Stretch-activated currents in ventricular myocytes: amplitude and arrhythmogenic effects increase with hypertrophy

A. Kamkin, I. Kiseleva, G. Isenberg

Department of Physiology, Martin Luther University, Magdeburgerstrasse 6, D-06097 Halle, Germany

Received 11 April 2000; accepted 24 July 2000

Abstract

Background: Mechanical dilation of the human ventricle is known to induce arrhythmias, the underlying ionic mechanisms, however, remain to be clarified. Methods: Ventricular myocytes isolated from human, guinea-pig or rat hearts were stretched between the patch electrode and a glass stylus. Results: Local stretch prolonged the action potential, depolarized the resting membrane and caused extra systoles. Under voltage-clamp conditions, stretch activated several ionic current components. The most prominent current was a stretch activated current ($I_{\text{SAC}}$) through non-selective cation channels. $I_{\text{SAC}}$ followed a linear voltage-dependence, reversed polarity close to 0 mV and was suppressed by 5 μM Gd$^{3+}$. During stretch, $I_{\text{SAC}}$ became steady within 200 ms. $I_{\text{SAC}}$ did not inactivate and it completely disappeared upon relaxation. Stretch-sensitivity was evaluated from the slope of $I_{\text{SAC}}$ versus amplitude of stretch. Stretch sensitivity was 75 pA/μm in myocytes from young (3 month), 143 pA/μm in myocytes from old (15 months), and 306 pA/μm in hypertrophied myocytes from old (15 months) spontaneously hypertensive animals. Stretch sensitivity was 262 pA/μm in hypertrophied myocytes from human failing hearts, and it was 143 pA/μm in guinea-pig ventricular myocytes. Conclusions: Local stretch of adult single ventricular myocytes can induce arrhythmias that resemble surface-recordings from whole hearts. Stretch modulates multiple current components, $I_{\text{SAC}}$ being the current with the largest arrhythmogenic potential. Stretch-sensitivity of $I_{\text{SAC}}$ is higher in hypertrophied than in control myocytes as can be expected from the observation that hypertrophy and failure increase the risk of stretch-induced arrhythmias. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Arrhythmia (mechanisms); Hypertrophy; Ion channels; Membrane currents; Membrane potential; Stretch/m–e coupling; Ventricular arrhythmias

1. Introduction

Mechanical dilation of the human ventricle is a well-known risk for stretch-induced ventricular arrhythmias with subsequent ventricular fibrillation and sudden cardiac death [1,2]. Despite this immense clinical relevance, the present understanding of the underlying mechanisms remains incomplete. Computer models attributed the stretch-induced arrhythmias to stretch activated channels (SACs) [3–5]. The models are based on evidence from embryonic chicken heart myocytes where mechanical stimulation induced single currents through SACs and increased the cytosolic $Ca^{2+}$ concentration [6,7].

To record intracellularly during stretch is a technically difficult task because the cell must remain without damage. Until now, the conclusions from those measurements are at controversy. When guinea-pig ventricular myocytes were attached with their two cell ends to carbon fibers, the stretch developed by the auxotonic contraction was reported to prolong the action potential (AP) without significant changes of the membrane currents [8–10]. Rat ventricular myocytes were fixed to suction pipettes, here, stretch between the two ends prolonged the AP and induced inward currents through SACs ($I_{\text{SAC}}$) [11]. Similarly, stretch of multicellular ventricular trabeculae was reported to prolong the AP [12].

The present study uses a method that stretches the isolated myocytes not end-to-end but locally by means of a glass stylus [7]. It demonstrates that stretch can prolong the action potential and can induce extra systoles and arrhyth-
mias. The present voltage-clamp analysis indicates that local stretch modulates not only \( I_{\text{SAC}} \) but also \( K^+ \) and \( Ca^{2+} \) currents. Our study finally presents data suggesting that the sensitivity to stretch increases with cardiac hypertrophy. We were surprised to find that hypertrophied human ventricular myocytes, isolated from explants of patients with heart failure, were much more stretch sensitive than myocytes from healthy guinea pigs. Since an inter-species comparison is not possible, we demonstrated for rat ventricular myocytes that stretch-sensitivity of \( I_{\text{SAC}} \) increases with aging (15 months versus 3 months old rats) and with hypertrophy (15 months normotensive versus 15 months hypertensive rats). Thus, the present results provide an explanation for the clinical experience that hypertrophic remodeling after infarction [13] increases the risk for stretch induced arrhythmias [14].

