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Mechanical Stretch of Atrial Myocyte Monolayer Decreases Sarcoplasmic Reticulum Calcium Adenosine Triphosphatase Expression and Increases Susceptibility to Repolarization Alternans

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Objectives The purpose of this study was to investigate the effect of stretch (the major risk factor for atrial fibrillation [AF]) on spatial and temporal alternations of action potential duration (APD-ALT) and calcium transient in cultured atrial myocyte monolayer.

Background How rapid firings or premature beats trigger AF is not completely understood. Discordant repolarization alternans, characterized by simultaneous prolongation and shortening of APD in different myocardial regions, is central to the genesis of ventricular fibrillation. We hypothesized that repolarization alternans also is central to the initiation of multiple re-entry circuits and AF.

Methods Confluent HL-1 atrial myocyte monolayer with spontaneous depolarization was cultured in silicone membrane and subjected to mechanical stretch. Rapid field pacing was used to induce alternans. A high-resolution dual optical mapping system was used to record action potentials and calcium transients.

Results High-rate pacing induced APD-ALT and calcium transient in atrial myocyte monolayer. Mechanical stretch significantly decreased the thresholds for APD-ALT and calcium transient. Mechanical stretch decreased the expression of sarcoplasmic reticulum adenosine triphosphatase 2, and thus slower calcium reuptake kinetics, which was responsible for the susceptibility to alternans. Mechanical stretch did not alter the APD restitution kinetics. Mechanical stretch also enhanced spatially discordant alternans. Overexpression of sarcoplasmic reticulum adenosine triphosphatase 2 reversed all the effects of stretch on susceptibility to alternans. In intact atrium, mechanical stretch also enhanced discordant alternans.

Conclusions Mechanical stretch increased the susceptibility to alternans in atrial myocytes, which may explain the susceptibility to AF in conditions of atrial stretch, such as mitral valvular heart disease, heart failure, and hypertension. (J Am Coll Cardiol 2011;58:2106–15) © 2011 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice and an important etiologic factor in stroke in the elderly; it also increases cardiovascular mortality (1). The presence of multiple simultaneous re-entry circuits has been considered central to the mechanism of AF (2). Recently, it was demonstrated that repolarization or action potential duration (APD) alternans (APD-ALT), particularly discordant alternans, which is characterized by simultaneous prolongation and shortening of APD in different myocardial regions, is central to the genesis of ventricular fibrillation (3,4). Because ventricular fibrillation and AF may share common pathogenesis and mechanisms, we hypothesized that spatially discordant APD alternans also may be the underlying mechanism of multiple re-entry circuits and initiation of AF. This hypothesis was based on

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a recent computer simulation study that dynamically induced discordant alternans underlied the induction of AF by atrial ectopic foci or atrial rapid firing (5).

Accordingly, in the present study, we attempted to identify factors that might induce APD-ALT in atrial myocytes and to investigate its molecular mechanism. First, we used a cellular model of HL-1 atrial myocyte culture and demonstrated that APD-ALT could be induced by high-rate pacing in this cellular model. Second, mechanical stretch is the common final stimulus to atrial myocytes in various cardiovascular diseases that are associated with AF, such as mitral valvular disease, hypertension, and congestive heart failure. We tried to investigate whether mechanical stretch increased the susceptibility to APD-ALT in atrial myocytes and to study its molecular mechanism.

Abbreviations and Acronyms

AF = atrial fibrillation
APD = action potential duration
APD-ALT = action potential duration alternans
Ca = calcium
Ca-ALT = calcium transient alternans
PR = pacing rate
SERCA2 = sarcoplasmic reticulum calcium adenosine triphosphatase 2

Methods

HL-1 myocyte culture, mechanical stretch, and pacing. The HL-1 myocytes were cultured and paced as previously described (6–8). The pacing cycle length gradually was shortened until loss of 1:1 capture. For mechanical stretch, myocytes were cultured in silicone membrane plates and were stretched in a Flexercell FX-4000 strain unit (Flexcell International, Hillsborough, North Carolina) to 105%, 110%, and 115% of resting length at a frequency of 1 Hz for indicated times.

Overexpression of sarcoplasmic reticulum adenosine triphosphatase 2. Mouse ATP2a2 gene complementary deoxyribonucleic acid (NM 001110140.3) was subcloned to pCMV-SPORT6 vectors (Open Biosystems, Huntsville, Alabama). Transient transfection of HL-1 myocytes was carried out using LipofectAMINE 2000 (Invitrogen, Life Technologies, Carlsbad, California) according to the manufacturer's instructions. We used Western blot to evaluate sarcoplasmic reticulum adenosine triphosphatase 2 (SERCA2) expression (6,7).

Optical mapping of HL-1 myocyte monolayer. High-resolution dual calcium (CA)-voltage mapping experiments were performed to record action potential and Ca transient. The cells were stained first with voltage-sensitive dye RH237 (5 μM, Molecular Probes Inc., Eugene, Oregon) for 10 min, and then Ca-sensitive dye Rhod-2 AM (5 μM, Molecular Probes) for 30 min. Action potentials and Ca transients were recorded by 2 Complementary Metal-Oxide-Semiconductor (CMOS) cameras (MiCam Ultima, Sci-Media, Tokyo, Japan), each with 100 × 100 pixels and acquiring images at 1,000 frames/s (9). Fluoresced light from both dyes were split by a fluorescence splitter (MiCam Ultima, Sci-Media). The longer-wavelength light (>690 nm) for action potential recording and shorter-wavelength light (585 nm) for Ca transient recording were directed to the 2 CMOS cameras.

Measurement of APD-ALT, Ca transient alternans, APD restitution, and Ca transient decay rate. Alternans of APD was determined by measuring differences in local APD on consecutive beats. The measurements were made on 6 serial consecutive beats, and the APD was averaged for the 3 even and 3 odd beats, respectively. The APD-ALT (in milliseconds) was plotted against the pacing cycle lengths. Dynamic APD restitution was measured by plotting APD as a function of diastolic interval (10). The restitution curve was fit to a single exponential function whose time constant, τ, was used to measure the kinetics of APD restitution (10). The spatial patterns and gradients of repolarization, or magnitude of APD-ALT, was represented as iso-alternans contour maps (11).

