Mechanisms of Stretch-Induced Changes in [Ca\(^{2+}\)]\(_i\) in Rat Atrial Myocytes

Role of Increased Troponin C Affinity and Stretch-Activated Ion Channels

Pasi Tavi, Chunlei Han, Matti Weckström

Abstract—To study the effects of stretch on the function of rat left atrium, we recorded contraction force, calcium transients, and intracellular action potentials (APs) during stretch manipulations. The stretch of the atrium was controlled by intra-atrial pressure. The Frank-Starling behavior of the atrium was manifested as a biphasic increase of the contraction force after increasing the stretch level. The development of the contraction force after step increase of the stretch (intra-atrial pressure from 1 to 3 mm Hg) was accompanied by the increase in the amplitude of the calcium transients \((P<0.05, n=4)\) and decrease in the time constant of the Ca\(^{2+}\) transient decay. The APs of the individual myocytes were also affected by stretch: the duration of the AP was decreased at positive voltages (AP duration at 15% repolarization level, \(P<0.001; n=13\)) and increased at negative voltages (AP duration at 90% repolarization level, \(P<0.01; n=13\)). To study the mechanisms causing these changes we developed a mathematical model describing [Ca\(^{2+}\)], and electrical behavior of single rat atrial myocytes. Stretch was simulated in the model by increasing the troponin (TnC) sensitivity and/or applying a stretch-activated (SA) calcium influx. We mimicked the Ca\(^{2+}\) influx by introducing a nonselective cationic conductance, the SA channels, into the membrane. Neither of the 2 plausible mechanosensors (TnC or SA channels) alone could produce similar changes in the Ca\(^{2+}\) transients or APs as seen in the experiments. The model simulated the effects of stretch seen in experiments best when both the TnC affinity and the SA conductance activation were applied simultaneously. The SA channel activation led to gradual augmentation of Ca\(^{2+}\) transients, which modulated the APs through increased Na\(^+/Ca\(^{2+}\)-exchanger inward current. The role of TnC affinity change was to modulate the Ca\(^{2+}\) transients, stabilize the diastolic [Ca\(^{2+}\)], and presumably to produce the immediate increase of the contraction force after stretch seen in experiments. Furthermore, we found that the same mechanism that caused the normal physiological responses to stretch could also generate arrhythmogenic afterpotentials at high stretch levels in the model. (Circ Res. 1998;83:1165-1177.)

Key Words: stretch ■ myocyte ■ Frank-Starling ■ Ca\(^{2+}\) ■ action potential

The stretch-induced changes in heart muscle are prototypically manifested as an increase in the contraction force, the well-known Frank-Starling mechanism. Although it is known that mechanical stimulation can influence the free intracellular calcium, contraction force, and electrical action of the myocytes,\(^7,8\) the underlying mechanisms have not been resolved. It has been reported that the stretch-dependent changes in the cardiac contraction force has 2 components.\(^9\)

Immediately after stretch the contraction force is increased. This increase is followed by additional slow increase in force. Increase of the muscle length produces a gradual increase in Ca\(^{2+}\) transient amplitude.\(^10\) The stretch sensitivity of troponin C (TnC)\(^11,12\) and mechanical changes in the crossbridge attachments, like the reduction of double thin-filament overlap\(^13-15\) might explain the fast increase in contraction force, causing additional buffering of Ca\(^{2+}\) by the contractile element and increase in contraction force. However, it is not possible to augment the Ca\(^{2+}\) transients by the increased sensitivity of TnC. A complementary explanation for the increase of the Ca\(^{2+}\) transients during stretch would be activation of stretch-activated (SA) channels, first reported by Guharay and Sachs,\(^16\) which have also been found in cardiac myocytes.\(^15-22\) The aim of the current study was to define the stretch-induced changes in the isolated rat atrium in which stretch could be controlled by intra-atrial pressure. From the physiological point of view the plausible candidates (TnC and SA channels) for the mechanosensation in the heart are not easy to study. Usually ion channel function is studied by blockers or activators of the channel in question. In the case of SA channels, selective blockers are not known presently. The SA channel blockers previously used are fairly unspecific. For example, gadolinium blocks SA channels\(^23\) but also L-type Ca\(^{2+}\) channels\(^24\) and delayed rectifier K\(^+\) channels.\(^25\)

The function of the TnC is also difficult to study. Usually ion channel function is studied by blockers or activators of the channel in question. In the case of SA channels, selective blockers are not known presently. The SA channel blockers previously used are fairly unspecific. For example, gadolinium blocks SA channels\(^23\) but also L-type Ca\(^{2+}\) channels\(^24\) and delayed rectifier K\(^+\) channels.\(^25\)

The function of the TnC is also difficult to study. Usually ion channel function is studied by blockers or activators of the channel in question. In the case of SA channels, selective blockers are not known presently. The SA channel blockers previously used are fairly unspecific. For example, gadolinium blocks SA channels\(^23\) but also L-type Ca\(^{2+}\) channels\(^24\) and delayed rectifier K\(^+\) channels.\(^25\)

The function of the TnC is also difficult to study. Usually ion channel function is studied by blockers or activators of the channel in question. In the case of SA channels, selective blockers are not known presently. The SA channel blockers previously used are fairly unspecific. For example, gadolinium blocks SA channels\(^23\) but also L-type Ca\(^{2+}\) channels\(^24\) and delayed rectifier K\(^+\) channels.\(^25\)
Semi-intact cardiac tissue. The central role of TnC in contraction makes it fairly inaccessible to study by physiological methods. It is also difficult to distinguish the effects of TnC on the function of the cardiac cells from other regulators of the contractile element (eg, troponins I and T).

