Effects of Applied Stretch on Native and Recombinant Cardiac Na+ Currents

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Chapter 7
Effects of Applied Stretch on Native and Recombinant Cardiac Na^+ Currents

Umberto Banderali, Robert B. Clark, Catherine E. Morris, Martin Fink and Wayne R. Giles

Abstract In the mammalian heart, electrical activity triggers and strongly modulates the contractions. In addition, under both physiological and pathophysiological conditions the mechanical activity of the heart may change tissue excitability, the action potential waveform and/or the pattern of conduction. In some cases, this mechanoelectrical feedback can alter the myocardium such that extrasystoles or rhythm disturbances are observed. It is thought that this sensitivity to mechanical perturbations is due to stretch-induced activation or alteration of ion channels which are expressed in the sarcolemma of cardiac myocytes. In the present manuscript, we describe studies on the effects of membrane stretch on the Na^+ channel alpha subunit, $Na_v1.5$ (which is predominant in the adult mammalian heart). Three different approaches have been considered: (i) recordings of Na^+ current from adult rat ventricular myocytes, (ii) studies of currents due to this Na^+ channel transcript expressed in a *Xenopus laevis* oocyte preparation, and (iii) integration of these findings, following appropriate alterations of the descriptors for this Na^+ current in a mathematical model of the human ventricular action potential. The results demonstrate that in both native mammalian myocytes and in the heterologous expression system, applied stretch causes the Na^+ current to activate at more negative membrane potentials. Stretch also significantly increases the Na^+ current density. When these effects are incorporated into a mathematical model of the human ventricular action potential, myocyte excitability is enhanced, and there is also a significant increase in the maximum rate of rise in the action potential. Thus, in the mammalian heart the effects of stretch on conventional time- and voltage-dependent intrinsic Na^+ currents need to be taken into account when attempting to understand either the basis for, or the consequences of mechanoelectrical feedback.

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7.1 Introduction

Cyclic contraction of the heart is a fundamental physiological event. Under most circumstances this excitation-contraction cycle is thought of as being unidirectional. That is, the contraction of the heart is triggered and modulated by the properties of the action potential, and the Ca^{2+} influx during the action potential initiates intracellular Ca^{2+} release, followed by myocyte shortening and/or tissue contraction. During relaxation, and in diastole, the myocardium essentially resets itself so that this excitation-contraction coupling phenomenon can be repeated. However, selected observations in the classical literature, and a number of detailed investigations within the last 20 years, have drawn attention to the fact that the mechanical activity of the heart itself can alter fundamental aspects of cardiac electrophysiology (Lab, 1998; Kohl et al., 2006). These alterations can occur both during the relatively long cardiac action potential and in the following resting or diastolic period (Horner et al., 1996). These electrophysiological changes are thought to be due to mechanical effects being transmitted to ion channels. Collectively, they have been termed "contraction-excitation coupling". Here, these effects will be referred to as "mechanotransduction" or "stretch-induced effects" (Horner et al., 1996; Isenberg et al., 2003; Eijsbouts et al., 2004; Nishimura et al., 2006; Taggart and Lab, 2008). Not surprisingly, although these effects can be recorded under physiological conditions in the adult heart they are more prominent in the embryonic and developing heart. Contraction-excitation coupling is also thought to be important in pathophysiological settings including heart failure, selected cardiomyopathies, and atrial dilation (Manios et al., 2006; Otway et al., 2007; Kuijpers et al., 2007; Taggart and Lab, 2008).

Papers published within the past decade have helped to define some aspects of the mechanisms and the consequences of mechanotransduction in the mammalian heart (Lab, 1998; Zhang et al., 2008a). Much of the initial focus and a significant amount of present effort is directed toward identification and characterization of so-called "stretch activated ion channels" (Hamill, 2006; Yaum et al., 2006). In general, these are thought to be nonselective cation channels having ion transfer functions (current-voltage curves) which are approximately linear (Kohl et al., 2006; Zhang et al., 2008b; Nishimura et al., 2008). However, in the fields of epithelial transport, neurophysiology, and cardiovascular sciences, it is now known that conventional ion selective time- and voltage-dependant channels undergo significant and selective biophysical changes in response to applied stretch (c.f. Tabarean and Morris, 2002; Laitko and Morris, 2004; Morris and Juranka, 2007a; Kalifa et al., 2007).