The calcium level was reported as F/F0, where F0 was the resting or diastolic fluorescence level (6). The measurements were made on 6 serial consecutive beats, and the F/F0 was averaged for the 3 even and 3 odd beats, respectively. To quantify further the rate of reuptake of intracellular Ca^{2+}, the decay portion of the Ca^{2+} transient (from 30% to 100% of decline phase) was fit to a single exponential function whose time constant, τ, was used to measure Ca^{2+} decay (11).

Mechanical stretch of rat left atrium. Wistar rats were anesthetized, the hearts and lungs were removed quickly, and the aorta was cannulated and perfused with oxygenated Tyrode solution. A self-made latex balloon was placed within the left atrium to stretch the left atrium. The nonstretched left atrium was used as the control group. Because the noise of Ca signal was large, we recorded only optical action potentials. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the National Taiwan University College of Medicine.

Statistical analysis. All data were expressed as mean ± SD. Data from independent group were compared using unpaired Student *t* test for continuous data and the Fisher exact test for categorical data. When there were more than 2 groups, variances were compared first using a 1-way analysis of variance and then the post hoc Student *t* test with Bonferroni corrections for the p values. A p value or post-hoc p value <0.05 was considered statistically significant.

Results

Mechanical stretch increases susceptibility to APD-ALT and Ca transient alternans in atrial myocytes. Dual CA-voltage mapping experiments were performed to evaluate the relationship between APD-ALT or Ca transient alternans (Ca-ALT) and PR in control and stretched myocytes. The effect of stretch (stretch 115% for 24 h) on suscepti-

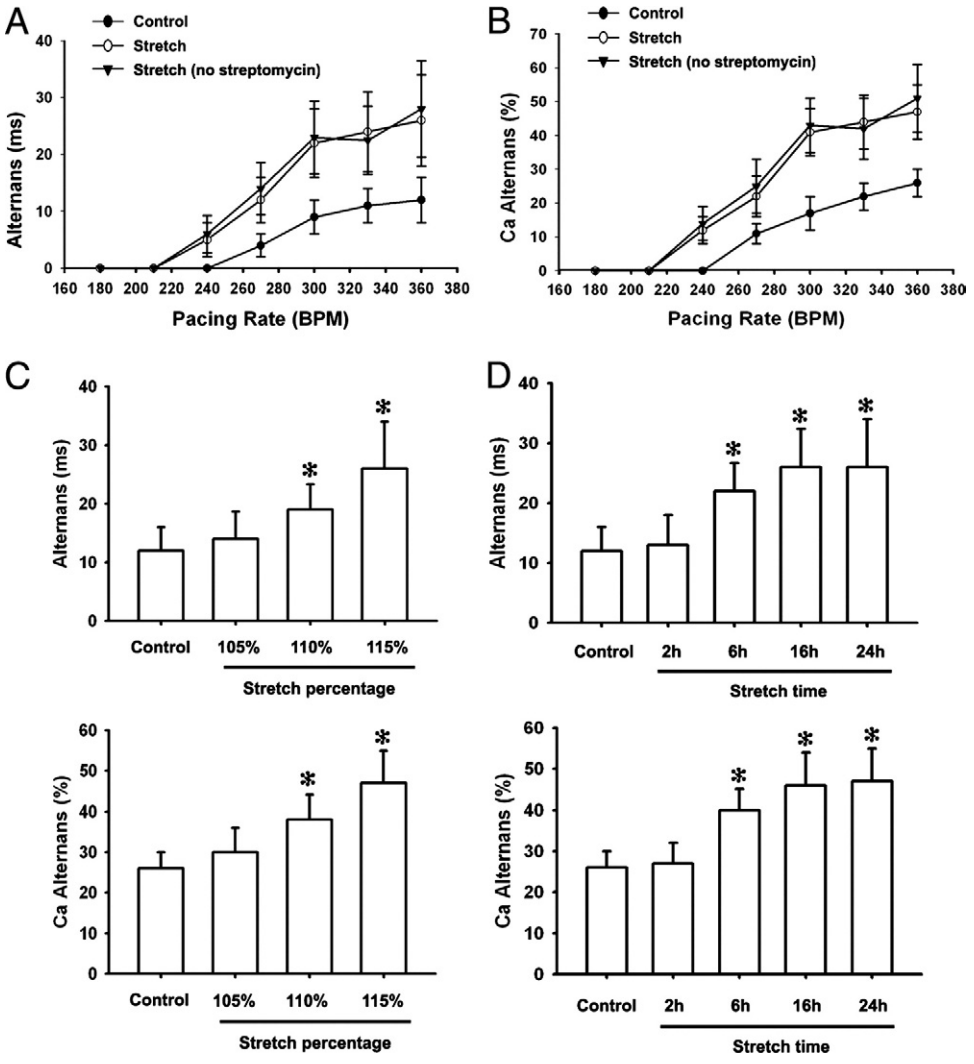

Figure 1 **Mechanical Stretch Increases the Susceptibility to Alternans in Atrial Myocytes**

Summary data for all optical mapping experiments demonstrating the relationship of pacing rate (PR) and alternans from control and stretched atrial myocytes. An additional group with stretch but no streptomycin (as an antibiotic in culture medium, but also a nonselective stretch-activated channel blocker) also is included. **(A)** In stretched atrial myocytes, more action potential duration alternans (APD-ALT) was observed at each PR tested, and stretch produced a leftward shift in the APD-ALT–to–PR relationship. No significant effect of streptomycin on stretch-induced APD-ALT was noted. **(B)** In stretched atrial myocytes, more calcium transient alternans (Ca-ALT) was observed at each PR tested, and mechanical stretch produced a leftward shift in the Ca-ALT–to–PR relationship. No significant effect of streptomycin on stretch-induced APD-ALT was noted. APD-ALT and Ca-ALT also were associated closely. **(C)** More stretch magnitude induced more APD-ALT and Ca-ALT (stretch to 105%, 110%, and 115% of resting length, respectively, for 24 h). **(D)** More stretch time induced more APD-ALT and Ca-ALT (stretch to 115% of resting length for indicated times). Data represent mean ± SD. *$p < 0.01$ versus control group. BPM = beats/min.

bility to APD-ALT is shown in Figure 1A. Optically recorded action potentials in control and stretched preparations were recorded while alternans was induced by rapid field pacing. In stretched myocytes, there was a leftward shift in the APD-ALT–to–PR relationship, indicating greater susceptibility to APD-ALT.

