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Stretch-induced voltage changes in the isolated beating heart: importance of the timing of stretch and implications for stretch-activated ion channels

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Abstract

Objectives: It is now well recognized that myocardial stretch can cause arrhythmias due to stretch-induced depolarizations. The effects of transient stretch applied during the various phases of the cardiac action potential have not been investigated. This study (1) examined the effects of short stretch pulses and sustained stretch on the monophasic action potential (MAP) repolarization time course and diastolic potential, (2) examined the arrhythmic response to differently timed stretch pulses, and (3) tested by comparison with computer simulations whether these effects are compatible with stretch-activated channel characteristics known from patch-clamp studies. *Methods:* We studied the MAP changes elicited by short transient stretch pulses applied at different times during the cardiac cycle to 8 isolated Langendorff-perfused rabbit hearts. The left ventricle (LV) was instrumented with a fluid-filled balloon, the volume of which was altered rapidly and precisely by means of a computer-controlled linear motor-driven piston. MAPs were recorded simultaneously from one right ventricular (RV) and two LV sites while short volume pulses of increasing amplitude were applied to the LV at variable delays after the last of 8 regular electrical pacing stimuli. The effect of pulsatile volume pulses applied at different phases of electrical systole and diastole was compared to the effect of sustained stretch pulses (60 s duration) of the same amplitude. The experimental results were compared with computer simulations of stretch-induced effects on the action potential to further validate the experimentally measured effects with theoretical predictions based on the Oxford Heart model with added stretch channel terms. *Results:* Stretch pulses applied during early systole caused a brief transient *re*polarization during the LV MAP plateau phase, with a maximal amplitude of $24 \pm 10\%$ of the total MAP amplitude. Stretch pulses at the end of the MAP caused a transient *de*polarization, with a maximal amplitude of $13 \pm 5\%$. These oppositely polarized stretch effects crossed over during a transitional range of repolarization (mean $65 \pm 9\%$ of repolarization) when stretch produced neither transient repolarizations nor depolarizations. Only stretch pulses applied at a mean repolarization level of $77 \pm 5\%$ or later led to arrhythmias, preceded by transient depolarizations. No corresponding *de-* or *re*polarizations were seen in MAPs recorded simultaneously from the unstretched RV. The effects of long pulses on the MAP waveform were nearly identical to an overlay plot of the effects of many differently timed short transient pulses. When the stretch-induced voltage changes in the MAP were plotted against the repolarization level at which they were produced, a linear relationship was found (mean correlation coefficient $r = 0.97$; $P < 0.0001$) with a reversal at approximately half the total MAP amplitude. The computer simulations of the influence of stretch-activated channels reproduced both the effects of short and sustained stretch seen in the MAP recordings. *Conclusions:* We demonstrated in the isolated beating heart that the electrophysiologic effects of sudden myocardial stretch depend on the timing of the stretch relative to electrical systole or diastole. These findings are in agreement with patch clamp studies on stretch-activated ion channels which showed a linear current/voltage relation with a reversal potential between -20 and -30 mV. Only stretch pulses applied at the end of the action potential or during diastole elicit ectopic beats as a result of transient depolarizations, while stretch pulses applied during phase 2 and 3 cause transient repolarizations or no effect, respectively.

Keywords: Stretch activated channels; Stretch; Mechano-electrical feedback; Membrane potential; Monophasic action potential

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1. Introduction

It is now well recognized that myocardial stretch can cause changes in electrophysiological properties of the heart, a phenomenon known as 'mechano-electrical' or 'contraction–excitation' feedback [1–4]. Most in vitro and in vivo experimental studies have found myocardial stretch to shorten action potential duration (APD) [3,5,6] and refractoriness [2,7,8] while some investigators have also reported a lengthening of APD [3,9–11]. These in part conflicting findings have not yet been reconciled or sufficiently explained. Load- or stretch-induced electrophysiological changes also differed depending on whether mechanical stretch was applied in the form of an increased preload or increased afterload [2,12,13]. For example, 'early afterdepolarizations' have been attributed to myocardial stretch mediated by increased afterload [3,14,15], but they were rarely seen during increased preload. It has never been ascertained if these stretch-induced 'afterdepolarizations' are triggered by the preceding action potential like 'classical' early or delayed afterdepolarizations, or if they simply coincide with myocardial stretch occurring at that moment.

Recently, the existence of stretch-activated ion channels (SACs) has been documented in cardiac cells [16–19]. These SACs may explain the above referenced electrophysiological changes, and more importantly, the genesis of stretch-activated arrhythmias. SACs are present in virtually every cell, serving a variety of functions [20–23]. Recent studies on myocardial SACs found a linear current–voltage relationship for this type of channel, with a reversal potential (potential where the response reverses its polarity) near 50–60% of repolarization [24].

The purpose of this isolated beating heart study was threefold: (1) to investigate the changes that occur in monophasic action potentials (MAPs) when short stretch pulses are applied during different phases of the cardiac action potential, (2) to compare these stretch pulse-induced changes to those produced by sustained stretch, and (3) to examine if the stretch-induced voltage changes in the MAP would be consistent with the expected effects introduced by known myocardial SAC characteristics, using a computer simulation.

2. Methods

2.1. Isolated rabbit heart preparation and experimental setup

The techniques to prepare isolated Langendorff-perfused rabbit hearts and the experimental setup have been described in detail previously [25]. The protocol was approved by the institutional review boards of Georgetown University and the Veterans Administration Medical Center in Washington, DC. In brief, 8 New Zealand white rabbits (3–3.5 kg) were anesthetized by means of administration of pentobarbital, and after complete suppression of their hindlimb reflexes, 500 IU i.v. heparin were injected. A thoracotomy was performed, and the heart was removed quickly and briefly immersed in ice-cold Tyrode's solution until cardiac arrest. The aorta was then cannulated and perfused with warm (37°C) Tyrode's solution. The perfusate was equilibrated with 95% O_2 and 5% CO_2, and the pH was adjusted to 7.4. The perfusion flow rate was adjusted to 45 ± 12 ml/min, maintaining a perfusion pressure of 50–70 mmHg. The cannulated and perfused hearts were then mounted on a modified Langendorff apparatus. To slow the intrinsic heart rate, the AV node was destroyed by radiofrequency energy applied with customized tweezers. The heart was paced via a bipolar platinum hookup electrode from the right ventricle (RV).

