

Factors governing mechanical stimulation in frog hearts

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Fasciano, Robert W., II, and Leslie Tung. Factors governing mechanical stimulation in frog hearts. *Am. J. Physiol.* 277 (*Heart Circ. Physiol.* 46): H2311–H2320, 1999.— Because stretch-induced activation may be important in generating clinically relevant arrhythmias in the heart, we delineated the ability of different types of stretches to activate ventricular tissue. Geometrically simple sheets of frog (*Rana catesbeiana*) ventricular tissue were mounted to allow stretches to be applied perpendicular to one edge. Every heart could be activated by a stretch pulse ($n = 25$), and several parameters were varied to determine their effects on mechanical activation threshold. At shorter coupling intervals, a larger stretch was needed to excite the tissue, and activation-recovery intervals were shorter, similar to previously published electrically probed strength-interval and restitution relations. Additionally, the tissue became easier to activate as the speed of the stretch increased from 0.09 to 2.6% length/ms. The increment in stretch needed for activation decreased as the baseline stretch increased from 0 to 6% length. Thus we show that mechanical activation is similar to electrical activation and that increasing uniquely mechanical parameters such as the speed of the applied stretch or baseline level of stretch can decrease the mechanical activation threshold.

stretch; mechanoelectric coupling; mechanotransduction; membrane currents; arrhythmia

SEVERAL CLINICAL STUDIES have shown that abnormal mechanical forces in the heart are important predictors of susceptibility to arrhythmia (29, 51) or mortality (6, 38). This evidence showing that mechanics plays an important part in some clinical outcomes has fueled mechanoelectric coupling (MEC) research. MEC is the phenomenon by which electrical changes in tissue are brought about by mechanical changes. In the heart, MEC exists in preparations as varied as mollusk heart cell patches (45) and the in situ human heart (47). One way in which MEC may lead to clinical arrhythmias is through induction of ectopic foci. For instance, ectopic foci in the heart can cause unidirectional block (30), which is a potential substrate for reentry (25) that may then progress into life-threatening fibrillation (18, 21). Because mechanically induced ectopic foci could have such serious clinical consequences, we undertook this study to further characterize whether different types of stretch can elicit activation in heart tissue.

We chose to apply the stretches to frog ventricular tissue for several reasons. Frog tissue is much easier to maintain than mammalian tissue. This ease allows recordings to be taken for long periods of time in the same tissue, which is necessary to obtain many data

points, particularly because, in these experiments, each data point can take up to 40 min to generate. This tissue has also been previously shown to have mechano-electrical sensitivity (32). Reduced significance of the sarcoplasmic reticulum in the frog (27) also allows the interpretation of the data to be simplified, because we can dismiss stretch-sensitive release of calcium from the sarcoplasmic reticulum (28) as an underlying MEC mechanism. Because the frog heart relies on superfusion for oxygenation, one may choose almost any desired shape for the tissue without worrying about perfusion limitations and tissue viability, as would be the case with mammalian hearts. We chose to use a rectangular sheet of tissue because this is a simplified tissue geometry, particularly in comparison with the complicated geometry of the whole heart. Although not a perfect two-dimensional structure, this shape allows us to approximate the strain pattern in the tissue as homogeneous and equal to a constant percent change from the original length. Additionally, similarly shaped tissues are capable of supporting reentry (9, 41), a potential area for future studies with this preparation. Several researchers (4, 16, 23, 32) have shown that mechanical perturbations can lead to electrical activation of heart tissue at the organ level. Thus once it is known in which ways a mechanical stimulus mimics an electrical one, it may be possible to envision or even design situations in which a mechanical perturbation can induce self-sustained electrical activity.

In this study, we used our ability to control the shape and timing of the stretch pulse that was applied to the tissue to determine the level of the mechanical activation threshold and its reproducibility. We also determined how the threshold varied as a function of stretch duration, rate of rise and fall of the stretch pulse, variation of baseline stretch, and refractory state of the tissue. Finally, we determined the effect of refractory state on the duration of the mechanically elicited electrical activity.

A preliminary version of this study was presented in abstract form (13).

METHODS

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85–23, Revised 1996).

Animal. Adult 5- to 6-in. American bullfrogs (*Rana catesbeiana*) of either sex were pithed and decapitated. The heart was excised and rinsed with Ringer solution so that no blood clots were lodged in the ventricle. The Ringer solution consisted of (in mM) 110 NaCl, 3 KCl, 10 HEPES, 10 glucose, and 1 CaCl₂, at pH = 7.26. The ventricle was dissected away from the rest of the heart, and potentially autorhythmic atrioventricular ring tissue was removed. The ventricle was cut along the shorter of the two remaining sides and spread flat. The tissue was trimmed to form a rectangle with approximate dimen-

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sions of 1.5×1.0 cm. The tissue was maintained at room temperature during the experiment.

Setup. After the tissue was allowed to recover in fresh Ringer solution for 30 min, the tissue was glued to the tissue supports as shown in Fig. 1 with a cyanoacrylate glue (262 Adhesive, Permabond, Englewood, NJ). The chamber was filled with Ringer solution. The left edge of the tissue was attached to a linear motor (model 203, Ling Dynamic Systems, Yalesville, CT) to apply stretch along a line perpendicular to the edge of the tissue (i.e., a linear stretch), and a displacement transducer (model 242, Trans-Tek, Ellington, CT) was used to measure the stretch. The stretch pulse applied to the tissue had a small overshoot of up to 2%, which lasted up to 20 ms. The tissue force in the direction of stretch was monitored with two force transducers (BG-25 & BG-50, Kulite, Leonia, NJ) via two 0.13-mm-diameter spring steel wires (SWGX-050, Small Parts, Miami Lakes, FL), and the sum of the forces was adjusted to 0.5 g, which defined our 0% stretch level. After the first few high-value stretches, this force may decrease due to some nonelastic deformation, so typically we ignored the first few trials in each experiment. This dual force-measurement system allowed twist to be measured in the tissue. Twist induced via stretch pulses was reduced by monitoring the difference in forces during a $67\text{-}\mu\text{m}$ stretch and then adjusting the positions of the force transducers. Two sintered silver-silver chloride pellet electrodes were positioned as shown in Fig. 1, one on the floor of the chamber and one on the left support (not visible in the diagram). These pellets measured the electrocardiogram (ECG) and were preferentially sensitive to propagation in the direction of a line joining the electrodes (*left to right* in Fig. 1).