In the present manuscript, our main goal is to present and evaluate the possibility that the main type of Na^+ channel which is expressed in the mammalian heart can be modulated by applied stretch. To carry out this work conventional cell-attached patch clamp recordings have been made from adult myocytes and from

a heterologous expression system, the *Xenopus* oocyte in which the predominant mammalian cardiac Na^+ channel alpha subunit isoform ($Na_v1.5$) was expressed. Effects of stretch were identified by developing and applying three part protocols in which parts 1 and 3 served as control recordings while part 2 involved selected and fixed amounts of applied negative pressure. This pressure was maintained for the entire duration of the data acquisition that was needed for obtaining an activation curve, or for delineation of the time course of the recovery of this transient inward Na^+ current (Morris and Juranka, 2007b). Data analysis and integration, and an illustration of the observed effects on excitability of the action potential of the mammalian ventricular myocyte was achieved by adapting the ten Tusscher model of the action potential of the human ventricular myocyte for this purpose (Ten Tusscher et al., 2004).

7.2 Effects of Stretch on Na^+ Currents

7.2.1 Modification by Stretch of Na^+ Current Density and Kinetics

The experimental work presented in this manuscript, and many of the other papers in this volume, provide evidence that either transient or steady-state stretch applied to biological cells can elicit significant electrophysiological responses. In our study the focus has been the human cardiac Na^+ channel. Our results demonstrate significant, reversible stretch-induced changes on the gating of this conductance. Specifically, stretch causes changes in the activation and inactivation processes which regulate opening and closing of this Na^+ channel. These changes are such that within a narrow window of time the Na^+ current is enhanced. The effects of membrane stretch on the biophysical properties of mammalian cardiac Na^+ channels, $Na_v1.5$, are illustrated in Fig. 7.1. Panels B and C in this figure show significant and reversible effects of stretch on both the kinetics and the peak size of Na^+ currents. In Panel B records were obtained from an adult rat ventricular myocyte. Very similar results were obtained in studies of the so-called recombinant Na^+ current records from *Xenopus* oocyte experiments (Panel C). In both sets of experiments the myocyte or oocyte was clamped at a holding potential approximately 75 mV more hyperpolarized than the resting potential. From this negative potential 50 ms depolarizing voltage clamp steps of either 95 (Panel B) or 90 (Panel C) mV were applied to activate macroscopic Na^+ currents. To assess reversibility of stretch effects on I_{Na}, this voltage clamp protocol was applied under control conditions, during stretch, and then after a recovery period in which no stretch was applied. The results consistently showed reversible increases in peak current as well as acceleration of the kinetics of activation and inactivation.

Our results also demonstrate that this enhancement in current is substantial only in a quite narrow range of membrane voltages. This range is very close to the negative slope region of the current-voltage relationship, and/or the voltage threshold for initiation of the action potential. In fact, the increase in peak current is

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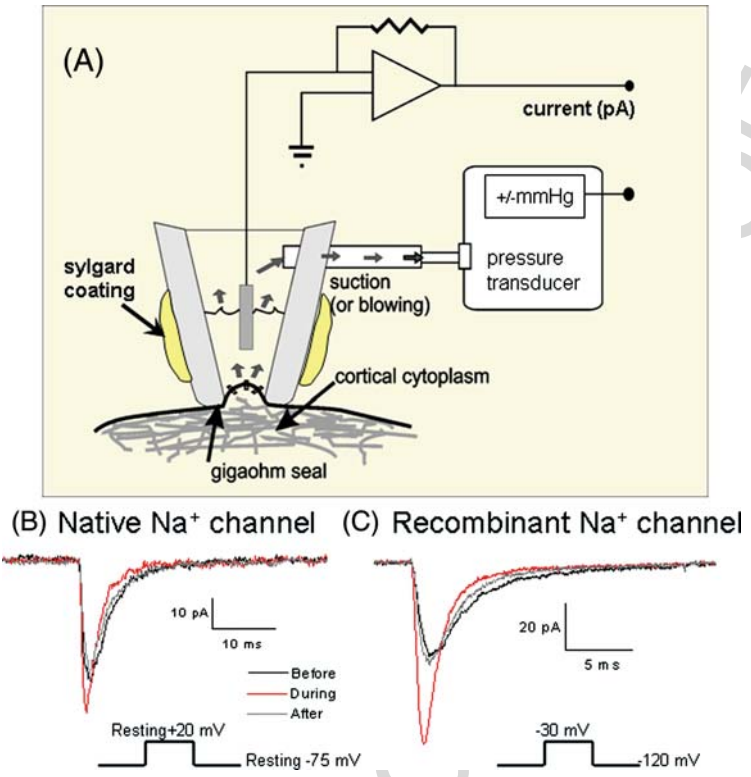