As was observed for APD-ALT, increased susceptibility to Ca-ALT in stretched myocytes also was evident by the significant reduction in PR required to induce Ca-ALT (Fig. 1B). Moreover, cells that exhibited the greatest magnitude of APD-ALT also exhibited the greatest magnitude of Ca-ALT, suggesting that cellular susceptibility to Ca-ALT is associated closely with susceptibility to APD-ALT in stretched myocytes. These results demonstrate the very close relationship between the development of Ca-ALT and APD-ALT and that Ca-ALT and APD-ALT are both highly rate dependent.

Streptomycin is known to be a nonselective stretch-activated channel blocker. Therefore, this antibiotic drug delivered to the cultures might have affected the overall stretch-induced pathophysiology. A group without streptomycin also was included. We found no significant difference of magnitude of stretch-

induced alternans between myocytes with or without streptomycin in the culture medium (Figs. 1A and 1B).

The dose response and the time course of stretch-induced alternans also were evaluated. The effect of stretch on alternans was observed only when the magnitude of stretch was more than 110% of the resting length (Fig. 1C) and the duration of stretch was more than 6 h (Fig. 1D).

Susceptibility to APD-ALT by mechanical stretch arises from Ca-ALT. We have shown that APD-ALT and Ca-ALT are related closely in both control and stretched preparations. Although it generally is believed that Ca-ALT is the passive response to beat-to-beat alternations in action potential (APD-ALT), accumulating evidence has shown that APD-ALT also may arise from defective intracellular Ca cycling or Ca-ALT (10,12).

To eliminate the possible primary effect of stretch-induced alternation in action potential or APD-ALT, additional experiments were performed where Ca transients were measured with repetitive, nonalternating action potential waveforms. In Figure 2A, the nonalternating action potential is shown in the upper panel. At this PR, no Ca-ALT was observed in the control myocytes, whereas in the stretched myocytes, significant Ca-ALT was observed. In Figure 2B, the Ca-ALT–PR relationship was shown, and it was demonstrated that stretch induced a similar leftward shift of the Ca-ALT–PR threshold even under conditions where APD-ALT was prevented. These data suggest that Ca-ALT is not entirely dependent on APD-ALT and reaffirm earlier reports that Ca-ALT is involved centrally in the genesis of alternans (10,12).

Mechanical stretch decreases SERCA2 expression and induces slower diastolic calcium decay and defective Ca cycling. Because defective intracellular Ca cycling or Ca-ALT may be responsible for the genesis of APD-ALT in stretched myocytes, we investigated whether mechanical stretch altered the expression of calcium handling proteins. We found that mechanical stretch decreased the expression of SERCA2 (Fig. 3A), without change in other calcium handling proteins in atrial myocytes, such as ryanodine receptor, sodium–calcium exchanger, calsequestrin, or phospholamban (data not shown). Accordingly, the Ca transient decay rate or Ca reuptake rate was slower, and the Ca transient amplitude was smaller in stretched myocytes when compared with those of control myocytes (Fig. 4).

Therefore, decreased SERCA2 levels may play a causative role in the susceptibility to Ca-ALT and APD-ALT in the stretched myocytes, which was compatible with the previous findings in the intact heart (12). This concept was reinforced further in that the time course of decreased SERCA2 expression by mechanical stretch was compatible with that of stretched induced alternans. Decreased SERCA2 expression was observed first after 6 h of stretch (Fig. 3A). Interestingly, cellular alternans also was observed first after 6 h of stretch (Fig. 1D).

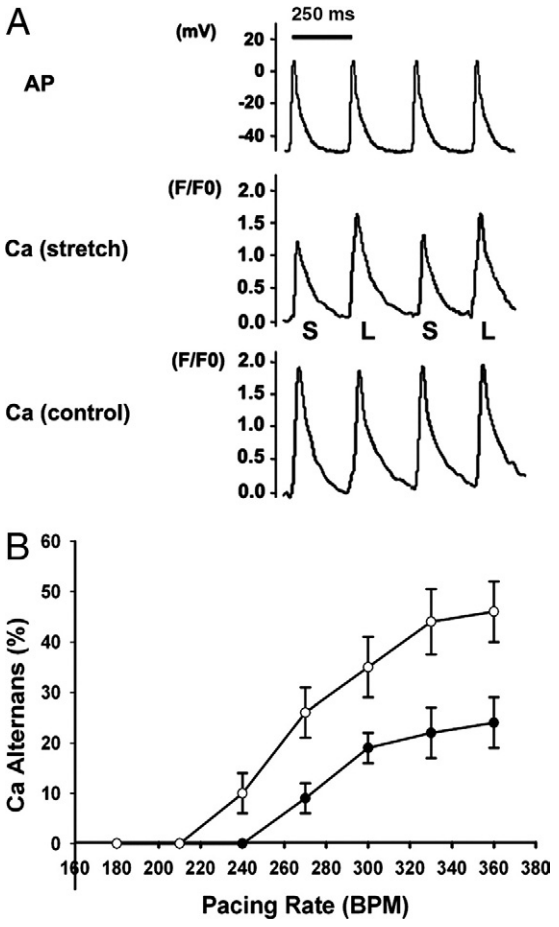

Figure 2 Mechanical Stretch Increases Susceptibility to Alternans Independent of Effects on the Action Potential

(A) The top tracing shows the nonalternating action potential (AP) waveform, and a representative example of calcium (Ca) transients from control and stretched myocytes are shown at a PR of 240 beats/min. In the stretched myocytes, but not in the control myocytes, Ca transient alternans (alternating small [S] and large [L] amplitudes) was induced under constant AP conditions, independent of AP alternans. Ca transients were expressed as F/F0 (fluorescence level [F] normalized to diastolic fluorescence level [F0]). **(B)** Data over a range of PR for control and stretched myocytes are shown. Stretch induced a similar leftward shift of the PR/Ca-ALT relationship. Abbreviations as in Figure 1.

