Intracellular sodium determines frequency-dependent alterations in contractility in hypertrophied feline ventricular myocytes

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Mills GD, Harris DM, Chen X, Houser SR. Intracellular sodium determines frequency-dependent alterations in contractility in hypertrophied feline ventricular myocytes. Am J Physiol Heart Circ Physiol 292: H1129–H1138, 2007. First published September 29, 2006; doi:10.1152/ajpheart.00375.2006.—Hypertrophy and failure (H/F) in humans and large mammals are characterized by a change from a positive developed force-frequency relationship (+FFR) in normal myocardium to a flattened or negative developed force-frequency relationship (−FFR) in disease. Altered Ca2+ homeostasis underlies this process, but the role of intracellular Na+ concentration ([Na+]i) in H/F and frequency-dependent contractility reserve is unclear. We hypothesized that altered [Na+]i is central to the −FFR response in H/F feline myocytes. Aortic constriction caused left ventricular hypertrophy (LVH). We found that as pacing rate was increased, contraction magnitude was maintained in isolated control myocytes (CM) but decreased in LVH myocytes (LVH-M). Quiescent LVH-M had higher [Na+]i than CM (LVH-M 13.3 ± 0.3 vs. CM 8.9 ± 0.2 mmol/l; P < 0.001) with 0.5-Hz pacing (LVH-M 14.9 ± 0.5 vs. CM 10.8 ± 0.4 mmol/l; P < 0.001) but were not different at 2.5 Hz (17.0 ± 0.7 vs. control 16.0 ± 0.7 mmol/l; not significant). [Na+]i, was altered by patch pipette dialysis to define the effect of [Na+]i, on contraction magnitude and action potential (AP) wave shape at slow and fast pacing rates. Using AP clamp, we showed that LVH-M require increased [Na+]i, and long diastolic intervals to maintain normal shortening. Finally, we determined the voltage dependence of contraction for Ca2+ current (I_{Ca})-triggered and Na+/Ca2+ exchanger-mediated contractions and showed that there is a greater [Na+]i dependence of contractility in LVH-M. These data show that increased [Na+]i is essential for maintaining contractility at slow heart rates but contributes to small contractions at fast rates unless rate-dependent AP shortening is prevented, suggesting that altered [Na+]i, regulation is a critical contributor to abnormal contractility in disease.

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acterized feline model of H/F. We (15, 26) previously showed that this model has fundamental contractile alterations that are similar to those demonstrated in human HF. Our present experiments show that feline H/F myocytes have elevated [Na\(^{+}\)], and a negative contraction-frequency relationship. We show that increased [Na\(^{+}\)] is essential for the maintenance of contractility at slow pacing rates in H/F myocytes but is unable to maintain their contractility at faster pacing rates unless rate-dependent AP shortening is prevented. These results suggest that changes in myocyte [Na\(^{+}\)] regulation are a critical part of contractility abnormalities in H/F.

METHODS

Aortic banding. Progressive pressure overload (PO) was induced by aortic constriction in 10 mongrel cats as previously described (4). All animal work was done with the approval and under the supervision of the Temple University School of Medicine Institutional Animal Care and Use Committee. Briefly, at 6–8 wk of age (0.9–1.1 kg), animals were sedated with ketamine and acepromazine (35 mg/kg and 0.5 mg/kg), intubated, and maintained on 1.0% isoflurane and 100% O\(_2\). Through a right thoracotomy at the third or fourth intercostal space, the pericardium was entered and a fixed, stationary band (12-mm circumference) was placed on the ascending aorta. This technique causes modest PO initially. However, as the animals grow, the band causes slow, progressive PO. Animals were banded for 6–12 wk after characterization of cardiac geometry and contractile function with transthoracic echocardiography.

Myocyte isolation. Left ventricular myocytes were isolated as previously described (34). After being washed with Krebs solution (in mmol/l: 12.5 glucose, 5.4 KCl, 1.0 lactic acid, 1.25 sodium sulfate, 130 NaCl, 1.2 sodium phosphate, 2.5 sodium bicarbonate, 2.0 sodium pyruvate, pH 7.4) containing 1% (w/vol) BSA, 10 mmol/l taurine, and 0.20 mmol/l CaCl\(_2\), cells were kept at room temperature with 5% CO\(_2\) blowby and used within 6 h of isolation. All experiments were performed on single rod-shaped myocytes with clear sarcromeric cross striations.

Na\(^{+}\) fluorescence measurement and calibration. [Na\(^{+}\)] was measured with the cell-permeant, sodium-sensitive fluorophore sodium-binding benzofuran isothiophtalic acid (SBFI; Molecular Probes, Eugene, OR). Cells were suspended in normal Tyrode solution (in mmol/l: 150 NaCl, 5 HEPES, 2 Na-pyruvate, 5.4 KCl, 1.25 magnesium chloride, pH 7.4) with 1 mmol/l CaCl\(_2\) and loaded with 10 mmol/l SBFI-AM for 90 min at room temperature in the presence of the nonionic surfactant Pluronic F-127 (0.05% wt/vol). The cells were then transferred to a 37°C water-jacketed chamber mounted on the stage of an inverted microscope (Zeiss Axiovert 10, Thornwood, NY), and perfused with normal Tyrode solution for 20 min to deesterify the fluorescence probe. Fluorescence was elicited by illumination with a 75-W xenon lamp filtered to obtain dual excitation measurements (340 and 380 nm, F340 and F380) with a Lambda 10B optical filter changer (Sutter Instruments, Novato, CA) at 4 Hz. Emitted light was filtered to 525 nm (filter set XF04 –2, Omega Optical, Brattleboro, VT) and recorded at each excitation wavelength, and a ratio was obtained (F340/F380). Cells were visualized at high magnification, and contractions were briefly elicited with field stimulation. Fluorescence recordings were then obtained while cells were quiescent for 5–10 min and while they were being paced at 0.5 and 2.5 Hz.