Diastolic threshold for the electrical S1 pulse was determined by stimulating at no faster than 0.1 Hz, with a total biphasic pulse width of 20 ms (10–10 ms) using an isolated pulse stimulator (model 2100, A-M Systems, Everett, WA). Biphasic pulses were used to eliminate polarization of the electrodes, which could result in artifactual shifts in excitation threshold. The stimulator voltage was increased from 1 V, in 0.25-V steps, until an action potential was elicited (as determined by ECG and force recordings). The S1 stimulus was then set to twice the diastolic threshold. The tissue was stimulated continuously at 0.5 Hz until both diastolic and developed force stabilized as monitored on an oscilloscope

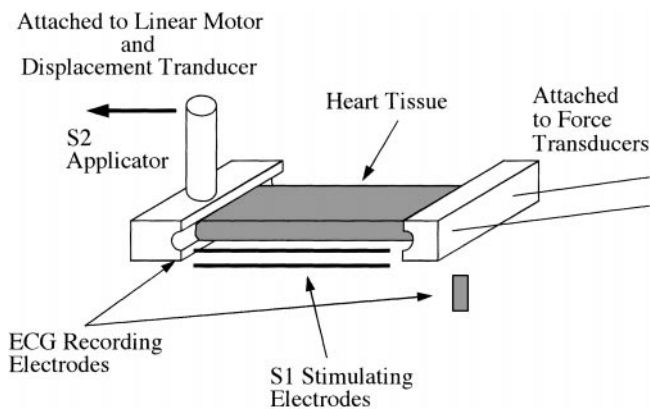


Fig. 1. Schematic representation of mounted experimental preparation. Frog ventricular tissue is shown at center, glued to two custom-machined acrylic supports. Right support is connected to two spring steel wires that lead to two force transducers. Left support is connected to a linear motor that applies mechanical stretches (S2) to the tissue. In front of the tissue are stimulating electrodes that apply electrical pulses (S1).

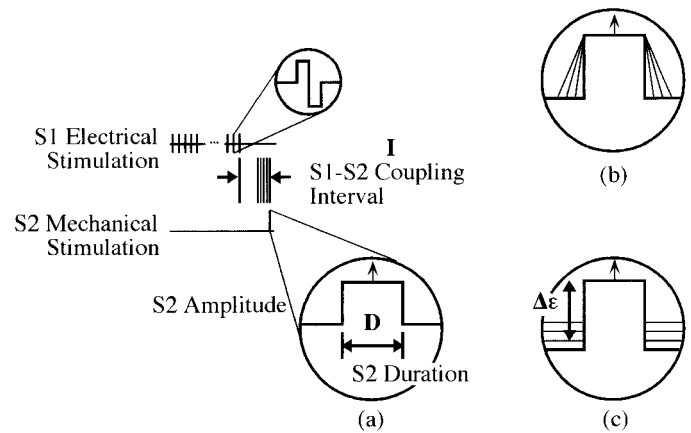


Fig. 2. Timing protocols for various experiments. General timing protocol used in these experiments is shown at left side. S1 stimulation was electrical, whereas S2 was a mechanical stretch of the tissue. Rectangular pulse shown in *inset a* was used for strength-duration, strength-interval, and restitution experiments. Duration of stretch pulse (D) was varied in strength-duration experiments. Interval (I) between last electrical stimulation and mechanical stretch was varied in strength-interval experiments. *Insets* show other types of stretches that were tested. Replacing *inset a* with *inset b* yields protocol for speed of stretch experiments. *Inset b* shows that the slopes of rates of rise and fall of stretch pulse were varied symmetrically. Protocol for baseline stretch experiments is shown in *inset c*, which shows that level of baseline stretch was changed. Also defined in *inset c* is quantity " $\Delta\epsilon$," the amplitude of stretch pulse relative to baseline stretch.

(model 2212, Tektronix, Beaverton, OR) after a period of ~ 40 min.

Protocol timing. Because the timing protocol was similar for the different sets of experiments, the protocol for the strength-duration experiments is described in detail, and then the aspects that varied for the other protocols are described. Timing for the protocols is shown schematically in Fig. 2.

Each strength-duration experiment was started by setting several parameters that remained unchanged during the experiment. A minimum stretch level (ϵ_{\min}) was set below the threshold level for mechanical stimulation at long durations (typically 6% of the tissue length). A maximum stretch level (ϵ_{\max}) was set to 26% of the tissue length. The coupling interval (I) between the last of a train of electrical stimuli and the mechanical test pulse was set to 2 s. The stretch pulse was rectangular as shown in Fig. 2, *inset a*, and had large rates of rise and fall (~ 26.7 cm/s). The first experimental trial started by reading in a stretch duration (D), which varied from 50 to 150 ms in a predetermined but randomized order. Starting at an amplitude of ϵ_{\min} , S2 stretches, preceded by at least 50 electrical S1 stimuli at a constant rate of 0.5 Hz to condition the tissue to a steady state, were applied at amplitude increments of ϵ_{inc} (2% of the tissue length in four experiments, 1% in one other) until an activation was elicited or ϵ_{\max} was reached, whichever came first. A new stretch duration was set, and a new trial was started.

