previous experiments, was able to quantitatively reproduce the rabbit results and give a good qualitative match with the biphasic CV observed in the cell cultures.

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References

1. Bayer J, Blake R, Plank G, Trayanova N. A novel rule-based algorithm for assigning myocardial fiber orientation to computational heart models. *Annals of Biomedical Engineering*. 2010; 40:2243–54. [PubMed: 22648575]
2. Bayly PV, KenKnight BH, Rogers JM, Hillsley RE, Ideker RE, Smith WM. Estimation of conduction velocity vector fields from epicardial mapping data. *IEEE Transactions on Biomedical Engineering*. 1998; 45:563–571. [PubMed: 9581054]
3. Belus A, White E. Streptomycin and intracellular calcium modulate the response of single guinea-pig ventricular myocytes to axial stretch. *The Journal of Physiology*. 2003; 546:501–509. [PubMed: 12527736]
4. Camelliti P, Gallagher JO, Kohl P, McCulloch AD. Micropatterned cell cultures on elastic membranes as an in vitro model of myocardium. *Nat Protoc*. 2006; 1(3):1379–1391. [PubMed: 17406425]
5. Camelliti P, McCulloch AD, Kohl P. Microstructured cocultures of cardiac myocytes and fibroblasts: a two-dimensional in vitro model of cardiac tissue. *Microsc Microanal*. 2005; 11(3):249–259. [PubMed: 16060978]
6. Franz MR. Mechano-electrical feedback in ventricular myocardium. *Cardiovascular Research*. 1996; 32:15–24. [PubMed: 8776399]

7. Healy SN, McCulloch AD. An ionic model of stretch-activated and stretch-modulated currents in rabbit ventricular myocytes. *Europace*. 2005; 7:S128–S134.
8. Hu H, Sachs F. Stretch-activated ion channels in the heart. *Journal of Molecular and Cellular Cardiology*. 1997; 29:1511–1523. [PubMed: 9220338]
9. Kohl P, Cooper PJ, Holloway H. Effects of acute ventricular volume manipulation on in situ cardiomyocyte cell membrane configuration. *Prog Biophys Mol Biol*. 2003; 82:221–227. [PubMed: 12732281]
10. Kuijpers N, Eikelder HT, Bovendeerd P, Verheule S, Hilbers TAP. Mechano-electric feedback leads to conduction slowing and block in acutely dilated atria: a modeling study of cardiac electromechanics. *Am J Physiol Heart Circ Physiol*. 2007; 292(6):H2832–H2853. [PubMed: 17277026]
11. Lee AA, Delhaas T, Waldman L, MacKenna DA, Villarreal FJ, McCulloch AD. An equibiaxial strain system for cultured cells. *American Journal of Physiology*. 1996; 271(4):1400–1408.
12. Li W, Gurev V, McCulloch AD, Trayanova NA. The role of mechanoelectric feedback in vulnerability to electric shock. *Progress in Biophysics and Molecular Biology*. 2008; 97(2–3):461–78. [PubMed: 18374394]
13. Mahajan A, Shiferaw Y, Sato D, Baher A, Olcese R, Xie LH, Yang MJ, Chen PS, Restrepo JG, Karma A, Garfinkel A, Qu Z, Weiss JN. A rabbit ventricular action potential model replicating cardiac dynamics at rapid heart rates. *Biophysical Journal*. 2008; 94:392–410. [PubMed: 18160660]
14. McNary TG, Sohn K, Taccardi B, Sachse FB. Experimental and computational studies of strain-conduction velocity relationships in cardiac tissue. *Progress in Biophysics and Molecular Biology*. 2008; 97:383–400. [PubMed: 18406453]
15. Mills RW, Narayan SM, McCulloch AD. Mechanisms of conduction slowing during myocardial stretch by ventricular volume loading in the rabbit. *Am J Physiol Heart Circ Physiol*. 2008; 295:H1270–H1278. [PubMed: 18660447]
16. Niederer SA, Kerfoot E, Benson AP, Bernabeu MO, Bernus O, Bradley C, Cherry EM, Clayton R, Fenton FH, Garny A, Heidenreich E, Land S, Maleckar M, Pathmanathan P, Plank G, Rodriguez JF, Roy I, Sachse FB, Seemann G, Skavhaug O, Smith NP. Verification of cardiac tissue electrophysiology simulators using an n-version benchmark. *Philosophical Transactions of the Royal Society A*. 2011; 369:4331–4351.
17. Niederer S, Mitchell L, Smith N, Plank G. Simulating Human Cardiac Electrophysiology on Clinical Time-Scales. *Frontiers in Physiology*. 2011; 2
18. Pfeiffer ER, Wright AT, Edwards AG, Stowe JC, McNall K, Tan J, Niesman I, Patel HH, Roth DM, Omens JH, McCulloch AD. Caveolae in ventricular myocytes are required for stretch-dependent conduction slowing. *Journal of Molecular and Cellular Cardiology*. 2014; 76:265–274. [PubMed: 25257915]
19. Reed A, Kohl P, Peyronnet R. Molecular candidates for cardiac stretch-activated ion channels. *Global Cardiology Science and Practice*. 2014; 2014(2):9–25. [PubMed: 25405172]
20. Roth BJ. Electrical conductivity values used with the bidomain model of cardiac tissue. *IEEE Transactions on Biomedical Engineering*. 1997; 44(4):326–328. [PubMed: 9125816]
21. Sachse F, Seemann G, Riedel C. Modeling of cardiac excitation propagation taking deformation into account. *Proceedings of BIOMAG*. 2002:839–841.
22. Sachse F, Steadman B, Bridge J, Punske B, Taccardi B. Conduction velocity in myocardium modulated by strain: measurement instrumentation and initial results. *Conference Proceedings of IEEE Engineering Medicine and Biological Society*. 2004; 5:3593–3596.
23. Sinha B, Kster D, Ruez R, Gonnord P, Bastiani M, Abankwa D, Stan RV, Butler-Browne G, Védie B, Johannes L, Morone N, Parton RG, Raposo G, Sens P, Lamaze C, Nassoy P. Cells respond to mechanical stress by rapid disassembly of caveolae. *Cell*. 2011; 144(3):402–413. [PubMed: 21295700]
24. Sokabe M, Sachs F, Jing Z. Quantitative video microscopy of patch clamped membranes stress, strain, capacitance, and stretch channel activation. *Biophys J*. 1991; 59:722–728. [PubMed: 1710939]

