Mechanisms Underlying the Increase in Force and Ca^{2+} Transient That Follow Stretch of Cardiac Muscle

A Possible Explanation of the Anrep Effect

Bernardo V. Alvarez, Néstor G. Pérez, Irene L. Ennis,
María C. Camilión de Hurtado, Horacio E. Cingolani

Abstract—Myocardial stretch produces an increase in developed force (DF) that occurs in two phases: the first (rapidly occurring) is generally attributed to an increase in myofilament calcium responsiveness and the second (gradually developing) to an increase in $[Ca^{2+}]_i$. Rat ventricular trabeculae were stretched from ≈88% to ≈98% of L_{max}, and the second force phase was analyzed. Intracellular pH, $[Na^+]_i$, and Ca^{2+} transients were measured by epifluorescence with BCECF-AM, SBFI-AM, and fura-2, respectively. After stretch, DF increased by 1.94±0.2 g/mm^2 ($P<0.01$, n=4), with the second phase accounting for 28±2% of the total increase ($P<0.001$, n=4). During this phase, $SBFI_{340/380}$ ratio increased from 0.73±0.01 to 0.76±0.01 ($P<0.05$, n=5) with an estimated $[Na^+]_i$ rise of ≈6 mmol/L. $[Ca^{2+}]_i$ transient, expressed as fura-$2_{340/380}$ ratio, increased by 9.2±3.6% ($P<0.05$, n=5). The increase in $[Na^+]_i$ was blocked by 5-(*N*-ethyl-*N*-isopropyl)-amiloride (EIPA). The second phase in force and the increases in $[Na^+]_i$ and $[Ca^{2+}]_i$ transient were blunted by AT_1 or ET_A blockade. Our data indicate that the second force phase and the increase in $[Ca^{2+}]_i$ transient after stretch result from activation of the Na^+/H^+ exchanger (NHE) increasing $[Na^+]_i$ and leading to a secondary increase in $[Ca^{2+}]_i$ transient. This reflects an autocrine-paracrine mechanism whereby stretch triggers the release of angiotensin II, which in turn releases endothelin and activates the NHE through ET_A receptors. **(*Circ Res*. 1999;85:716-722.)**

Key Words: myocardial stretch ■ Ca^{2+} transient ■ Anrep effect ■ pH_i ■ Na^+/Ca^{2+} exchanger

In 1912, von Anrep[1] observed that when aortic resistance was elevated abruptly in the heart-lung preparation, the ventricular volume at first increased, but, within 1 or 2 minutes, a positive inotropic effect followed and ventricular volume subsequently returned toward normal. Experiments by Rosenblueth et al[2] indicated that an increase in coronary perfusion pressure was not necessarily concomitant with the return of the heart to its initial volume. Sarnoff et al[3] coined the term "homeometric autoregulation" to denote the secondary slow increase in myocardial performance that follows the initial stretch of the beating heart. Subsequently, the fact that an increase in heart volume precedes a time-dependent increase in contractility was also reported in different preparations and with different approaches to assess myocardial performance.[4–6]

Since the in vitro work of Parmley and Chuck,[7] Allen and Kentish,[8] Nichols et al,[9] Lakatta et al,[10] and Kentish and Wrzosek[11] more recently, it is known that when the length of the cardiac muscle is increased, there is first a rapid and then a slow increase in twitch force (first and second phases, respectively). The first phase, thought to be due to an increase in myofilament calcium responsiveness, constitutes the basis of the Frank-Starling mechanism and is not the focus of the present study. The second force phase develops over the next 10 minutes or so and seems to be due to a slow rise of $[Ca^{2+}]_i$.[12] The mechanism by which this slow rise in the $[Ca^{2+}]_i$ transient develops after an increase in muscle length remains obscure.

In the experiments presented herein, we demonstrate that the slow increases in force and $[Ca^{2+}]_i$ transient after the stretch of rat trabeculae are due to an autocrine-paracrine mechanism involving activation of the Na^+/H^+ exchanger (NHE) by endothelin (ET).

Materials and Methods

Rat trabeculae isolated from the right ventricle were used. All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23). Animals were supplied by the Animal Care Facility of La Plata School of Medicine. The muscles were mounted in a perfusion chamber placed on the stage of an inverted microscope for epifluorescence measurements and superfused with either HEPES- or HCO_3^-/CO_2-buffered solution at a constant rate (5 mL/min) as previously described.[13] The dimensions of the muscles were (in mm) 2.35±0.11 long, 0.34±0.02 wide, and 0.14±0.01 thick (n=51). Cross-sectional area (calculated as 0.75 of the product of thickness by width) was 0.044±0.005 mm^2 (n=51), and it was used to normalize the force records obtained with a silicon strain gauge

Received May 17, 1999; accepted August 9, 1999.

From Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Argentina.