2.1.1. Application of stretch

A latex balloon was inserted into the left ventricle (LV) and prefilled with a volume of 1 ml water. The balloon volume could be changed by a linear motor-driven piston pump. The pump was controlled from a Macintosh IIfx computer using custom-designed LabVIEW software (National Instruments, Austin, TX). The accuracy of the volume pulse was controlled by comparing the volume command signal with a feedback signal which represented the instantaneous position of the piston [25]. Although LV volume itself was not measured, it was made sure that the volume returned spontaneously to the pre-pulse volume after release of the long stretch pulse by means of equilibration with the atmospheric pressure. Additional software (also written in LabVIEW language) generated pacing stimuli sent to a stimulus isolator driving the heart at a constant cycle length of 400 ms from the RV hook electrode.

2.1.2. MAP recordings

A plastic ring with 3 evenly spaced cantilever arms holding silver–silver chloride contact MAP electrodes was mounted around the pump casing. Constant contact pressure of the electrodes was maintained by springs in the cantilever arms Electrodes were placed on the RV, the midanterior LV wall, and the lateral LV wall. Recordings obtained with these contact MAP electrodes have been shown to reproduce the time course of the transmembrane action potential with high accuracy [26,27]. In addition to an online printout on a strip chart recorder (Gould Electronics, Model TA 4000), all acquired data (MAPs, volume command and volume feedback signals) were stored in digital form (1000 samples/s).

2.2. Protocols

2.2.1. Short stretch pulses

After electrophysiological and mechanical steady-state was reached, the heart was subjected to short transient

stretch pulses of 50 ms duration. The durations of the upstroke and downstroke of this pulse were 20 ms, respectively, while the plateau duration of the pulse was 10 ms. The effects of differently timed stretch pulses were studied by changing the output delay of a fixed amplitude pulse (750 μl) relative to the pacing stimulus. First, starting from the pacing stimulus, systole was scanned in 5 ms increments. Where necessary the steps were decreased to 1 ms. During diastole, when little changes were observed between pulses, scanning steps were increased to 25 and 50 ms. In 5 experiments, the stretch pulse amplitude was varied from 200 to 750 μl.

2.2.2. Sustained stretch

Long static pulses were studied to compare their effects with those of the short pulses. While an overlay plot of an infinite number of feedback signals from short pulses with different timing would look identical to a single long pulse with the same amplitude, the movement velocity of the pump and thus also of the MAP electrodes is completely different. Accordingly, a comparison between the effects of short and long pulses with the same amplitude would allow important insight into whether the stretch-induced voltage changes might be a movement artifact or not. Long stretch pulses were applied with an amplitude of 750 μl, for a total duration of 60 s, and with slow rise and fall times of 2 s.

2.3. Data analysis

Digitally stored MAPs and pulse signals were displayed on screen for measurements with cursors. Superimposition was performed with another customized LabView software program and the MAP subjected to stretch was compared