Overexpression of SERCA2 reverses stretch-induced defective Ca cycling and decreases the susceptibility to APD-ALT and Ca-ALT. We then attempted to test whether targeted SERCA2 (ATP2a2) overexpression might prevent stretch-induced slower Ca reuptake, and thus decrease the susceptibility to cellular alternans. Overexpression of SERCA2 induced a robust increase of cellular SERCA2 protein (Fig. 3B), but did not alter the expression of other calcium handling proteins, such as ryanodine receptor, sodium–calcium exchanger, calsequestrin, or phospholamban (Fig. 3C). We found that overexpression of SERCA2 prevented stretch-induced decreased SERCA2 expression (Fig. 3D) and prevented stretch-induced slower Ca reuptake and smaller Ca

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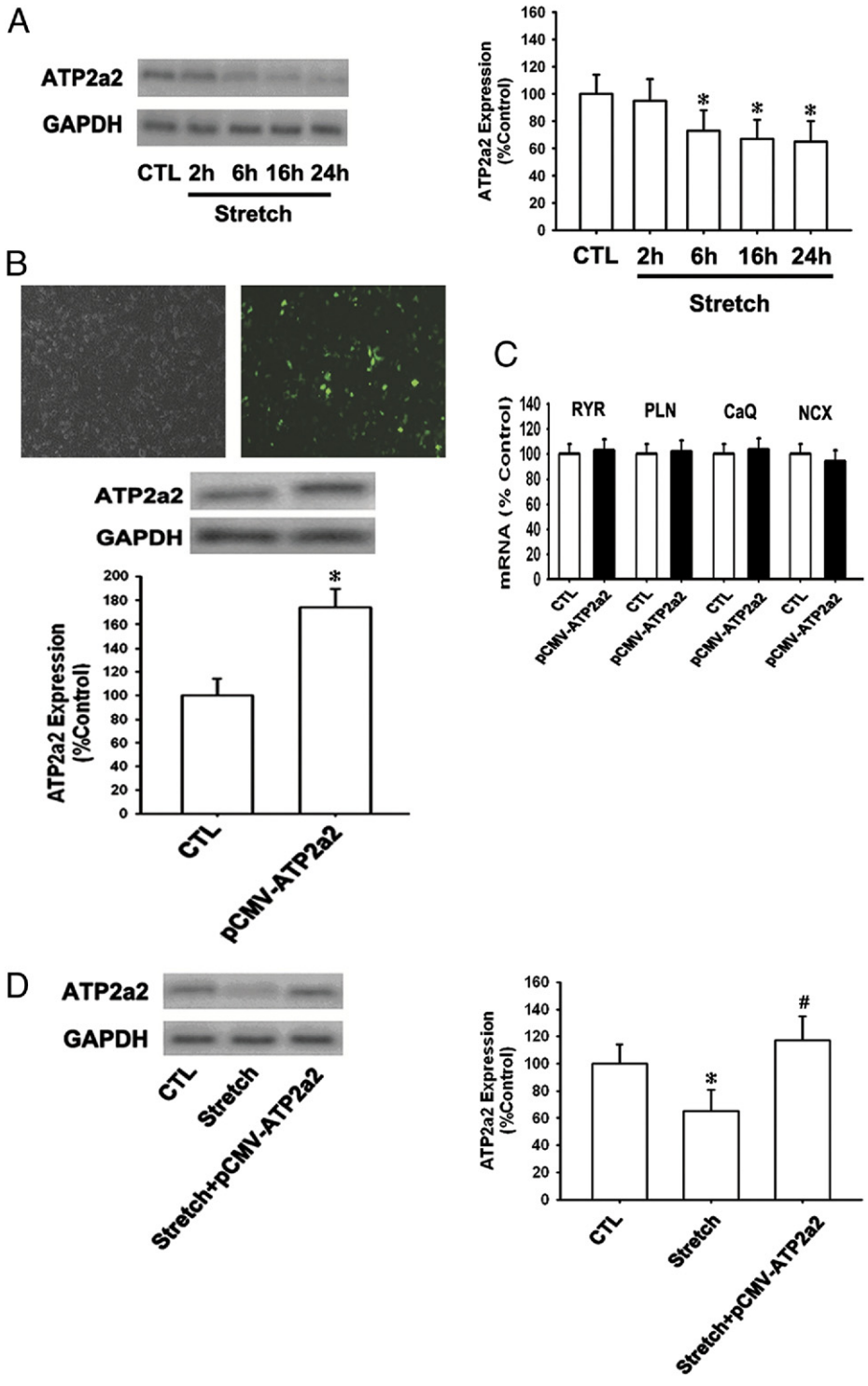

Figure 3 **Mechanical Stretch Decreases ATPA2 Expression in HL-1 Atrial Myocytes, Which Is Prevented by Sarcoplasmic Reticulum Calcium Adenosine Triphosphatase Expression Overexpression**