2. Methods

2.1. Cell isolation and solutions

Ventricular myocytes (VM) were dispersed by standard collagenase dissociation technique [15]. Human myocytes were prepared from tissue-chunks of explanted hearts from patients suffering from dilated cardiomyopathy (54±8 years age). The study was approved by the Ethical Committee of the University of Halle. Guinea-pigs and rats were killed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). During the experiment, the cells were continuously superfused with a 37°C warm physiological bath solution composed of (in mM) 150 NaCl, 5.4 KCl, 1.8 CaCl\(_2\), 1.2 MgCl\(_2\), 20 glucose, 5 HEPES/NaOH (pH 7.4). Whole cell patch clamp recordings were performed with patch pipettes (1.5 MΩ tip resistance) filled with K\(^+\) electrode solution composed of (in mM) 140 KCl, 5 Na\(_2\)ATP, 5.5 MgCl\(_2\), 0.01 EGTA, 10 HEPES/KOH adjusted to pH 7.2. In experiments where K\(^+\) channel currents were suppressed, Cs\(^+\) ions substituted the K\(^+\) ions in both bath and electrode solution.

2.2. Measurement of membrane potential and whole cell currents

Membrane potentials and currents were recorded with an RK400 amplifier (Biologic, Echirolle, France) connected to a personal computer via a CED-1401 interface (Cambridge Instruments, Cambridge, UK). Data were filtered at 2 kHz, sampled at 5 kHz and evaluated by customer written software. The 140-ms pulses were applied at 1 Hz and started from a holding potential of −45 mV to inactivate the TTX-sensitive Na\(^+\) channel current. The current through L-type Ca\(^{2+}\) channels (\( I_{\text{Ca-L}} \)) was estimated as difference negative peak current minus late current at the end of the 140 ms pulse [16]. Currents in response to trains of short 5 mV pulses (applied at −45 mV) were evaluated in terms of the membrane capacitance (time integral) and the access resistance (time constant divided by time integral [17]). During the ~15 min of the experiment, the access resistance as well as the seal resistance remained constant (see Results).

2.3. Ventricular hypertrophy

The first experimental series worked with VM from human failing hearts (VMHF). On average, these cells were 155±16 \( \mu \)m long, 32±7 \( \mu \)m wide and had a membrane capacitance of 279±22 pF (mean±S.D., \( n=12 \)). Control myocytes from healthy humans hearts were not available. Instead, we measured the response of VM from young guinea-pigs (VMGP, 3 months age). The guinea-pig ventricular myocytes were 105±10 \( \mu \)m long, 19±4 \( \mu \)m wide and had a membrane capacitance of 155±11 pF (\( n=16 \)). When we discovered that VMHF were more sensitive to stretch than VMGP, we extended the analysis to VM from young Wistar–Kyoto (WKY) rats (3 months), old WKY rats (15 months) and rats with spontaneous hypertension (SHR, 15 months). The morphological dimensions of myocytes from young WKY rats were 125±8 \( \mu \)m in length and 25±5 \( \mu \)m in width, the membrane capacitance was 142±12 pF. The counterparts for myocytes from old WKY rats were 136±9 \( \mu \)m in length, 26±6 \( \mu \)m width and 161±18 pF capacitance. VM from old SHR were 158±18 \( \mu \)m long and 31±9 \( \mu \)m wide, their membrane capacitance was 218±21 pF. In myocytes from old SHR the plate area (product length times width) was 57% greater and the membrane capacitance was 51% greater than in myocytes from young WKY suggesting that the cells from old SHR were hypertrophied. Cellular dimensions and membrane capacitance suggest that also the VMHF are hypertrophied; although a direct comparison with control cells is not available, this assumption is in line with the literature [18,19].

2.4. Mechanical stimulation.

After seal formation and whole cell access of the patch pipette, a fire-polished glass stylus was attached to the membrane [6,7]. For successful attachment, the stylus had to be freshly polished and the surface membrane of the cell had to be clean (success rate ≈50%). The attached part of the cell followed the stylus when this was lifted by 2 \( \mu \)m. The small lift was done with the idea that the coverslip should not ‘scratch’ the bottom surface of the cell during stretch. A motorized micromanipulator (Sutter MP 285, Novato CA, accuracy 0.2 \( \mu \)m) increased the distance between patch pipette (P) and glass stylus (S) by 2–10 \( \mu \)m, the patch-electrode being the fix-point (Fig. 1). The stretch–relaxation experiment could be repeated on the same cell three times, on average.
Fig. 1. Mechanical stimulation: local stretch of a ventricular myocyte from the guinea pig (VMGP). (A) Before, (B) during stretch. Left: original microphotographs. Right: labels for glass stylus S, microbead B attached to the cell surface, patch pipette P, and a line connecting S over B to P. Numbers indicate the sarcomere length. Increasing the distance S±P by 7 μm (connecting line from 31 to 38 μm or by 22%) increases distance S±B and B±P by the same extent of 22%. The sarcomere length was 1.83±0.01 during control (n=18, analyzed between S and P from top to bottom). Stretch increased the sarcomere length to 1.94±0.08 (B), the large S.D. caused by sarcomere inhomogeneities. As indicated by numbers, sarcomere length increased up to 2.09 μm between S and P, however, only to 1.95 μm at the peripheral regions. Left to S and on the right to P, the sarcomere length did not change significantly.