Correspondence to Dr Horacio E. Cingolani, Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Calle 60 y 120, 1900 La Plata, Argentina. E-mail cicme@atlas.med.unlp.edu.ar

Circulation Research **is available at http://www.circresaha.org**

(model AEM 801, SensoNor). Mean resting force and developed force (DF) for the overall used muscles were 0.205±0.019 and 1.619±0.129 g/mm^2, respectively (n=51). After mounting, the slack length was determined, and the trabeculae were progressively stretched to the length at which they developed maximal twitch force (L_{max}). After a few minutes at L_{max}, the trabeculae were shortened to obtain the 95% of the maximal twitch force (length that approximates 98% of L_{max}). The muscles were then shortened to 88% of L_{max} and maintained at this length until the beginning of the experimental protocol, which consisted of a one-step stretch from 88% to 98% of L_{max}. All experiments were performed at room temperature (22°C to 24°C). In the experiments in which NHE inhibition or blockade of AT_1 or ET_A receptors was induced, drugs were added ≈20 minutes before stretch.

For pH_i measurements, the trabeculae were loaded with the ester form of the dye BCECF-AM as previously described.[13] Emitted fluorescence (excitation wavelengths of 440 and 495 nm) was collected after passage through a 535±5-nm filter. At the end of each experiment, fluorescence emission was calibrated in vivo by the high K^+-nigericin method.[14] For $[Na^+]_i$ measurements, the trabeculae were loaded with sodium-binding benzofuran isophthalate (SBFI-AM), using a modification of a described technique.[15] The excitation wavelengths were 340 and 380 nm, and the emitted fluorescence was monitored after passage through a 535±5-nm filter. The 340/380-nm ratio fluorescence signals linearly increased as $[Na^+]_i$ increased from 4 to 16 mmol/L, consistent with previous reports.[15,16] In the present study, changes in $[Na^+]_i$ were assessed by the changes in SBFI fluorescence ratios. Fura-2 pentapotassium salt was microinjected iontophoretically to measure $[Ca^{2+}]_i$, according to the method of Backx and ter Keurs.[17] The excitation wavelengths were 340 and 380 nm, and the emitted fluorescence was monitored after passage through a 510±5-nm filter. Values of $[Ca^{2+}]_i$ will be presented as 340/380-nm fluorescence ratios. Because we were primarily interested in the relative changes of $[Ca^{2+}]_i$, we did not perform in vivo calibrations[17] to convert fluorescent ratios to $[Ca^{2+}]_i$.

Statistics

Data are expressed as mean±SEM. One-way ANOVA was used to identify significant differences between prestretch and poststretch values,[18] and two-way ANOVA was used to compare poststretch data in the absence and in the presence of drugs. A value of $P<0.05$ was considered to be significant.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results

Typical changes in DF and pH_i after muscle stretch are shown in Figure 1. Figure 1A shows a representative experiment in a rat trabecula in which an increase in length of ≈10%, from ≈88% to ≈98% of L_{max}, was performed in HCO_3^--free buffer. The force record shows both the rapid and the slow force responses to the length change. The changes in myocardial pH_i during the course of the experiment are shown below the force record; the increase in pH_i reflects the stretch-induced activation of NHE.[19] In rat trabeculae, the average increase in pH_i after stretch was 0.09±0.01 pH units ($P<0.01$, n=4). Figure 1B shows the force response to stretch in a rat trabecula bathed with a buffer containing bicarbonate. The increase in DF after stretch shows the first and second force phases. However, as shown in Figure 2B, the change in pH_i is minimal in the presence of bicarbonate (0.03±0.01 pH units; not significant [NS], n=3). Therefore, the slow increase in DF after stretch occurs even when the underlying changes in pH_i are not significant. Figure 2 also shows the increase in myocardial $[Na^+]_i$ after stretch in the presence of bicarbonate. The fast increase in DF accounted

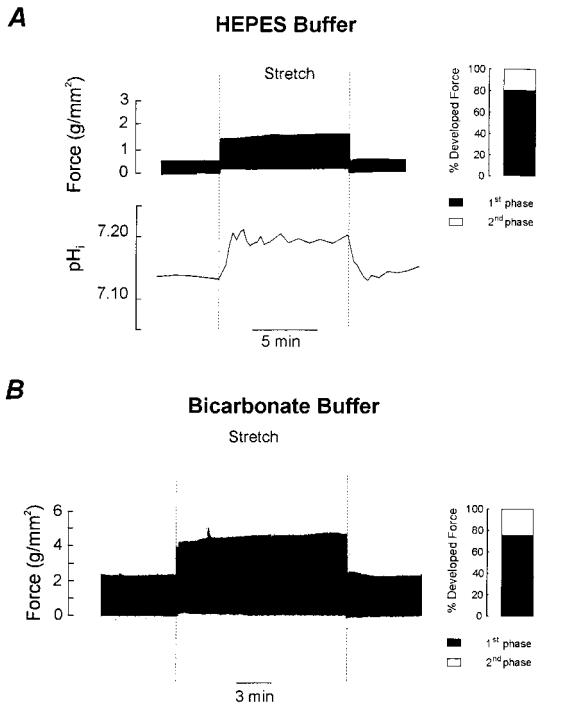

Figure 1. A, DF and pH_i in a typical experiment in the absence of bicarbonate. The increase in DF shows the 2 typical phases. The force tracing was scanned digitally from the original record. The column on the right shows the relative magnitudes of the first (in black) and the second (in white) phases of the increase, expressed as a percentage of the total increase in DF. Myocardial pH_i is shown below the force tracing. B, Typical changes in DF after stretching a rat trabecula in a bicarbonate-buffered medium. The first and the second phases are clearly detected. The column on the right shows the relative magnitudes of the two phases. The absence of changes in pH_i is shown in Figure 2.

for 72±2% of total increase, and the subsequent slow force response accounted for the remaining 28±2% (n=4). For the sake of clarity, only the slow force response is shown in Figure 2A. Figure 2C shows the overall results of the changes in $[Na^+]_i$ after stretch. The slow rise in DF after stretch was associated with a highly significant increase in $[Na^+]_i$ as a result of the activation of NHE. From the slope of the SBFI fluorescence ratio calibrated in parallel experiments, this increase in $[Na]_i$ was estimated at ≈5.5±0.5 mmol/L (53±11% of baseline).