The timing for the strength-speed experiments was similar to the strength-duration timing and is shown in Fig. 2, *inset b*. Instead of the duration, the rates of rise and fall of the pulse were varied, symmetrically, between 1.07 and 26.7 cm/s. The duration of the pulse (measured at peak amplitude) was fixed at 50 ms, ϵ_{inc} at 2% of the tissue length, and coupling interval at 2 s.

In the protocol used to explore the dependence of mechanical threshold on baseline stretch (Fig. 2, *inset c*), the baseline stretch was varied from 0 to 6% of the tissue length in increments of 0.25%. Stretch duration was 50 ms, rates of rise and fall were 26.7 cm/s, and the coupling interval was 2 s. ϵ_{inc} of between 0.3 and 2% of the tissue length were used in different experiments.

The timing for the strength-interval experiments is also described by Fig. 2, *inset a*. The coupling interval (I) was typically varied between 0.9 and 2.9 s. In a few experiments, coupling intervals were as short as 0.5 s. The stretch duration was 50 ms, rates of rise and fall were 26.7 cm/s, and ϵ_{inc} was 2% of the tissue length. Data from these experiments were also analyzed to generate restitution curves.

Data acquisition and sampling. The signals were sampled at 1 kHz by a data acquisition board (Lab-NB, National Instruments, Austin, TX), controlled by a custom-designed LabVIEW interface running on a desktop computer (7100/80AV, Apple Computer, Cupertino, CA). The ECG signals were amplified by monolithic instrumentation amplifiers (AD620, Analog Devices, Norwood, MA). The displacement transducer signal was filtered at 500 Hz by an eight-pole, low-pass, switched capacitor Bessel filter (MAX292, Maxim, Sunnyvale, CA) set to remove noise from the transducer's internal oscillator.

Statistical analysis. Data from the strength-duration, strength-speed, and baseline stretch experiments were first fit with a least squares regression line for each trial. A two-sided t -test for linear regression tested the null hypothesis that the slope of the line is equal to zero. To adjust for variability between different hearts, y offsets were added (preserving slope) to the data from individual hearts so that regression lines of individual experiments passed through the same point in the middle of the x range as did the regression line of the grouped data. Data among experiments of each type were then combined. In all slope tests, a P value of <0.05 was considered to be significant.

Data from the strength-interval experiments were combined by normalization to the stretch level needed to activate the tissue at long coupling intervals (rheobase). The data were then binned, and single-sided Student's t -tests were performed to compare the mean of each bin to the stretch at long coupling intervals, with the null hypothesis that the bin mean is less than or equal to the mean at long coupling intervals. A single-sided test was done because the threshold was expected to increase at shorter coupling intervals (by analogy to electrical strength-interval relations). A P value of <0.05 was considered to be significant.

RESULTS

Can a linear stretch activate cardiac tissue? In every heart preparation used in this study ($n = 25$), activation could be elicited via mechanical stimulation. Figure 3 compares the ECG (Fig. 3A) and force (B) recordings elicited by an electrical and a mechanical stimulus in the same preparation. The downward arrows indicate the start of both the electrical and mechanical stimulation pulses. The electrically elicited ECG recording was obtained 2 s before the mechanically elicited one. Both ECG traces in Fig. 3A are similar. After a latency of ~ 200 ms, the polyphasic deflection indicates the occurrence of tissue depolarization. Approximately 1 s later, a monophasic deflection occurs, which indicates tissue repolarization. Estim-

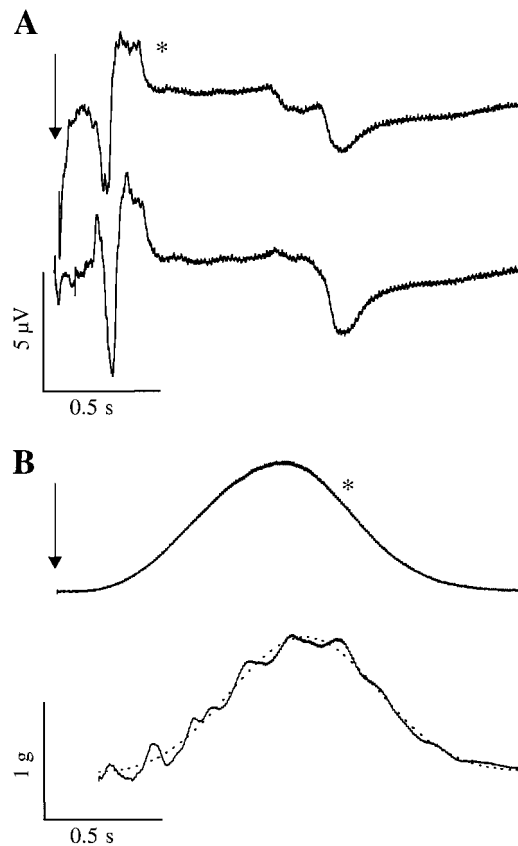


Fig. 3. Comparison of electrical and mechanical stimulation of tissue. *A*: electrocardiograms (ECGs) elicited from same preparation, with electrically stimulated response (indicated by *) occurring 2 s before mechanically stimulated response. Electrically stimulated response is last of a train of 100 S1s. Mechanical stimulus was a 9% tissue length, 86-ms duration stretch. Timing of applied stimulation is indicated by downward arrow. *B*: forces of contraction recorded simultaneously with these ECG traces. First 190 ms of force trace elicited mechanically was contaminated by motion of bath fluid in response to stretch pulse and has been removed for clarity. Dashed line in *B* shown in this force trace is a modified Nwasokwa curve fit (see Eq. 1 in text), which allowed a better estimate of time of peak contraction. Curve-fit parameters are the following: $A_e = 1.000$, $B_e = 3.1$, $C_e = 1.18$, $D_e = 0.079$, $E_e = 0.34$; $A_m = 1.032$, $B_m = 3.2$, $C_m = 1.1$, $D_m = -0.073$, $E_m = 0.36$ for electrically (e) and mechanically (m) elicited contractions, respectively (see Eq. 1 in text for definitions of parameters). Both force traces were digitally filtered at 100 Hz by a 20-pole, finite impulse response, low-pass filter.