Calibration of SBFI was accomplished by exposing each myocyte to 0, 10, and 20 mmol/l extracellular Na\(^{+}\) concentration ([Na\(^{+}\)]\(_{o}\)) in the presence of 10 mmol/l gramicidin D and 100 mmol/l strophanthidin, as described previously (9). Two solutions of equal ionic strength were prepared, one containing 145 mmol/l Na\(^{+}\) (30 mmol/l NaCl and 115 mmol/l sodium gluconate) with no K\(^{+}\) and the other containing 145 mmol/l K\(^{+}\) (30 mmol/l KCl and 115 mmol/l potassium gluconate) with no Na\(^{+}\). Both contained (in mmol/l) 10 HEPES, 10 glucose, and 2 EGTA (pH 7.2, adjusted with Tris base). These were mixed to prepare the final calibration solutions. Cells were exposed to 0, 10, and 20 mmol/l [Na\(^{+}\)], for 15, 10, and 10 min, respectively, or until the fluorescence signal was stable.

Cell physiology. Suction-type patch pipettes were prepared from borosilicate glass (1B150F, World Precision Instruments, Sarasota, FL) with a two-stage pipette puller (model P-87, Sutter Instruments, Novato, CA). The pipette tip was heat polished before use and had a tip resistance of 2–5 MΩ. The pipette was attached to a patch-clamp amplifier (Axoclamp II, Axon Instruments, Foster City, CA). Cells were studied at 37°C and were perfused with normal Tyrode solution (in mmol/l: 150 NaCl, 5 HEPES, 2 Na-pyruvate, 5.4 KCl, 1.25 magnesium chloride, pH 7.4) with 1 mmol/l CaCl\(_2\).

The perforated patch technique was used where noted to gain electrical access to the cell and induce contraction, minimizing cell dialysis (8). The pipette solution contained (in mmol/l) 10 HEPES, 110 potassium aspartate, 5 dipotassium-ATP, 0.001 Tris-GTP, 2 magnesium chloride, 20 potassium chloride, 10 sodium chloride, 1 calcium chloride, and amphotericin B (240 µg/ml in DMSO), pH 7.2 with potassium hydroxide. Amphotericin B formed aqueous channels in the cell membrane, permitting electrical access to the cell while preventing the dialysis of cellular components that could alter cell function. After seal formation, increases in the capacitive current response to a –10-mV step were monitored to determine cellular access (3–5 min). To initiate action potentials, a square current pulse was applied at 1.0 Hz. Contractions were recorded by video edge detection (VED-104, Crescent Electronics, Sandy, UT). pCLAMP 8.2 software (Axon Instruments) was used for data acquisition and analysis.

To obtain AP recordings and induce contraction, the ruptured-patch technique was used to gain electrical access to the cell (8). Three pipette solutions were used to dialyze cells with 0, 10, or 20 mmol/l Na\(^{+}\) ([Na\(^{+}\)]\(_{o}\)). All solutions contained (in mmol/l) 10 HEPES, 110 potassium aspartate, 5 dipotassium-ATP, 0.001 Tris-GTP, 2 magnesium chloride, 20 potassium chloride, and 0 NaCl-20 N-methyl-D-glucamine (NMDG), 10 NaCl-10 NMDG, or 20 NaCl-NMDG to maintain ionic balance. Cells were randomly assigned to each group, and cells from each animal were included in each sodium group. After high-resistance seal formation was obtained, the patch was ruptured and the pipette solution was allowed to dialyze for 10 min. APs were initiated with a square current pulse was applied at 0.5 and 2.5 Hz for 5 min each. Contractions were recorded by video edge detection (VED-104, Crescent Electronics). pCLAMP 8.2 software (Axon Instruments) was used for data acquisition and analysis.

The AP voltage-clamp technique was used to study AP duration (APD)-independent effects of rate on contractility. A command voltage profile was modeled after a 250-ms AP recorded from a representative control feline left ventricular (LV) myocyte under perforated-patch ( nondialyzed) conditions. Pipette filling solutions and dialysis protocols used were identical to those in the AP recording protocol described above. Current recordings and contractions were recorded during the AP clamp step at 0.5 and 2.5 Hz. Subsequently, each cell was tested with 250-ms square depolarizing steps to –30 to +70 mV from a holding potential of –40 mV, and early peak and late plateau currents and contractions were recorded to approximate the voltage sensitivity of whole cell Ca\(^{2+}\) current, whole cell NCX current, and contraction.