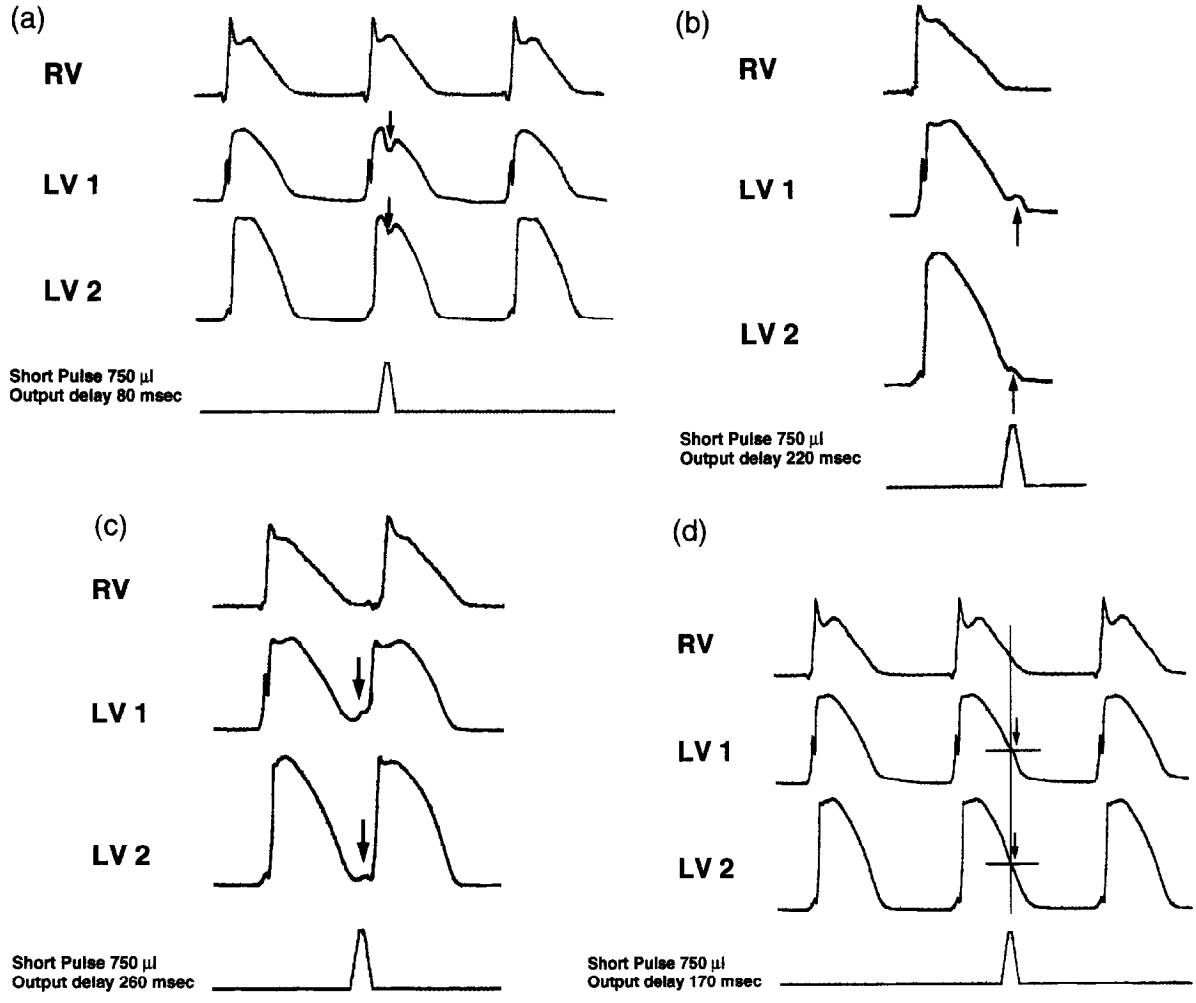


Fig. 1. *Panel A:* Example of a stretch-induced repolarization during early systole. Note that the timing of the repolarization is exactly coincident with the stretch pulse. No change is seen in the right ventricular monophasic action potential recording. *Panel B:* Example of a stretch-induced depolarization during late systole/early diastole. *Panel C:* Example of a stretch-induced premature ventricular contraction (PVC). Note that the highest amplitude is seen in the tracing where activation of the premature beat occurs earlier (LV2). *Panel D:* Example of an absent response to myocardial stretch during phase 3 of depolarization. Note also that due to the slightly different shape of the two stretched action potentials (LV1 and LV2) there is a minimal depolarization left in LV1 whereas in LV2 there is already a minimal repolarization. When studying these individual leads these responses could be further minimized by changing output delays in steps of 1 ms.

to the preceding normal MAP. Utilizing this method, the changes in the MAP subjected to a short stretch pulse could be easily discerned. The amplitude of a stretch-induced deflection was defined as its maximal vertical deviation from the superimposed normal signal and expressed as a percentage of the total MAP amplitude. Repolarization levels were determined as a percentage of complete repolarization from the crest of the action potential plateau. While stretch-induced voltage changes were usually seen in both LV MAPs, the greater of these two responses was quantified and is given in the results section. Data are expressed as mean ± standard deviation. A paired Student's *t*-test was used to compare the amplitude of the MAP before and under a long stretch pulse. Pearson's correlation coefficients were calculated for the relationship between voltage changes and repolarization level or pulse amplitude.

2.4. Computer simulation of stretch-induced responses

To test whether the stretch-induced changes in the MAP could be explained by known characteristics of SACs, a number of simulations were carried out. The appropriate 'Hodgkin-Huxley' equations were integrated using the program HEART (Oxsoft Ltd., Oxford, UK) as described in [28]. We chose to use the guinea pig model since it was the most complete and appeared not unlike the rabbit electrophysiology. We made several simplifications and assumptions in performing the simulations, but the goal was whether the basic picture was reasonable, not whether quantitative details can be duplicated. The main assumptions made were that (1) isopotential rather than conducted action potentials were a reasonable representation, (2) mechanical stresses were constant over the cell, and (3) a model for the stretch-induced conductance (below). The code was modified to include a stretch-activated current controlled by the sarcomere length L according to:

$$I_{sac} = -(V - V_{rev})\,\gamma\rho A/\left[1 + K\exp(-\alpha(L - L_0))\right] \quad (1)$$

where V is the membrane potential, V_{rev} is the reversal potential, γ is the single channel conductance, ρ is the channel density, A is the cell area, L_0 is a minimal sarcomere length, K is an equilibrium constant controlling the amount of current at L_0, and α is a parameter controlling stretch sensitivity. This is an equilibrium Boltzmann relationship for a non-inactivating three-state kinetic model (see Hamill and McBride [29] for examples of inactivation). In the absence of experimental information, we assumed the channel kinetics were instantaneous. With stimulation durations > 50 ms, this is probably reasonable for many SACs, but since the time constants are stress-dependent, at low stress the intrinsic time constants may become significant [30].

Eq. 1 is over-parametrized in terms of known constants, but we found it useful to express it using some values from the literature and to use a physically reasonable model for length dependence [31,32]. The channel conductance was taken to be 25 pS, a typical value for non-selective SACs [33]. The density was taken to be $0.3/\mu m^2$ [18]. The cell area was 2×10^4 μm^2, calculated from the 200 pF capacitance used in HEART and resulted in a maximal conductance of 0.15 μS/cell. The equilibrium constant K (nominally 100) and the stretch sensitivity α (nominally 3) were the only parameters adjusted to optimize the simulations over changes in length. L_0 was taken arbitrarily to be 1 μm. Simulations were done by using a stimulation interval of 0.3 s and measurements were made on the third action potential in the train so that initial conditions could relax to steady-state values.

3. Results

3.1. Effect of short stretch pulses during early systole

When transient stretch pulses of 50 ms duration were applied during the plateau of the action potential (Fig. 1A), a transient *re*polarization was seen in both LV recordings. The onset, overall duration, and end of this transient repolarization mirrored the waveform of the transient stretch pulse. No change was seen in the RV MAP recording. While the amplitude was different, the two LV MAPs showed the same polarity in response to a stretch pulse. The amplitude of the stretch-induced repolarization in the two LV MAP recordings was 24 ± 10% of the total MAP amplitude in the 8 hearts studied.