The explanation for the different behavior of myocardial pH_i in the absence and presence of HCO_3^- arises from the mechanism proposed to explain the increase in myocardial pH_i caused by stretch.[19] Myocardial stretch induces the release of angiotensin II (Ang II), which in turn causes the release of ET and activates the NHE. The expected intracellular alkalization is minimized in the presence of HCO_3^- because Ang II also activates the Na^+-independent Cl^-/HCO_3^- exchanger (AE). However, an increase in $[Na^+]_i$ would be expected to be caused by hyperactivity of the NHE after stretch, despite the compensation in myocardial pH_i by the simultaneous activation of the AE. We have previously reported in cat papillary muscles this effect of Ang II on the AE[20] as well as the lack of significant changes in pH_i in the presence of bicarbonate.[21]

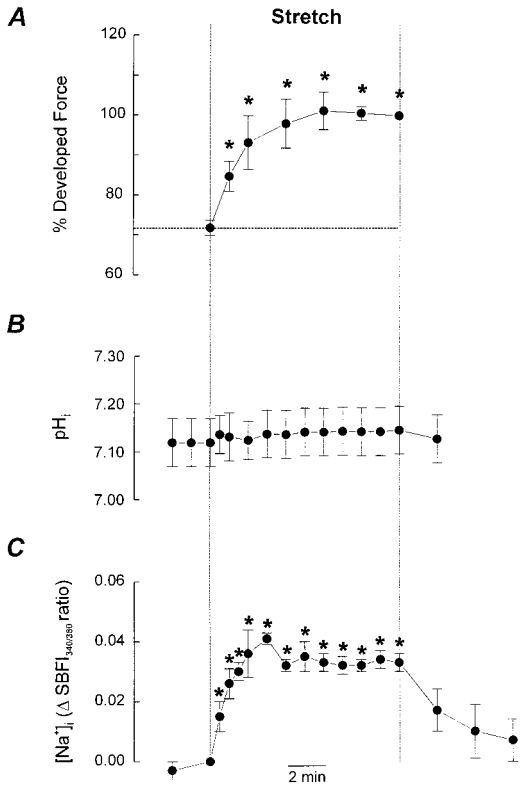

Figure 2. The increase in DF that follows myocardial stretch is accompanied by a significant increase in $[Na^+]_i$, without changes in pH_i in the presence of bicarbonate. Only the slow response in DF is shown in panel A. The total increase in DF was normalized to 100%, and the slow response was expressed as a percentage of this value. *$P<00.5$ vs initial fast phase, n=5, ANOVA. B and C, Values of myocardial pH_i and changes in SBFI fluorescence ratios. Although pH_i did not change (B, n=3), $[Na^+]_i$ increased by 53±11% of baseline value (C, n=5). *$P<0.01$ vs prestretched control value, n=5, ANOVA.

Similar experiments to those shown in Figure 2, performed after NHE inhibition [5-(*N*-ethyl-*N*-isopropyl)-amiloride, EIPA, 1 μmol/L], demonstrated that the increase in $[Na]_i$ after stretch is due to the activation of NHE. Figure 3 shows that when the muscles were stretched in the presence of EIPA, the increase in $[Na^+]_i$ was not significant (ANOVA) and the slow force response was blunted. Therefore, the slow increase in DF occurs when there is an increase in $[Na^+]_i$, and its suppression also prevents the slow force response. These results would suggest that the increase in the $[Ca^{2+}]_i$ transient detected by other investigators during the slow force response could be secondary to the increase in $[Na^+]_i$. The simplest explanation is that the increase in $[Na^+]_i$ induced by the activation of the NHE causes a secondary increase of the $[Ca^{2+}]_i$ transient via Na^+/Ca^{2+} exchange.

We have already demonstrated that the stretch of cat myocardium activates an autocrine-paracrine system,[19] in which the chain of events is as follows: stretch → release of Ang II → release and/or increase in the synthesis of ET → PKC activation → NHE activation. Assuming that our previous results in cat myocardium can be extrapolated to rat trabeculae, we could speculate that the second phase of the increase in DF after stretch is the result of this