Statistical methods. All data are presented as means ± SE. Statistical difference between the mean values for two groups was evaluated with paired or unpaired Student’s t-test, and two-way ANOVA was used to determine the effects of multiple factors on the overall variance within a data set. Post hoc analyses of individual group comparisons were performed with the Bonferroni posttest method. Values of P < 0.05 were considered significant.
RESULTS

Aortic constriction (banding) causes an increase in LV thickness (anterolateral wall of LV: 0.61 ± 0.04 cm vs. control 0.44 ± 0.04 cm; *P < 0.05) and end-diastolic LV (1.42 ± 0.07 cm vs. control 1.31 ± 0.06 cm; not significant) and peak systolic LV (0.82 ± 0.09 cm vs. control 0.64 ± 0.02 cm; *P < 0.05) chamber diameter with, on average, preserved baseline (resting) contractility (fractional area change ratio derived from 2-dimensional images of the LV chamber area during diastole and systole: 0.59 ± 0.04 vs. control 0.65 ± 0.04; not significant) as evaluated by transthoracic echocardiography. This model was further characterized in a previous study from this laboratory (21). Most animals had compensated LV hypertrophy (LVH) based on the absence of clinical signs of HF (shortness of breath with exertion and pulmonary effusions or ascites at death), but four animals, with large end-diastolic chamber dimensions, exhibited these signs of early HF. This model represents a continuum of severe hypertrophy and early HF. Myocytes from all of these animals were used in the experiments described below. Banded animals had an increased heart weight-to-body weight ratio of 9.0 ± 0.9 vs. control (4.1 ± 0.5).

Rate-dependent fractional shortening. The frequency dependence of LV myocyte contractility was measured in LV myocytes (LVH-M) and control myocytes (CM). These studies were performed with perforated patch techniques to minimize cellular dialysis and preserve the native intracellular environment. Contraction were measured immediately after gaining electrical access to the cell. Fractional shortening (FS) was not significantly different at 0.5 Hz [Fig. 1B; LVH-M 9.1 ± 1.4% vs. CM 7.5 ± 1.1% resting cell length (RCL); n = 8 in each group]. As pacing rate was increased, contraction magnitude was maintained in CM but decreased significantly in LVH-M [1.5 Hz: 9.0 ± 1.0 vs. 5.7 ± 0.8% RCL (*P < 0.05); 2.0 Hz: 8.6 ± 1.0 vs. 5.3 ± 0.8% RCL (*P < 0.05); 2.5 Hz: 9.3 ± 1.4 vs. 5.2 ± 0.5% RCL (*P < 0.05)]. These experiments document that feline LVH-M (exhibiting a spectrum of severe hypertrophy and early HF) have a fundamental contractility defect similar to that observed in the failing human heart and animal models with less severe disease (10, 28). We next investigated the role of altered [Na+]i regulation in the –FFR. In these experiments, myocytes were paced at 0.5 and 2.5 Hz to explore the limits of the frequency ranges that can be routinely used in feline myocytes.

[Na+]i in quiescent and paced LV myocytes. [Na+]i, was measured in LVH-M and CM with the Na+-fluorescent dye SBFI. After 20 min of deesterification and dye washout, fluorescence was recorded at 340 and 380 nm in quiescent (nonpaced) myocytes until SBFI fluorescence reached a steady state. Myocytes were then paced at 0.5 and 2.5 Hz (Fig. 2, A and B, show representative 340 to 380 nm ratios from CM and LVH-M). Each cell was then calibrated with bath solutions with 0, 10, and 20 mmol/l Na+ with the Na+ ionophore gramicidin D and the Na+ pump inhibitor strophanthidin. Quiescent LVH-M had significantly higher [Na+]i than CM (Fig. 2C; LVH-M 13.3 ± 0.3 vs. CM 8.9 ± 0.2 mmol/l; *P < 0.001; n = 15 and 10, respectively). [Na+]i, increased with pacing rate in both groups, and at 0.5 Hz was significantly higher in banded animals (LVH-M 14.9 ± 0.5 vs. CM 10.8 ± 0.4 mmol/l; *P < 0.001) but was similar at 2.5 Hz (LVH-M 17.0 ± 0.7 vs. CM 16.0 ± 0.7 mmol/l; not significant). The absolute increase in [Na+]i was smaller in LVH-M vs. CM (LVH-M 3.7 ± 0.5 vs. CM 7.1 ± 0.5 mmol/l increase at 2.5-Hz stimulation; *P < 0.001). These experiments show that [Na+]i is significantly elevated in LVH-M at slow pacing rates, but the greater increase in [Na+]i, with rate in CM is such that there are no significant differences between groups at fast pacing rates. The role of these differences in rate-dependent changes in myocyte contractility was next studied by controlling [Na+]i; with cellular dialysis through low-resistance pipettes.

Effect of dialysis with 0, 10, and 20 mmol/l Na+ on contractility and AP. [Na+]i, was altered by dialysis with pipette solutions containing 0, 10, or 20 mmol/l Na+; [Na+]i pip had significant effects on myocyte contractility and AP morphology in both groups. Myocyte shortening increased with [Na+]i pip in both groups and at slow (0.5 Hz) and fast (2.5 Hz) pacing rates (Fig. 3A). Two-way ANOVA comparing the contribution of group (LVH-M vs. CM) and [Na+]i pip with the overall data variability was significant (P < 0.01 for both
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Fig. 2. Intracellular Na\(^+\) concentration ([Na\(^+\)]\(_i\)) measurement with sodium-binding benzofuran isophthalate in isolated quiescent and paced CM and LVH-M myocytes. Representative recordings of ratio of fluorescence at 340 and 380 nm (F\(_{340}/F_{380}\)) from CM (A) and LVH-M (B) when quiescent (Q) and when paced at 0.5 and 2.5 Hz followed by calibration with perfusates containing 0 (0 Na\(^+\)), 10 (10 Na\(^+\)), and 20 mmol/l Na\(^+\) (20 Na\(^+\)), gramicidin D, and strophanthidin.