Fig. 7.1 **Panel A** Experimental setup for patch-clamp experiments involving applied stretch. The diagram illustrates a patch pipette sealed to the plasma membrane of a *Xenopus laevis* oocyte or a cardiac myocyte. The pipettes used in the experiments were pulled from thick-walled glass tubes and pipette tips were coated with sylgard to reduce electrical noise. A pressure transducer connected to the patch pipette allowed control and monitoring of the positive or negative pressure applied to the membrane patch. With the oocyte preparation, a high-K^+ solution containing (in mM) 89 KCl, 0.4 $CaCl_2$, 5 HEPES, 0.8 $MgCl_2$ (pH 7.4 with KOH) was used as extracellular medium. In the recording pipette, the KCl was substituted with 89 mM NaCl (pH adjusted to 7.4 by adding NaOH). $LaCl_3$ (250 μM) was added to the pipette solution to block the endogenous stretch activated cation channels. The La^{3+} ion at this concentration completely abolished the endogenous stretch activated currents and had only a small blocking effect of Nav1.5 (Bustamante 1987; Nathan et al., 1988). The human heart wild type Nav1.5 plasmid (pSP64T-hH1) was kindly provided by Dr. Al George (Makita et al., 1996). In the experiments in which native cardiac Na^+ currents were studied, adult rat ventricular myocytes were prepared for patch recordings as described previously (Ward and Giles, 1997). The same solution containing (in mM) 150 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 5.5 glucose (pH 7.4 with NaOH) was used both in the bath and in the pipette. No ion channel inhibitors were added. All other experimental conditions were identical to the experiments on recombinant channels. (For more details on experimental methods, see Morris and Juranka, 2007b) **Panel B** Cell-attached patch-clamp recording of sodium currents from an adult rat cardiac myocyte. The membrane was held at a voltage 75 mV more hyperpolarized than its resting potential (approx. –80 mV). A rectangular voltage clamp step to 20 mV more depolarized than resting potential elicited a transient inward current. Three current records are superposed: before application of negative pressure to the pipette (*black*), during application of –30 mmHg suction

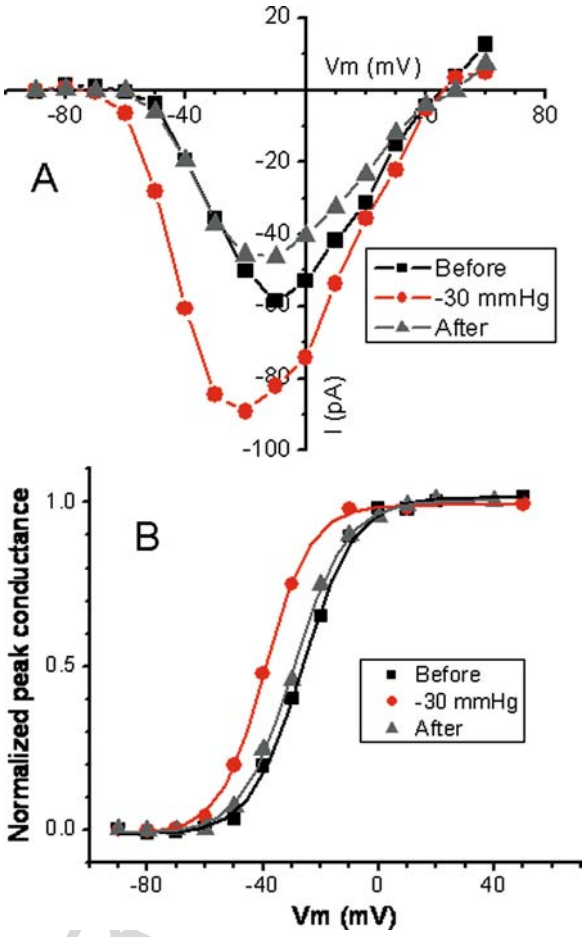

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Fig. 7.2 **Panel A** Peak current vs. voltage (I/V) relations for the recombinant Nav1.5 channel recorded (i) before application of negative pressure to the patch pipette, (ii) during application of –30 mmHg suction and (iii) after return to the non stretch conditions. Note that membrane stretch induces a reversible increase in peak currents which is more evident at the "foot" of the I/V curve, and throughout the voltage range of increasing conductance. **Panel B** Steady state activation curve for I_{Na}. Note that stretch shifts the $V^1/_2$ of the conductance vs. voltage relation approx 11 mV in the hyperpolarizing direction. These recordings were made using 0.25 mM La^{3+} ion in the patch pipette as inhibitor of the oocyte endogenous stretch activated channels. The conductance values in the steady-state activation curve were obtained dividing the peak currents in the I–V curve by the Na^+ ion driving force and normalizing the results to the maximum