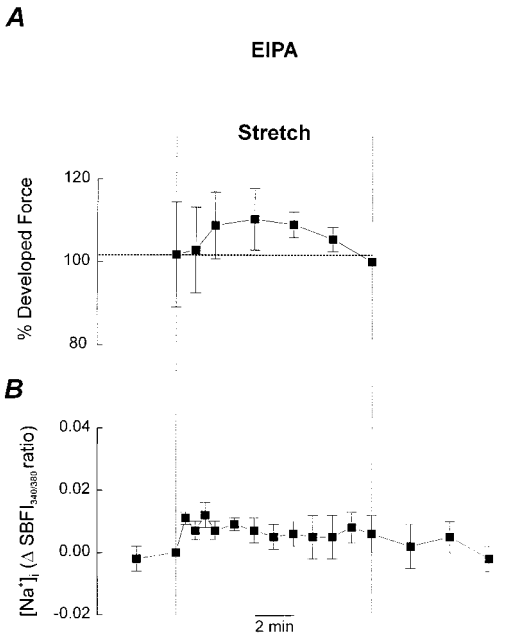

Figure 3. When the muscle is stretched in the presence of 1 μmol/L EIPA, the increase in $[Na^+]_i$ is attenuated and so is the slow force response. EIPA was added ≈20 minutes before stretch.

autocrine-paracrine mechanism in which Ang II and ET play crucial roles. If this were the case, the second phase of the increase in DF as well as the increase in $[Na^+]_i$ and $[Ca^{2+}]_i$ transient during this phase should be abolished by blocking either of the two receptors involved in this pathway, namely AT_1 or ET_A.

Figure 4 shows the effect of stretch in muscles exposed to losartan, a highly selective AT_1 blocker. Note that the slow

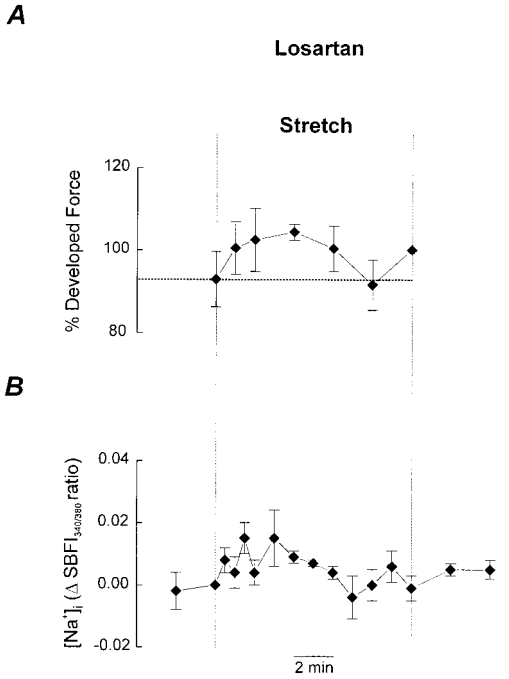

Figure 4. Preincubation of the muscles with the specific AT_1 blocker losartan (1 μmol/L) attenuated the slow force response (A) and suppressed the rise in $[Na^+]_i$ (B). Losartan was added ≈20 minutes before stretch.

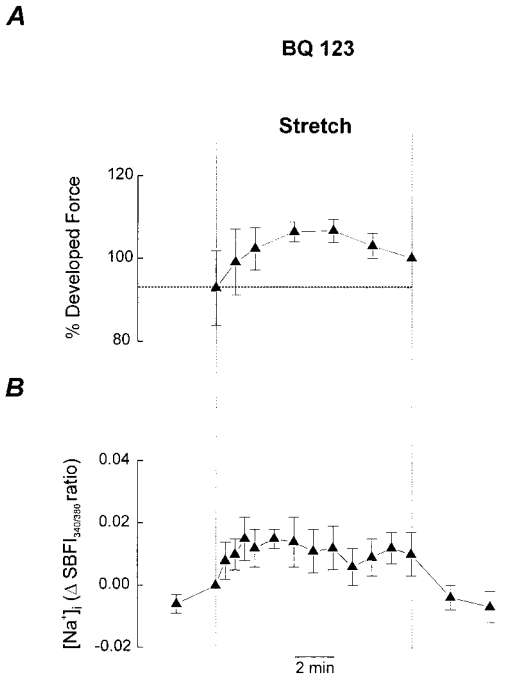

Figure 5. The specific blockade of the ET_A receptor (BQ123, 300 nmol/L) abolished the slow force response (A) and also reduced the increase in $[Na^+]_i$ (B). BQ123 was added ≈20 minutes before stretch.