(A) HL-1 atrial myocytes were subjected to mechanical stretch for indicated times. The levels of ATP2a2 in the cellular extracts were analyzed by Western blot, which was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). **(B)** The **upper panel** shows the high transfection efficiency of HL-1 myocytes, as evaluated by green fluorescent protein (GFP) reporters (**Left**: phase contrast image; **Right**: green fluorescence image). The **lower panel** shows ATP2a2 was overexpressed by the cytomegalovirus (CMV) promoter in control myocytes (pCMV-ATP2a2), as evaluated by Western blot. **(C)** Overexpression of ATP2a2 did not alter the expression of other calcium handling proteins, such as ryanodine receptor (RYR), phospholamban (PLN), calsequestrin (CaQ), and sodium/calcium exchanger (NCX), as evaluated by reverse-transcriptase polymerase chain reaction. **(D)** ATP2a2 was overexpressed by the CMV promoter in stretched myocytes (stretch + pCMV-ATP2a2). Data represent mean ± SD. *$p < 0.01$ versus controls. #$p < 0.05$ versus stretch-treated cells. CTL = control cells without stretch.

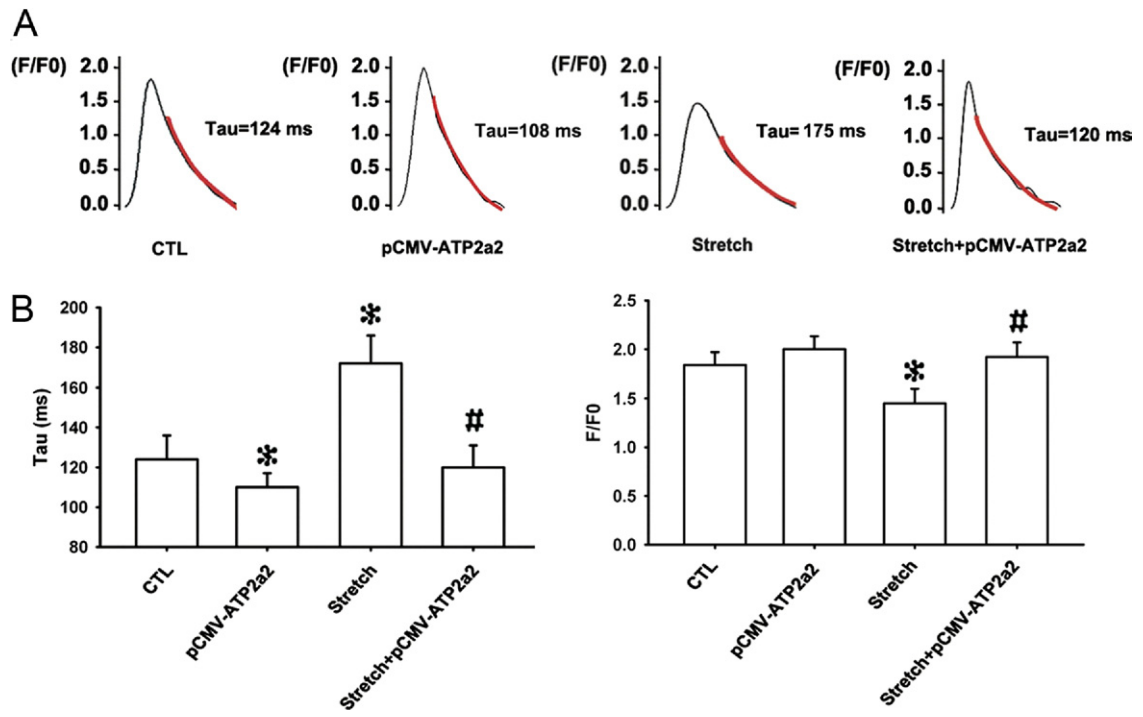

Figure 4 **Mechanical Stretch Induces Slower Calcium Reuptake Kinetics, Which Is Prevented by ATP2a2 Overexpression**

(A) Representative calcium transients from control (CTL), ATP2a2 overexpression (pCMV-ATP2a2), stretched, and stretched with ATP2a2 overexpression (stretch + pCMV-ATP2a2) myocytes are shown. Calcium transients were expressed as F/F0. **(B)** Summary data of time constant tau (τ) and calcium transient amplitude in CTL, pCMV-ATP2a2, stretch, and stretch + pCMV-ATP2a2 myocytes at a baseline PR of 180 beats/min. As expected, overexpression of ATP2a2 increased the decay rate of Ca transient in control myocytes (pCMV-ATP2a2 vs. CTL), with a smaller tau (τ) value. The decay rate of calcium transient was significantly slower in stretched myocytes than control myocytes (stretch vs. CTL). This was reversed by ATP2a2 overexpression (stretch + pCMV-ATP2a2 vs. stretch). The calcium transient amplitude also was significantly smaller in stretched myocytes than in controls, which was reversed by ATP2a2 overexpression. Data represent mean ± SD. *$p < 0.01$ versus controls. #$p < 0.05$ versus stretch-treated cells. Abbreviations as in Figures 1, 2, and 3.

transient amplitude (Fig. 4). As expected, overexpression of SERCA2 also increased the rate of Ca reuptake in control myocytes (Fig. 4). Finally, overexpression of SERCA2 successfully prevented stretch-induced susceptibility to APD-ALT and Ca-ALT (Figs. 5A and 5B).

Effect of mechanical stretch on spatial discordant alternans. Figure 6 shows representative action potentials and Ca transients in different regions from control, stretched, and stretched with SERCA2 overexpression myocytes. The magnitude and phase of APD-ALT is also shown by iso-alternans maps. In control myocytes, cells alternated in phase at PR 360 beats/min, with each alternating in a long-short-long-short pattern for APD and large-small-large-small pattern for Ca transient (concordant alternans) (Fig. 6B). However, at the identical PR in the stretched myocytes, cells alternated in the opposite phase, with a region alternating in a long-short-long-short pattern and the other in a short-long-short-long pattern (discordant alternans) (Fig. 6A). Discordant alternans in stretch preparations were prevented with SERCA2 overexpression (Fig. 6C).