The role of SA channels in the generation of the pathological effects of stretch has been widely studied previously. From the physiological point of view, if SA channels exist in the cardiac myocytes, the major function of these channels would be the participation in the normal stretch-dependent changes in the heart muscle (such as Frank-Starling [F-S] relation), rather than generation of pathological phenomena. On the basis of this hypothesis, we studied the influence of stretch on the APs, contraction force, and intracellular [Ca\(^{2+}\)] in the rat atrium. To keep the stretch in the range of the rising phase of the Frank-Starling relation, we used only small stretch stimuli. To complement the experimental part of the study, we developed a mathematical model by which we could further study the role of TnC and SA channels in the stretch-induced changes at the level of individual myocytes. We found that during the stretch activation of rat atrium, which included prolongation of APs, biphasic increase of the contraction force, and augmentation of the Ca\(^{2+}\) transients, at least 2 different mechanisms were involved. We show that the model could produce similar changes in Ca\(^{2+}\) balance and APs as seen in experiments only when a stretch-dependent Ca\(^{2+}\) influx (mimicking SA channel activation) was included in the simulation. The model simulations resembled the experiments closest when stretch was simulated by SA channel activation and increased Ca\(^{2+}\) affinity of TnC.

Materials and Methods

Animals, Preparation, and Superfusion

Male Sprague-Dawley rats (n = 60) weighing 290 to 400 g were used. The care and use of animals were approved by the committee of laboratory animal experimentation at the University of Oulu. They were kept at 20°C to 22°C and had free access to tap water and standard food. The rats were decapitated, and their hearts were rapidly removed and placed in oxygenated cool (25°C) buffer consisting of (in mmol/L) NaCl 137, KCl 5.6, CaCl\(_2\) 2.2, MgCl\(_2\) 1.2, HEPES 5.0, glucose 2.5 (pH 7.4), which was also used for superfusion of the atrial preparation.

The experimental model used in this study was the isolated rat atrial appendix, prepared as described previously. Briefly, an X-branch polyethylene adapter was inserted into the lumen of the left auricle, and the tissue was placed in a constant-temperature (37°C) organ bath. Another tube with smaller diameter was inserted inside the adapter to carry perfusate inflow into the lumen of the auricle. The outflow from the lumen came from 1 cross-branch of the X-cannula. The stretch of the atrium was produced by changing intra-atrial pressure. Pressure inside the atrium was increased by inflow and outflow (3 mL/min) both to the auricle and to the organ bath. The outflow was conducted with photomultiplier tubes (Hamamatsu). The emission signal was further amplified (×8) and filtered with an adjustable Kemo filter (Kemo Corp) at 50 Hz. The Indo 1 emission ratio (405/495) was calculated online from an A/D-converted (Data Translation) signal by Testpoint (Capital Equipment Corp) customized software. The atrium was paced with 2 platinum electrodes at 1 Hz.

Loading the Atria With Indo 1

For Ca\(^{2+}\) measurements the left atrial preparation was attached, prior to loading, to the perfusion system to measure the autofluorescence from each atrium. The autofluorescence at both emission wavelengths was determined and then subtracted from the signals after loading. Together with the autofluorescence the contraction force (developed pressure) was measured at the low pressure (1 mm Hg), which was compared with the contraction force after loading. For Indo 1 AM loading, the preparation connected to the plastic tube was attached to a separate loading chamber. In this chamber, the atria were superfused for 25 to 40 minutes (flow 7 mL/min) with HEPES buffer (4 mL) containing 10 μmol/L Indo 1 AM dissolved to 100 μmol/L DMSO with 20% Pluronic, 0.5 mmol/L probenecid, and 1.5% BSA. To avoid loading of the intracellular organelles such as sarcoplasmic reticulum (SR) and mitochondria, the temperature in the chamber was kept between 25°C and 30°C during the entire loading period. After loading the fluorescence was ~20 times greater than the autofluorescence before loading. The contraction force (developed pressure), which was 1 mm Hg before loading, was 3.1 ± 0.4 and 3.17 ± 0.4 mm Hg after loading (NS, n = 15). This indicates that Indo itself does not buffer calcium ions enough to influence the contraction. It has been shown previously that when epifluorescence of the heart muscle is used in estimation of the intracellular calcium of the myocytes, part of the fluorescence signal may originate from cells other than myocytes, forming a possible source of error. When cardiac tissue is loaded with fluorescence indicators such as Fura-2 and Indo 1 through coronary arteries, a great portion of the epifluorescence comes from the endothelial cells. Here we tried to avoid this by not using the coronary circulation in loading; instead we used direct perfusion of the tissue. The source of fluorescence was also under visual control (ie, when the atria were attached to the perfusion system the areas of clearly greater fluorescence intensity were avoided, and the atria were measured from the areas with low total fluorescence intensity and no visually detectable “hot spots”). In some preparations the contraction and stretch of the tissue caused a prominent movement artifact, and these atria were not used.
Mathematical Model

The model used in this study is based on that developed by Luo and Rudy.30–32 Since the original model was designed to model the guinea pig ventricular cell, we had to make extensive modification to fit the model to our and previously published experimental results from rat atrial myocytes. Together with the species-specific modifications (see Appendix), we introduced a more complex method of Ca\(^{2+}\) handling into the model on the basis of recent reports. The calcium release in atrial myocytes is a combination of the release from 2 different compartments of the SR.33,34 The basic idea of this 2-step Ca-release model is that the calcium entering through L-type channels triggers calcium release from the first release compartment (peripheral SR), and the Ca\(^{2+}\) released from the first compartment triggers release from the second compartment (corbular SR) in an all-or-none manner. The trigger threshold of the second compartment was set at the value equal to the half of the calcium peak of normal release from the first compartment, with the time constants of activation and inactivation 40 and 10 ms, respectively. Time delay between release from first and second compartments was 5 ms based on the calcium wave propagation velocity and calcium gradients in atrial myocytes.33,35,36 These adjustments made the calcium release from the second compartment slower than from the first compartment, consistent with experimental data.34

Simulation of Stretch by the Model

The SA channels in the cardiac myocytes have reportedly a near linear voltage dependence,29 no adaptation,30 and nonselectivity over cations.30 The reversal potential of the SA channel current is −3.2 mV in rat atrial myocytes.19 The SA channel formalism was adapted from previous models,32 with small modifications.

