Intracellular Na\(^+\) Concentration Is Elevated in Heart Failure But Na/K Pump Function Is Unchanged

Sanda Despa, PhD; Mohammed A. Islam, PhD; Christopher R. Weber, PhD; Steven M. Pogwizd, MD; Donald M. Bers, PhD

**Background**—Intracellular sodium concentration ([Na\(^+\)]) modulates cardiac contractile and electrical activity through Na/Ca exchange (NCX). Upregulation of NCX in heart failure (HF) may magnify the functional impact of altered [Na\(^+\)].

**Methods and Results**—We measured [Na\(^+\)] by using sodium binding benzofuran isophthalate in control and HF rabbit ventricular myocytes (HF induced by aortic insufficiency and constriction). Resting [Na\(^+\)], was 9.7±0.7 versus 6.6±0.5 mmol/L in HF versus control. In both cases, [Na\(^+\)] increased by ≈2 mmol/L when myocytes were stimulated (0.5 to 3 Hz). To identify the mechanisms responsible for [Na\(^+\)] elevation in HF, we measured the [Na\(^+\)], dependence of Na/K pump–mediated Na\(^+\) extrusion. There was no difference in \(V_{\text{max}}\) (8.3±0.7 versus 8.0±0.8 mmol/L/min) or \(K_m\) (9.2±1.0 versus 9.9±0.8 mmol/L in HF and control, respectively). Therefore, at measured [Na\(^+\)], levels, the Na/K pump rate is actually higher in HF. However, resting Na\(^+\) influx was twice as high in HF versus control (2.3±0.3 versus 1.1±0.2 mmol/L/min), primarily the result of a tetrodotoxin-sensitive pathway.

**Conclusions**—Myocyte [Na\(^+\)], is elevated in HF as a result of higher diastolic Na\(^+\) influx (with unaltered Na/K-ATPase characteristics). In HF, the combined increased [Na\(^+\)], decreased Ca\(^2+\) transient, and prolonged action potential all profoundly affect cellular Ca\(^2+\) regulation, promoting greater Ca\(^2+\) influx through NCX during action potentials. Notably, the elevated [Na\(^+\)], may be critical in limiting the contractile dysfunction observed in HF. (*Circulation*. 2002;105:2543-2548.)

Key Words: sodium ■ heart failure ■ calcium

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Cardiac contractile dysfunction in heart failure (HF) is due largely to altered Ca\(^2+\) transport. Specifically, HF cardiac myocytes exhibit depressed and prolonged Ca\(^2+\) transients. These changes can be explained by reductions in sarcoplasmic reticulum (SR) Ca\(^2+\)-ATPase and increased Na/Ca exchange (NCX).

Intracellular sodium concentration ([Na\(^+\)]) affects excitation-contraction coupling by modulating pH and [Ca\(^2+\)], through Na/H exchange and NCX, respectively. The upregulated NCX in HF may increase the functional impact of altered [Na\(^+\)]. Recent reports indicate that [Na\(^+\)] is increased in hypertrophy; however, HF data are limited. Preliminary data in human HF suggest some increase in [Na\(^+\)], but a slight decrease in [Na\(^+\)], was found in pacing-induced HF rabbits.

Higher [Na\(^+\)], could be explained by lower Na/K pump activity, consistent with reports of decreased Na/K pump expression and isoform shifts in some HF models. However, functional studies in HF ventricular myocytes are sparse and contradictory. Enhanced Na\(^+\) influx could also raise [Na\(^+\)], and this explains the higher [Na\(^+\)], in rat versus rabbit ventricular myocytes.

Our first aim was to determine whether [Na\(^+\)], is altered in a nonspecific HF model that we have extensively characterized. 