The effects of mechanical stimulation on the sarcomere pattern were imaged by means of a slow scan CCD camera (Princeton Instruments, ST138) and evaluated by MetaMorph software (Universal Imaging, West Chester, PA). Sarcomere length was estimated from the distance between ten sarcomeres lying on the same axial myofila-ment, on average the sarcomere length was 1.825±0.014 μm (>10 counts on 12 VMGP).

The stretch experiments started by positioning stylus and patch electrode at ≈40 μm distance apart before attaching them to the cell. By this procedure, the area of stretched membrane was restricted to approximately one third of the cell length (see first part in the Results). To monitor changes of the surface membrane, microbeads of 4 μm diameter (Spherotech, Libertville, IL) were added to the cells 20 min in advance of the stretch experiment.

If one compares the larger amplitudes of currents that were recorded from hypertrophied myocytes with those from control cells, one worries how much of this difference is due to the increase in membrane area with hypertrophy. The effect of an increased cell length is without influence because the distance between the glass tools was constant. Since myocytes from SHR have larger diameter (31±9 μm) than those one from control rats (25±6 μm), this could increase the stretched membrane area by 25%, an effect that is small in relation to the 100–200% changes indicated by the measurements. Furthermore, we will show in Section 3.1. that the glass stylus stretches the surface mostly within a stripe adjacent to the line connecting the stylus with the patch pipette. Due to this spatial decrement of the deformation, the influence of cell diameter on the area of stretched membrane is probably much smaller than 25%. Since the membrane capacitance would measure the total and not the stretched membrane, the stretch-induced currents were not normal-ized by the membrane capacitance.
2.5. Statistics

Values are given as mean±S.D. Significances were determined by Student’s t-test. Significance was assumed at P>0.05.

3. Results

3.1. Local stretch by means of the attached glass stylus

The present protocol moved away the cell attached glass stylus (S) from the patch pipette (P) (Fig. 1: from 31 to 38 μm or by 22%). The resulting stretch increased the distance between S and the microbead (B) and the distance between B and P at the same extent (22% in Fig. 1B), suggesting that the stylus did not slip on the surface membrane. The sarcomere length (SL), however, did not follow the glass stylus as expected. On average, SL before stretch was 1.83±0.01 μm (Fig. 1A: n=13 evaluations in the area on top or below the line S–B–P). During local stretch, the average SL increased to 1.92±0.08 μm or by 5% (Fig. 1). The different lengthening, 5% SL versus 22% surface (microbead) and the increased S.D. of the SL are a first indication that the stylus induced surface deformation spread with spatial decrement.

In seven experiments, multiple beads had been attached, and the stylus induced changes of the surface area could be analyzed both within and outside the S–P line. When the lengthening between S and P was 25±5%, this stretched the cell surface by 24±6% when the bead had attached in the S–P line, by 22±5% at points 2 μm away, by 20±8% at a 6-μm distance, and by only 15±9% when the beads were 10 μm away from the S–P line. These results suggest that the glass stylus stretched the cell surface with spatial gradients, i.e. the local deformations decay from the S–P line to the neighbored surface sarcolemma.

In Fig. 1, the local stretch increased the SL in the line S–B–P by 14% (from 1.83 to 2.09 μm), however, 10 μm above or below this line, the SL increased by only 6% (from 1.83 to 1.95 μm, numbers indicated in Fig. 1B2). Results similar to those of Fig. 1 were obtained in 16 experiments. The maximal increase in SL was always in the axis between S and P, whilst SL at the margin of the cell increased only by 20–50% of this maximum. Furthermore, the maximal stylus induced lengthening of SL was always smaller than the stretch of the cell surface (63±12%, n=16). We postulate a spatial decrement also for the spread of deformation from the cell surface to the inner cell that generates the SL pattern.