The SA channel current is given elegantly by Sachs34 by the following equation:

\[
I_{\text{AC}} = \frac{(V - V_{\text{rev}})g}{1 + \exp(\frac{-\alpha(L-L_0)}{V})}
\]

where \(I_{\text{AC}}\) is the current density (\(\mu\text{A}/\text{cm}^2\)), \(V\) is membrane potential, \(V_{\text{rev}}\) is reversal potential (−3.2 mV), \(K\) is equilibrium constant (100), \(L\) is sarcomere length, \(L_0\) is minimum sarcomere length (1 \(\mu\text{m}\)), \(\alpha\) is the parameter defining the stretch sensitivity (3), \(\rho\) is channel density (0.015/\(\mu\text{m}^2\)), and \(\gamma\) is single-channel conductance (25 pS). The iteration of the SA current was based on our experimental data. To simulate the stretch effects seen in our experiments, only 5% of the previously suggested value38 (0.3/\(\mu\text{m}^2\)) of the SA channel current was needed in the model. To simulate stretch of the rat atrium caused by the increase of the intra-atrial pressure from 1 to 3 mm Hg, increase of the current density during diastole from −0.03 to −0.32 \(\mu\text{A}/\mu\text{F}\) was used. In our experiments we have not measured the sarcomere lengths. However, in the model (using the definitions of Sachs34), this SA channel current increase corresponds to an increase of sarcomere length from 1.2 to 1.9 \(\mu\text{m}\), increasing the open probability of the SA channels from 0.03 to 0.16.

Gulati et al12 showed that the calcium affinity of the contractile element increased after a length change, whereby \(K_t\) (for Ca\(^{2+}\)) of the TnC decreased 42% when the sarcomere length of skinned cardiac muscle was increased from 1.7 to 2.2 \(\mu\text{m}\), which almost corresponds to the overall sarcomere length change during the rising phase of the F-S relation in rat trabeculae.35 According to these observations, we approximated that the overall increase of the TnC affinity during the rising phase of the F-S relation is ∼50%. Knowing that the rising phase of the F-S relation in rat left atrium is within intra-atrial pressure between 0 and ∼10 mm Hg,66 we approximated that the increase of the intra-atrial pressure in our experiments from 1 to 3 mm Hg produces a 25% decrease of the \(K_t\) in the TnC. This value was used in modeling the stretch caused by increase of the intra-atrial pressure from 1 to 3 mm Hg (Figure 1). The simulation of the stretch in the model was based on the following assumptions. First, the relation of the TnC affinity change between 0% and 50% increase and sarcomere length is linear. Secondly, the SA channel current density (open probability of the channel) increases with increased sarcomere length, giving the maximum open probability of the channel at a sarcomere length that produces maximal developed tension. Thus, the current density increase from 0% to 100% is achieved during the rising phase of the F-S relation in the model. Figure 1 shows the SA current density and TnC affinity change at different sarcomere lengths in the model. In extrapolating to pathological stretch levels we used SA channel current density from 0.2 to 1.54 \(\mu\text{A}/\mu\text{F}\), and at the same time the TnC affinity was increased from 20% to 50%. The assumptions are based on the scarce published data on the mechanisms, and, if the relationships between the modeled components of stretch sensitivity are more complex, this would probably introduce more complex behavior into the model but does not change the basic results.

Materials

HEPES was obtained from Sigma; KCl, glucose, CaCl\(_2\), and MgCl\(_2\) from Merck; NaCl and potassium acetate from FF-Chemicals AB; Indo-1 AM, poloxamer, and probenecid (p-dipropylsulfamoyl)-benzoic acid) from Molecular Probes Europe BV.

Statistical Analysis

Statistical testing was done by the SPSS (SPSS Inc) and SigmaStat programs (Jandel Scientific). The AP data were tested with 1-way ANOVA. The data from contraction and Indo fluorescence were tested with a paired t test. In all cases, \(P\) values less than 0.05 were considered statistically significant. Variances are expressed as ±SEM.

Stretch-Induced Changes in AP Parameters

<table>
<thead>
<tr>
<th></th>
<th>1 mm Hg (n=9)</th>
<th>3 mm Hg (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP, mV</td>
<td>−75.1±0.7</td>
<td>−77.3±0.7</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>95.9±1.2</td>
<td>99.3±1.4</td>
</tr>
<tr>
<td>APD(_{90}), ms</td>
<td>4.8±0.2</td>
<td>3.3±0.3***</td>
</tr>
<tr>
<td>APD(_{90}), ms</td>
<td>17.3±0.2</td>
<td>17.5±0.6</td>
</tr>
<tr>
<td>APD(_{90}), ms</td>
<td>43.4±0.8</td>
<td>53.4±2.3**</td>
</tr>
</tbody>
</table>

Variances were tested by 1-way ANOVA. Asterisks denote the statistical significance compared with AP parameters in 1 mm Hg (**\(P<0.01\); ***\(P<0.001\)).
Results

Stretch-Induced Changes in APs

In the first series of experiments we investigated the effects of a sustained moderate stretch on APs of rat atrial myocytes. The tissue was stretched by the intra-atrial (diastolic) pressure. Criteria for acceptable experiments were a stable recording with a resting potential (RP) of at least –70 mV and an overshoot of the AP of at least 10 mV. The atria were kept at constant pressure (1 or 3 mm Hg) for 5 minutes until recordings were started. At low pressure (1 mm Hg), myocytes with RP of –75.1 ± 0.7 mV generated APs (n = 9) with amplitude of 95.8 ± 1.2 mV. When the pressure was 3 mm Hg, AP duration at 90% repolarization level (APD$_{90}$) and APD$_{90}$ decreased (Table, Figure 2).