In 90% of animals having nonsustained ventricular tachycardias (10% incidence of sudden death), NCX expression is 2-fold increased, and contractions, Ca\(^2+\) transients, and SR Ca\(^2+\) load are reduced. We measured [Na\(^+\)], in ventricular myocytes at 37°C, using the fluorescent indicator sodium binding benzofuran isophthalate (SBFI) and validated calibration methods. We found that [Na\(^+\)], is higher in HF versus control myocytes at rest and during stimulation. A second aim was to determine why [Na\(^+\)], is higher in HF (altered extrusion or influx). Using Na\(^+\) loading/recovery protocols to measure Na\(^+\) influx by the Na/K pump, we found that the maximal Na\(^+\) transport rate (\(V_{\text{max}}\)) and [Na\(^+\)], for half-maximal stimulation (\(K_m\)) are comparable in control and HF myocytes. On the other hand, resting Na\(^+\) influx (measured as the initial rate of [Na\(^+\)], rise on abrupt Na/K pump inhibition) was significantly higher in HF than control. Therefore, higher [Na\(^+\)], is due to elevated diastolic Na\(^+\) influx rather than altered Na/K pump characteristics in this HF model. We also show how the elevated [Na\(^+\)], in HF has major functional consequences for calcium flux during the action potential (AP).

**Methods**

**Rabbit HF Model and Myocyte Isolation**

HF was induced in rabbits by aortic insufficiency, followed 2 to 4 weeks later by aortic constriction as described. Rabbits were
studied ~5 months later, when the left ventricular end-systolic dimension exceeded 1.20 cm (University of Illinois Animal Studies Committee–approved protocols). Myocytes were isolated as described,19 with back-flow across the incompetent aortic valve in HF rabbits blocked by an inflated balloon-tipped catheter. Data were obtained from 14 control and 11 HF rabbits.

[Na\(^+\)]\text{\textregistered} Measurements
Myocytes were plated on laminin-coated coverslips and incubated with 10 \(\mu\)mol/L SBFI-AM and Pluronic F-127 (0.05% w/v) for 90 minutes at room temperature. After washout, SBFI-AM was allowed to deesterify for 20 minutes. The normal Tyrode’s solution contained (in mmol/L): 140 NaCl, 4 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\), 10 HEPES, and 10 glucose (pH 7.4). Fluorescence excitation at 340 and 380 nm (F\(_{340}\) and F\(_{380}\), alternating at 100 Hz) was by a 75-W xenon lamp, and emission was recorded at 535±20 nm. F\(_{340}/F_{380}\) was calculated after background subtraction and converted to [Na\(^+\)]\text{\textregistered} at the end of each experiment (using divalent-free solutions with 0, 10, or 20 mmol/L extracellular [Na\(^+\)]). [Na\(^+\)]\text{\textregistered} was measured in normal Tyrode’s solution, first at rest and then stimulated at 3 Hz for 10 minutes at room temperature. After washout, SBFI-AM was allowed to deesterify for 20 minutes. The normal Tyrode’s solution contained (in mmol/L): 145 NaCl, 2 EGTA, 10 HEPES, and 10 glucose (pH=7.4). The maximal pump-independent Na\(^+\) efflux rate (passive) was higher in HF (5.2±0.2 mmol/L/min, n=5, versus 3.2±0.1 mmol/L, n=6, in control, P<0.001). Na/K pump–mediated Na\(^+\) efflux (total minus passive) is plotted versus [Na\(^+\)]\text{\textregistered} in contracting myocytes was 11.3±0.9 mmol/L (n=15) in HF and 8.0±1.1 mmol/L (n=12) in control.

Na\(^+\) Efflux Through the Na/K Pump
Na/K pump flux was determined as the rate of pump-mediated [Na\(^+\)]\text{\textregistered} decline.18 Myocytes were Na\(^+\)-loaded by inhibiting the Na/K pump in a K\(^-\)-free solution containing (in mmol/L): 145 NaCl, 2 EGTA, 10 HEPES, and 10 glucose (pH=7.4). [Na\(^+\)]\text{\textregistered} was measured in pump reactivation in solution containing (mmol/L): 140 TEA-Cl, 4 KCl, 2 EGTA, 10 HEPES, and 10 glucose (pH=7.4). Since cell volume did not change with this protocol (n=4), [Na\(^+\)]\text{\textregistered} reflect Na\(^+\) influx. The rate of [Na\(^+\)]\text{\textregistered} decline was plotted versus [Na\(^+\)]\text{\textregistered} and fitted with a Hill expression to derive [Na\(^+\)]\text{\textregistered} decline (V\(_{max}\)/(1+(K\text{\textregistered}/[Na\(^+\)]\text{\textregistered}))\text{\textsuperscript{\textcircled{d}}}).