Fig. 7.1 (*dark grey*) and after return to non stretch conditions (*grey*). The purpose of Before-During-After protocols was to ensure that stretch effects on I_{Na} were reversible. The color code will be the same in all figures. **Panel C** Cell attached patch clamp recording of sodium currents on a *Xenopus laevis* oocyte which expressed the human cardiac Nav1.5. Na^+ current holding potential was –120 mV and a rectangular voltage pulse to –30 mV elicited the transient current. The same experimental protocol as in **Panel B** was performed. The results obtained on the recombinant Nav1.5 were very similar to those observed when studying native sodium currents in cardiac myocytes. Specifically, a reversible increase in peak currents accompanied by reversible acceleration of both activation and inactivation kinetics of the channel in response to stretch

caused by changes in the absolute rates of activation and inactivation. The consequence of these changes in Na^+ kinetics is most pronounced at membrane voltages near the foot of the I–V relation. This effect can be seen in the I–V relation presented in Fig. 7.2a. As shown near the foot of the I–V relation, stretch produces an approximately three fold increase in peak current. In contrast, there is very little effect at more depolarized voltages when the Na^+ conductance is near maximum even though stretch still accelerates the current kinetics (as explained in Morris and Juranka, 2007b). This effect, an increase in transient current which is limited to the membrane potential range where the current is first activated and which saturates when conductance is maximal, is more likely explained by changes in kinetics than by a stretch-induced increase in the number of Na^+ channels in the patch. Consistent with this hypothesis, and as shown in Fig. 7.2b, the steady-state activation curve for I_{Na} is shifted approximately 10 mV toward more hyperpolarized membrane voltages during stretch.

7.2.2 *Changes in Biophysical Properties of I_{Na} Depend on Amount of Stretch*

Figure 7.3 consists of a family of I_{Na} recordings from a *Xenopus* oocyte in which six different amounts of negative pressure were applied to determine whether the effects of stretch shown in Fig. 7.2 were graded. The voltage clamp protocol is described in the figure legend. All of these changes were reversible with the first significant alterations being observed at approximately –5 mmHg pressure, and maximal effects obtained at approximately –30 mmHg. In this figure all current traces have been normalized to the value of peak current with no stretch. The superimposed records illustrate the changes in both activation and inactivation kinetics which are

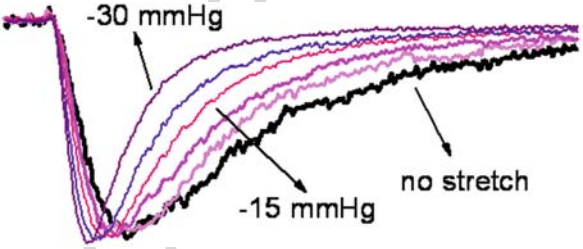

Fig. 7.3 The stretch-induced effects on recombinant Nav1.5 currents appear to be proportional to the negative pressure which is applied. Six recordings from the same patch are plotted each with different applied negative pressures, respectively: 0 (*black*), –5, –10, –15, –20 and –30 mmHg (colors from *light grey* to *grey*). The holding potential was –120 mV and a rectangular voltage clamp step to –30 mV elicited each transient increased current. The amplitude of each current peak has been normalized to the control (no stretch) current in order to illustrate the effects of progressively increasing stretch on the Na^+ channel kinetics. Note that gradual increases in negative pressure accelerate both activation and inactivation of Nav1.5 current

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both accelerated by stretch. In most experiments, negative pressures larger than –40 mmHg caused seal rupture.

7.3 Pathophysiological Implications

7.3.1 Sodium Channels and Stretch-Induced Arrhythmias

Previous works have demonstrated that the antiarrhythmic compound, flecainide, can alter the response of isolated ventricular myocytes to stretch (Kalifa et al., 2007), and this effect can derive from its ability to block Na^+ current. Similarly, gadolinium has been reported to be a blocker of stretch-induced arrhythmias (Li and Baumgarten 2001) and, in this case too, the antiarrhythmic effect may be ascribed to the inhibitory action of gadolinium on Na^+ currents.