Because the sustained stretch changed the AP shape, we recorded APs during the pressure manipulations to observe the time course of the changes. These recordings were found to be extremely difficult, and only a very few of the attempts were successful. Before each recording the atrium was kept at steady pressure (1 mm Hg) until acceptable impalement (stable recordings, RPs at least –70 mV and overshoot at least 10 mV) was achieved. After the recording had been stable for at least 20 s, pressure inside the atria was gradually increased from 1 to 3 mm Hg over 10 seconds and then kept constant (3 mm Hg) for at least 1 minute. Figure 3 shows a representative trace (out of 3 successful experiments, in which the impalement was still stable after at least 1 minute of stretch) of the changes that increased stretch (from 1 to 3 mm Hg) causes in the APs of myocytes. Immediately after the increase of stretch the shape of the APs was changed. The duration decreased at positive voltages (at 10% repolarization level from 3.75 to 3.25 ms) and increased at negative membrane potentials (at 90% repolarization level from 43 to 47 ms). After 60 seconds of onset of stretch, some of the changes in AP were even more prominent (APD$_{90}$, 52.5 ms). All cells studied were able to maintain fairly constant RP during pressure manipulations. Depolarization of the measured potential (during diastole) was always associated with the changes in the cell-electrode impalement (resistance and capacitance) caused by the electrode leaving the cell.

To see whether the AP lengthening corresponds to the development of the contraction force after stretch, the APD$_{90}$ was plotted together with the change in contraction force. Figure 4 shows that the development of the slow increase of force (within 1 minute) in rat atrial tissue is accompanied by the lengthening of the AP (APD$_{90}$) of the individual myocytes with a similar time course.
The main findings of the experimental part of the study can be summarized as follows. Moderate stretch causes increase in the amplitude of the Ca²⁺ transients and decrease of the time constant of the decay of the transients without a significant
change in the diastolic $[Ca^{2+}]_{i}$. These changes were accompanied by lengthening of the APs of the myocytes and a biphasic increase of the contraction force of the atrium. In the second part of the study we tried to reproduce similar changes in the Ca $^{2+}$ balance and APs by modulating the TnC affinity and/or calcium influx mimicking SA channel activation in the model.

Role of TnC

One candidate for mechanosensation in heart cells is the TnC, calcium-binding part of the contractile machinery, which is known to be sensitive to stretch.\textsuperscript{11,12} We increased the TnC affinity to $Ca^{2+}$ by decreasing the dissociation constant ($K_{d}$) of TnC by 25% in our model (see Materials and Methods). This caused a biphasic behavior of the $Ca^{2+}$ transients. First transients were smaller, but amplitude stabilized near the control value (Figure 7A) within 1 minute. Even when the systolic $[Ca^{2+}]_{i}$ had returned to the control value, the decay of the $Ca^{2+}$ transient was faster (time constant decreased from 118 to 105 ms; Figure 7C). When control APs were compared with steady-state APs after a simulated stretch, only modest changes were observed, the most prominent change being the small increase of the APD$_{90\%}$ ($12.4$ ms; Figure 7B). The increased $Ca^{2+}$ affinity of the TnC modulated slightly the $Ca^{2+}$ dependent currents, the peak value of the L-type $Ca^{2+}$ current (Figure 7D) was increased from $-10.3$ to $-10.5 \mu A/\mu F$, and the inward current of the Na$^{+}/Ca^{2+}$ exchanger (Figure 7E) was decreased from $-1.09$ to $-1.05 \mu A/\mu F$. This result indicates that the TnC affinity change alone cannot produce similar changes as observed in experiments, since the TnC affinity change could not augment the $Ca^{2+}$ transients or lengthen the APs.

Role of SA Channels

When stretch of the myocyte was simulated by applying the SA channel conductance into the cell membrane in our model, the amplitude of the calcium transients were gradually increased (Figure 8A). The activation of a cation-selective SA channel increased the systolic $[Ca^{2+}]_{i}$ gradually from $0.88$ to $1.04 \mu mol/L$ within 1 minute ($+15.4\%$). The decay of the calcium transients decreased (as measured by the time constant of the 1-exponential fits) from 115 to 114 ms (Figure 8C). Only a small change in the diastolic $[Ca^{2+}]_{i}$ was observed (Figure 8A). Greater $Ca^{2+}$ transients increased the late duration of the APs (Figure 8B), the time course of the AP lengthening being related to the increase of the amplitude of the calcium transients. The APD$_{90\%}$ increased from 52.2 to 61.4 ms within 1 minute after the increased SA channel current. This was probably due to the increase of the inward current carried by Na$^{+}/Ca^{2+}$ exchanger (Figure 8E), which was increased from $-1.09$ to $-1.16 \mu A/\mu F$. Bigger $Ca^{2+}$ transients decreased the peak value of the L-type $Ca^{2+}$ current (by increasing inactivation) from $-10.3$ to $-10.1 \mu A/\mu F$ (Figure 8D) and made the decay of the current a little faster.

Combination of SA Channel and TnC Effects

Neither of the 2 candidates of the mechanosensation (SA channels and TnC) could faithfully produce similar changes in the model cell as seen in experiments. The TnC affinity change could not produce the gradual increase of the amplitude of the $Ca^{2+}$ transients or increase of the APD$_{90\%}$. The
activation of the SA channels produced both effects, but not the prominent change in the decay of the transients. Furthermore, the SA channel activation cannot explain the fast increase of the contraction force seen in experiments, since it caused a slow and gradual augmentation of Ca^{2+} transients during stretch. To better fit the model predictions to experimental findings, we combined these 2 mechanisms in the next round of modeling (Figure 9). When the stretch was simulated by increasing the TnC affinity together with the activation of the SA current, the Ca^{2+} transient amplitude gradually increased by 20.2% from 0.88 to 1.09 mM (Figure 9A and 9C). The increase of the amplitude of the Ca^{2+} transients was accompanied by the decrease of the time constant of the decay of the transients from 119 to 96 ms (Figure 9C).

Increased calcium mobilization during the AP caused a simultaneous lengthening of the AP (Figure 9B). The APD_{90\%} increased from 52.2 to 63.8 ms within 1 minute, and time course of the AP lengthening was related to the increase of the calcium transients. Since the peak value and the decay of the L-type Ca^{2+} current was unaffected (Figure 9D), we may conclude that the increase of the inward current carried by Na^+/Ca^{2+} exchanger from –1.09 to –1.19 μA/μF (Figure 9E) increased the late duration of the APs.