In some experiments, this protocol was repeated with the use of whole-cell voltage clamp or current clamp. 5 to 10 MO\(\) pipettes were filled with (in mmol/L): 30 KCl, 110 K-aspartate, 5 NaCl, 10 HEPES, 5 MgATP, 0.72 MgCl\(_2\), (1 free [Mg\(^++\)]), 3 BAPTA, 1.15 CaCl\(_2\) (100 mmol/L free [Ca\(^++\)]), and 0.2 SBFI, pH 7.2. In some experiments, further isolation of the Na/K pump current (I\(_{p}\)) was achieved by isoosmotic replacement of (mmol/L) 20 internal KCl with TEA-Cl, and 7 external NaCl or TEA-Cl with 5 NiCl\(_2\), and 2 BaCl\(_2\), with E\(_{m}\)=-30 mV to inactivate sodium channels.

Resting Na\(^+\) Influx
Resting Na\(^+\) influx was taken as the initial rate of [Na\(^+\)]\text{\textregistered} rise after abrupt Na/K pump inhibition with strophanthidin (200 \(\mu\)mol/L), with physiological [Na\(^+\)]\text{\textregistered} and [Ca\(^++\)]\text{\textregistered}. Some measurements were made with tetrodotoxin, HOE 642 (provided by Dr J. Punten, Aventis Pharma, Frankfurt, Germany), and/or Ni\(^2+\) present.

Statistical Analysis
Data are expressed as mean±SEM, and the Student’s unpaired \(t\) test was used.

Results

[Na\(^+\)]\text{\textregistered} at Rest and During Stimulation
[Na\(^+\)]\text{\textregistered}, was measured in control and HF myocytes at rest and during stimulation at 0.5 to 3 Hz. Figure 1, A and B, shows a control cell in which [Na\(^+\)]\text{\textregistered}, was measured in normal Tyrode’s solution, first at rest and then stimulated at 3 Hz for 10 minutes, reaching a new steady state in 3 to 5 minutes. When stimulation was stopped, [Na\(^+\)]\text{\textregistered}, returned to baseline (Figure 1A, inset shows SBFI calibration).18 Resting [Na\(^+\)]\text{\textregistered}, was significantly higher in HF (9.7±0.7 mmol/L, n=20) versus control (6.6±0.5 mmol/L, n=24; Figure 1D and the Table). On stimulation, [Na\(^+\)]\text{\textregistered}, rose by similar amounts in control and HF at all frequencies (Figure 1C). Thus, [Na\(^+\)]\text{\textregistered}, remained significantly higher in HF during stimulation.
V_{\text{max}}, K_m, \text{ and } n_{\text{Hill}} \text{ (Table). These data show that Na/K pump characteristics are similar in control and HF, so elevated } [\text{Na}^+]_i \text{ in HF is not due to a lower Na/K pump rate. Moreover, Figure 2C shows that at resting } [\text{Na}^+]_i \text{ (6.6 and 9.7 mmol/L for control and HF), Na}^+ \text{ efflux mediated by the Na/K pump is } \approx 2 \text{ times higher in HF versus control (Table). Since Na}^+ \text{ influx and efflux must be equal and opposite at steady state, this suggests that resting Na}^+ \text{ influx is also higher in HF; this could cause the higher } [\text{Na}^+]_i \text{ in HF myocytes.}