3.2. Effect of short stretch pulses during late systole, early diastole, and subsequent arrhythmia induction

When transient stretch pulses were administered during late systole or diastole, a *de*polarization was seen, again coinciding in time with the stretch pulse (Fig. 1B). The maximal amplitude of this depolarization occurred during phase 3 and 4 of repolarization and was in almost all cases followed by a premature ventricular contraction (PVC) (Fig. 1C), sometimes by couplets or short runs of ventricular tachycardia. The amplitude of this stretch-activated depolarization averaged 12 ± 8% of the total MAP amplitude. While the incidence of a stretch-activated PVC was 100% throughout diastole with the greatest stretch pulses (750 μl) administered, the earliest point in diastole when all of a series of 5 stretch pulses with the same output delay led to a PVC averaged 96 ± 3% of repolarization. With an incidence of at least one in 5 stretch pulses, PVCs were induced as early as 75 ± 5% of repolarization.

3.3. Response to stretch pulses applied during phase 3 of the action potential

A gradual crossover was seen between the two oppositely polarized stretch effects as described above (Fig.

1D). When applying the stretch pulse during early phase 3 of the MAP, the repolarizing deflection was less than during the plateau of the action potential. Similarly, stretch-activated depolarizations became smaller when the pulse was moved from full repolarization towards a less complete repolarization level. By moving the output delay of the stretch-pulse in 1 ms steps relative to the last pacing stimulus of the basic drive train a certain output delay could be found at which no or only a minimal change in the MAP (slight repolarization with transition into a depolarization due to the 50 ms width of the pulse) was seen. This 'neutral' response was calculated as the repolarization level at which the peak of the pulse intersected the action potential (Fig. 1D) and averaged 64 ± 10% repolarization for all experiments.

3.4. Superimposition of responses with different timing of stretch

To summarize and visualize the different responses in MAP amplitude and polarity when transient stretch pulses were administered at different levels of repolarization, voltage changes induced by transient stretch together with their respective stretch pulses were digitally superimposed for one experiment (Fig. 2A). Because of the gradual transition of the effects it was possible to connect the peak amplitudes with an enveloping line. Another transition between voltage changes with different polarity occurs during the upstroke phase of the MAP. When the transient stretch pulse was placed directly before the MAP upstroke, a stretch-activated depolarization was seen; a stretch pulse with an output delay only milliseconds longer led to a repolarizing deflection during the early plateau phase. With the stretch pulse timed exactly coincident with the upstroke, no response was noted.

3.5. Estimation of SAC current–voltage relationship from MAP data

The fact that the magnitude of stretch-induced negative deflections (transient *re*polarizations) and positive deflections (transient *de*polarizations) was greatest toward the maximum systolic and diastolic MAP voltage, respectively, with a crossover halfway in between, suggests that the current passing through SACs increases with voltage to both sides from a reversal potential. In order to relate the MAP data to a current–voltage relationship on the cellular level we plotted the amplitude of the stretch-induced MAP voltage changes (with a fixed pulse amplitude of 750 μl) against the repolarization level at which the peak of the stretch pulse occurred. Fig. 3A shows a representative example of this type of analysis from one experiment and suggests a linear 'current–voltage' relationship. While the average correlation coefficient was high with 0.97 ± 0.02, there were slightly different slopes between experiments. On average the linear regressions fitted an equation where

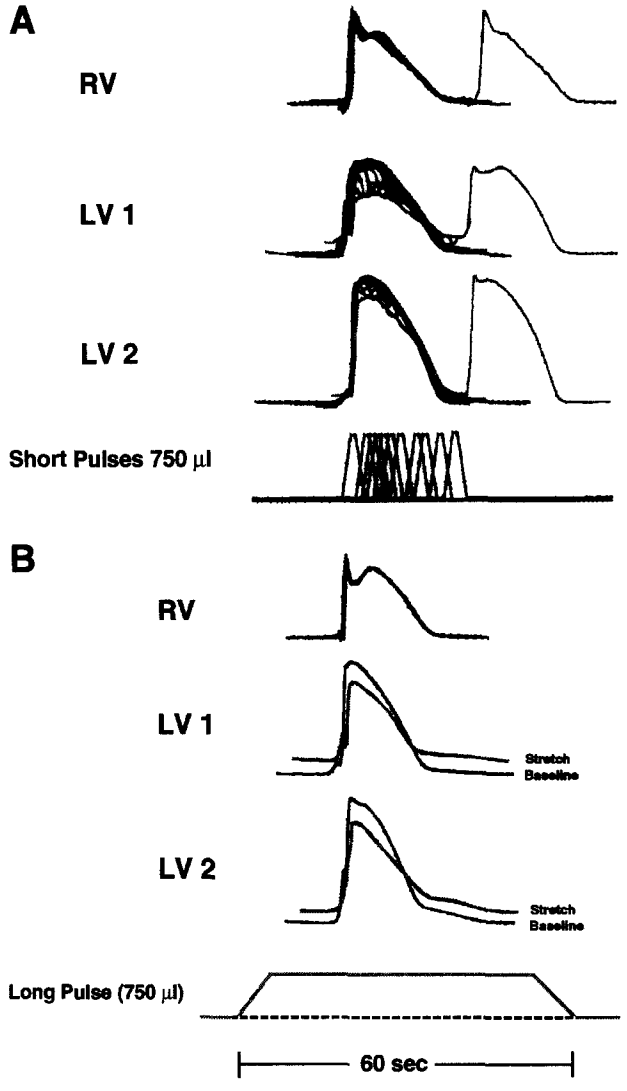


Fig. 2. Plot of baseline MAP recordings with overlay of several MAPs during short transient stretch pulses (A) and the steady-state MAP during a long static stretch pulse (60 s duration, B) of the same amplitude. Note that the enveloping line of the transient stretch pulses corresponds exactly to the real MAP during a long stretch pulse.

the slope was 0.36 ± 0.14 and the intercept was −23.1 ± 9.9 (Table 1).