3.2. Stretch induces membrane depolarization and action potential prolongation

Ventricular myocytes from human failing hearts responded to 2 μm stretches with a depolarization of 10±3 mV (mean±S.D., n=6). With 2-μm stretches, the AP duration (APD, measured at 90% repolarization) increased from 341±15 to 571±68 ms. Stretch accelerated the repolarization during the initial phase 1 of the AP but retarded it during the later phase 2 (Fig. 2A). Changes in the repolarization resembling those in Fig. 2 have been recorded with surface electrodes from intact animal hearts [3] or tissue strips [12,20] and reported to cause early after-depolarizations and arrhythmias in humans [2].

Myocytes from healthy guinea-pig ventricles (VMGP) responded to stretch in a similar way as the VMHF did. However, to obtain comparable changes in resting potential (RP) and AP, the mechanical stimuli had to be 2- to 4-fold larger in VMGP than in VMHF. The 2-μm stretches modified neither RP nor AP (n=8) of VMGP; 4 μm stretches depolarized the resting membrane by 3±1 mV (n=5), and 8 μm stretch depolarized by 6±2 mV. The 8-μm stretches prolonged the APD from 360±14 to 502±27 ms (n=8, compare Fig. 2C); 8-μm stretches (n=3) and 10-μm stretches (n=5) triggered extra APs.
(synonymous extra systoles, ES) that started from the diastolic depolarization (label 3 in Fig. 2C). The AP following the ES was abbreviated.

### 3.3. Stretch modulation of the net membrane current

In VMHF, the membrane potential was clamped to −70 mV, a value close to the resting potential of these cells. At −70 mV, 2 μm stretches reduced the current from +126±32 to −28±41 pA (n=4 VMHF). In VMGP clamped to −70 mV (Fig. 3A), 8-μm stretch reduced the current from +150 pA (trace C) to −120 pA (trace S). At −70 mV, the stretch-induced difference current (ΔI, −270 pA, trace D in Fig. 3A1) was nearly time-independent and slightly more negative than the holding current at −45 mV (−270 vs. −210 pA, Fig. 3A1 trace D: initial 20 ms versus currents at later times).

The local stretch may have induced currents that do not flow through channels but through a leak. Following the assumption that the stretch could have broken the seal between the patch electrode and the surface membrane, we had repeated the stretch experiments with the patch pipette in the cell attached configuration (n=7). Essentially, the seal resistance remained constant, i.e. it was 1.6±0.3 GΩ before and 1.5±0.4 GΩ during the stretch. The local stretch did not induce single channel currents in the cell attached patch. Also, the access resistance and the membrane capacitance remained unmodified (n=6 VMHF, 12 VMGP). Hence, the stretch-induced inward current should be attributed to the activation of an ionic current and not to a leakage around the seal.

At 0 mV, stretch shifted the current in the positive direction, i.e. ΔI, was +240 pA (Fig. 3A2). Stretch attenuated the L-type Ca²⁺ channel current I_{Ca,L} (Fig. 3A2 negative current wave from light gray, control, to dark gray, during stretch). At 0 mV, the difference current decayed from a positive peak (Fig. 3A2 trace D), as if stretch-suppressed time-dependent I_{Ca,L} superimposed with the time-independent I_{SAC} (see next paragraph). Fig. 3B compares the voltage-dependence of the membrane cur-

![Fig. 3. Modulation of net membrane currents by 8 μm stretch, VMGP. I_{Ca,L} not suppressed. Net membrane currents before (label C) and during stretch (label S) together with stretch-induced difference currents (label D, trace at the top). (A) Starting from a holding potential of −45 mV, 140 ms steps went to −70 mV (A1) or 0 mV (A2). Note: the current in presence of stretch is more negative than the control current at −70 and −45 mV, however, more positive at 0 mV. Before and after each voltage-step, currents in response to a 10-ms 10-mV voltage ramp command measured cell membrane capacitance. (B) Current voltage relation of the currents measured at the end of pulse (late current I, triangles) and peak Ca²⁺ channel currents (I_{Ca,L} = negative peak current minus I, circles). Empty symbols before, filled symbols during the 8-μm stretch. (C) The stretch induced difference current follows an outwardly rectifying voltage dependence and reverses polarity at E_{rev} = −16 mV.](https://academic.oup.com/cardiovascres/article-abstract/48/3/409/485463)
rents before (empty symbols) with those during stretch (filled symbols). The modest change in the curve through the circles indicates the stretch mediated attenuation of peak $I_{\text{Ca-L}}$ (for quantified data, see next paragraph). More importantly, the ‘late currents’ measured at the end of the 140 ms pulses ($I_L$, triangles) were reduced at negative and increased at positive potentials. The stretch induced changes in the late currents ($\Delta I_L$) followed an outwardly rectifying voltage-dependence with a reversal potential ($E_{\text{rev}}$) of $-16$ mV (Fig. 3D). Stretch induced changes in net membrane currents similar to those in Fig. 3 were observed in 7 VMGP (8 and 10 μm stretch) and 2 VMHF (2 μm stretch). The changes suggest that stretch modulates not a single but several ionic current components (see below).