Membrane potential (E_m) was not controlled in Figure 2. Although Na/K pump activity is nearly voltage-insensitive in Na\textsuperscript{+}-free solution,\textsuperscript{20} non-pump-mediated Na\textsuperscript{+} efflux could vary with E_m, complicating our analysis. To test this, we repeated Na\textsuperscript{+} efflux experiments under voltage clamp and current clamp conditions. Figure 3A shows a typical experiment (n=4) in which a myocyte was first clamped at constant E_m (−80 mV), whereas [Na\textsuperscript{+}], decline was measured in the presence and absence of strophanthidin. We then switched to current clamp and repeated the protocol. The cell depolarized to −25.7 mV during Na\textsuperscript{+} loading in K\textsuperscript{+}-free solution (Figure 3A, lower trace). However, with 4 mmol/L K\textsuperscript{+} during Na\textsuperscript{+} efflux measurements, E_m repolarized to −85.3 and −86.5 mV (with and without strophanthidin, respectively). This is consistent with a very small Na/K pump contribution to resting E_m in ventricular myocytes.\textsuperscript{21,22} Figure 3B shows that the rate of [Na\textsuperscript{+}]_i decline is similar under voltage clamp and current clamp, both with and without strophanthidin. Thus, results shown in Figure 2 are not affected by lack of voltage control. In voltage-clamp experiments in which ionic conditions were chosen to isolate I_p with simultaneous [Na\textsuperscript{+}]_i measurement, we compared the [Na\textsuperscript{+}]_i dependence of I_p and pump d[Na\textsuperscript{+}]_i/dt. Since these curves are comparable (Figure 3C), Na\textsuperscript{+} efflux rate measured by d[Na\textsuperscript{+}]_i/dt reports Na/K pump function much like I_p (although converting I_p to d[Na\textsuperscript{+}]_i/dt requires an assumed surface-to-volume ratio).

**Resting Na\textsuperscript{+} Influx**

To test the hypothesis that resting Na\textsuperscript{+} influx is higher in HF, we measured resting Na\textsuperscript{+} influx as the initial rate of [Na\textsuperscript{+}]_i increase...
on abrupt Na/K pump inhibition (Figure 4A). As expected, Na\(^+\) influx was twice as high in HF (2.26 ± 0.31 mmol/L/min, n = 9) versus control (1.13 ± 0.15 mmol/L/min, n = 10) (Figure 4B).

Next we investigated various Na\(^+\) entry pathways. First, we measured Na\(^+\) influx with Na\(^+\)/K channels blocked (30 μmol/L tetrodotoxin, TTX). Figure 4B shows that TTX abolishes the HF versus control difference and that TTX-sensitive Na\(^+\) entry is significantly higher in resting HF versus control cells. This higher TTX-sensitive Na\(^+\) entry accounts for ≈85% of the difference in the total Na\(^+\) entry. In another series of experiments, we measured Na\(^+\) influx in the presence of TTX (30 μmol/L), HOE 642 (2 μmol/L, to block the Na/H exchange), and Ni\(^{2+}\) (5 mmol/L, to block NCX). The presence of all these blockers did not completely abolish Na\(^+\) influx, suggesting that other mechanisms may contribute to Na\(^+\) entry (eg, TTX-insensitive background Na\(^+\) leak channel or Na/K/2Cl cotransport). Subtracting the influx measured in the presence of all three blockers from that in the presence of TTX gives the Ni\(^{2+}\)- and HOE 642-sensitive pathways, which are not significantly altered in HF (Figure 4B).

![Figure 4](image)

**Figure 4.** Resting Na\(^+\) influx in control (Ctl) and HF. A, Representative HF cell in which Na\(^+\) influx is taken as the initial rate of [Na\(^+\)]\(i\) rise on abrupt Na/K pump inhibition with 200 μmol/L strophanthidin, in the absence and presence of TTX. B, Average data for control and HF. TTX-sensitive component of Na\(^+\) influx accounts for most of the difference between control and HF.