Role of SR
If the Ca^{2+} release kinetics is not altered, the amount of Ca^{2+} in SR contributes prominently to the released Ca^{2+} during excitation.42 Figure 10 shows how different strategies of the stretch simulation (TnC affinity, SA channels, or both) influence the amount of Ca^{2+} in the SR. When stretch was simulated solely by increasing the TnC affinity by 25% (Figure 10A), the SR Ca^{2+} content was affected only slightly. The diastolic Ca^{2+} in the SR increased from 1.65 to 1.69 mM within 1 minute. Activation of SA channels without any change in TnC affinity (Figure 10B) increased the diastolic SR Ca^{2+} content from 1.65 to 1.79 mM. The effects of TnC affinity change and activation of SA channels were additive (as shown in Figure 10C); SR calcium content increased from 1.65 to 1.88 mM. This modeling result indicates that augmentation of the Ca^{2+} transient in the model during simulated stretch is due to the increased amount of Ca^{2+} in the SR. Furthermore, SA channel activation does not cause direct increase of the Ca^{2+} transient, but the effect is mediated by the Ca^{2+} release from SR.

Electrical Changes Caused by the Simulation of the Stretch
Since the combination of SA channel activation and the increased affinity of TnC simulated fairly well the effects of stretch observed in the experiments, we investigated whether this approach causes simultaneous changes in Ca^{2+} transients and APs and whether the time course of this change is similar to those in the experiments. Figure 11 shows the AP train (A), calcium transients (B), and APD_{90\%} (C) when the stretch was simulated by increasing the SA channel current and calcium affinity of the TnC. The augmentation of the Ca^{2+} transient lengthened the APs by 10 ms within 1 minute, as indicated by the APD_{90\%}, in line with our experimental findings (Figures 2 through 4).

Pathological Effects of Stretch
In addition to the physiological effects, the stretch of the cardiac tissue also causes pathological phenomena. It is known that intensive stretch can trigger arrhythmias caused...
by early or delayed afterdepolarizations of the membrane potential. The mechanism suggested to be responsible for these arrhythmias is the calcium overload caused by stretch. We investigated whether the same mechanisms that cause the physiological effects of stretch could be responsible for the pathological stretch-induced changes in the function of the heart. To do this, we simulated the effects of incremental stretch on the function of the model cell. It is natural to assume that if a cationic current through SA channels, with reversal potential more positive than the RP, is activated, it should cause a depolarization of the membrane potential. Because SA channels also pass Ca\(^{2+}\) ions, the depolarization would be followed by an increase of the [Ca\(^{2+}\)]. Both of these effects were seen when SA current density was increased gradually from 0.1 to 1.6 mA/mF (Figure 12A and 12B; see also Materials and Methods for details), at the same time that the TnC affinity was increased from 20% to 50%, respectively. The RP depolarized up to –68 mV (from –80.5 mV) at the same time when diastolic [Ca\(^{2+}\)], was increased from 0.1 to 0.16 \(\mu\)mol/L.

Figure 11. Comparison of modeled train of APs (A), Ca\(^{2+}\) transients (B), and APD at 90% repolarization level (C) when the stretch was simulated by increased TnC affinity and increased SA current density.

Figure 12. The model predictions of the more severe (possibly pathophysiological) stretch on the RP (A) and the diastolic free Ca\(^{2+}\) (B). The numbers in parentheses refer to the estimation of the SA current density and affinity change of the TnC given in Figure 1.

Figure 13 shows that the model can produce an arrhythmogenic effect when the SA channel current density is 8 times as high as needed in the model to produce the physiological effects seen in our experiments. Similarly, as in other simulations, augmentation of the Ca\(^{2+}\) transients (Figure 13B) modulated the APs (Figure 13A) through increased Na\(^+\)/Ca\(^{2+}\)-exchanger current (Figure 13D). When 80% of the channels present in our model were open, the augmented Ca\(^{2+}\) transient reduced the L-type Ca\(^{2+}\) current (Figure 13C) from –10.4 to –8.2 \(\mu\)A/\(\mu\)F. Because the Na\(^+\)/Ca\(^{2+}\)-exchanger inward current was increased from –1.2 to –2.6 \(\mu\)A/\(\mu\)F, it generated an afterpotential that resembles the early afterdepolarizations of the atrial myocytes. These results show that the same mechanisms that mimic the physiological effects of stretch can also produce the arrhythmogenic changes in the function of myocytes.

Discussion
The present study demonstrates that stretch, in this case diastolic pressure, has a wide variety of actions in isolated rat atrium. The experimental part of the study shows that contractile function has a biphasic response to stretch, associated with augmentation of the Ca\(^{2+}\) transients, without a significant change in the diastolic [Ca\(^{2+}\)]. Diastolic calcium concentration remains fairly constant because the Ca\(^{2+}\) buffering power of the cell is increased, indicated by the faster decay of the Ca\(^{2+}\) transients during stretch. APs of the myocytes reflect the changes in Ca\(^{2+}\) transients and contraction force. The most prominent change in APs during stretch was the increase of the late duration of the APs. The modeling part of the study demonstrates that both affinity change of the TnC and activation of the SA channel current are needed to reproduce these effects seen in experiments. The model also suggests that SA channels do not directly modulate the function of the myocytes but instead increase the SR calcium stores, causing greater release of Ca\(^{2+}\) during systole. A bigger Ca\(^{2+}\) transient modulates the membrane voltage during...
APs through increased Na+/Ca2+-exchanger inward current. The same mechanisms can also induce arrhythmogenic after-potentials, a rise in diastolic [Ca2+], and depolarization of the RP when high enough amounts of SA channels are activated in the model.