A: mean [Na\(^+\)]\(_i\) for CM and LVH-M while quiescent and when paced at 0.5 and 2.5 Hz. Data are expressed as mean ± SE [Na\(^+\)]\(_i\). CM: n = 10; LVH-M: n = 15. ***P < 0.001; NS, not significant.

Duration of contraction (or relaxation time) was measured as the time from the stimulus to the time when contraction recovered to 50% maximum FS (Fig. 3B). In both groups (LVH-M and CM), stimulation rate had a significant effect on the time to 50% relaxation (P < 0.05), but there was no effect of [Na\(^+\)]\(_{\text{pip}}\) on the overall variation in the data. In CM, the relaxation time shortened with increasing [Na\(^+\)]\(_{\text{pip}}\) at slow and fast pacing rates. However, in banded animals, contractions were prolonged at 10 and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) at 0.5 Hz and relaxation time at 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) was significantly longer in banded animals compared with CM (banded 420 ± 26 vs. CM 299 ± 5 ms, P < 0.001; n = 18 and 28, respectively).

APD, the time from stimulus to 75% repolarization, was measured in each group (Fig. 3D). APD was shortest at fast pacing rates and with high [Na\(^+\)]\(_{\text{pip}}\). Two-way ANOVA revealed a group effect (CM vs. LVH-M, P < 0.001) at each rate and a significant effect of [Na\(^+\)]\(_{\text{pip}}\) on overall data variability (P < 0.001). Post hoc analysis revealed a significantly longer AP at 0 mmol/l [Na\(^+\)]\(_{\text{pip}}\) in LVH-M compared with CM at the same rate and [Na\(^+\)]\(_{\text{pip}}\) (LVH-M 433 ± 61 vs. CM 280 ± 116 ms, P < 0.01; n = 15 and 36, respectively). These data suggest a significant effect of [Na\(^+\)]\(_{\text{pip}}\) on both contractility and the electrophysiological properties of LV myocytes (LVM) and suggest that there are significant differences between the effect of altering [Na\(^+\)]\(_i\) in LVH-M and CM. The major electrophysiological difference was that the APD of LVH-M was significantly more sensitive than that of CM to reduced [Na\(^+\)]\(_{\text{pip}}\).

Effect of dialysis with 0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) on contractility during AP clamp. The experiments presented above document that changes in both stimulation rate and [Na\(^+\)]\(_{\text{pip}}\) have significant effects on APD, which is known to have an independent effect on contractility. To more clearly define the direct effect of [Na\(^+\)]\(_{\text{pip}}\) on rate-dependent differences in contractility in LVH-M vs. CM, experiments were performed using AP voltage clamp to prevent APD changes during rate alterations and with different [Na\(^+\)]\(_{\text{pip}}\). Cells from control and banded animals from all groups at slow and fast pacing rates were stimulated with the same 250-ms (APD75%) AP waveform derived from a control myocyte stimulated at an average (1.5 Hz) pacing rate with perforated patch (i.e., with...
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Figure 4A shows the stimulus waveform and representative steady-state contractions from a CM dialyzed with 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) that was paced at 0.5 Hz (solid line) and 2.5 Hz (dashed line). Myocyte shortening increased with increasing [Na\(^+\)]\(_{\text{pip}}\) and with increasing rate in both CM and LVH-M (Fig. 4B). Two-way ANOVA showed a significant contribution of both group (CM vs. LVH-M) and [Na\(^+\)]\(_{\text{pip}}\) at each frequency to the overall data variance. CM shortening was significantly greater with 0 and 10 mmol/l [Na\(^+\)]\(_{\text{pip}}\) at slow and fast pacing rates (P < 0.01). Myocyte shortening was greater in LVH-M dialyzed with 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) vs. LVH-M dialyzed with 0 mmol/l [Na\(^+\)]\(_{\text{pip}}\) at both slow (6.9 ± 1.0 vs. 0 mmol/l: 1.3 ± 0.2% RCL, P < 0.001; n = 18 and 15, respectively) and fast (8.7 ± 1.3 vs. 0 mmol/l: 1.7 ± 0.3% RCL, P < 0.001; n = 18 and 15, respectively) pacing rates. CM dialyzed with 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) also had greater shortening compared with CM dialyzed with 0 mmol/l [Na\(^+\)]\(_{\text{pip}}\) at 2.5 Hz (9.6 ± 1.3 vs. 0 mmol/l: 5.4 ± 0.8% RCL, P < 0.05; n = 21 and 18, respectively) but not at 0.5 Hz. These results show that contractility in LVH-M has an increased dependence on [Na\(^+\)]. Rate-dependent decreases in contractility were not present in LVH-M at any [Na\(^+\)]\(_{\text{pip}}\) under these experimental conditions.

Relaxation time was also measured in cells dialyzed with 0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) at low and high pacing rates (Fig. 4C). There was a small, but insignificant, increase in the time to 50% relaxation with increasing [Na\(^+\)]\(_{\text{pip}}\) in CM at both pacing rates, with high rate causing shorter contractions. Two-way ANOVA revealed a significant effect of both group (control vs. banded, P < 0.01) and [Na\(^+\)]\(_{\text{pip}}\) (P < 0.01) at each rate. LVH-M had longer contractions than CM when dialyzed with 10 mmol/l at both low (462 ± 24 ms vs. CM 362 ± 29 ms, P < 0.01; n = 15 and 20, respectively) and high (327 ± 4 vs. CM 281 ± 11 ms, P < 0.05; n = 6 and 21, respectively) rates.