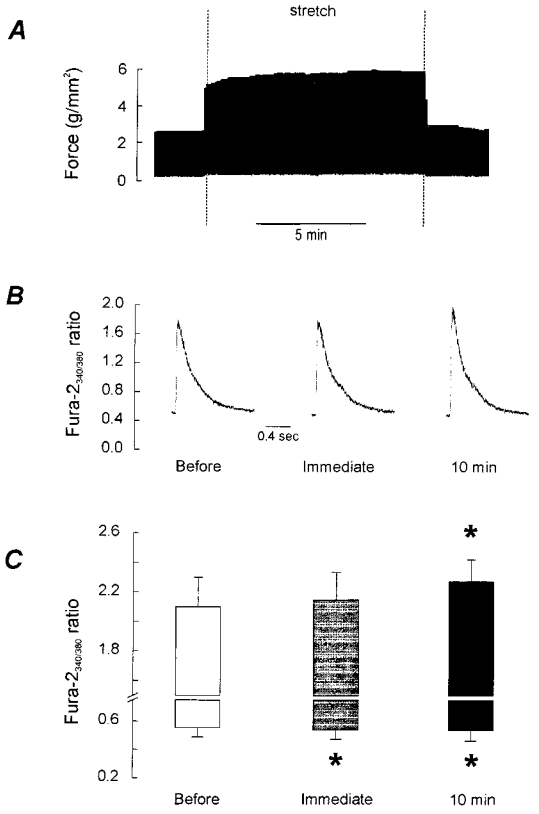

Figure 6. A, The first and the second force phases from a typical experiment. B, Values of fura-$2_{340/380}$ ratios at 3 different times: before (control), immediately, and 10 minutes after stretch. C, Floating columns show the averaged (±SEM) peak systolic (top of the column) and diastolic ratios (bottom of the column) before, immediately, and 10 minutes after stretch. The $[Ca^{2+}]_i$ transient did not change during the fast response but significantly increased during the slow force response. A small but significant decrease in diastolic $[Ca^{2+}]_i$ was detected immediately after stretch, with no further changes during the course of the slow force response. *$P<0.05$ vs control.

increase in DF was blunted. The absence of the slow phase was accompanied by a suppression of the rise in $[Na^+]_i$ that occurred during stretch under control conditions.

Figure 5 shows that no significant change in $[Na^+]_i$ with respect to the prestretched value could be detected after stretch when the ET_A receptors were selectively blocked with BQ123. The slight increase in the SBFI ratio observed under this condition was significantly lower than in control conditions ($P<0.001$). The results shown in Figures 2 through 5 indicate that an increase in $[Na^+]_i$ is a requirement for the development of the slow force response. Taken together, these results support the idea that the increase in the $[Ca^{2+}]_i$ transient, which underlies the slow force response, is secondary to the rise in $[Na^+]_i$.

To test this idea further, we determined the effects of muscle stretch on $[Ca^{2+}]_i$. Figure 6 shows that no significant changes in the $[Ca^{2+}]_i$ transient were detected during the fast response in DF, whereas the slow force response was accompanied by an increase in both peak systolic $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ transient of 6.5±2.6% and 9.2±3.6%, respectively ($P<0.05$, n=5). The increase in the $[Ca^{2+}]_i$ transient is in agreement with published results[11,12] and rationalizes the increase in contractility that takes place during the slow phase. A small decrease in diastolic $[Ca^{2+}]_i$ was detected immediately after the stretch (from 0.555±0.065 to 0.539±0.065 fura-$2_{340/380}$ ratio unit, $P<0.05$), and no further changes in diastolic $[Ca^{2+}]_i$ were detected during the development of the second phase in force response. Although the slight decrease in diastolic $[Ca^{2+}]_i$ detected immediately after stretch is perhaps irrelevant (estimated to be ≈5 nmol/L), it is statistically significant. Similar small changes in diastolic $[Ca^{2+}]_i$ after stretch were recently reported[11] and are consistent with an increase in Ca^{2+} binding to troponin C.[22] The lack of change in diastolic $[Ca^{2+}]_i$ during the slow force response is also in agreement with recently published results.[11]

Figure 7 shows that both the slow force response and the accompanying changes in the $[Ca^{2+}]_i$ transient were suppressed in rat trabeculae exposed to 1 μmol/L losartan. Losartan per se, applied during the 20 minutes before stretch, slightly increases the $[Ca^{2+}]_i$ transient from 1.062±0.14 to 1.233±0.109 $fura_{340/380}$ ratio unit ($P<0.05$). The data of Figure 7 also support the results of the parallel experiments shown in Figure 4, in which both the slow force response and the increase in $[Na^+]_i$ were suppressed by exposing the rat trabeculae to the AT_1 blocker.

Figure 8 shows that, like AT_1 blockade, exposure to an antagonist of the ET_A receptor (BQ123) prevented the slow force response and the changes in the $[Ca^{2+}]_i$ transient. The ET_A blocker applied during the 20 minutes before stretch did not modify significantly the $[Ca^{2+}]_i$ transient (from 1.661±0.35 to 1.699±0.32 $fura_{340/380}$ ratio unit, NS). These results support those obtained from parallel experiments (Figure 5) showing that the slow force response and the increase in $[Na^+]_i$ were both canceled by ET_A blockade.

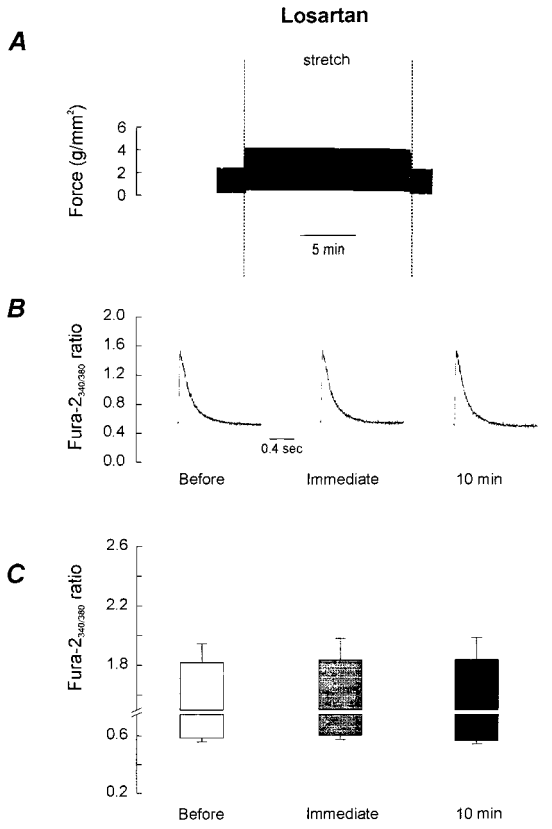

Figure 7. A, Absence of the slow force response when the action of Ang II on AT_1 was prevented with 1 μmol/L losartan. The changes in the $[Ca^{2+}]_i$ transient during the slow force response were suppressed by the blockade (B and C). Losartan was added ≈20 minutes before stretch.