Properties of the Model
A mathematical model based on the Luo-Rudy model30–32 was used to support the experimental study of the stretch-dependent mechanisms. The model could reproduce the phenomena observed in the experiments. First, it has to be clarified to what extent the model reflects the function of the cardiac myocytes. It is clear that a model cannot possess all the components in the real cells, since many of them are not known in detail presently. Despite the scarcity of facts, we have tried to obtain a holistic point of view as possible in the development of the model. Therefore, the validity of the model could be judged by how well it can reproduce documented physiological behavior. Our model can produce fairly similar APs as recorded from rat atrial myocytes, which are also modulated by calcium transients in the model. Because we wanted to study the Ca2+ balance of the myocytes during stretch, the model parameters relevant to the Ca2+ handling are essential. In modeling the calcium-induced calcium release (CICR), it has usually been assumed that there is only 1 cytosolic pool of calcium, the concentration of which controls the release of calcium from the SR. To this pool, calcium enters from both the SR and sarcoplasmic calcium current.45 The CICR model we used is a common pool model with 2 different release sites. The release from the first compartment is controlled by the L-type current, whereas the release from the second compartment is controlled by the release from the first compartment (see Materials and Methods and Appendix for details). In this respect, our model differs from all the other models used previously to study the stretch-dependent changes in the cardiac myocytes. The well-documented role of TnC in the stretch-dependent changes of the heart muscle11,12 has been previously modeled to evaluate TnC-induced changes during stretch by several authors.13–16,46 The works by Landesberg and Sideman,13,14 Landesberg,15 and Katsnelson and Markhasin46 have thoroughly investigated, by modeling, the role and function of the length-dependent changes in the contractile apparatus, including increase of calcium affinity and reduction of the double thin-filament overlap. Their results show similar changes in the [Ca2+], as in our work. In contrast to those works, we wanted to investigate the [Ca2+] in terms of time-dependent changes in the function of the myocytes, including the APs, Ca2+ transients, and membrane currents. Although in the modeling we had to approximate the TnC affinity change during stretch, our model produced changes very similar to those observed in the experiments. The SA channel formalism used in our model is similar to what has been used before.38

[Ca2+]i During Stretch
A considerable agreement exists that mechanical stimulation of the heart muscle11,12 has been previously modeled during stretch, the model parameters relevant to the Ca2+ concentration during stretch.45 Concerning systolic [Ca2+], Allen et al.24 suggested that augmentation of the Ca2+ transients during stretch might be due to a change in diastolic [Ca2+]. Other studies indicate that Ca2+ transients are augmented without a change in diastolic [Ca2+], in isolated rat myocytes.47 This was also the finding in isolated rat atria in the present study. On the other hand, the development of the increase of the amplitude of the Ca2+ transients over a period of several minutes47 indicates that even small changes in diastolic calcium concentration might contribute to these changes. The fluorescent Ca2+ indicators have relatively high Kd values when compared with the low [Ca2+] during diastole. This suggests that small changes in diastolic [Ca2+] are hard to detect with these indicators. In general, a prominent rise in diastolic calcium concentration in heart myocytes indicates that Ca2+ buffers are unable to remove or bind the calcium. This is known to happen in several pathological situations such as ischemia,39 in which the Ca2+ buffers of the cell are incapacitated. Usually this leads to a Ca2+ overload, causing abnormal electrical and contractile behavior of the tissue. It is clear that stretch can be severe enough to be pathological and cause the calcium overload. However, a rise in diastolic Ca2+ concentration may lead also to an increase in resting tension of the tissue that may reduce stretch of the cell membrane. The present study shows, experimentally, that a small amount of stretch increases the amplitude of the Ca2+ transients without any detectable change in diastolic calcium concentration. Our model simulations support the idea, since they can reproduce significant increase in Ca2+ transients without prominent change in diastolic [Ca2+].

Role of TnC
The calcium-binding part of the contractile machinery, the TnC,38 is known to be sensitive to muscle length.11,12 In addition to biochemical studies, many physiologically oriented reports suggest the prominent role of TnC in the length-dependent activation of the cardiac tissue of different species.5,3,53–55 If Ca2+ sensitivity of TnC increases with stretch, this might influence the function of the myocytes in several ways, as follows. First, fast increase of the affinity would increase the contraction force and decrease the systolic [Ca2+]. Secondly, the decay of the Ca2+ transients during stretch would be faster than what would be expected on the basis of enzyme kinetics alone, as previously demonstrated,56 because the calcium binding “eats into” the calcium transient. Thirdly, the affinity change would favor the formation of the TnC-Ca2+ complex, leading to a slower off-rate of the complex, and so the time course of the contraction would be prolonged. Our experimental data demonstrate that stretch decreases the decay of the calcium transients significantly, and the model simulation shows that TnC affinity increase, alone or in combination with SA channel activation, leads to similar changes (Figures 7 and 9). The decline of the Ca2+ transients immediately after the onset of stretch was not seen

isolated cardiac preparations10 and also in isolated rat cardiac myocytes.47 The results concerning the change of the diastolic [Ca2+], are somewhat controversial. Some studies show a clear increase in diastolic [Ca2+],34,56 but others indicate that cardiac myocytes can maintain constant diastolic calcium concentration during stretch.47 Concerning systolic [Ca2+], Allen et al.24 suggested that augmentation of the Ca2+ transients during stretch might be due to a change in diastolic [Ca2+]. Other studies indicate that Ca2+ transients are augmented without a change in diastolic [Ca2+], in isolated rat myocytes.47 This was also the finding in isolated rat atria in the present study. On the other hand, the development of the increase of the amplitude of the Ca2+ transients over a period of several minutes47 indicates that even small changes in diastolic calcium concentration might contribute to these changes. The fluorescent Ca2+ indicators have relatively high Kd values when compared with the low [Ca2+] during diastole. This suggests that small changes in diastolic [Ca2+] are hard to detect with these indicators. In general, a prominent rise in diastolic calcium concentration in heart myocytes indicates that Ca2+ buffers are unable to remove or bind the calcium. This is known to happen in several pathological situations such as ischemia,39 in which the Ca2+ buffers of the cell are incapacitated. Usually this leads to a Ca2+ overload, causing abnormal electrical and contractile behavior of the tissue. It is clear that stretch can be severe enough to be pathological and cause the calcium overload. However, a rise in diastolic Ca2+ concentration may lead also to an increase in resting tension of the tissue that may reduce stretch of the cell membrane. The present study shows, experimentally, that a small amount of stretch increases the amplitude of the Ca2+ transients without any detectable change in diastolic calcium concentration. Our model simulations support the idea, since they can reproduce significant increase in Ca2+ transients without prominent change in diastolic [Ca2+].
Role of SA Channels