Our voltage clamp findings suggest that the threshold for initiation of the action potential moves in the hyperpolarizing direction, becoming closer to the resting potential. Our preliminary computational work, which is illustrated in the next section, is consistent with this. A second prediction is that the larger net inward Na^+ current could result in increased conduction velocity, at least within tissue segments experiencing relatively large stretch or displacement. However, this supposition remains to be documented.

7.3.2 Mathematical Simulations of the Human Ventricular Action Potential

The pattern of results illustrated in Figs 7.2–7.4 was observed consistently in both of the experimental preparations utilized in this study. These findings raise fundamental questions concerning the functional significance of stretch-induced changes in the biophysical properties of I_{Na}. Mathematical modeling can, in principle, be useful in integrating these findings and illustrating the consequences of either a set of assumptions, or in this case a subset of experimental findings. Figure 7.4 shows the output of simulations of the human ventricular action potential waveform carried out using the ten Tusscher model, as described in our recent papers (Fink et al., 2006, 2008). When the mathematical descriptors of the Na^+ current in this model are changed from control values to values consistent with our experimental findings, significant changes in excitability were observed. Specifically as shown in Fig. 7.4b the maximum rate of rise of the action potential increased substantially and the latency between the applied stimulus and the regenerative depolarization was reduced. Consistent with this effect, and as expected, the strength duration curve for the membrane action potential was altered in such a way that the simulated stretch-induced changes would be expected to reduce the threshold for excitation of the action potential and therefore increase the excitability of the ventricular myocardium.

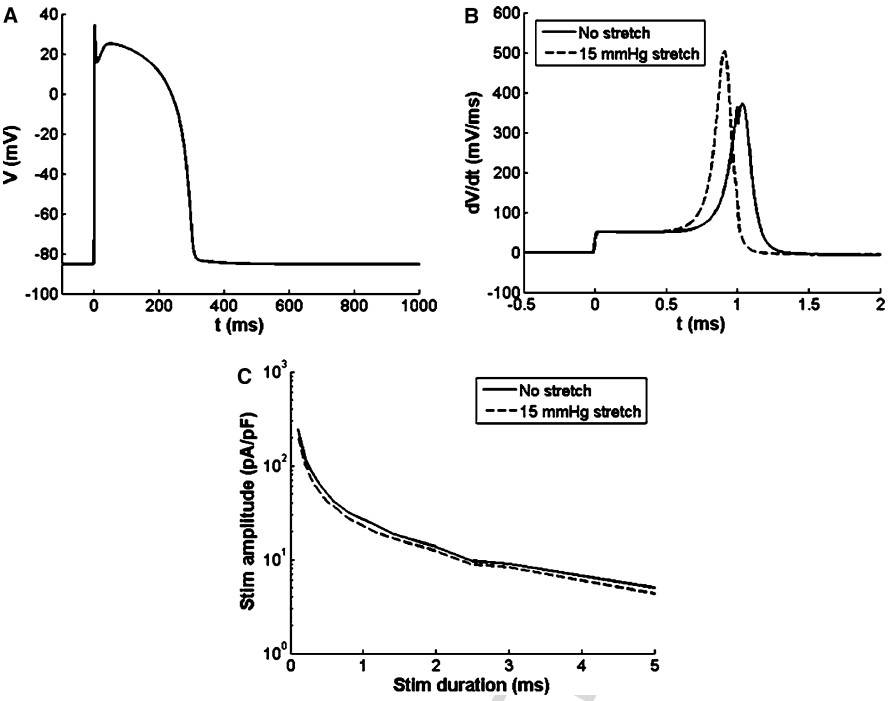

Fig. 7.4 Mathematical simulation of the changes in excitability and/or action potential waveform in a human ventricle myocyte. The changes in the size and kinetics of I_{Na} (identified in Figs. 7.2–7.4) were incorporated in the ten Tusscher model of the human ventricular action potential and action potentials were elicited at 1 Hz. These changes included: (i) a hyperpolarizing shift of the m-gate (i.e., activation) kinetics by 0.5 mV per 1 mmHg of stretch and (ii) acceleration of all gating kinetics by a factor of 1.011 stretch (mmHg). In **Panel A,** two action potentials are superimposed. The control action potential and the action potential computed assuming –15 mmHg negative pressure are virtually identical. **Panel B** Plot of the first derivative of the voltage with respect to time, i.e., the maximum "upstroke velocity". As expected from the underlying increase in I_{Na} the dV/dt max increases with stretch, which is 371mV/ms for the control and 503 mV/ms in the presence of stretch. Note also that the activation starts earlier for stretch. **Panel C** Plot of the excitability thresholds: the stimulus amplitudes necessary to elicit an action potential vs. the stimulus duration on a semilogarithmic scale. The two curves are quite distinct. Note that in the control case approx. 1.16 times more stimulus is required than during applied stretch