Effect of mechanical stretch on APD restitution. To investigate the primary effect of stretch on APD restitution kinetics, dynamic restitution curves also were compared between the control, stretched, and stretched with SERCA2 overexpression myocytes (Fig. 7). Figures 7A shows the dynamic restitution curves of representative examples from the 3 groups. Interestingly, all 3 groups showed similar kinetics (with a similar time constant) and similar maximum APD restitution slope (Fig. 7B), reaffirming that susceptibility of stretched myocytes to alternans is not determined by its APD restitution properties.

Mechanical stretch increases susceptibility to alternans in intact atria. Finally, we tried to validate the finding that mechanical stretch increases susceptibility to alternans in the intact whole atria. The rat left atrium was stretched mechanically by a latex balloon. At a high pacing rate (pacing cycle length: 100 ms), discordant APD alternans was observed only in the stretched left atria, but not in the control nonstretched left atria (Fig. 8).

Discussion

Main findings. The present investigation shows that APD-ALT and Ca-ALT are inducible in atrial myocytes and are rate dependent. Mechanical stretch significantly

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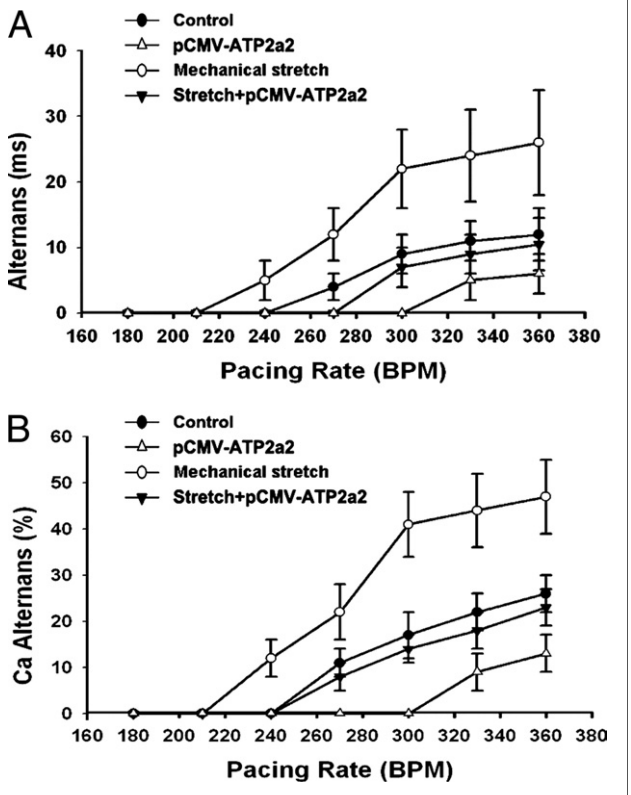

Figure 5 **Stretch-Induced Increase of Susceptibility to Alternans in Atrial Myocytes Is Reversed by ATP2a2 Overexpression**

Summary data for all optical mapping experiments demonstrating the relationship of PR and alternans from control, ATP2a2 overexpression (pCMV-ATP2a2), stretched, and stretched with ATP2a2 overexpression (stretch + pCMV-ATP2a2) myocytes are shown. **(A)** In stretched atrial myocytes, more APD-ALT was observed at each PR tested, and stretch produced a leftward shift in the APD-ALT–to–PR relationship, which was prevented by ATP2a2 overexpression. **(B)** In stretched atrial myocytes, more Ca-ALT was observed at each PR tested, and mechanical stretch produced a leftward shift in the Ca-ALT–to–PR relationship, which also was prevented by ATP2a2 overexpression. Abbreviations as in Figures 1 and 3.

increases the susceptibility to Ca-ALT, and thus APD-ALT and discordant alternans, by decreasing SERCA2 expression and by slower diastolic Ca reuptake kinetics. Targeted SERCA2 gene overexpression reverses the effect of stretch on increasing the susceptibility to alternans. This is also the first report to provide the direct evidence that stretch induces alternans and that stretch-induced alternans can be targeted by therapies aimed at overexpressing SERCA2 in cardiomyocytes.

Alternans and mechanism of AF. It has been demonstrated that APD-ALT is linked to a mechanism of arrhythmogenesis, where spatially discordant alternans between myocytes amplifies repolarization gradients to produce conduction block and re-entrant excitation (3). It also has been shown that premature beats or rapid firings induce repolarization alternans, including discordant alternans (13,14). These findings provide the theoretical basis