Effect of dialysis with 0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) on voltage dependence of contraction. Alterations in myocyte contractility in H/F are known to include changes in excitation-contraction coupling (6). We examined whether alterations in [Na\(^+\)]\(_{\text{pip}}\) could contribute to these changes. The voltage dependence of Ca\(^{2+}\) influx-induced SR Ca\(^{2+}\) release (via ICa), which peaks at 0–10 mV, and Ca\(^{2+}\) influx-mediated contractions (via NCX), which are primarily seen at strongly positive voltages where ICa is small, were determined. The early and late whole cell currents (I\(_{\text{Early}}\) and I\(_{\text{Late}}\)) as well as early and late contraction peaks were used as indexes of ICa-triggered and direct, NCX-mediated, contractions. An example of the protocol and data from a representative CM dialyzed with 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) is shown in Fig. 5A. I\(_{\text{Early}}\) was not different in CM vs. LVH-M at any [Na\(^+\)]\(_{\text{pip}}\), and there was no clear [Na\(^+\)] dependence to this current in either group (data not shown).

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Fig. 3. Effect of dialysis with 0, 10, and 20 mmol/l pipette Na\(^+\) concentration ([Na\(^+\)]\(_{\text{pip}}\)) on contractility and action potential (AP) duration (APD). A and B: fractional shortening (%RCL; A) and relaxation time (time to 50% relaxation; B) in CM and LVH-M after pipette dialysis stimulated at 0.5 Hz and 2.5 Hz. C: representative AP waveforms from 3 LVH-M after pipette dialysis with 0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\). D: APD (time to 75% repolarization) in CM and LVH-M at 0.5 and 2.5 Hz. Data are expressed as means ± SE; n, CM, 36, 33, and 28; LVH-M, 15, 21, and 18 (0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\)). CM vs. LVH-M within treatment: *0 vs. 10 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M); ‡10 vs. 20 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M); ‡0 vs. 20 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M).
early contraction vs. voltage relationships in CM vs. LVH-M are shown in Fig. 5B. LVH-M with 0 mmol/l [Na\(^+\)]\(_{\text{pip}}\) have very small, almost immeasurable, contractions at any voltage compared with CM, which had some contraction at more positive potentials (although not statistically significant). With 10 mmol/l [Na\(^+\)]\(_{\text{pip}}\) contractions were smaller in LVH-M vs. CM (\(P < 0.05\)), but contractions with 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) were no different between groups. These results are very similar to those with AP clamp and further document that LVH-M are more sensitive to [Na\(^+\)]\(_{\text{pip}}\) than CM, with smaller contractions at lower [Na\(^+\)]\(_{\text{pip}}\). These results suggest that LVH-M require higher than normal [Na\(^+\)]\(_{\text{pip}}\) to maintain contractility.

Late contractions are shown in Fig. 5C. Late current was inward at negative voltages, crossing the 0 pA/pF point at \(-20\) mV in CM at all [Na\(^+\)]\(_{\text{pip}}\) and peaking at \(-20\) pA/pF at \(+80\) mV (data not shown). Late contractions were smallest with 0 mmol/l and largest with 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) in both groups. At 0 and 10 mmol/l [Na\(^+\)]\(_{\text{pip}}\), contractions were smaller in LVH-M vs. CM at positive potentials. Late contractions were similar in size in CM and LVH-M dialyzed with 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\). These results suggest that Ca\(^{2+}\) entry via the NCX support these late, tonic-type contractions and again show that LVH-M have a greater [Na\(^+\)]\(_{\text{pip}}\) dependence than CM.

DISCUSSION

Increased [Na\(^+\)]\(_{\text{pip}}\) is a potentially important factor in maintaining cardiac function in hypertrophy and HF. While the present literature regarding the issue of [Na\(^+\)]\(_{\text{pip}}\) in various models of hypertrophy and HF is species- and technique

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**Fig. 4.** Effect of dialysis with 0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) on contractility during AP clamp. **A:** representative command voltage waveform (top) and contraction tracings (bottom) from LVH-M dialyzed with 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) simulated at 0.5 Hz (solid line) and 2.5 Hz (dashed line). **B** and **C:** mean fractional shortening (B) and relaxation time (C) in CM and LVH-M after pipette dialysis at 0.5-Hz and 2.5-Hz stimulation rate. Data are expressed as means ± SE; \(n\): CM, 18, 21, and 21; LVH-M, 15, 21, and 18 (0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\)). \(\ast\) CM vs. LVH-M within treatment; \(\ast\) 0 vs. 10 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M); \(\dagger\) 10 vs. 20 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M); \(\ddagger\) 0 vs. 20 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M).