7.3.3 Experimental and Computational Limitations

Our computations are made on the basis of non conducted or membrane action potentials. Moreover, the expression levels of the Na^+ current may not be uniform within the ventricle either in the base-to-apex axis, or in the transmural aspect of the left ventricle (Ashamalla et al., 2001; Antzelevitch and Belardinelli 2006; Brette and Orchard 2006; Stones et al., 2007). It is also known that the stress-strain relationships in both the left ventricle and the right ventricle vary with time during the cardiac cycle (Nishimura et al., 2006; Hoshijima 2006).

7.3.4 Effects of Stretch on I_{Na}, in the Context of Heart Function

Identification of significant electrophysiological effects of stretch on the biophysical properties of the Na^+ channel isoform which is predominant in the mammalian heart should also be considered in the context of chamber-specific effects within the heart. Although chamber pressure differentials are much smaller in the right ventricle than in the left, the relatively thin wall of the right ventricle may result in it being more susceptible to stretch under physiological conditions including exercise onset (Reddy et al., 2007), or in the setting of pulmonary hypertension. Somewhat similarly both the right and the left atria are highly deformable compared to the left ventricle. This property and the fact that the resting potential in the atrium is relatively depolarized, may combine to make the stretch-sensitive effects on the Na^+ current very important in atrial physiology/pathophysiology (c.f. Kneller et al., 2005). The relatively depolarized resting potential of atrial tissue (or ventricular tissue in the setting of hypoxic or ischaemic challenge) could also result in the effects of stretch on the time course of reactivation of the Na^+ current becoming relevant.

7.3.5 Stretch Alters the Kinetics of Reactivation of Cardiac Na^+ Current

A well known property of mammalian Na^+ channels is that both inactivation and reactivation (recovery) show strong voltage-dependence within the range of membrane potentials of physiological interest. This property plays an important role in modulating the refractory period in the mammalian heart. It therefore was of considerable interest to explore the effects of stretch on reactivation or recovery kinetics of I_{Na}. Figure 7.5 consists of data obtained from the *Xenopus* oocyte preparation. In these experiments the membrane potential was held at either –120 mV, or –80 mV and two short (15 ms) depolarizing voltage clamp pulses were applied at selected inter-pulse intervals. The first pulse served to activate the Na^+ current and allow it to inactivate. As a result, the peak amplitude of the current during the second pulse, taken as a ratio to the current elicited by the first pulse provides a measurement of the relative amount of recovery from inactivation. Raw data illustrating the current records at a holding potential of –120 and –80 mV are shown in Panels 5A and 5B respectively. Panel C in Fig. 7.5 consists of aggregate data which illustrates the entire time-course of reactivation at these two holding potentials in the absence, and in the presence of significant (30 mmHg) stretch. Note that while at the very hyperpolarized holding potential there is little effect of stretch on recovery kinetics; the preliminary data obtained at –80 mV suggest that stretch may slow the recovery process.

Previous findings agree with our own in showing that extreme hyperpolarized membrane voltages stretch has no effect on the time-course of reactivation of the Na^+ current (Li and Baumgarten, 2001). However, and in contrast, when the holding potential is in the range of that of the resting potential of the atrium, applied stretch can alter reactivation (and thus the refractory period) quite substantially.