Stretch-induced changes in heart function might be possible to explain by SA ion channels located in the plasmalemma of cardiac myocytes. These channels have been described, and their open probability has been found to depend on mechanical stress of the membrane. After the initial discovery of SA channels, several types of SA channels have been documented in isolated adult cardiocytes and in cultured neonatal cardiocytes. The majority of SA channels seems to be of a type with considerable permeability to both monovalent and divalent cations. In isolated myocytes mechanical loading increases intracellular Ca\(^2+\) concentration, and interestingly, this increase can be inhibited by gadolinium (Gd\(^3+\)), a blocker of SA channels. Our model reproduced a gradual increase of the calcium transients only when an additional calcium influx through plasma membrane was activated. The SA channel we used in our model has a permeability ratio of 1:1:1 for Na\(^+\):Ca\(^2+\):K\(^+\), similar to what has been reported earlier from rat atrial myocytes. Activation of the SA channel leads to an increase in [Ca\(^2+\)], by direct Ca\(^2+\) influx. Since the SA channel is permeable to Na\(^+\), increased sodium leads to the activation of the Na\(^+\)/Ca\(^2+\) exchanger and to an additional accumulation of Ca\(^2+\) ions into the cell. The increased [Ca\(^2+\)] is pumped to the SR by the Ca\(^2+\) pump. Increased amount of calcium in SR causes a greater release of Ca\(^2+\) during systole. In this scheme, potassium ions may serve as a countercurrent mechanism that stabilizes the membrane potential during diastole. Our study does not rule out the possibility that the additional calcium flux comes via channels or exchangers other than the SA channels. Since the stretch-dependent increase of the contraction force in the heart muscle preparations has even isolated cells has 2 distinct components (fast part and slow part), one has to consider the [Ca\(^2+\)] changes as a 2-step process. If the TnC affinity change and the overlap of the actin and myosin filaments are responsible for the fast part as previously suggested, the additional activation of Ca\(^2+\) flux would contribute to the slow part by modulating the [Ca\(^2+\)] of the cells. In fact, as shown in Figure 6, the increase of the contraction force after stretch seems to consist of the 2 components, increased TnC affinity and increase of the Ca\(^2+\) transients, of which the latter is much slower than the former.

Role of SR

CICR in cardiac myocytes can be modulated by the trigger, the L-type current through membrane, or by the release of the Ca\(^2+\) from SR. On the basis of our results, the amount of the trigger current is not significantly changed by stretch. This leads to the conclusion that the Ca\(^2+\) transient augmentation is mediated by the release of the Ca\(^2+\) from the SR. It has been reported that SR calcium content is increased slowly after a step increase of cardiac muscle length. Our model produced an increase in SR content concomitantly with the augmentation of the Ca\(^2+\) transient amplitude when SA channels were introduced to the model cell (Figure 10). However, SR calcium content increased only 12.2%, whereas amplitude of calcium transients increased 20.2%, indicating a nonlinear correlation between SR calcium content and the amount of calcium released during systole. The reason for this is probably the positive feedback in CICR, caused by the fact that with a higher amount of calcium in SR a bigger proportion is released during systole. On the premise that our model, which has simpler Ca\(^2+\) dynamics than the real cells, can reproduce this effect, we can assume that this positive feedback is a fundamental feature of the CICR. On the basis of our experimental and modeling data, we can conclude that during the normal stretch-dependent activation in rat atrium, the SR has a crucial role in augmentation of the Ca\(^2+\) transients.

Electrical Activity of the Myocyte During Stretch

Since calcium transient modulates the AP\(^65\) and vice versa, the changes in [Ca\(^2+\)], during stretch should change the shape of the APs of the myocytes. It is known that inotropic interventions, such as stretch, increase the late duration of the APs\(^67\) in cardiac myocytes with a short plateau. This happens apparently via the increased Na\(^+\)/Ca\(^2+\)-exchanger inward current that is boosted by augmentation of the calcium transients. In rat myocytes, the Na\(^+\)/Ca\(^2+\)-exchanger current is proportional to [Ca\(^2+\)]. The present study demonstrates that stretch influences the APs of the rat atrial myocytes. The APD\(_{90}\) was lengthened during the slow phase of the contraction development. The model simulated similar changes in APs when the simulation included SA channel activation. The mechanism suggested by the modeling was the increased Na\(^+\)/Ca\(^2+\)-exchanger inward current generated by bigger Ca\(^2+\) transients. The data support the idea that SA channels do not, during moderate stretch, directly modulate APs, but instead, the effects are mediated by the SR through the increased Ca\(^2+\) release during systole. Many reports indicate that stretch causes a depolarization of the RP of cardiac myocyte. In the present study the RP of the myocytes was not significantly altered by moderate stretch, neither in experiments nor in the model. The reason for this was that only a very small SA current (depolarizing current) is needed to reproduce the normal physiological time-dependent changes in rat atrial function.

Pathological Changes Caused by Stretch

The effects of stretch on the function of the heart muscle have previously been studied on the premise that stretch can cause many pathological phenomena. These effects include changes in conduction, excitability, and generation of afterpotentials. We show that the same mechanism that produces typical Frank-Starling responses during moderate
stretch in the rat atrium can also induce pathological changes in cardiac myocytes when they are subjected to a more intense stretch. These effects were manifested in our model as depolarization of the RP and increase of the diastolic [Ca\(^{2+}\)]\. During the stretch-induced Ca\(^{2+}\) overload, the Ca\(^{2+}\)-induced inactivation of the L-type Ca\(^{2+}\) current opens a time window in which the increased Na\(^{+}/Ca\(^{2+}\) current can trigger early afterpotentials.