**Fig. 5.** Effect of dialysis with 0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\) on the voltage dependence of contraction. **A:** representative experiment with command waveform (top), current (middle), and contraction (bottom). Early whole cell current (\(I_{\text{Early}}\)) was defined for each voltage step as the current occurring at the same time point as the peak inward current found at \(-20\) mV. Late whole cell current (\(I_{\text{Late}}\)) was defined as the current present at 25 ms from the end of each voltage step. These time points were then used to define early and late contractions at each voltage. **B** and **C:** early contraction (B) and late contraction (C) (%RCL) in CM and LVH-M after dialysis with 0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\). All data are expressed as means ± SE; \(n\): CM, 4, 6, and 3; LVH-M, 3, 11, and 7 (0, 10, and 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\)). \(\ast\) CM vs. LVH-M within treatment; \(\ast\) 0 vs. 10 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M); \(\dagger\) 10 vs. 20 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M); \(\ddagger\) 0 vs. 20 [Na\(^+\)]\(_{\text{pip}}\) within group (CM or LVH-M). FS, fractional shortening.
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A

20 pA/pF

5% RCL

Early Late

2000 ms

B

CM

Early Contraction

FS (%RCL)

Voltage (mV)

Banded

FS (%RCL)

Voltage (mV)

C

Late Contraction

FS (%RCL)

Voltage (mV)
dependent and sometimes contradictory, the available data support the idea that [Na\(^+\)]\(_i\) is elevated in these disease states (36). In addition, there is extensive literature examining alterations in Na\(^+\) influx and efflux pathways in H/F that support the idea that [Na\(^+\)]\(_i\) would consequently be increased. Studies in normal myocardium have shown that increasing [Na\(^+\)]\(_i\) causes increased SR load but also alters the timing of SR loading such that long diastolic intervals are necessary to maintain SR load, resulting in a negative force-frequency response (19). This latter effect is compounded by the shortened APs caused by high [Na\(^+\)]\(_i\), (23). There is little information concerning the effect of altering [Na\(^+\)]\(_i\) on myocyte contractile function in the context of hypertrophy and HF. Therefore, while it is known that [Na\(^+\)]\(_i\) is an important modulator of cardiac function, its role in disease is unclear and was the topic of this study.

In the present investigation, we studied the role of altered [Na\(^+\)]\(_i\) in a model of PO hypertrophy and early HF. We examined the frequency dependence of contractility in isolated myocytes from banded and control animals and then determined the resting [Na\(^+\)]\(_i\), and rate-dependent change in [Na\(^+\)]\(_i\), in both groups with the Na\(^+\)-sensitive dye SBFI and a previously validated calibration method. Finally, by dialyzing banded and control myocytes with 0, 10, and 20 mmol/l pipette filling solutions ([Na\(^+\)]\(_{\text{pip}}\)), we determined the effect of artificially lowering and raising [Na\(^+\)]\(_i\), on contractility and APD as well as the AP-independent effect of Na\(^+\) on contraction and the voltage dependence of contraction in the context of banded and control myocyte environments. Our major findings are that 1) LVH-M have a negative frequency-shortening response compared with CM; 2) [Na\(^+\)]\(_i\), is elevated in LVH-M compared with CM and the increase in [Na\(^+\)]\(_i\), with pacing is reduced; 3) increasing [Na\(^+\)]\(_i\), by cellular dialysis increases myocyte shortening to a smaller degree in LVH-M at fast rates but not slow rates of stimulation; 4) APD shortens with increasing [Na\(^+\)]\(_i\), at slow rates more than at fast rates; 5) under conditions in which the APD is prevented from changing with increases in rate, the effect of [Na\(^+\)]\(_i\), on shortening is more pronounced and normalizes contractions at high rates in LVH-M; 6) the voltage dependence of contraction is altered in LVH-M, and contractions are smaller than in CM when dialyzed with 0 and 10 mmol/l [Na\(^+\)]\(_{\text{pip}}\), but similar at 20 mmol/l [Na\(^+\)]\(_{\text{pip}}\); and 7) the &#956;early-voltage relationship is left-shifted in LVH-M. These results show that LVH-M have a higher than normal reliance on [Na\(^+\)]\(_i\), for the maintenance of basal contractility. We suggest that this increased [Na\(^+\)]\(_i\), helps support contractions in LVH-M that would otherwise be reduced because of depressed SR function (5). Altered [Na\(^+\)]\(_i\), is one element that contributes to the FFR demonstrated in this model of hypertrophy and early HF.

Measurements of [Na\(^+\)]\(_i\), in hypertrophy and HF have been made in several model systems in a few species. The data from these disease models are largely consistent, showing elevated [Na\(^+\)]\(_i\), in diseased myocytes. Two studies in aortic-banded/aortic-insufficiency rabbits have shown an increase in quiescent LVH-M [Na\(^+\)]\(_i\), compared with control by 63% and 47%, respectively (2, 9). However, another study with pacing-induced HF in rabbits showed a small decrease from 5.2 to 4.4 mmol/l [Na\(^+\)]\(_i\), with disease (38). In dogs, atrioventricular block-induced hypertrophy (AVB) caused [Na\(^+\)]\(_i\), to increase from 3.2 to 7.9 mmol/l. [Na\(^+\)]\(_i\), has also been measured in hypertrophied human myocardial sections with Na\(^+\)-sensitive microelectrodes and was shown to be elevated compared with control myocardium taken from patients undergoing mitral valve repair (14.2 vs. 11.9 mmol/l) (14). Finally, a study done in collaboration with our laboratory found elevated [Na\(^+\)]\(_i\), in paced failing human myocytes and papillary muscles by two separate methods (28). Taken together, these data suggest that [Na\(^+\)]\(_i\), is elevated in hypertrophy and HF. Our present data in the feline model of hypertrophy and early HF clearly show that [Na\(^+\)]\(_i\), is elevated at rest and with pacing at slow rates. However, at fast rates there was no difference in [Na\(^+\)]\(_i\), in LVH-M vs. CM. The possible mechanisms for these differences were not explored directly but are discussed below.