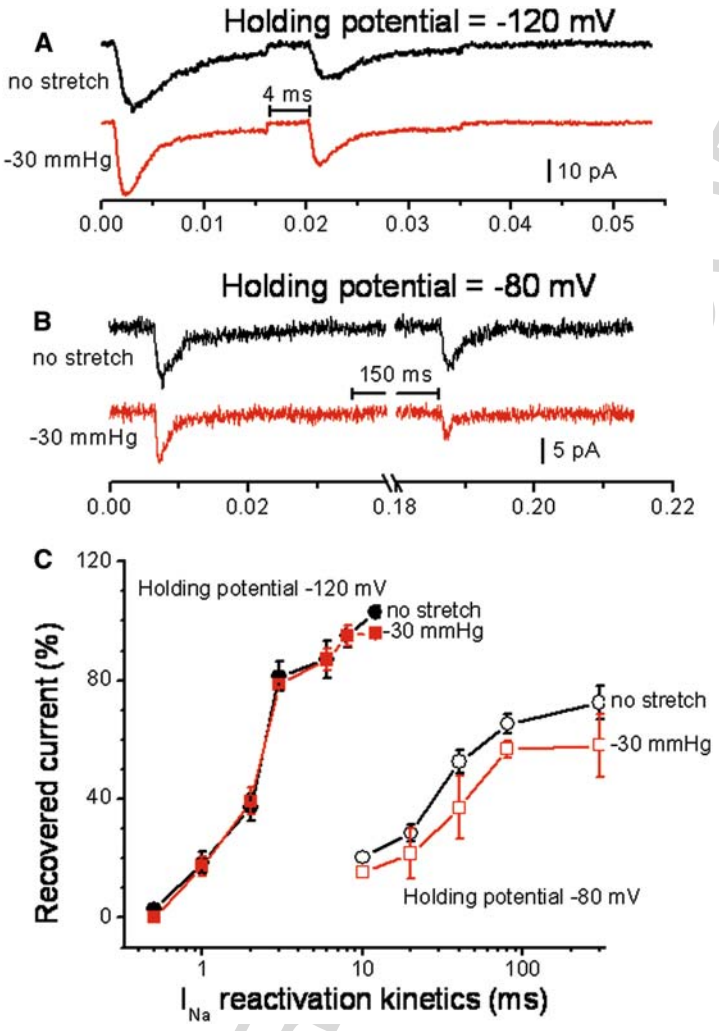

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Fig. 7.5 Effects of stretch on I_{Na} reactivation. **Panel A** Recordings of a reactivation protocol when Nav1.5 was studied at a holding potential of –120 mV. Two successive rectangular voltage clamp steps to –40 mV separated by a time interval which varied between 1 and 15 ms were applied. In the example shown here, an interval of 4 ms was used. The black trace is the recording before application of negative pressure. Peak current recovery was approx. 53%. The grey trace shows the results when the experiment was repeated with a –30 mmHg stretch. Peak current recovery was approx 54%. **Panel B** Recordings of a reactivation protocol of Nav1.5 from a holding potential of –80 mV. Two successive rectangular voltage clamp steps to –20 mV were separated by a time interval which varied between 10 and 300 ms. In the example shown here, the time interval is 150 ms. In black the recording before application of negative pressure. Peak current recovery was 82%. In *grey*, the experiment repeated with a –30 mmHg stimulus. Peak current recovery was 40%. **Panel C** Semilog plot of the percentage of current recovered vs. recovery time for Nav1.5 at holding potentials of –120 (*filled symbols*) and –80 (*open symbols*) mV. Note that at holding potential –120 mV, recovery is much faster than at –80 mV and at –120 mV stretch has no effect (n = 9). In contrast, at –80 mV stretch (–30 mmHg) appears to slow the recovery from inactivation (n = 2). In this graph, the stretch points are averages of the data before and after stretch

7.4 What is the Nature of the Mechanotransduction?

Our results, and previous work, raise questions concerning the microanatomical features of the mechanotransduction system or mechanism which is responsible for the biophysical changes which we have observed. It is known that at least one of the classical components of mechanotransduction sensors, the integrin family of multifunctional proteins, are expressed in surface indentations or cavaeolae in mammalian hearts (Hoshijima, 2006; Dyachenko et al., 2008). It is also known that a significant fraction or subpopulation of cardiac Na^+ channels are expressed or targeted to these cavaeolae (Yarbrough et al., 2002; Vatta et al., 2006). This microdomain signaling complex may serve as an important element in mechanotransduction in the myocytes of mammalian heart. The innervation of the heart includes intracardiac ganglia which may also serve a role in mechanotransduction. Interestingly, the nerve cell bodies in these ganglia appear to express low levels of the sodium channel isoform, $Na_v1.5$ (Scornik et al., 2006).