ing the onset of activation to be at the time of maximum absolute change in the voltage during the activation interval (2), we find that in this experiment the onset of the mechanically induced activation occurred 23 ms later than the electrically induced activation (264 vs. 241 ms). Estimating the end of the action potential to be at the maximum deviation from isoelectric voltage, we find that the mechanically induced repolarization ended 5 ms later than the electrically induced repolarization (1,240 ms vs. 1,245 ms). In Fig. 3B, the corresponding contractions are shown. The small oscillations in the mechanically stimulated contraction trace were due to fluid motion of the bath after the stretch. To determine time to peak contraction, we fit each curve

with the following equation, modified from Nwasokwa (40)

$$F = C \left(\frac{t - D}{A} \right)^B e^{1 - [(t - D)/A]^B} + E \quad (1)$$

where F is force, t is time, the sum of A and D is time to peak activity, B is a shape parameter, C is developed force, and E is diastolic force. These curve fits show the time to peak contraction to be longer in the mechanical activation than in the electrical activation (1.079 vs. 0.959 s), which was typically seen even when the duration of the stretch pulse was accounted for ($P < 0.05$, $n = 10$). This difference in time to peak contraction was much greater than the difference in latency times seen in the ECG traces.

Does width of stretch pulse change mechanical threshold? Next, we explored the effect of varying the width of the stretch pulse on the mechanical activation threshold. Compiled, binned data from five hearts are shown in Fig. 4 for durations from 50 to 150 ms. Other parameters were kept at nominal values, i.e., the stretch pulse was given 2 s after the start of the previous electrical stimulation; the speed of the rise and fall of the stretch was ~ 26.7 cm/s; and the baseline stretch was 0%. The linear regression line appears to be flat. The slope of this line for the grouped data (slope = 0.0029% length/ms) was not significantly different from zero ($P > 0.99$).

Does speed of stretch pulse alter mechanical threshold? The dependence of mechanical threshold on symmetric variation of the rates of rise and fall of the stretch pulse (Fig. 2, inset b) was also tested over a range of 1.07–26.7 cm/s. Data compiled from seven experiments are shown in Fig. 5. The mechanical threshold decreased at the faster speeds of stretch, as shown by the linear regression line [slope of -3.6% length/(% length/ms)]. The slope of this line is statistically different from zero ($P < 0.002$). For each of the individual experiments, the slope of the regression line was also statistically different from zero ($P < 0.01$).

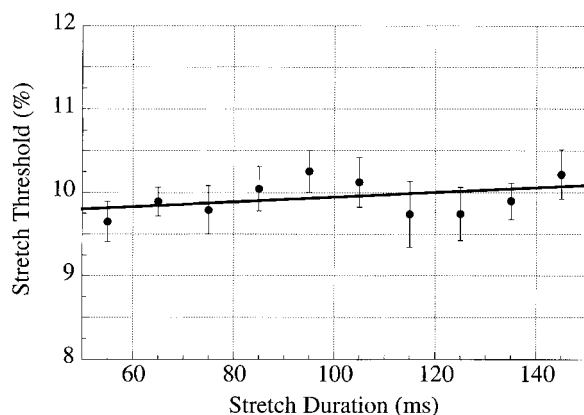


Fig. 4. Strength-duration results showing no effect of stretch duration on mechanical thresholds. Data were grouped into 10-ms bins, and means \pm SE are plotted. Data from different hearts were combined after an offset was added to each data set to make the linear fit to data set pass through same activation threshold at 100 ms (see *Statistical analysis*). Linear regression line shown ($y = -0.00286x + 9.66$) is fit to the combined data.

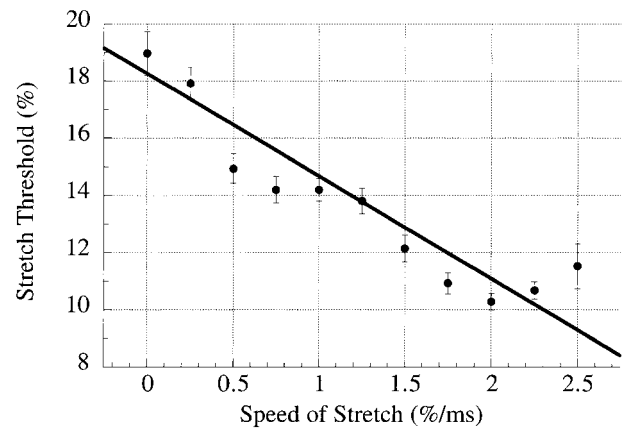


Fig. 5. Strength-speed results showing dependence of stretch thresholds on speed of applied stretch. Data from individual hearts were combined as described in Fig. 4 legend, except that individual experiments were shifted to pass through the same activation threshold at 1.5% length/ms (instead of 100 ms). Data were grouped into 0.25% length/ms bins. Means \pm SE are plotted. Linear regression line shown ($y = -3.59x + 18.3$) is fit to the combined data.