There are two broad mechanisms for increased [Na\(^+\)]\(_i\), in hypertrophy and HF: increased influx, via multiple pathways including the NCX, the Na\(^+\) channel, and Na\(^+\)/H\(^+\) exchanger (NHE), or decreased efflux via the Na\(^+\)/K\(^+\)-ATPase. Several studies have shown decreased expression of the Na\(^+\)/K\(^+\)-ATPase in hypertrophy and HF, and others have shown altered expression of the Na\(^+\)/K\(^+\)-ATPase isoforms, coinciding with a shift in Na\(^+\) affinity (29). There are data showing reduced Na\(^+\)/K\(^+\)-ATPase activity in HF rats and reduced [Na\(^+\)]\(_i\), affinity for the Na\(^+\)/K\(^+\)-ATPase in AVB canines (33, 35). These studies support a role for decreased Na\(^+\)/K\(^+\)-ATPase function as a cause for increased [Na\(^+\)]\(_i\), in HF. More recent data from a rabbit HF model found increased [Na\(^+\)]\(_i\), but unaltered Na\(^+\)/K\(^+\)-ATPase function, with strong data suggesting a role for increased Na\(^+\) influx through TTX-sensitive Na channels (10). In the same model, another group found similar Na\(^+\)/K\(^+\)-ATPase function and increased Na\(^+\) influx via the NHE, which was upregulated at the protein level as well (3). Our study was not designed to determine the cause of the increased [Na\(^+\)]\(_i\), we observed but rather to study the consequences of this difference. Nevertheless, the fact that [Na\(^+\)]\(_i\), in LVH-M starts at a higher level in quiescent LVH-M and eventually reaches the same level in LVH-M and CM at fast pacing rates seems most consistent with the idea that Na\(^+\) influx is greater in resting LVH-M vs. CM. That [Na\(^+\)]\(_i\), increases but normalizes in LVH-M after pacing suggests that the Na\(^+\)/K\(^+\)-ATPase is working normally in these myocytes and AP-related Na\(^+\) fluxes are equivalent, leading to normalization of [Na\(^+\)]\(_i\),. In addition, this observation may be due to a reduction in Na\(^+\) influx in LVH-M with reduced Na\(^+\)/K\(^+\)-ATPase activity or Na\(^+\) sensitivity causing normalization of [Na\(^+\)]\(_i\), at higher pacing frequencies. This issue needs to be addressed in future studies.

Functional consequences of increased [Na\(^+\)]\(_i\),. Elevated [Na\(^+\)]\(_i\), would be expected to cause both increased Ca\(^{2+}\) influx via the NCX during systole (at more positive potentials) and slowed Ca\(^{2+}\) efflux via the NCX during diastole (at more negative potentials), thereby enhancing SR load but potentially causing diastolic dysfunction. This effect should be similar to the observed effect on contractility of inhibiting the Na\(^+\)/K\(^+\)-ATPase with cardiac glycosides. We and others (1, 7) have documented the [Na\(^+\)]\(_i\), dependence of NCX function and shown that the NCX plays an important, possibly adaptive, role in hypertrophy and HF. Other studies have shown the [Na\(^+\)]\(_i\), dependence of other Na\(^+\) carriers (such as the NHE) in normal myocardium, but we are aware of no studies examining the functional role of altered [Na\(^+\)]\(_i\), in the context of a diseased myocyte.
[Na\(^+\)]_, plays an central role in the FFR in myocardium (36). Data from our laboratory (31) in right ventricular trabeculae from feline hearts show a positive force-frequency response in control myocardium but a negative frequency response in H/F. The SR Ca\(^{2+}\) load of quiescent mammalian myocytes from large mammals is very low largely because they have low [Na\(^+\)]_ that maintains low [Ca\(^{2+}\)]_ and minimizes the amount of Ca\(^{2+}\) available for SR loading (15). The increase in contraction in muscle preparations (or preserved peak shortening in isolated myocytes) with high pacing rates in normal, large mammalian myocardium such as felines, is due to increased Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channel with less time for Ca\(^{2+}\) extrusion via the NCX during diastole. This is coupled with accumulated [Na\(^+\)]_, from increased time-averaged influx via the Na\(^+\) channel and reduced time for the relatively slow process of Na\(^+\) removal by the Na\(^+\)-K\(^+\)-ATPase. This increase in [Na\(^+\)]_ reduces NCX-mediated diastolic Ca\(^{2+}\) efflux, promoting SR loading and increased force development. A negative frequency response occurs when the SR loading at slow pacing rates is very high. Rodent cardiac muscle, typically shown to have high resting [Na\(^+\)]_ compared with that in larger mammals, normally has a more negative force-frequency response (7). The elevated [Na\(^+\)]_ in these myocytes leads to increased cellular Ca\(^{2+}\) loading at rest, with high SR Ca\(^{2+}\) loading and spontaneous SR Ca\(^{2+}\) release (25). Pacing leads to reduced SR Ca\(^{2+}\) because it results in increased Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channel (11). The results presented in this report suggest that the increased [Na\(^+\)]_ in quiescent feline ventricular myocytes enhances SR loading at slow pacing rates, even though these myocytes have reduced SR function (5).