3.6. Effects of long, static stretch pulses

In addition to applying short *transient* stretch pulses over a range of different output delays with respect to the MAP waveform, all hearts were also submitted to long, *static* stretch pulses. A close similarity was found between the effect of a *long* stretch pulse (60 s, Fig. 2B) and the envelope which summates the effects of all individual *short* stretch pulses (Fig. 2A). During long static stretch the LV MAP amplitude uniformly decreased within 5–10 beats to 72.3 ± 15.0% of the baseline ($P = 0.001$) and returned to a value of 102.1 ± 5.7% after stretch. There

was a non-significant change in the RV MAP amplitude to 94.8 ± 4.7% during long stretch with a value of 97.8 ± 2.7% after stretch. In general, no PVCs were seen with the slow ramp pulse delivered in this protocol. It was obvious from the MAP recordings that the onset of stretch effects was gradual over the first seconds of the long stretch pulse and that the effects on the MAP were maintained without any change throughout the 60 s of the stretch pulse. Directly after release of the long stretch pulse the MAP shape and duration returned to the pre-stretch state.

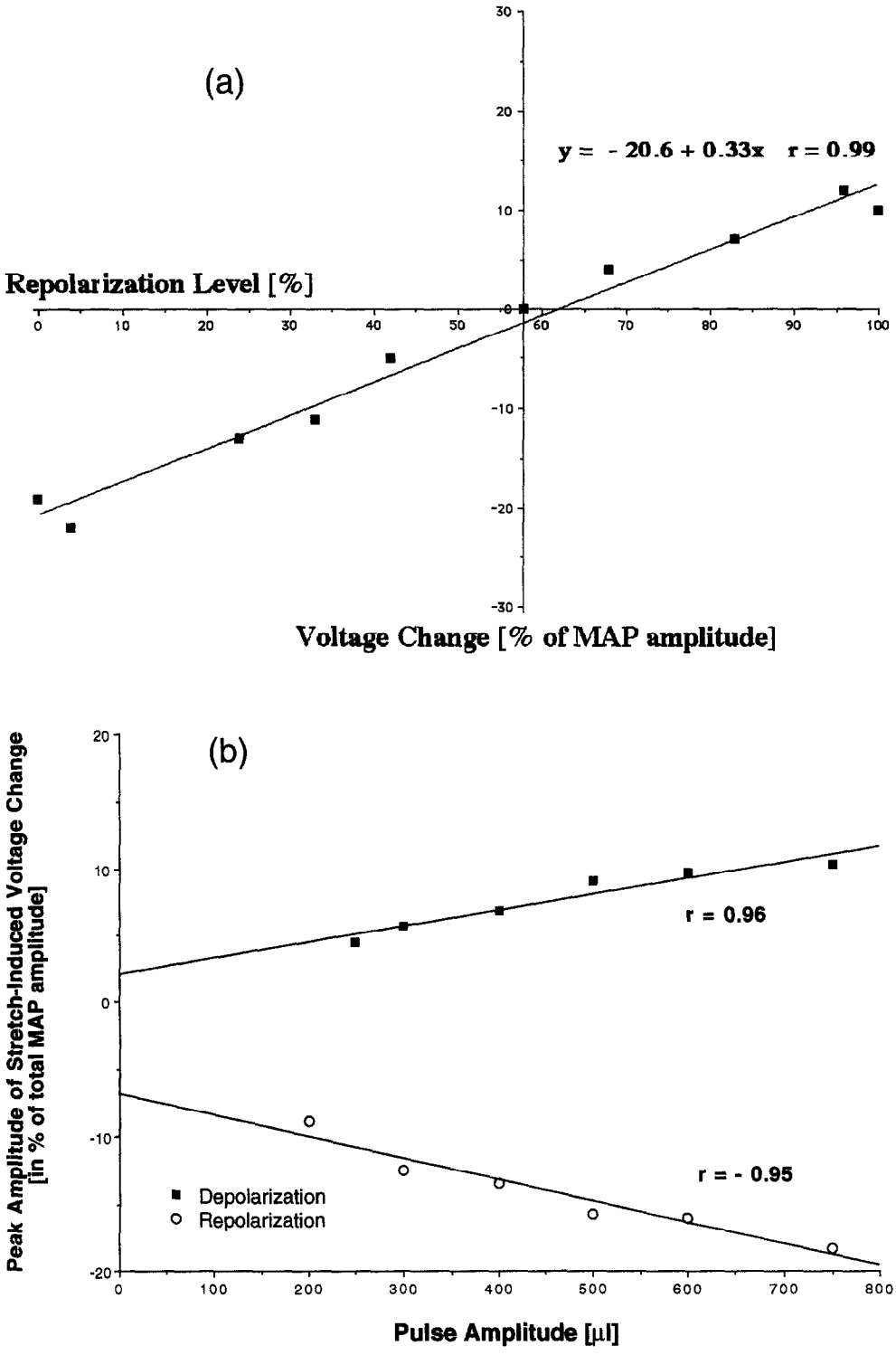
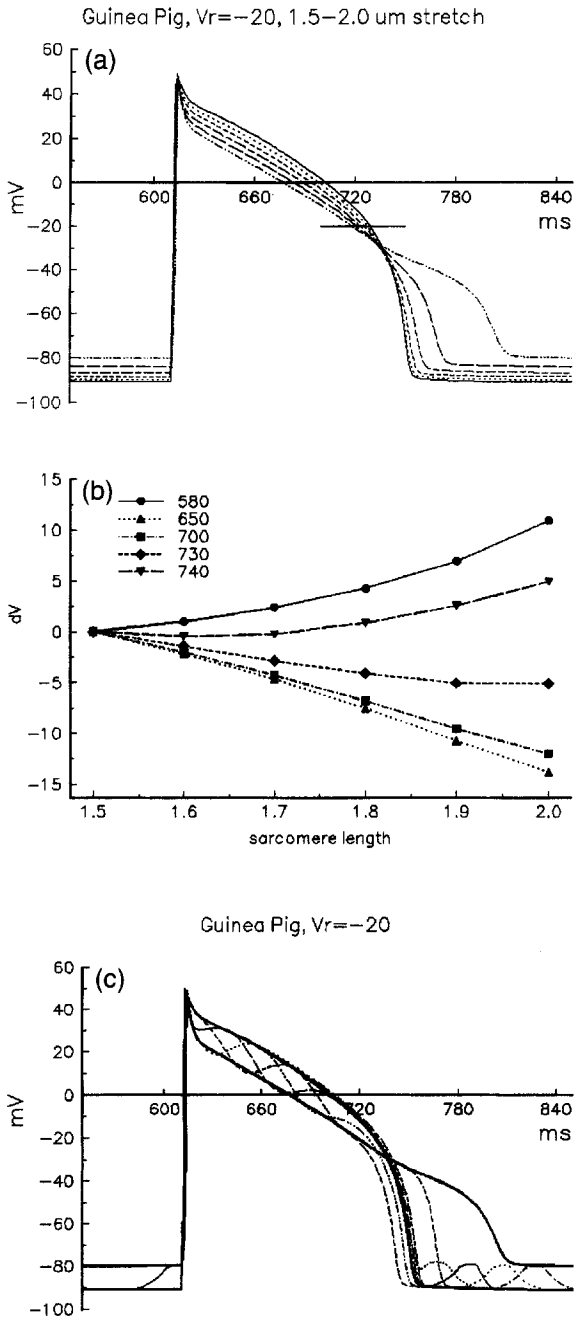