### 3.4. The stretch activated current $I_{\text{SAC}}$

To simplify the separation of net currents into current components, currents through inwardly rectifying K+ channels ($I_{\text{Kir}}$) were suppressed by substituting K+ by Cs+ ions, in the cytosol by dialysis of Cs+ electrode solution and in the bath by replacing 5.4 mM KCl by 5.4 mM CsCl (stretch-activated currents recorded under these conditions will be labeled as $I_{\text{SAC}}$). In VMHF, 4 μm stretch induced a late difference current that followed an almost linear voltage-dependence and crossed the voltage axis at a reversal potential $E_{\text{rev}}$ of $-16$ mV (Fig. 4A and C). On average, $E_{\text{rev}}$ was $-11 \pm 5$ mV (5 VMHF). The stretch-induced current was insensitive to substitution of Cl− ions by aspartate− ions (n=5), hence $I_{\text{SAC}}$ is likely to be carried by cations and not by Cl− ions. Stretch-activated $I_{\text{SAC}}$ was suppressed by Gd3+ (Fig. 4D, compare Refs. [21,22]), by 100 μM within 1 min and by the usual 5 μM Gd3+ within 7–10 min. In the presence of stretch, the late currents measured in absence and presence of Gd3+ crossed at a reversal potential of $-1 \pm 4$ mV (n=5 VMHF). Linear voltage-dependence, $E_{\text{rev}} = -11$ mV and Gd3+ sensitivity suggest that stretch activated $I_{\text{SAC}}$ flows through mechanically activated non-selective cation channels (SACs, compare Refs. [6,11,23]). The late membrane currents in absence of stretch were slightly but not significantly increased by Gd3+ (5 VMHF, 6 VMGP, compare the

![Fig. 4. VMHF: Stretch activation of Gd3+-sensitive non-selective cation currents, 4 μm stretch, $I_{\text{Ca-L}}$ suppressed. (A) Stretch modulation of membrane currents during 140 ms steps from $-45$ to $-30$, $-20$, and 0 mV (B) Voltage-dependent of peak $I_{\text{Ca-L}}$ (circles) and late current (triangles) before (empty symbols) and during stretch (filled symbols). (C) Stretch induced difference current $I_{\text{SAC}}$. (D) Stretch-induced late (triangles) and peak inward currents $I_{\text{SAC}}$ before (filled) and after addition of 5 μM Gd3+ (empty symbols). Note: Late currents in absence and presence of Gd3+ cross at a reversal potential of 0 mV. Gd3+ also attenuates $I_{\text{Ca-L}}$ (from filled to empty circles).](#)
empty triangles between Fig. 4B and D; compare non-selective background currents in rat atrial myocytes [24]). At the usual concentration of 5 μM Gd3+, a 10% reduction of the delayed rectifier K+ current can be expected [25], this possible effect was neglected.

In VMGP with blocked inward rectifier K+-current (I\textsubscript{K1}), stretches of 8 μm induced inward currents for all negative potentials (Fig. 5). The stretch induced currents followed a linear voltage dependence and reversed at \( E_{rev} = -5 \pm 3 \) mV (n=6). The result that \( E_{rev} \) was closer to zero mV in myocytes from healthy guinea-pig than in those failing human hearts may be attributed to the smaller cell size of VMGP than of VMHF that permitted more complete block of K+ currents by cell dialysis of Cs+ ions.

3.5. Ion-selectivity of \( I_{SAC} \)

Replacement of extracellular 150 mM NaCl by 75 mM CaCl\(_2\) reduced the stretch-induced inward currents at negative potentials, however, did not abolish them (Fig. 6A,B). At the holding potential of −45 mV, the ion substitution reduced the inward currents to 32±6%. Substitution of Na\(^+\) by Ca\(^{2+}\) ions shifted the reversal potential of the stretch induced current from −5 to +25±8 mV. The difference current followed an outwardly rectifying voltage-dependence, that is, the inward current remained almost constant when the Ca\(^{2+}\) diving force was increased by setting the clamp steps to potentials to potentials more negative than −20 mV. The results suggest that Ca\(^{2+}\) ions can permeate through the SACs, that their permeability is smaller than the permeability for Na\(^+\) ions, and that the Ca\(^{2+}\) ions may interact with the SAC-protein during permeation (fast flickering block of SACs by Ca\(^{2+}\) and by Gd\(^{3+}\) [21,26]).