Does baseline level of stretch change mechanical threshold? Figure 6 shows that as the level of baseline stretch applied to the tissue increased from 0 to 6% of the tissue length, the increment in stretch ($\Delta\epsilon$) needed to elicit an activation by a 50-ms stretch pulse decreased. The linear regression line shown has a slope of -0.46 and is significantly different from a slope of zero ($P < 0.002$). The slopes of individual experiments also were all statistically different from zero ($P < 0.05$). These results suggest that increased baseline stretch facilitates mechanical activation. Replotting the data as total stretch (sum of baseline and $\Delta\epsilon$) against baseline stretch (not shown) showed an increase in mechanical threshold at higher baseline stretches for the grouped data ($P < 0.002$ for test of slope = 0). Therefore, baseline stretch affects stretch threshold viewed as either a relative or absolute value.

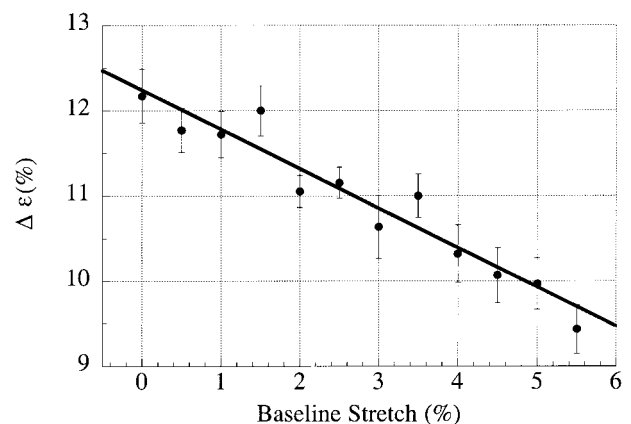


Fig. 6. Effect of baseline stretch on stretch thresholds. x -Axis is baseline stretch applied to tissue, and y -axis is incremental stretch ($\Delta\epsilon$) from baseline that elicited activation of tissue. Individual experiments were combined as described in Fig. 4, except that individual experiments were shifted to pass through same activation threshold at 3% baseline stretch (instead of 100 ms). Bin size was 0.5%. Means \pm SE are plotted. Linear regression line shown ($y = -0.463x + 12.24$) is fit to the combined data.

How does refractoriness of tissue alter mechanical threshold? The dependence of mechanical threshold on the coupling interval between the final electrical S1 stimulation and a subsequent mechanical S2 stimulation was tested, and the compiled results from all of the experiments are shown in Fig. 7. Figure 7A shows the

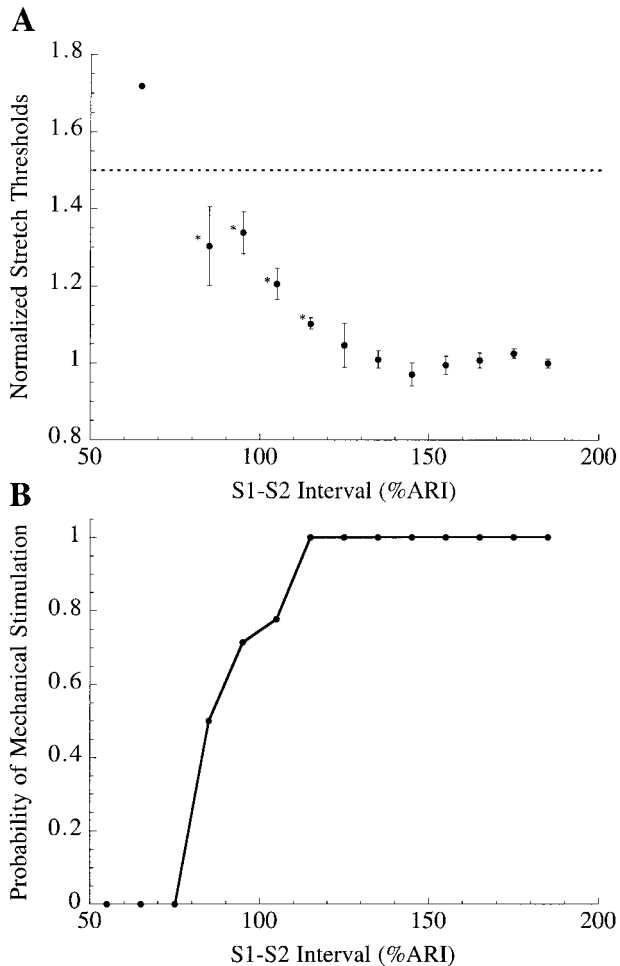


Fig. 7. Strength-interval results showing dependence of stretch thresholds on S1-S2 coupling interval. In both panels, x-axis is normalized to electrically stimulated activation-recovery index (ARI), which was estimated as time from onset of stimulus pulse to peak of T wave read from both ECG. Data were placed in bins of 5% of ARI (extending 2.5% before and after plotted point), except for value at 185%, which represents all points >180%. Stretch thresholds of each experiment were normalized to their rheobase value (mean of values >180% ARI). A: compiled binned results of 12 individual experiments. Plotted values are means \pm SE. * P -values < 0.0005 calculated by using a one-sided t -test to compare mean bin value to mean rheobase value. Point shown at 65% ARI could not be statistically checked because it represents only one trial. All other unmarked points were not statistically different from mean rheobase value ($P > 0.05$). Trials in which tissue was not excited by any stretch up to and including ϵ_{\max} are not included in A but are included in B. B: data plotted in terms of probability of mechanically activating tissue. To generate this graph, each of the individual trials summarized in the data shown in A are categorized as a 1 if stretch threshold of that trial is determined to be <50% over rheobase stretch (corresponding to dotted line at $y = 1.5$ in A) and as a 0 otherwise. Trials from all 12 tissues are combined and binned, and for each bin, plotted probability of mechanical stimulation is simply the average of the 1s and 0s that occur during that bin. Therefore, this graph represents probability that a stretch at 50% above rheobase will cause activation of tissue. Bin size for both panels was 10% of ARI.