**Appendix**

The Mathematical Model of the Rat Atrial Myocyte

The mathematical model we used is a further development of the one by Luo and Rudy.\(^{30,32}\) Like their model, it includes the ionic currents responsible for generation of the AP (Na\(^{+}\), K\(^{-}\), and Ca\(^{2+}\)) and the Na\(^{+}/Ca\(^{2+}\)-exchanger current. It also includes the Ca\(^{2+}\) release mechanisms and intracellular buffers. The model has been built with MATLAB software in a UNIX workstation and uses the optimized integration algorithms therein to solve numerically the resulting multiple differential equations. The integration algorithm used to solve the differential equations was based on hybrid methods.\(^{6,7}\) An adaptive time step was used to shorten calculation times from 500 to 10 \(\mu s\). Before any interventions or observations, the model was allowed to run until a steady state was reached.

A full description of the model is beyond the scope of this paper, but certain crucial modifications as compared with the Luo-Rudy model have to be recapitulated. The cell geometry has been changed to correspond to that of the rat atrial myocytes, including cell size and the size of the SR compartment. Ionic concentrations have been modified to be the same as those used in our experimental work. Concerning the ionic currents, the only current that has been fundamentally changed is the K\(^{-}\) current, which has been modeled with 2 time- and voltage-dependent currents (based on fast and slow inactivating channels) and 1 time-independent (noninactivating) current. The Ca\(^{2+}\) balance has been modeled using the knowledge in that rat about 90\% of the calcium transient comes from the intracellular stores (SR), and only about 10\% comes directly via L-type Ca\(^{2+}\) channels. The calcium release from the stores also includes 2 phases, the first directly coupled to the L-type Ca\(^{2+}\) current (release from the junctional SR) and the second triggered by this initial release from neighboring (nonjunctional or network) SR.

The Depolarization-Activated Outward Current

The depolarization-activated outward current \((I_{\text{out}})\) was the main repolarization-inducing current. The subtypes of the outward current have been described in detail previously.\(^{7,8}\) In our model, the \(I_{\text{out}}\) is modeled with 3 components, the rapidly inactivating \((I_{\text{fast}})\), slowly inactivating \((I_{\text{slow}})\), and nonactivating \((I_{\text{ref}})\) K\(^{-}\) current. We adapted the values of forward and reverse activation rate constants given by Boyle and Nerbom\(^{8}\) and VanWagoner\(^{8}\) with the MATHCAD modeling tool to get the activation and inactivation into the format used in H-H type formulae. The \(I_{\text{out}}\) is given according to the H-H model, as follows:

\[
I_{\text{out}} = \frac{G_{\text{m,ca}} G_{\text{r}} (V - E_{\text{k}})}{\left(1 + G_{\text{m,ca}} (V - E_{\text{k}}) / G_{\text{r}}\right)}
\]

where \(G_{\text{m,ca}}\) is the maximum conductance of \(I_{\text{fast}}\), \(G_{\text{r}}\) is activation gate, \(G_{\text{m,ca}}\) is inactivation gate for \(I_{\text{fast}}\) and \(G_{\text{m,ca}}\) is inactivation gate for \(I_{\text{slow}}\); \(k_{\text{con}}\) and \(k_{\text{act}}\) are fractional for \(I_{\text{act}}\), \(I_{\text{slow}}\), and \(I_{\text{ref}}\), respectively. Please note that the first-order kinetics of the opening gate was used instead of the fourth order,\(^{2,6}\) since the duration of AP generated by using the fourth-order power did not match the experimental data.

Parameter Details

- **Cell geometry (largely after Schaper et al\(^{10}\))**
  - Cell volume: \(V_{\text{c}} = 12 \times 10^{6} \, \mu \text{L}\)
  - SR volume: \(V_{\text{sr}} = V_{\text{c}} \times 0.1\)
  - JSR volume: \(V_{\text{jsr}} = V_{\text{c}} \times 0.02\)
  - NSR volume: \(V_{\text{nsr}} = V_{\text{c}} \times 0.98\)
  - Capacitive membrane area: \(A_{\text{m}} = 0.7 \times 10^{4} \, \text{cm}^{2}\)
  - Extracellular membrane area: \(A_{\text{ac}} = 0.27 \, \text{m}^{2}\)
  - Calcium release from second release compartment: \(\tau_{\text{rel}} = 5 \, \text{ms}\)
  - The triggering threshold of calcium release from second compartment is 5 ms
  - First calcium release compartment: maximum conductance, \(G_{\text{ac}} = 6 \, \text{ms}^{-1}\)
  - Second calcium release compartment: maximum conductance, \(G_{\text{mr}} = 0.046 \, \text{ms}^{-1}\)
  - L-type Ca\(^{2+}\) channel: \(P_{\text{m}} = 1.62 \times 10^{4} \, \text{cm/s}\)
  - \(K_{\text{rel}} = 0.27 \, \mu \text{mol/L}\)
  - Voltage-dependent outward current \(I_{\text{act}}\):
    - Maximum conductance: \(G_{\text{r}} = 0.154 \, \sqrt{V_{\text{k}} / 5.4}\)
    - Voltage-independent \(K^{+}\) current:
      - Maximum conductance: \(G_{\text{ac}} = 0.382 \, \sqrt{V_{\text{k}} / 5.4}\)
  - Initial values:
    - \(K_{\text{m}} = 140 \, \text{mol/L}; \, K_{\text{rs}} = 131.4 \, \text{mol/L}; \, C_{\text{m}} = 0.07 \, \mu \text{mol/L}\)
    - \(C_{\text{cm}} = 1.73 \, \mu \text{mol/L}; \, C_{\text{ca}} = 1.73 \, \mu \text{mol/L}; \, V_{\text{c}} = -82.1 \, \text{mV}\)
  - Pacing stimulus:
    - Intensity = -80 \, \mu A/\mu F
    - Duration = 0.5 ms

References

1176 Stretch and Atrial Function


Mechanisms of Stretch-Induced Changes in $[Ca^{2+}]_i$ in Rat Atrial Myocytes: Role of Increased Troponin C Affinity and Stretch-Activated Ion Channels

Pasi Tavi, Chunlei Han and Matti Weckström

_Circ Res._ 1998;83:1165-1177
doi: 10.1161/01.RES.83.11.1165

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/83/11/1165