As expected for a non-selective cation channel, substitution of 150 mM extracellular Na\(^+\) by 150 mM tetraethyl ammonium (TEA\(^+\)) reduced \( I_{SAC}(−45 \text{ mV}) \) to 31±6% and shifted the reversal potential to −20±6 mV (Fig. 6C). Substitution of Na\(^+\) with the larger cation N-methyl-D-glucosamine (NMDG\(^+\)) reduced \( I_{SAC}(−45 \text{ mV}) \) to 20±3% and shifted the reversal potential to −42±6 mV (n=5 VMGP). The results indicate that both TEA\(^+\) and NMDG\(^+\) can permeate through SACs although with a permeability that is low in comparison to the permeability of Na\(^+\) or Ca\(^{2+}\) ions.
Cs+ ions. They further suggest that the retarded repolarization and the diastolic depolarization are caused by Na+ influx through SACs.

3.6. Stretch modulates additional current components

The comparison of Fig. 3D with Fig. 5C indicates that stretch-activated currents follow a linear voltage-dependence only when the K+ currents had been suppressed. Subtraction of stretch-induced currents recorded after Cs+ block from those recorded before Cs+ block of K+ channels suggests that stretch activated an inwardly rectifying Cs+ sensitive current with a reversal potential at −90 mV, i.e. a K+ current. The present evidence for stretch activation of $I_{k1}$ confirms an early report on stretch-effects on VMGP [27].

During stretch, the sustained inward current $I_{SAC}$ of approximately −500 pA should cause intracellular accumulation of Na+ and Ca2+ ions, and this ion accumulation is expected to secondarily modify other current components [10,23,25,28]. Indeed, our results from Cs+ dialyzed cells (suppression of $I_{k1}$) show that stretch suppressed the L-type Ca2+ channel current $I_{Ca-L}$ (traces at 0 mV in Figs. 4 and 5). Evaluation of the current–voltage relations indicated that stretch suppressed $I_{Ca-L}$ by 13±6% in VMHF ($n=5$) and by 15±3% in VMGP ($n=8$). This reduction of $I_{Ca-L}$ was not recorded when cell dialysis of 5 mM BAPTA preceded the stretch for a 5-min period of
time ($n=4$, not illustrated), whilst stretch-induction of $I_{SAC}$ remained unmodified. The effect BAPTA is likely due to chelation of $Ca^{2+}$ ions, hence, the observed stretch-induced reduction of $I_{Ca,L}$ can be explained as ‘$Ca^{2+}$ inactivation’ due to intracellular $Ca^{2+}$ accumulation [26,29]. In summary, the present analysis indicates that stretch modulates the resting and action potential not by a single but by several ion current components. A comparison of the amplitude of these stretch-sensitive current components let us conclude that membrane depolarization and prolongation of the AP are dominated by the stretch-activation of $I_{SAC}$.

3.7. Time-course and reversibility of $I_{SAC}$

The present method applied the mechanical stretch within $\approx 200$ ms, the rate of stretch was limited by the rate of the stepping motor. Activation of $I_{SAC}$ ($-45$ mV) completed within these 200 ms (Fig. 7, similar results from 6 VMFH, 18 VMGP, 14 VM from WKY rats). The rapid activation of $I_{SAC}$ confirms the recent report of Zeng et al. [11] where both ends of the rat ventricular myocyte were stretched by means of a piezo-unit. During the stretch, $I_{SAC}$ ($-45$ mV) was constant for several minutes, i.e. inactivation or adaptation were not observed (see Fig. 7). The absence of inactivation during continuous stretch was true for ventricular myocytes from humans ($n=6$), guinea-pigs ($n=18$) and rats ($n=14$ WKY, $n=8$ SHR). The absence of $I_{SAC}$ inactivation is in line with recent results from rat atrial [24] and rat ventricular myocytes [30].