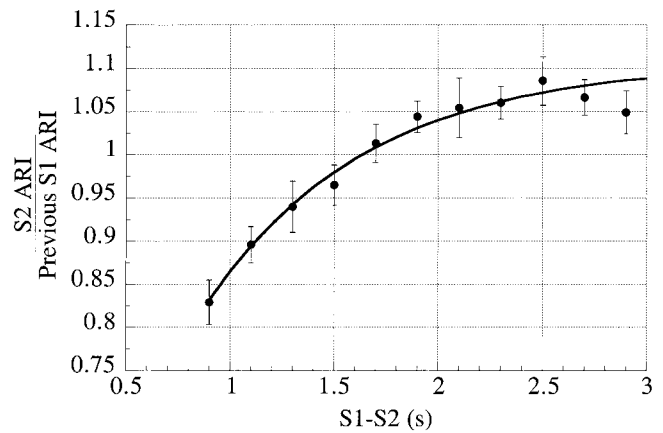


Fig. 8. Restitution results showing effect of varying coupling interval between an electrical (S1) and mechanical (S2) stimulation of tissue on ARI elicited by mechanical stimulation. Means \pm SE are plotted. Along the y-axis are data values normalized to ARI elicited by preceding S1 electrical stimulus. Data were grouped into 0.2-s bins. ARI was estimated from onset of stimulation to peak of the repolarization signal read from ECG recording and included a small latency between application of stimulus pulse and onset of activation. An exponential best fit is shown.

mean thresholds plotted against coupling interval. In general, at shorter coupling intervals the threshold for mechanical stimulation increased. The asterisks indicate that the thresholds between 80 and 120% of the activation-recovery interval (ARI) are all significantly greater than the rheobase value (shown to the far right of Fig. 7A). Shown in Fig. 7B is the probability of eliciting an activation via a stretch fixed in amplitude to be 50% over the rheobase value. The trend toward a zero probability at shorter coupling intervals is further evidence that it is more difficult to elicit activation with a mechanical pulse at shorter coupling intervals.

How does refractoriness of tissue alter response to a mechanical pulse? The effect on the ARI of changing the coupling interval between the last of a train of electrical stimuli and a subsequent mechanical stimulus is shown in Fig. 8. Data are binned and compiled from six hearts. The mechanically activated ARI was obtained at the mechanical activation threshold. It is clear that the ARI increases at longer coupling intervals, closely following the exponential curve drawn in the figure. The ARI at a 2-s coupling interval has nearly the same value as the last electrically stimulated ARI.

DISCUSSION

This study shows that different types of controlled linear stretch can elicit activation in heart tissue, much like electrical stimulation. Specifically, mechanical activation thresholds decreased significantly with longer coupling intervals, greater rates of rise, and greater baseline stretch. No significant effect of stretch duration was found over the range tested.

Width of stretch pulse does not change mechanical threshold. Although the strength-duration relation shown in Fig. 4 does not indicate any change over the range of values tested, this result is similar to electrical strength-duration relations. In isolated frog ventricular myocytes, the strength-duration relation increases

significantly in threshold only at values <5 ms (48). In dogs, the electrical strength-duration relation increases in threshold only at durations less than ~ 10 ms (39). These shorter durations were not possible to attain in our experiments due to mechanical limitations. The analogy between mechanically derived and electrically derived strength-duration relations has not been shown previously. However, volume pulses applied during the relative refractory period of Langendorff-perfused dog hearts are less likely to induce a contraction when the duration of the volume pulse is decreased from 500 to 50 ms (23).

Faster stretch pulses activate tissue more easily. The speed of stretch experiments (Fig. 5) shows that pulses with greater rates of rise and fall make activation easier. One possible explanation of this result is that slower pulses may allow time for sodium channels to inactivate, making the tissue less excitable. Another explanation is that slow pulses might allow stretch-sensitive membrane currents to inactivate (22, 24), although Sasaki et al. (44) showed that stretch-activated currents recorded from guinea pig myocytes are constant over several seconds. One possible complication of our strength-speed experiments is that pulses with slower slopes can be viewed as having a longer effective duration. However, we have already shown that the effect of increasing the width of the pulse is insignificant at widths >50 ms (Fig. 4).

Experiments by Franz et al. (16, 53) on Langendorff-perfused rabbit ventricles are less clear in analogous studies of the rates of rise and fall of a volume pulse. Their earlier results showed that as the rate of rise of a volume pulse increased from 1 to 10 ml/s, the probability of eliciting an activation increased (16). However, more recent results are somewhat contradictory and show that the effect of a volume pulse on an electrically elicited action potential recorded via a monophasic action potential electrode depends on the amplitude of the pulse, irrespective of the rates of rise and fall (53). Because these stretches were applied while the tissue was refractory, we cannot easily extrapolate to how these pulses would cause activation at rest in the nonlinear heart tissue; therefore, these findings do not necessarily contradict our results.

Increased levels of baseline stretch make mechanical stimulation easier. The results in Fig. 6 imply that a higher baseline level of tissue stretch makes it easier to bring the tissue to threshold in terms of an additional stretch pulse ($\Delta\epsilon$). One explanation for these results comes from the fact that stretch can cause diastolic depolarization (50, 53). It has been proposed that small depolarizations decrease the excitation threshold (43, 49).