**Effect of [Na\(^+\)]_ on AP.** Small changes in [Na\(^+\)]_ have the potential to cause large changes in contractility (12, 18). However, there is a complex interaction between NCX function and [Na\(^+\)]_ and membrane potential (E_m). Therefore, it is important to consider the effect of [Na\(^+\)]_ on the AP in the context of a hypertrophied myocyte when considering the potential role of this finding in the physiology of these muscle cells. We examined the multifaceted effect of altering [Na\(^+\)]_, by dialyzing control and banded LVM with known concentrations of Na\(^+\) through large-bore pipette loading. This technique should sufficiently raise or lower the true [Na\(^+\)]_, toward the [Na\(^+\)]_pip. We found that APD shortened with increasing [Na\(^+\)]_pip in both groups and that APD was shortest at high rates. APD was significantly longer in LVH-M vs. CM at low [Na\(^+\)]_, likely because of the differences in K\(^+\) currents that are known to be present in these cells (16). Longer APD is likely to be involved in the prolongation of contraction duration, because Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channel and the NCX can take place during this time (37). When we eliminated differences in APD with AP clamp, contractions were significantly smaller in LVH-M than CM at 0 and 10 mmol/l [Na\(^+\)]_, strongly supporting an increased reliance of LVH-M Na\(^+\)-dependent Ca\(^{2+}\) fluxes.

**Effect of [Na\(^+\)]_ on voltage dependence of contraction.** We examined the effect of dialysis with 0, 10, and 20 mmol/l [Na\(^+\)]_pip on voltage-dependent early and late currents and contractions. Our data using SBFI to record [Na\(^+\)]_, in CM and LVH-M suggested an approximate range of values between 9 and 16 mmol/l [Na\(^+\)]_. We chose to significantly reduce the amount of Na\(^+\) in the cell by dialysis with 0 mmol/l [Na\(^+\)]_pip and increase the amount of Na\(^+\) with 20 mmol/l [Na\(^+\)]_pip, not only to encompass the in vivo range but also to determine the extremes of [Na\(^+\)]_-dependent dynamic cell physiology. Under our conditions, with normal bath [Na\(^+\)]_, we observed an early component to the contractions that had a bell-shaped voltage relationship with the typical shape and distribution of the voltage-Ca\(^{2+}\) current relationship. We previously documented (26) that this is due to Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR. We recorded whole cell current tracings at each voltage and showed a voltage- \(I_{\text{early}}\) relationship. A second, late component to the contraction was identified that increased exponentially at more positive potentials, following the typical voltage- \(I_{\text{NCX}}\) relationship of contractions mediated by the NCX. Our data show that when control cells are dialyzed with 10 mmol/l [Na\(^+\)]_pip (higher than the native [Na\(^+\)]_, in control LVM determined by SBFI fluorescence), they have a significantly greater FS at the peak of the voltage-contraction relationship than banded LVM dialyzed with 10 mmol/l [Na\(^+\)]_pip. In addition, shortening in banded LVM was negligible when dialyzed with 0 mmol/l [Na\(^+\)]_pip with respect to both early and late contractions. A key finding in this study is that LVH-M dialyzed with 10 mmol/l had smaller contractions at −20 to +20 mV than CM dialyzed with the same [Na\(^+\)]_pip. Under these conditions, both [Na\(^+\)]_, and the duration of depolarization are fixed between groups. When dialyzed with 20 mmol/l [Na\(^+\)]_pip, however, banded LVM early and late contractions were robust and similar to controls. These data suggest that the effects of [Na\(^+\)]_ on SR load during \(I_{\text{L}}\)-mediated contractions and NCX-mediated contractions are different in banded LVM. The basis of this difference is not clear from our study. Previous data from our laboratory (21) show no difference in the abundance of NCX in banded myocardium, suggesting that differences in NCX abundance may not be the explanation.

**Altered [Na\(^+\)]_ and rate reserve.** There is strong evidence in the literature that shows that [Na\(^+\)]_ plays an important role in frequency-dependent contractile reserve (14, 28). Our data show that [Na\(^+\)]_ in single cells increases during pacing in a frequency-dependent manner. Given the effect of [Na\(^+\)]_ on the APD, the effect of rate on APD, and the complex interaction between [Na\(^+\)]_, E_m, and NCX function, a balance of factors needs to be in place for the observed increase in [Na\(^+\)]_ in H/F to enhance contractility: normal FS at low pacing rates is dependent on the duration of the AP and on the diastolic interval to load the SR. The longer APD of LVH-M prolongs the time available for reverse-mode NCX (and Ca\(^{2+}\) influx through the L-type Ca\(^{2+}\) channel) but shortens the diastolic interval, which, in the presence of high [Na\(^+\)]_, would limit Ca\(^{2+}\) influx through forward-mode NCX and further Ca\(^{2+}\) load the SR. Our data show that, at a slow pacing rate and in the context of cells isolated from H/F animals, contractility is not different from controls regardless of high, low, or normal [Na\(^+\)]_. However, at high rates, even with artificially increased [Na\(^+\)]_, there is a defect in FS that is reversed when the APD is normalized by AP clamp. These data suggest that the adaptive effect of [Na\(^+\)]_ on contractility at high pacing rates is offset by rate-dependent AP shortening.

In summary, our study shows that [Na\(^+\)]_ is elevated in LVM isolated from pressure-overloaded feline myocytes and that this alteration is an important adaptive mechanism to support contractions at slow heart rates but may limit contractility at high heart rates. Further investigations will be necessary to study the complex role of the NCX in this model of hypertrophy and early HF.
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