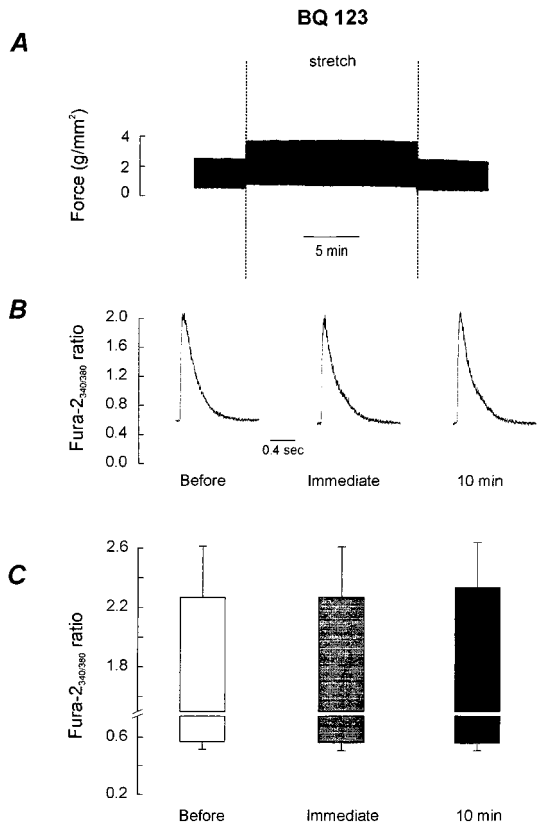

Figure 8. In the presence of the ET_A blocker (BQ123, 300 nmol/L), the slow increase in DF and the changes in the $[Ca^{2+}]_i$ transient were abolished. BQ123 was added ≈20 minutes before stretch.

The data presented herein, therefore, show that the increase in the $[Ca^{2+}]_i$ transient after muscle stretch is a consequence of the increase in $[Na^+]_i$. The increase in $[Na^+]_i$ itself results from NHE activation by an autocrine-paracrine mechanism.

Discussion

It is well-documented that the rapid increase in force that follows an increase in muscle length is due to an increase in myofilament Ca^{2+} sensitivity and in maximal force. However, the mechanism underlying the increase in the $[Ca^{2+}]_i$ transient causing the slow force response still remains obscure. An important and recent contribution was the finding that the slow force response and the change in the $[Ca^{2+}]_i$ transient were not altered under conditions designed to inhibit the sarcoplasmic reticulum (SR).[11] These results not only discount the SR as a central player in the second force phase but also argue against a previously proposed mechanism suggesting that stretch increases diastolic $[Ca^{2+}]_i$, which in turn loads the SR and increases Ca^{2+} release during contraction.[23,24]

There are contradictory data in the literature about diastolic $[Ca^{2+}]_i$ after changes in muscle length.[11,23–26] Allen et al[23] were unable to detect changes in diastolic $[Ca^{2+}]_i$ using aequorin, but a possible criticism to this report is the relative insensitivity of aequorin to $[Ca^{2+}]_i$ at diastolic levels. On the other hand, Kentish and Wrzosek,[11] using fura-2 to measure Ca^{2+}, reported, in agreement with our findings, that the second force phase and the increase in the $[Ca^{2+}]_i$ transient occur without any increase in diastolic $[Ca^{2+}]_i$.

Regarding the mechanism(s) involved in the increase in the $[Ca^{2+}]_i$ transient during the slow force response, three possibilities should be discussed: first, an increase in Ca^{2+} influx through the L-type Ca^{2+} channels; second, a decrease in Ca^{2+} efflux through the sarcolemmal Ca^{2+} ATP_{ase} and/or the Na^+/Ca^{2+} exchanger working in forward mode; and third, an increase in Ca^{2+} influx through the Na^+/Ca^{2+} exchanger working in reverse mode. The first possibility seems to be unlikely given the results of Hongo et al,[27] who found no changes in Ca^{2+} currents after stretch. Regarding the second possibility, a diminished activity of the Ca^{2+} ATP_{ase} is also unlikely, given that the increase in the $[Ca^{2+}]_i$ transient is secondary to an increase in $[Na^+]_i$. Moreover, the fact that during the slow force response diastolic $[Ca^{2+}]_i$ does not increase, and the SR does not participate, eliminates the possibility that a decreased Ca^{2+} efflux through the Na^+/Ca^{2+} exchanger could increase SR loading and augment Ca^{2+} release. Therefore, the possibility of an increased direct *trans*-sarcolemmal Ca^{2+} influx through the Na^+/Ca^{2+} exchanger working in reverse mode should be considered as the main mechanism for the increase in the $[Ca^{2+}]_i$ transient during the slow force response. In connection with this, Bers et al[28] were able to demonstrate that the Na^+/Ca^{2+} exchanger working in reverse can influence contractility without an

active SR. The prolongation of the action potential duration (APD) by muscle stretch[29] could favor reverse-mode Na^+/Ca^{2+} exchange and thereby increase Ca^{2+} influx.