These results are consistent with previous work done in our laboratory, which showed increased electrical excitability with stretch in the single frog myocyte (49). Likewise, other researchers have found that increasing the diastolic volume from 10 to 30 ml caused a decrease in the additional stretch needed to elicit an action potential in a dog ventricle (23). Others (50) have reported a decrease in excitability with baseline stretch of single guinea pig

ventricular myocytes. These contradictory results may be explained by the fact that a larger baseline stretch may result in a larger depolarization and make the tissue less excitable via inactivation of inward sodium channels or activation of outward potassium channels (8, 10).

The stretch threshold plotted in Fig. 6 was presented as a change in stretch ($\Delta\epsilon$) relative to the baseline stretch. This definition was used because it is analogous to the clinically seen volume-overloaded hearts, where additional stretch over the baseline level is important. We also analyzed our data in terms of absolute stretch thresholds (sum of baseline and $\Delta\epsilon$). If the baseline stretch were inconsequential, and there was simply an absolute stretch threshold at which the tissue activated, a regression line fit to the absolute stretch data should not have a slope significantly different from zero. However, this was not the case ($P < 0.002$). Thus the results from these baseline stretch experiments cannot be attributed solely to an absolute stretch threshold. Some combination of the diastolic depolarization and absolute stretch contributes to the final relation that we measured.

Also note that the test pulses in the baseline stretch experiments were applied several minutes after the baseline stretch level was set, because we wanted this procedure to mimic a steady-state volume load for the whole heart. However, it has been shown that the effect of uniaxial stretch on action potentials of single frog ventricular myocytes (49) and stretch-induced current in single guinea pig ventricular myocytes (44) declines on the time scale of minutes. Therefore, shorter delays in the application of the test pulse might unveil a greater effect of baseline stretch and increase the magnitude of the slope of the relation shown in Fig. 6.

Refractory tissue is more difficult to mechanically activate. The strength-interval relation shown in Fig. 7A shows that as the delay between the last electrical stimuli and the mechanical stimuli is shortened, it becomes more difficult to mechanically activate the tissue. This is shown both in Fig. 7A, where the thresholds at intervals between 80 and 120% ARI are statistically different from the rheobase, and in Fig. 7B, which directly plots the probability that a stretch at 50% above the rheobase will cause activation. The shorter intervals at which it is more difficult to mechanically excite the tissue are consistent with the location of the relative refractory period of the frog ventricular tissue. This relation in Fig. 7A is analogous to the hyperbolic relation typically obtained from purely electrical stimuli (36).

In two Langendorff-perfused whole rabbit hearts, Franz et al. (16) found a mechanical strength-interval relation qualitatively similar to ours. As the coupling interval shortened from 800 to 150 ms, the volume needed to activate the heart increased from 300 to 900 μ l. However, Franz et al. did not employ any means to measure the strain level in the tissue; therefore, it is difficult to compare his results with ours. Had they reported the size of these two hearts, it might have been possible to estimate how their threefold increase in volume relates to our stretch thresholds. For example,

assuming a thin-walled, spherical left ventricle with an initial volume of 1 cm³, the change in volume represents an increase in circumferential stretch of 9–24%. Assuming also that 9% is the stretch needed to activate the tissue at rheobase, these numbers would correspond to an increase in normalized stretch threshold from 1 to 2.7 on the *y*-axis in Fig. 7A, a range of normalized stretches greater than those tested in our experiments. One notable difference in Franz et al.'s data is that their relations were much steeper at shorter coupling intervals. The differences in the data may be a reflection of the minimum coupling interval in our experiments, which was limited by the maximum stretch levels that we allowed in our experiments. Therefore, it is conceivable that we, too, would have observed a steeper relation had we probed even shorter coupling intervals.

Earlier work by Brooks et al. (4) showed that when a thrust from a solenoid was applied to the epicardial surface of an *in vivo* canine heart, action potentials could sometimes be elicited in the tissue. Because the intensity of their mechanical pulse was not variable, they presented their data as the probability of eliciting an action potential at different coupling intervals. Our results of Fig. 7B are consistent with those of Brooks et al. and show that as the coupling interval shortens to the absolute refractory period for mechanical stimulation, the probability for stimulation decreases from 1 to 0.

Comparison of electrical and mechanical activation. In addition to showing that a linear stretch can excite heart tissue, our study also made direct comparisons of the ECG and contraction responses when the tissue was activated mechanically instead of electrically, as shown in Fig. 3. The ECG traces for the two forms of stimulation in Fig. 3A look remarkably similar. The onsets of activation differed by only 23 ms, the repolarization times differed by only 5 ms, and the overall morphology was similar. The difference in activation times were within the 86-ms width of the stretch pulse that was applied to the tissue. Therefore, it is likely that, in this example, the parts of the tissue first excited by the two forms of stimuli were in close proximity. By analogy to electrical stimulation, the stretch pulse could be causing the membrane to depolarize and reach threshold late in the pulse. However, the time of peak contraction (as determined by a curve fit) occurred significantly later in the mechanically stimulated contraction than in the electrically stimulated contraction (120 ms difference in Fig. 3B). One possibility is that this effect is related to the transient decrease in force that can occur after a stretch. Le Guennec et al. (33) have shown that a decreased baseline stretch causes a lengthening of time to peak contraction. Because the decreased stretch in the cells Le Guennec et al. studied would also indicate a decreased force, their result may explain the delay noted here. Our findings that tissue can be mechanically activated are consistent with previous studies (4, 16, 20, 23, 26, 32), although none of the studies compared electrically

induced ECG and force responses to those induced mechanically.