A significant missing data set in forming a working hypothesis concerning mechanotransduction in the ventricle could be obtained if semiquantitative mechanical measurements and manipulations could be combined with measurements of membrane potential and/or underlying ionic currents. Recently, studies in which some of these results have been obtained have been published. In general, it would appear that a 15–20% lengthening of an isolated myocyte results in a significant depolarization of the resting potential in the ventricular myocardium (Nishimura et al. 2008). It has even been suggested that this amount of applied stretch can alter the permeability properties of the stretch-activated, nonselective cation channels which are presumed to be activated. However, this interpretation assumes that nons-

Table 7.1 Mechanic stimuli can affect voltage gated channels (VCGs). Studies on recombinant VGCs expressed in heterologous systems have shown that both stretch (**A**) and shear stress (**B**) can modify the behaviour of these channels

Channel type	Details about channel	Effects of stretch	Comments on stimulus	References
A. Reversible effects of membrane stretch on recombinant VGCs				
Kv1	Shaker-IR (Shaker, fast inactivation removed)	Increased NP_{open} (macroscopic and single channel I_K), accelerated activation	Oocyte patch stretch	Gu et al. (2001)
Kv1-5aa	Shaker-IR-5aa: rate limiting step = independent activation motions of V-sensors	Accelerated activation and inactivation (same-fold), left-shifted g/V	Oocyte patch stretch	(Laitko and Morris 2004)

Table 7.1 (continued)

Channel type	Details about channel	Effects of stretch	Comments on stimulus	References
Kv-ILT	Shaker-IR-ILT: rate limiting step = concerted pre-pore opening	Slower activation, smaller steady-state I_K, right-shifted g/V	Oocyte patch stretch	Laitko et al. (2006)
Kv3.2	Shaw2 mutant F335A	Steady-state I_K increases, no change of activation speed	Oocyte patch stretch	Laitko et al. (2006)
Cav2.2	N-type (α_{1B} subunit co-expressed with several auxiliaries)	Peak I_{Ba} increases transiently, no change of activation speed, inactivation accelerates	Whole-cell inflation by positive pressure HEK cell	Calabrese et al. (2002)
Cav2.2	N-type (α_{1B} subunit only)	Unitary current NP_{open} increases	Patch stretch HEK cell	Calabrese et al. (2002)
Cav1.2	L-type	Peak I_{Ba} increases	Increased intracell. Pressure	Farrugia et al. (1999)
HCN2	Homotetramers	Depolarization-induced deactivation and hyperpolarization-induced activation both accelerate	Oocyte patch stretch	Lin et al. (2007)
Nav1.5	α subunit only	Activation and inactivation accelerate to the same extent	Oocyte patch stretch	Morris and Juranka (2007b)
B. Effects of shear flow and osmotic swelling/shrinking on VGCs				
Kv7.1 KCNQ1	Co-expressed in oocytes with KCNE β-subunit and aquaporin AQP1	Reversible increase of steady-state current.	Two electrode clamp, hypo-osmotic swelling	Grunnet et al. (2003)
Kv7.1 KCNQ1	LQT1-related mutant compared to WT expressed in HEK cells	Faster activation, left-shift of g/V	Hypotonic swelling	Otway et al. (2007

Table 7.1 (continued)

Channel type	Details about channel	Effects of stretch	Comments on stimulus	References
Nav1.5	SCN5A α subunit in human interstitial intestinal cells	Overall increase in transient currents elicited by flow	Whole-cell recording Stimulus: no flow-flow- no flow	Strege et al. (2003)
Cav1.2	L-type	Overall increase in transient currents elicited by flow	Whole-cell recording Stimulus: no flow-flow- no flow	Farrugia et al. (1999)
HCN2	Homotetramers coexpressed in oocytes with aquaporin AQP1	Increase in currents with no modification of kinetics	Two electrode clamp, hypoosmotic or isosmotic swelling	Calloe et al. (2005)

elective cation channels, as opposed to specific alterations in conventional time- and voltage-dependent conductances, are the main targets for applied stretch in excitable membranes (von Lewinski et al., 2003; Lin et al. 2007). Table 7.1 summarizes the effects of stretch and/or shear stress on different types of recombinant voltage gated channels that have been reported so far.

Our results, and many of the other findings in this volume, provide meaningful starting points for beginning to attempt to understand the roles of the human cardiac Na^+ current in triggering or maintaining cardiac rhythm disorders, including some types of genetic mutations known to target cardiac Na^+ channels (Bennett et al., 1995; Dumaine et al., 1996; Clancy and Rudy 1999; Ruan et al., 2007).

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