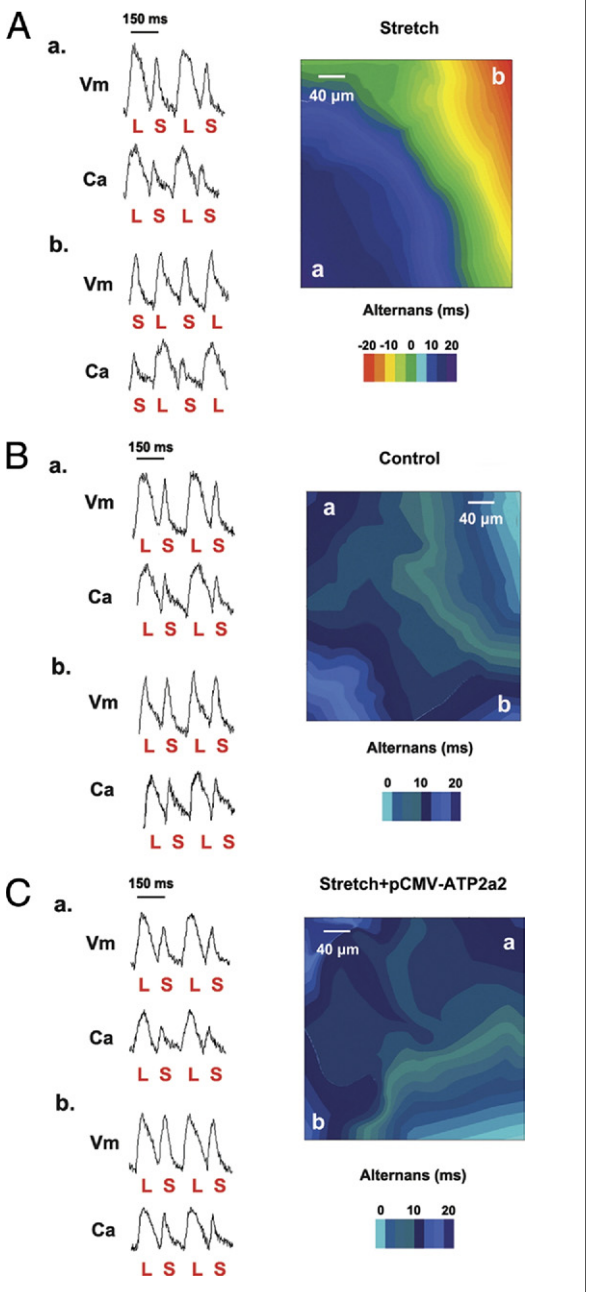

Figure 6 **Mechanical Stretch Enhances Susceptibility to Spatially Discordant Alternans, Which Is Prevented by ATP2a2 Overexpression**

(A) In mechanically stretched myocytes, alternans, which is out of phase between site a and site b (discordant alternans), is observed at pacing rate of 360 beats/min. The **right panel** shows the contour map of APD-ALT magnitude (contour intensity) and phase (contour color: **red** is +phase, **blue** is −phase). At the 360 beats/min pacing rate, discordant alternans is observed, because APD of myocytes at site a and site b alternate with opposite phase, depicted by the presence of both **red** and **blue** contours. **(B)** In control myocytes, alternans that is in-phase (concordant alternans) between site a and site b is observed at 360 beats/min pacing rate. In the contour map, concordant alternans is distributed across the entire map region (both site a and site b). **(C)** In stretched myocytes with ATP2a2 overexpression, discordant alternans is no longer observed at pacing rate 360 beats/min. Vm = membrane action potential; other abbreviations as in Figures 1 and 2.

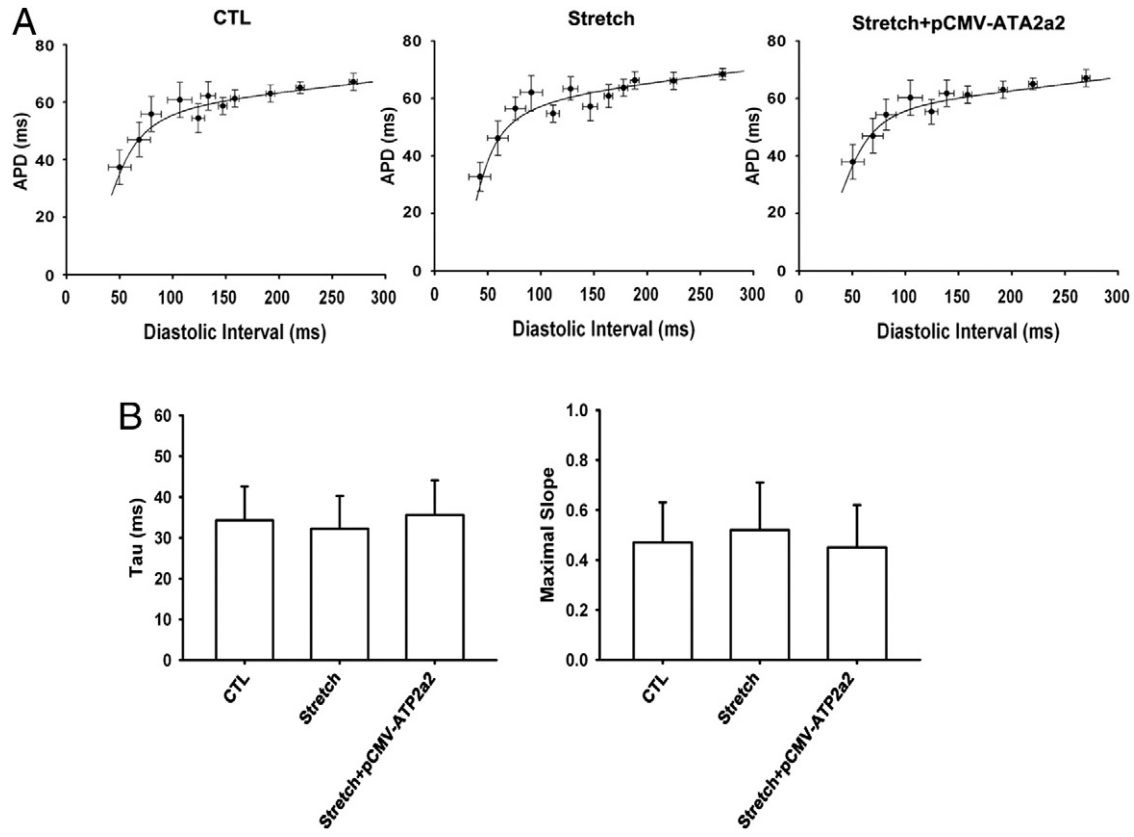

Figure 7 The Effect of Stretch on APD Restitution Kinetics

Dynamic restitution curves are compared between the CTL, stretched, and stretched with ATP2a2 overexpression (stretch + pCMV-ATP2a2) myocytes. **(A)** The dynamic restitution curves of representative examples from the 3 groups are shown. **(B)** Summary data of time constant tau (τ) and maximal slope of the APD restitution curve for the 3 groups. All the 3 groups showed similar kinetics (with a similar time constant) and similar maximum APD restitution slope. Abbreviations as in Figures 1 and 3.

regarding how premature beats or rapid firings from the thoracic veins or atria proper trigger AF (5). As such, recently it has been shown that rapid atrial pacing induced discordant alternans and was associated with the initiation of AF in humans (15).