There is reason to wonder whether, in species in which the contribution of the Na^+/Ca^{2+} exchanger to Ca^{2+} influx is greater, the slow force response might also be larger. Data collected (but not published) from a previous study of our laboratory on cat papillary muscles[19] revealed that the contribution of the second and slow phase to the total increase in force after stretch was $36\pm6\%$ ($n=5$, data not shown). In the present study, we have shown that in the rat myocardium the contribution of the second phase to the increase in force was lower (28%). The difference could be the result of a different APD. A greater APD, like the one found in the cat, will favor the reverse-mode Na^+/Ca^{2+} exchanger activity. However, we should be cautious in comparing the increases in force when different magnitudes of forces are being measured and the compliance of the systems used to measure force could be different.

The question of whether the diastolic term of the stretch or the stretched state during the action potential is the determinant that triggers the chain of events leading to the increase in the $[Ca^{2+}]_i$ transient was not analyzed in our study. However, the experiments by Nichols et al[9] would suggest that the increase in diastolic length is the factor primarily underlying the slow force response.

We show evidence that the chain of events accounting for the slow force response after myocardial stretch involves the release of Ang II and ET through an autocrine-paracrine mechanism, the activation of NHE by ET, and a consequent increase in $[Na^+]_i$. We would like to emphasize, as our results demonstrate, that the increase in pH_i is not a necessary phenomenon in this chain of events. This is particularly important because, although the rise in pH_i is prevented, the rise in $[Na^+]_i$ is not affected by the compensation of pH_i changes through the AE. The enhanced NHE activity with the resulting increase in Na^+ influx is able to cause a rise in $[Na^+]_i$ sufficient to increase Ca^{2+} influx without any participation of the SR.

We were able to dissect the chain of events leading to the increase in the $[Ca^{2+}]_i$ transient that follows muscle stretch, although the source of Ang II and ET remains to be determined. The rat ventricular trabecula is a multicellular preparation. Myocytes, fibroblasts, and endothelial cells have been shown to produce both Ang II[30–32] and ET[33–35] under in vitro conditions. However, the work of Sadoshima et al[30] suggests that cardiac myocytes are the source of Ang II. Furthermore, the fact that isolated myocytes show the second slow force response after stretch[36] suggests that the origin of Ang II and ET is probably the myocyte itself. However, we cannot rule out a contribution of nonmyocyte cells to the underlying mechanism.

Acknowledgments

B.V. Alvarez is a recipient of a predoctoral fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. I.L. Ennis is a recipient of a predoctoral fellowship from La Plata University, Argentina. N.G. Pérez, M.C. Camilión de Hurtado, and H.E. Cingolani are Established Investigators of CONICET.