The restitution curve provides additional evidence of the similarity of electrical and mechanical stimulation (Fig. 8). The fact that the mechanically elicited ARI at a 2-s coupling interval is similar in value to the last electrically stimulated ARI is not surprising, because the S1-S1 coupling interval of the S1 train was 2 s. The generally accepted hypothesis for the observed shape of the restitution curve is that the shorter coupling interval interrupts either recovery of the slow inward calcium current or the decay of the potassium outward current (17). Whatever the mechanism may be, it still appears to operate when the tissue is stimulated with a mechanical pulse. Note that in typically reported electrical-electrical restitution curves, a single strength is used for all of the coupling intervals. The data presented here for the electrical-mechanical restitution curve is at threshold strength, which varies over the coupling interval used (as indicated in Fig. 7A). If we had used a constant stimulus strength, we might expect the curve to flatten out due to two effects. The first effect would be due to the finite conduction velocity of the tissue and could cause the restitution curve to decrease at longer S1-S2 coupling intervals. Because stimuli at strengths higher than threshold could initially excite more of the tissue, dispersion of electrical activity in the tissue would decrease and would cause the repolarization of the tissue as a whole to occur more quickly. The second effect could shift the curve upward at shorter coupling intervals and arise because action potentials elicited during the refractory period of the tissue at higher stimulus intensities tend to be longer than those elicited at the same coupling interval at threshold (46). The exponential rise in ARI with increasing coupling intervals resembles the results of other researchers (1, 37), who characterized purely electrical restitution curves.

Implications. This study shows the ability of various types of mechanical pulses to active ventricular tissue, with clinical implications in arrhythmogenesis. Modeling studies have indicated that at end systole, the circumferential stress at an infarct border zone immediately after an infarction can be four times higher than that in the rest of the myocardium far from the infarct (3). This increase in stress corresponds roughly to an increase in end-systolic strain of 10% in the border zone. Figures 4–6 all show that this strain is at a level that can cause excitation. Note that this abnormal mechanical load would be occurring at a time when the tissue is recovering excitability. The strength-interval relation could help predict if this strain would cause extrasystoles in this region and could explain the higher incidence of clinical arrhythmias in the time period immediately after an infarction (31). Recent modeling work suggests that stretch-induced currents can create reentry in a homogeneous two-dimensional sheet of myocardium (42).

Another method of reentry induction that can occur in a homogeneous, isotropic piece of myocardium has been proposed by Winfree (52). He proposed that a

reentrant circuit can be formed around a location that is defined by the intersection of a critical timing and a critical strength contour. The strength-interval relation that we have characterized may help to define the placement of these contours. The rheobase of the curves would help to determine the amount of stretch that must be applied to the tissue to generate this type of arrhythmia. Depending on how the stretch is applied and the tissue's mechanical properties, this stretch will create a critical strength contour somewhere on the tissue. The coupling interval at which the strength-interval curve starts to turn up from the rheobase, coupled with the conduction velocity of the tissue would dictate how long after the previous electrical excitation the stretch should be delivered (critical timing contour). Our strength-interval relation starts to curve up at 100% of the ARI. Assuming a conduction velocity of 50 cm/s (12) and an action potential duration of 250 ms (15), our data suggest that in a normal mammalian heart, the mechanical stimulus would have to be applied at least 12.5 cm behind the leading edge of excitation.

The speed of stretch experiments have several potentially important implications. First, it may be the case that any mechanical perturbation is even more focal than that predicted simply on the basis of the geometry of the mechanical contact. For example, a catheter pressing on the endocardial surface of the heart may create a strain field that falls off inversely with the radius. However, because the tissue is viscoelastic (11), it is likely that during the initial contact the effective speed of the applied stretch falls off with distance from the impact site. This would cause the amount of tissue that is directly excited to be smaller and also cause the gradient of mechanical stimulation to be larger. Sharper gradients in excitation responses can facilitate the creation of propagated waves of activity (14), at least for electrical stimuli. Second, the higher incidence of arrhythmias seen in the sympathetically stimulated hearts may be related to the speed of stretch effect. If the underlying cause of an extrasystole is from the stretch of tissue, caused perhaps by ischemic tissue being stretched by adjacent healthy tissue, faster contraction due to sympathetic stimulation may be proarrhythmic, even in the absence of a change in the amount of contraction. Third, the precordial thump is currently recommended by the American Heart Association as an optional technique in Advanced Cardiac Life Support for a witnessed cardiac arrest where the patient is pulseless and a defibrillator is not immediately available (7). Our results suggest why the location, distance, and force of the thump need to be controlled (5, 19) and why a minimum ventricular pressure may be necessary (54). Mechano-electrical transduction has been previously recognized to be a potential mechanism underlying "thump-version" (35), and it is possible to pace the heart with a mechanical device that is suitably calibrated (55). Finally, the experimental studies of commotio cordis show that the

probability of causing ventricular fibrillation increases with the hardness of the object that strikes the chest (34). These results could be explained by our speed of stretch results, because a harder object will cause a faster change in strain in the heart and therefore is more likely to cause an initiating ectopic beat.

Limitations. One limitation in these experiments was the in-plane variability of strain in the tissue perpendicular to the direction of applied stretch. Gross differences were eliminated by monitoring the differential forces (twist) induced in the resting tissue, but small differences may have persisted. Another limitation in these experiments was the limited frequency response of the system that applied the stretch. In particular, the strength-duration experiments could not be probed at durations <50 ms. As was noted in the DISCUSSION, large changes might not be expected to occur in the strength-duration relation except at durations less than ~5 ms. A final limitation of these experiments was that the strain distribution in the tissue may not have been perfectly uniform, perhaps due to edge effects, the nonuniformity of the underlying fiber structure, or variations in the tissue thickness. Even with slight changes in strain throughout the tissue, however, we would expect that the trends in the change in thresholds with our set of mechanical perturbations would be unaffected.

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