25. Spear JF, Moore EN. Stretch-induced excitation and conduction disturbances in the isolated rat myocardium. *J Electrocardiol.* 1972; 5(1):15–24. [PubMed: 5030643]
26. Sreejit P, Kumar S, Verma RS. An improved protocol for primary culture of cardiomyocyte from neonatal mice. *Vitr Cell Dev Biol - Anim.* 2008; 44(3–4):45–50.
27. Sundnes J, Lines GT, Tveito A. An operator splitting method for solving the bidomain equations coupled to a volume conductor model for the torso. *Mathematical Biosciences.* 2005; 194:233–248. [PubMed: 15854678]
28. Sundnes J, Wall S, Osnes H, Thorvaldsen T, McCulloch A. Improved discretisation and linearisation of active tension in strongly coupled cardiac electro-mechanics simulations. *Computer methods in biomechanics and biomedical engineering.* 2012; 0:1–12.
29. Sung D, Mills R, Schettler J, Narayan SM, Omens JH, McCulloch AD. Ventricular filling slows epicardial conduction and increases action potential duration in an optical mapping study of the isolated rabbit heart. *J Cardiovasc Electrophysiol.* 2003; 14(7):739–749. [PubMed: 12930255]
30. Taggart P, Sutton PM. Cardiac mechano-electric feedback in man: clinical relevance. *Progress in Biophysics and Molecular Biology.* 1999; 71:139–154. [PubMed: 10070214]
31. Tavi P, Weckstrom MLM. Effect of gadolinium on stretch-induced changes in contraction and intracellularly recorded action- and after potentials of rat isolated atrium. *Br J Pharmacol.* 1996; 118:407–413. [PubMed: 8735645]
32. Usyk TP, LeGrice IJ, McCulloch AD. Computational model of three-dimensional cardiac electromechanics. *Computing and Visualization in Science.* 2002; 4:249–257.
33. Vetter FJ, McCulloch AD. Mechanoelectric feedback in a model of the passively inflated left ventricle. *Annals of Biomedical Engineering.* 1998; 29:414–426. [PubMed: 11400722]
34. Wall ST, Guccione JM, Ratcliffe MB, Sundnes JS. Electromechanical feedback with reduced cellular connectivity alters electrical activity in an infarct injured left ventricle: a finite element model study. *AJP: Heart and Circulatory Physiology.* Dec; 2011 302(1):H206–H214. [PubMed: 22058157]
35. Zhang Y, Sekar RB, McCulloch AD, Tung L. Cell cultures as models of cardiac mechanoelectric feedback. *Prog Biophys Mol Biol.* 2008; 97(2–3):367–382. [PubMed: 18384846]
36. Zhu WX, Johnson SB, Brandt R, Packer JBDL. Impact of volume loading and load reduction on ventricular refractoriness and conduction properties in canine congestive heart failure. *J Am Coll Cardiol.* 1997; 30(3):825–833. [PubMed: 9283547]

Figure 1.

Hill equations were chosen to describe the relationships of both the capacitance and the space constants with strain. To match biphasic strain-CV relations reported in the literature, the space constant was chosen to increase quickly with small strains, while the capacitance shows a step-like behavior. The two curves are compared with measurements of capacitance and cross-fiber space constant for 4% strain, from [15].

Figure 2. Normalized CVs obtained on the cuboid simulations with stretch in the fiber direction and SAC based MEF mechanisms. A biphasic strain-CV relation is obtained in the model, but only for much larger strains than those observed in the experiments.

Figure 3.

Normalized CVs obtained on the cuboid simulations with stretch in the fiber direction comparing the effect of capacitance (SDC) and space constant (SDSC) based MEF mechanisms with SAC based changes. SDC and SDSC are the dominating mechanisms for changes in CV and provide the closest biphasic match to obtained experimental data.

Figure 4. Fiber (left) and Cross-fiber (right) strains on the left ventricle free wall after passive loading to 30mmHg (4.0 kPa).

Figure 5.

Normalized CVs obtained in the simulations using the rabbit geometry. Each point in these curves represents the CV obtained in a simulation including the specified MEF mechanisms and conductances for the SACs.

Figure 6.

Normalized CV on the rabbit geometry including both the SDC and SDSC stretch feedback. Similar results are seen on the complex geometry as compared to the simplified cuboid. The model parameters have been adjusted to match the experimental data measured at 30mmHg.