References

1. von Anrep G. On the part played by the suprarenals in the normal vascular reactions on the body. *J Physiol (Lond)*. 1912;45:307–317.
2. Rosenblueth A, Alanais J, Lopez E, Rubio R. The adaptation of ventricular muscle to different circulatory conditions. *Arch Int Physiol Biochim*. 1959;67:358–373.
3. Sarnoff SJ, Mitchell JH, Gilmore JP, Remensnyder JP. Homeometric autoregulation in the heart. *Circ Res*. 1960;8:1077–1091.
4. Gilmore JP, Cingolani HE, Taylor RR, McDonald RH Jr. Physical factors and cardiac adaptation. *Am J Physiol*. 1966;211:1219–1226.
5. Lew WYW. Mechanisms of volume-induced increase in left ventricular contractility. *Am J Physiol*. 1993;265:H1778–H1786.
6. Burkhoff D, De Tombe PP, Hunter WC, Kass DA. Contractile strength and mechanical efficiency of left ventricle are enhanced by physiological afterload. *Am J Physiol*. 1991;260:H569–H578.
7. Parmley WW, Chuck L. Length-dependent changes in myocardial contractile store. *Am J Physiol*. 1973;224:1195–1199.
8. Allen DG, Kentish JC. The cellular basis of the length-tension relationship in cardiac muscle. *J Mol Cell Cardiol*. 1985;17:821–840.
9. Nichols CG, Hanck DA, Jewell BR. The Anrep effect: an intrinsic myocardial mechanism. *Can J Physiol Pharmacol*. 1988;66:924–929.
10. Lakatta EG. Length modulation of muscle performance. Frank-Starling law of the heart. In: Fozzard HA, ed. *The Heart and Cardiovascular System*. 2nd ed. New York, NY: Raven; 1992:1325–1351.
11. Kentish JC, Wrzosek A. Changes in force and cytosolic Ca^{2+} concentration after length changes in isolated rat ventricular trabeculae. *J Physiol (Lond)*. 1998;506:431–444.
12. Allen DG, Kurihara S. The effects of muscle length on intracellular calcium transients in mammalian cardiac muscle. *J Physiol (Lond)*. 1982; 327:79–94.
13. Pérez NG, Alvarez BV, Camilión de Hurtado MC, Cingolani HE. Intracellular pH regulation in myocardium of the spontaneously hypertensive rat: compensated enhanced activity of the Na^+/H^+ exchanger. *Circ Res*. 1995;77:1192–1200.
14. Thomas JA, Buchsbaum RN, Zimniak A, Racker E. Intracellular pH measurements in Ehrlich ascites tumor cells utilizing spectroscopic probes generated in situ. *Biochemistry*. 1979;18:2210–2218.
15. Frampton JE, Harrison SM, Boyett MR, Orchard CH. Ca^{2+} and Na^+ in rat myocytes showing different force-frequency relationships. *Am J Physiol*. 1991;261:C739–C750.
16. Harrison SM, Frampton JE, McCall E, Boyett MR, Orchard CH. Contraction and intracellular Ca^{2+}, Na^+, and H^+ during acidosis in rat ventricular myocytes. *Am J Physiol*. 1992;262:C348–C357.
17. Backx PH, ter Keurs HEDJ. Fluorescent properties of rat cardiac trabeculae microinjected with fura-2 salt. *Am J Physiol*. 1993;264(pt 2):H1098–H1110.
18. Snedecor GW, Cochran WG. *Statistical Methods*. 8th ed. Ames, Iowa: Iowa State University Press; 1989.
19. Cingolani HE, Alvarez BV, Ennis IL, Camilión de Hurtado MC. Stretch-induced alkalinization of feline papillary muscle: an autocrine-paracrine system. *Circ Res*. 1998;83:775–780.
20. Camilión de Hurtado MC, Alvarez BV, Pérez NG, Ennis IL, Cingolani HE. Angiotensin II activates Na^+-independent Cl^--HCO_3^- exchange in ventricular myocardium. *Circ Res*. 1998;82:473–481.
21. Mattiazzi A, Pérez NG, Vila-Petroff MG, Alvarez BV, Camilión de Hurtado MC, Cingolani HE. Dissociation between positive inotropic and alkalinizing effects of angiotensin II in feline myocardium. *Am J Physiol*. 1997;272:H1131–H1136.
22. Hofmann PA, Fuchs F. Bound calcium and force development in skinned cardiac bundles: effect of sarcomere length. *J Mol Cell Cardiol*. 1988; 20:667–677.
23. Allen DG, Nichols CG, Smith GL. The effects of changes in muscle length during diastole on the calcium transient in ferret ventricular muscle. *J Physiol (Lond)*. 1988;406:359–370.
24. Nichols CG. The influence of "diastolic" length on the contractility of isolated cat papillary muscle. *J Physiol (Lond)*. 1985;361:269–279.
25. Steele DS, Smith GL. Effects of muscle length on diastolic $[Ca^{2+}]_i$ in isolated guinea-pig ventricular trabeculae. *J Physiol (Lond)*. 1993; 467:328P. Abstract.
26. Craelius W. Stretch-activation of rat cardiac myocytes. *Exp Physiol*. 1993;78:411–423.
27. Hongo K, White E, Le Guennec J-Y, Orchard CH. Changes in $[Ca^{2+}]_i$, $[Na^+]_i$ and Ca^{2+} current in isolated rat ventricular myocytes following an increase in cell length. *J Physiol (Lond)*. 1996;491:609–619.

28. Bers DM, Christensen DM, Nguyen TX. Can Ca entry via Na-Ca exchange directly activate cardiac muscle contraction? *J Mol Cell Cardiol*. 1988;20:405–414.
29. Allen DG. On the relationship between action potential duration and tension in cat papillary muscle. *Cardiovasc Res*. 1977;11:210–218.
30. Sadoshima J, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell*. 1993;75:977–984.
31. Dostal DE, Rothblum KN, Conrad KM, Cooper GR, Baker KM. Detection of angiotensin I and II in cultured rat cardiac myocytes and fibroblasts. *Am J Physiol*. 1992;263:C851–C863.
32. Fischer TA, Ungureanu-Longrois D, Singh K, de Zengotita J, de Ugarte D, Alali A, Gadbut AP, Lee MA, Balligand JL, Kifor I, Smith TW, Kelly RA. Regulation of βFGF expression and ANG II secretion in cardiac myocytes and microvascular endothelial cells. *Am J Physiol*. 1997;272: H958–H968.
33. Shah AM. Paracrine modulation of heart cell function by endothelial cells. *Cardiovasc Res*. 1996;31:847–867.
34. Yamazaki T, Komuro I, Kudoh S, Zou Y, Shiojima I, Hiroi Y, Mizuno T, Maemura K, Kurihara H, Aikawa R, Takano H, Yazaki Y. Endothelin-1 is involved in mechanical stress-induced cardiomyocyte hypertrophy. *J Biol Chem*. 1996;271:3221–3228.
35. Harada M, Itoh H, Nakagawa O, Ogawa Y, Miyamoto Y, Kuwahara K, Ogawa E, Igaki T, Yamashita J, Masuda I, Yoshimasa T, Tanaka I, Saito Y, Nakao K. Significance of ventricular myocytes and nonmyocytes interaction during cardiocyte hypertrophy: evidence for endothelin-1 as a paracrine hypertrophic factor from cardiac myocytes. *Circulation*. 1997; 96:3737–3744.
36. White E, Boyett MR, Orchard CH. The effects of mechanical loading and changes of length on single guinea-pig ventricular myocytes. *J Physiol (Lond)*. 1995;482:93–107.