In the present study, we showed that the stretch of atrial myocytes increased susceptibility to cellular alternans, including discordant alternans. This finding implicates that stretching of the atria, such as in conditions of heart failure, mitral valvular diseases, or hypertension, increases susceptibility to alternans and conduction block, and thus the initiation of AF in the presence of premature beats or rapid firing foci. We also showed that this mechanism was through decreased SERCA2 expression. Therefore, the results of the present study indicate that SERCA2 may be a reasonable therapeutic target for treatment of stretch-related AF, such as AF in heart failure, mitral valvular diseases, or hypertension.

In addition to stretch, structural barriers are also one of the important triggering factors for discordant alternans (16). Atrial fibrosis or structural remodeling is one of the major mechanisms of AF. Although it is generally believed that atrial fibrosis results in conduction slowing or block and thus initiation of AF (17), it also is very likely that atrial fibrosis produces regional heterogeneity of Ca handling or repolarization, and thus increases the susceptibility for discordant alternans.

Mechanical stretch, SERCA2, and alternans. Previously, it was shown that targeted SERCA2 overexpression in "normal" heart decreases the susceptibility to ventricular arrhythmia (18). Herein, we provided the first evidence that targeted SERCA2 overexpression decreases the susceptibility to cellular alternans in stretched cardiomyocytes. We also showed that stretch did not alter the membrane APD restitution kinetics. These findings suggest that susceptibility of stretched atrial myocytes to alternans is determined by its Ca kinetics, but not by its membrane APD restitution properties, compatible with the findings in the intact whole heart (18).

How stretch induces spatially discordant alternans remains unknown. Narayan et al. (19) first reported the existence of atrial decoupling, resulting in regionally nonuniform APD alternans. We also observed regionally nonuniform magnitude of APD alternans in control HL-1 myocyte monolayers at a high pacing rate, indicating the

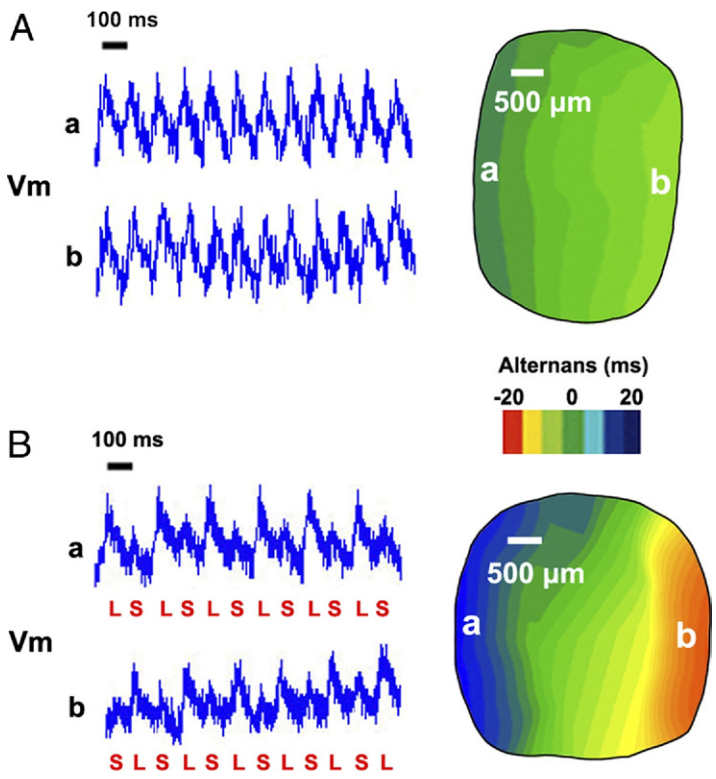

Figure 8 **Mechanical Stretch Increases Susceptibility to Discordant Alternans in Intact Rat Atria**

(A) In the control atrium, no significant APD alternans was noted at pacing rate of 600 beats/min. **(B)** In the stretched atrium, APD alternans that is out of phase between site a and site b (discordant alternans) is observed at pacing rate of 600 beats/min. The **right panel** shows the contour map of APD alternans at the recorded area. At the 600 beats/min pacing rate, discordant alternans is observed, because APD of myocytes at site a and site b alternate with opposite phase, depicted by the presence of both **red** and **blue** contours. Abbreviations as in Figures 1, 2, and 6.

existence of decoupling. It is possible that this decoupling of the HL-1 myocyte monolayer may be amplified by stretch and is responsible for the spatially discordant alternans.

Study limitations. In the present study, we used rapid field pacing to promote alternans (we failed to do point pacing in HL-1 myocyte monolayer at higher rates). During field pacing, all the myocytes were captured at the same time; thus, no conduction pattern, such as reentrant conduction or conduction block, could be observed. However, conduction block is the key factor to link alternans to arrhythmia (20). Therefore, we could not induce any arrhythmic activity or fibrillation during high-rate field pacing. Nevertheless, our primary goal was to evaluate the cellular mechanism of alternans at a high depolarization rate, and we have demonstrated that at a high depolarization rate, atrial myocytes also are susceptible to alternans, even in the setting of in vitro culture, as observed in the intact heart. Second, how stretch decreases SERCA2 expression is unknown. It has been shown that angiotensin II decreases SERCA2 expression (21). Mechanical stretch may activate the cellular renin–angiotensin system or other autocrine systems (22). We speculate that stretch-induced decreased SERCA2 expression is related to the effect of autocrine factor(s).

Conclusions

The results of the present study prove the concept that mechanical stretch decreases the expression and function of SERCA2 in atrial myocytes, thus increasing the susceptibility to alternans. These results also explain the susceptibility to AF in conditions of atrial stretch, such as mitral valvular heart disease, heart failure, and hypertension.

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Key Words: atrial fibrillation ■ atrial myocyte culture ■ optical mapping ■ repolarization alternans.