Fig. 3. (A) Plot of stretch-induced voltage changes (in % of MAP amplitude) against level of repolarization. A stretch pulse amplitude of 750 µl was applied. (B) Relationship between the amplitude of short transient stretch pulses and the amplitude of stretch-induced depolarizations and repolarizations. The x-intercept is the level of repolarization at which stretch induces no change in potential.

Table 1
Intersect, slope and correlation coefficient for the linear regression of voltage changes vs. respective repolarization level in each experiment

Experiment no.	Intersect	Slope	r-Value	P-value	No. of observations
1	−15.7	0.22	0.98	< 0.0001	11
2	−22.3	0.37	0.96	< 0.0001	9
3	−39.5	0.56	0.99	< 0.0001	9
4	−14.1	0.22	0.93	< 0.0001	11
5	−37.4	0.57	0.99	< 0.0001	11
6	−20.6	0.33	0.99	< 0.0001	10
7	−18.5	0.32	0.98	< 0.0001	8
8	−17.5	0.24	0.95	< 0.0001	10



3.7. Correlation between amplitude of stretch-pulses and stretch-induced voltage changes

While stretch-induced voltage changes induced by a high amplitude (750 μl) volume pulse were shown to be dependent on the timing and the instantaneous repolarization level, we also studied the effects of volume pulses of variable amplitudes administered during the diastolic baseline and the plateau of the MAP in 4 experiments. Stretch-activated depolarizations could be induced even with an absent basic drive train (i.e., in an asystolic heart). The

Fig. 4. Computer simulations. *Panel A:* Superimposed traces of guinea pig action potentials obtained at different degrees of steady-state stretch, 0.1 μm increments from 1.5 to 2.0 μm. The longest actions potentials are the most stretched. The horizontal bar near 720 ms represents the reversal potential for the stretch-activated current. Notice that at the crossover potential (the potential that is minimally affected by stretch) is about 10 mV negative to the true reversal potential. $V_r = -20$ mV. The large increase in duration with the large stretch is not observed in the MAP. This may represent the difference between the isopotential response (the simulation) and the conducted response (the MAP). In addition, the MAP represents a spatial average of the conducted response that will smooth out variations in the time course of repolarization. *Panel B:* Proportionality between the magnitude of stretch and the change in voltage at various times during the simulated action potential (same data as Fig. 4A). The ordinate dV represents the change in voltage (relative to an action potential calculated with a sarcomere spacing of 1.5 μm) at the times indicted in the legend (in milliseconds corresponding to Fig. 4A). $T = 0$ represents the beginning of the simulation. Notice that for diastole and much of the plateau, the relationship is surprisingly linear despite the voltage dependence of many of the rate constants. Compare to Fig. 3B. Although the physiological experiments were carried out by volume injection rather than linear strain, the calculated linearity will not be affected. In the crude approximation of the ventricle as a thin-walled sphere, the linear wall strain is: $1/1_0 = 3D((V/V_0)^{1/3}$ where V/V_0 is the relative change in volume. In the range of $V/\mathrm{i}V_0 = 3D1{-}1.5$, the linear strain is almost exactly proportional to V/V_0 with a correlation coefficient > 0.999. *Panel C:* Comparison of the transient and steady-state effects of stretch on the action potential. Trapezoidal pulses (20 ms rise and fall time, 10 ms plateau) were applied every 20 ms beginning at 580 ms into the simulation. The thick lines represent the steady-state responses while the multiple thin lines represent the pulsatile responses. As before, the longest action potentials arise from the most stretched cell. $V_r = -20$ mV.
The crossover of the action potentials near −30 mV is a characteristic feature of stretch-induced effects in many experiments [33].

preparation exhibited stretch-induced beats when the stretch-activated depolarizations reached threshold with a pulse volume of 500 μl. Fig. 3B shows the peak amplitude of the stretch-activated depolarizations and repolarizations (in % of total MAP amplitude) plotted against the increase in volume. Both relationships exhibited a linear or inversely linear correlation, respectively ($r = 0.96$ and $r = -0.95$).