3.8. Stretch sensitivity increases with age and hypertension

The amplitude of $I_{SAC}$ increased with the amplitude of stretch. In VMHF with suppressed $I_{K1}$ and at $-45$ mV, 2 µm stretch induced a current of $-116\pm 25$ pA ($n=6$) and 4 µm stretch an $I_{SAC}$ of $-483\pm 28$ pA ($n=3$). In VMGP $I_{SAC}$($-45$ mV) was zero for 2 µm stretch ($n=19$), $-65\pm 21$ pA for 4 µm ($n=2$), $-300\pm 89$ pA for 6 µm ($n=6$), $-557\pm 78$ pA for 8 µm ($n=7$), and $-1050\pm 190$ pA for 10 µm stretch ($n=3$). Thus, the VMHF had a more sensitive stretch response than the VMGP. The high stretch sensitivity of VMHF could have been attributed to a species difference or to the hypertrophy as it occurs during the development of cardiac failure [27].
context, cellular hypertrophy is indicated by the morphological dimensions and membrane capacitance that were nearly twice as high in VMHF than in VMGP (see Methods).

To test whether augmented stretch sensitivity does indeed correlate with ventricular hypertrophy, we studied the effects of stretch on rat VM from young and old healthy animals (strain WKY) as well as from those with spontaneous hypertension (strain SHR). The SHR did not show signs of cardiac failure, however, their hearts were substantially hypertrophied as indicated by a ratio of cardiac weight to body weight that was twice as large as in young control WKY rats as well as by nearly the doubled cellular dimensions and membrane capacitance. With blocked K\(^+\) currents, VM from rats responded to stretch with an \(I_{\text{SAC}}\) that closely resembled the one described above for VMGP, that is, \(I_{\text{SAC}}\) followed a linear voltage-dependence, had a reversal potential of \(E_{\text{rev}}=-4 \pm 3\) mV and was suppressed by 5 \(\mu\)M Gd\(^{3+}\). In VM from 3 months young rats (WKY), stretches of 2 or 4 \(\mu\)m did not induce measurable \(I_{\text{SAC}}\) (Fig. 7D, filled circles). The 8–10 \(\mu\)m stretch induced inward currents of \(269 \pm 40\) pA (\(-45\) mV, \(n=5\)). In VM from 15 month old rats (WKY), 8 \(\mu\)m stretch induced a significantly larger \(I_{\text{SAC}}\) (\(-460 \pm 55\) pA, \(n=6, P<0.01\); filled squares in Fig. 7D). In VM from 15 month old rats with spontaneous hypertension (SHR, filled triangles in Fig. 7D), 2 \(\mu\)m stretches induced currents of \(-420 \pm 110\) pA, 4 \(\mu\)m stretches induced \(I_{\text{SAC}}=-1205 \pm 110\) pA (\(n=5\)) and 6 \(\mu\)m stretches currents as large as \(-1500\) pA (\(n=2\)).

The stretch-sensitivity can be evaluated from the position and the slope of the points that characterize \(I_{\text{SAC}}=-45\) mV as a function of stretch (Fig. 7D). As a first approximation, stretch-sensitivity was evaluated as the slope at stretches greater than 4 \(\mu\)m (linear regression). Regarding the rat ventricular myocytes, the stretch sensitivity was 77 pA/\(\mu\)m in young (3 month) and 132 pA/\(\mu\)m in old (15 months) WKY, however, 270 pA/\(\mu\)m in myocytes from old (15 month) SHR. The changes suggest that the stretch sensitivity increases with the extent of hypertrophy as it develops moderately during aging but strongly with the hypertension [31]. The stretch-sensitivity estimated in myocytes from failing human hearts was 260 pA/\(\mu\)m resembling the value obtained in myocytes from SHR. In summary, the data of Fig. 7D suggest that the modest stretch-sensitivity of myocytes from young healthy animals increases when the heart undergoes left ventricular hypertrophy.

4. Discussion

The results of this study demonstrate that myocytes isolated from ventricles of men, guinea-pigs or rats respond to mechanical stimulation with membrane depolarization, prolongation of the action potential and extra systoles (Fig. 2). The present voltage-clamp analysis suggests that \(I_{\text{SAC}}\) is the major cause of these stretch-induced events. At negative potentials \(I_{\text{SAC}}\) was negative and carried by influx of Na\(^+\) ions, and it was this negative \(I_{\text{SAC}}\) that retarded repolarization phase 2 and induced diastolic depolarization. The negative \(I_{\text{SAC}}\) was the only component that could generate extra systoles, if the depolarization reached threshold.

The present results are in conflict with several reports where stretch, produced during the auxotonic contractions, did not change the membrane currents (ends of guinea-pig ventricular myocytes attached to carbon fibers [8–10]). One could argue that activation of \(I_{\text{SAC}}\) would need spatial force gradients such as suggested by Fig. 1 and speculate that the channel protein would ‘sense’ the force between the superficial sarcolemma and the cytoskeleton linked to the inner structures of the z-lines. However, such a speculation is unlikely because results nearly identical to the present ones, prolongation of the action potential and induction of \(I_{\text{SAC}}\), were measured when rat ventricular myocytes were stretched in the end-to-end fashion without visible inhomogeneities in the sarcomere pattern [11]. We hope the present controversy may be clarified by future experiments.

Local stretch modulated not only \(I_{\text{SAC}}\) but also other current components. The stretch-activation of inwardly rectifying K\(^+\) currents is thought to attenuate the effects of \(I_{\text{SAC}}\) on the resting potential. The faster repolarization phase 1 and the shift of the plateau the action potential to more negative potentials (Fig. 2; phase 1) may be attributed to positive \(I_{\text{SAC}}\) (efflux of K\(^+\) ions through SACs), activation of \(I_{\text{K1}}\), and reduction of \(I_{\text{Ca,L}}\). Since stretch-inhibition of \(I_{\text{Ca,L}}\) was prevented by BAPTA-loading the cell, we interpret this effect not as a direct but as ‘secondary effect’ mediated by a stretch-induced Ca\(^{2+}\) accumulation (compare Ref. [26]). The Ca\(^{2+}\) accumulation could have been caused by Ca\(^{2+}\) influx through SACs, and in addition by the stretch-induced Na\(^+\) influx that causes Na\(^+\) accumulation and modulation of Na\(^+\),Ca\(^{2+}\)-exchange in turn [32,33]. The stretch induced Na\(^+\) accumulation should also have modulated the currents due to Na\(^+\),Ca\(^{2+}\)-exchange and Na\(^+\),K\(^+\)-ATPase, details about these ‘secondarily stretch-modulated currents’ are under present investigation.

How stretch activates SACs is not yet clear. The SACs of vertebrates have not yet been cloned, and the molecular pathways transducing the mechanical stimulus to the channel activation are essentially unknown. Signaling of stretch could occur via integrin receptors [34], activation of protein kinases phosphorylating and opening SACs. The present results do not favor this speculation because the stylus was free of matrix proteins (fire-polished glass). According to the literature, activation SACs could be gat ed by the tension developed in cytoskeleton structures coupled to the channel protein or by the tension developed in the lipid bilayer membrane [35].
The present study compared the response to stretch between myocytes from control and hypertrophied ventricles. In both cell types, $I_{\text{SAC}}$ did not flow in the absence of stretch. That is, the present study did not reveal a significant contribution of a persistently activated Gd$^{3+}$ sensitive $I_{\text{SAC}}$ as the swelling-activated inwardly-rectifying $I_{\text{SAC}}$ described hypertrophied canine ventricular myocytes [36,37]. In the present study, hypertrophied myocytes from hypertensive rats or patients with terminal failure responded more sensitive to stretch than myocytes from young healthy guinea-pig or rat hearts, that is, activation of $I_{\text{SAC}}$ required a smaller mechanical stimulus, and the amplitude of $I_{\text{SAC}}$ changed more steeply with the extent of stretch. The literature reports that hypertrophy of ventricular myocytes goes along with the re-expression of fetal proteins, for example F-type pacemaker channels [38]. In analogy, one could speculate that hypertrophy of ventricular cells could have augmented the expression of SACs thereby increasing channel density and amplitude of maximal $I_{\text{SAC}}$. Such a speculation may be supported by the observation that single channel recordings from SAC have repeatedly been published for embryonic and neonatal, however, never for adult ventricular myocytes [11]. Alternatively, one could attribute the higher stretch-sensitivity to a more effective transduction of the mechanical stimulus (surface deformation) to the channel protein because hypertrophy is known to stiffen the submembraneous cytoskeleton by increasing the concentration of extramyofilament microtubules in this region [39]. The speculations indicate that our understanding of mechanically activated ion channels in heart ventricular cells is still rudimentary. Nevertheless, the result of this study, activation of $I_{\text{SAC}}$ and induction of arrhythmias requiring smaller mechanical stimuli in hypertrophied than in normal myocytes, is in line with the observation that stretch induced arrhythmias occur more easily in hypertrophied and failing than in healthy hearts [1,2].

Acknowledgements

We thank Professor Dr E. Silber, Klinik für Herz- und Thoraxchirurgie, University Halle, for the human material, Drs B. Husse and F. Rudolf for their help with the preparation of myocytes and the Deutsche Forschungsgemeinschaft for financial support.

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