3.8. Computer simulation of stretch-induced responses

The first simulations were directed at looking at the effect of steady stretch on the action potential shape. Fig. 4A shows a set of simulations done at 0.1 μm intervals from 1.5–2.0 μm. Stretch depolarizes the diastolic potential, speeds the early phase of repolarization and delays the later phase of repolarization. The crossover (i.e., the potential of minimum stretch effect) occurs near −30 mV and is about 10 mV negative to the reversal potential of −20 mV (horizontal bar near 720 ms) used in this simulation. These results were similar to the experimental data shown in Fig. 2B. We varied the reversal potential and found that negative to −30 mV, crossover occurred almost at the end of repolarization. At −25 mV the results were similar to −20 mV but with a decreased duration of late repolarization, and at 0 mV, surprisingly, there was again no crossover until the end of repolarization. Thus, to reproduce the effects of crossover near the middle of repolarization, the reversal potential of the stretch-induced effect had to be in the range of −20 to −25 mV. This compares well with the -23.1 ± 9.9 mV crossover found in the experimental studies.

In analogy with Fig. 3B, we examined the amplitude response at different phases of the action potential to look for the observed linearity in what is a very non-linear system. To simplify the analysis, steady-state stretches were used. With the data from Fig. 4A, the change in voltage (relative to the response at 1.5 μm) was plotted at selected times as a function of the degree of stretch. Fig. 4B shows the results. Over a wide range of times during diastole and early systole, the response is surprisingly linear. However, during the late phase of repolarization, the response is clearly non-linear since the action potentials have differing duration (data not shown, but the effect is clear from Fig. 4A). In general, there is a good correspondence between the experimental and simulated data.

To examine the effect of transient stretches (in analogy with Fig. 2A), we drove the simulation with trapezoidal pulses having 20 ms rise and fall times and 10 ms plateaus as well as using steady-state maximal and minimal stretches. Fig. 4C shows the results of one study. The dark traces outline the steady-state responses with the longest response belonging to the stretched cell. The thin lines show the transient responses administered at 20 ms increments. The steady-state responses (static stretch) form an envelope for the transient responses during diastole and much of early repolarization. Over the latter third of repolarization, the transient responses leave the envelope.

4. Discussion

4.1. Stretch-induced voltage changes and timing of myocardial stretch

One key result of this study in the intact isolated rabbit heart is that the occurrence of stretch-induced voltage changes in the MAP, depolarization or repolarization, as well as stretch-activated arrhythmias are clearly dependent on the timing of the stretch pulse with respect to the action potential phase. A short transient stretch pulse elicited either transient depolarizations when applied during late systole or diastole, or transient repolarizations when applied during the plateau of the MAP. Thus, stretch-induced voltage changes vary in polarity depending on the timing of stretch. A stretch pulse placed towards the end of the MAP caused depolarizations which mimicked 'early afterdepolarizations' or, if placed after the MAP, 'delayed afterdepolarizations'. In contrast to 'classical' early or delayed afterdepolarizations [34,35] the stretch-induced depolarizations did not depend on the trigger of a preceding action potential. The time relationship between these depolarizations and the preceding action potential depended solely on the output delay of the stretch pulse. Action potential duration was not affected by the short pulses used in this study.

4.2. Amplitude and polarity of stretch-induced voltage changes

It has been demonstrated recently that the amplitude of a stretch-induced *de*polarization during diastole is linearly correlated with the amplitude of the underlying stretch-pulse [14]. These findings were confirmed in this study (Fig. 3B). We could further extend these data by showing that the amplitude of a stretch-related *re*polarization during the plateau phase of the MAP exhibits a similar linear relationship to the stretch pulse amplitude. Approximately halfway between these opposing responses, a gradual transition occurred with a neutral response at about 50% repolarization. Our study confirms previous data by Lab [10] in isolated strips and from whole frog ventricles which showed opposite electrophysiological stretch effects, depending on the action potential phase. Action potential changes in this study were assessed by the insulated (sucrose)-gap technique which is not applicable to an intact heart, or by suction electrodes in intact beating heart which are more prone to artifact as compared to the contact electrode method used in this study [36]. These initial data were subsequently not explored in a mammalian heart.

Our study is the first to systematically study the effects of pulsatile stretch on simultaneously recorded MAPs in a whole heart preparation, to make plausible the concept of stretch-induced electrophysiological changes which are opposite to each other depending on the timing of the stretch pulse and with regard to the instantaneous action potential time course. This study demonstrates in an intact mammalian heart preparation the phenomenon that there is a crossover or reversal potential for stretch effects.

4.3. Comparison of stretch-induced changes between the two LV electrodes

Apparently, the LV myocardium is not subjected to the same stretch along its circumference which is most likely explained by a varying compliance of the LV wall. Fig. 1D shows that the earliest activation of the stretch-activated PVC is seen with the LV electrode that demonstrates the greatest amplitude of the stretch-activated depolarization. The site of origin of a stretch-activated PVC therefore does not seem to be random but is likely the site subjected to the highest level of stretch. The emergence of a stretch-induced PVC from a 'take-off' potential reaching threshold has been reported previously [14,37]; however, this group reported a second pattern of PVC induction, namely a delayed induction of a PVC which was explained with an accelerated phase 4 depolarization of Purkinje fibers. This pattern was not observed in our study. A possible explanation is that one exploring MAP electrode as used by Stacy et al. [14] would rarely be positioned by chance at the LV site where the PVC is probably induced. It could well be that more simultaneous MAP electrodes could give more insight into this phenomenon.

4.4. Interpretation of whole heart studies in view of reported SAC characteristics

SACs have been demonstrated in various non-cardiac tissues [20–23,38–42] and, recently, also in myocardial tissue [16–19]. While more cellular studies are needed, it is likely that the myocardium contains SACs capable of providing both inward and outward currents [16–19]. The only available whole cell recordings of mechanosensitive currents in heart indicate a reversal potential in the range of -15 to -18 mV at room temperature [43]. Simulating the behaviour of the action potential with inclusion of SAC characteristics from patch-clamp studies demonstrated a remarkable similarity between the simulation and the actual results. Activation of SACs during the diastolic resting potential should result in an inward current which leads to a depolarization. Conversely, SAC activation during the MAP plateau should result in an outward current which leads to a repolarization. Both voltage changes are actually seen in this study. The reversal potential of whole cell mechanosensitive currents, -15 to -18 mV [42], agrees well with the experimental results shown here. According to the simulations, the crossover potential (between depolarizing and hyperpolarizing effects of stretch) is actually about 10 mV negative to the SAC reversal potential (Fig. 4A). Although the simulation results do not mimic all the effects of stretch as anticipated by the MAP recordings, it is surprising how well the results agree. Deviations between our experimental data and the computer simulations occur mainly in late repolarization. Comparing the experimental results of Fig. 2B and the simulation results of Fig. 4A reveals that the simulation shows a wide dispersion in the time course of late repolarization whereas the data show a narrow dispersion. It is difficult to assess the significance. The time course of late repolarization is very sensitive to small changes in current. Since MAP recordings arise from propagating action potentials averaged in space (defined by the dimension of the exploring MAP electrode) [36], it is possible that inhomogeneities in the late phases of repolarization are averaged out, reducing dispersion. We anticipate that multidimensional modelling in both electrical and mechanical parameters of the heart [43] under these conditions will show a reduced variability of the volume-conducted action potential. It is beyond the scope of this paper to exactly model all the expected and observed changes in the action potential during stretch. However, the addition of the computer modeling to the intact heart results allows the suggestion that the observed effects of stretch on the MAP can be explained by a reasonable modification to existing models of heart action potentials.

Because the MAP is almost identical in shape and changes to the transcellularly recorded action potential [26,27], one can assume a reasonably good correlation between voltage changes in the MAP and ion flow on a cellular level given that all other factors remain constant. A remarkable similarity is then found between the voltage change to voltage relationship exhibited in this study (Fig. 3) and what appears to be a linear current–voltage relationship reported for many SACs [16–19]. The computer simulations suggest that in the isopotential case, this relationship is not truly linear.

4.5. Interpretation of the findings in view of other mechano-electrical feedback studies

Although very short stretch pulses do not affect action potential duration, the voltage-dependent changes in the polarity of the stretch-induced change on the action potential may help explain why myocardial stretch has been reported to shorten, lengthen or not change APD, depending on the various experimental settings and methods of data analysis [3,5–11], and possibly reconcile controversial views in the past. It is conceivable from the results of this study why APD can be modulated by stretch in opposite directions. Stretch shortens APD at early repolarization levels (repolarization), does not change it at midway repolarization (neutral response) and lengthens APD at final

repolarization levels (depolarization). These observations can occur independently or at the same time depending on the duration and timing of stretch. The latter has been shown in an earlier study by Franz et al. [3]. Moreover, the differences between the mechano-electrical feedback effects of an increased preload versus those of an increased afterload [12,13] may now be in part explained. While an increased afterload increases mechanical stretch predominantly during the time of peak systolic pressure or slightly later, corresponding to repolarization levels of 50–80%, one would expect no change or an 'afterdepolarization' as a response. In contrast, an increased preload would lead to mechanical stretch throughout the cardiac cycle, leading to shortening of the action potential at early repolarization levels and possible lengthening at final repolarization. Similar observations were made by Hansen et al. [12]. Earlier data from this laboratory [25] show that the rate of change (of the stretch pulse) plays an independent role for the generation of stretch-induced arrhythmias. The current study compared extremely short stretch pulses with long stretch pulses of the same amplitude. The fact that the short pulses with a fast rate of change and the long pulses with a slow rate of change lead to similar voltage changes in the MAP suggests a more important role of stretch amplitude rather than change in amplitude.

4.6. Possibility of movement artifacts in MAP recordings

In this study, as in previous publications [3,14,25], the question arises whether some of the stretch-induced voltage changes represent movement artifacts. In the intact beating heart no 'gold standard' technique is available to absolutely prove that MAP recordings are artifact-free. However, for the following reasons we believe that the electrical changes recorded are not artifacts. First, the RV MAP electrode, mounted on the free wall, never showed significant changes in response to the stretch pulse which increased the volume of the LV and led to voltage changes in both LV MAP recordings (see Fig. 1A–C, 2A,B). This occurred although the RV electrode, riding on the LV, was subjected to a similar movement compared to the LV electrodes. Second, the similarity between effects of short and long stretch pulses (Fig. 2A,B) is important. There are no sudden mechanical perturbations of the heart during static volume loading, yet the net effect on the MAP waveform is the same as with short stretch pulses. Third, it is not clear why a movement artifact should change its polarity. Fourth, the arrhythmias seen in diastole and emerging from a 'take-off' potential support the notion that these potentials are real. Additional arguments were given in Franz et al. [3,14,25].

4.7. Conclusions

Both amplitude and polarity of stretch-induced voltage changes in the monophasic action potential depend on the amplitude and the timing of the administered stretch. Stretch-induced voltage changes vary in polarity depending on the timing of stretch. Even though they may mimic early or delayed after depolarizations if stretch is applied in late systole or early diastole, they differ from them by not requiring a preceding action potential as a trigger. The voltage changes recorded from an intact heart by MAP technique match reported characteristics of stretch-activated ion channels and are adequately predicted by a computer simulation, further supporting the existence of these channels in the myocardium. These data may have widespread clinical implications in scenarios where ventricular myocardium is exposed to regional or global ventricular wall stretch.

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