Discussion

We studied Na\(^+\) regulation in myocytes from a rabbit model of HF. Using SBFI, we determined [Na\(^+\)]\(i\), the [Na\(^+\)]\(i\) dependence of Na\(^+\) transport by the Na/K pump, and the rate of Na\(^+\) influx. We found that (1) [Na\(^+\)]\(i\) is higher in HF versus control, both at rest and during stimulation, (2) [Na\(^+\)]\(i\) dependence of the Na/K pump is unchanged in HF, (3) resting Na\(^+\) influx and efflux are greater in HF, and (4) increased Na\(^+\) influx in HF is largely TTX-sensitive.

![Figure 5](image)

**Figure 5.** Outward h\(_{\text{NCX}}\) is favored during HF (simulations). A, Typical control (Ctl) and HF AP at 1 Hz (control AP is from Pogwizd et al\(^{10}\); HF AP was simulated by extending plateau phase by 42 ms).\(^{19}\) B, [Ca\(^{2+}\)]\(i\) from typical control and HF calculated as described\(^{24}\) from typical control and HF Ca\(^{2+}\) transients. Actual Ca\(^{2+}\) transient from control was used\(^{19}\); for HF, the peak was reduced by 30%.\(^{4}\) C, h\(_{\text{NCX}}\) calculated in HF and control by means of the h\(_{\text{NCX}}\) equation described by Weber et al.\(^{24}\) Em from Figure 5A, [Ca\(^{2+}\)]\(i\) from Figure 5B, [Na\(^+\)]\(i\) = 8.0 or 11.3 mmol/L, and V\(_{\text{maxNCX}}\) = 6 (control) and 11 A/F (HF). All other parameters were as used in Weber et al.\(^{24}\) D, Cumulative h\(_{\text{NCX}}\) Ca flux based on h\(_{\text{NCX}}\) integral and cytosolic volume of 27 pL (Ctl) and 55 pL (HF).

[Na\(^+\)]\(i\) is higher in HF Myocytes: Physiological Consequences

[Na\(^+\)]\(i\), is ≈3 mmol/L higher in resting as well as contracting HF myocytes. Higher [Na\(^+\)]\(i\), has been reported in cardiac hypertrophy,\(^{7-10}\) but the only published [Na\(^+\)]\(i\) measurements in HF\(^{12}\) showed a slight decrease (by 0.8 mmol/L) in rabbits with pacing-induced HF. However, this HF model differs significantly from the model used here because the density of the NCX current was significantly reduced, whereas in our model\(^{14,19}\) and in human HF,\(^{3,5}\) NCX expression is increased. Higher [Na\(^+\)]\(i\), in HF has very important implications for NCX function and consequently on Ca\(^{2+}\) transients and contractility. Figure 5 shows simulations of how NCX current (I\(_{\text{NCX}}\)) may be expected to vary during a steady-state AP in HF and control, with the use of values of [Na\(^+\)]\(i\), measured here (8.0 and 11.3 mmol/L in control and HF myocytes, respectively). I\(_{\text{NCX}}\) was calculated by means of the equation described by Weber et al.\(^{24}\) and we accounted for enhanced NCX expression, AP prolongation by 42 ms (Figure 5A), and reduced [Ca\(^{2+}\)]\(i\) transients, as previously recorded in HF.
and control rabbit myocytes. Because NCX actually senses the submembrane [Ca\(^{2+}\)], \([\text{Ca}^{2+}]_{\text{sa}}\), which can differ from the bulk \([\text{Ca}^{2+}]_{\text{sm}}\), we approximated \([\text{Ca}^{2+}]_{\text{sa}}\) by using the procedure described by Weber et al.\(^{24}\) We assumed that submembrane \([\text{Na}^{+}]\), \([\text{Na}^{+}]_{\text{sa}}\), is equal to bulk \([\text{Na}^{+}]_{\text{sm}}\). However, this may not be the case, because \([\text{Na}^{+}]_{\text{sa}}\) may be elevated transiently as the result of \([\text{Na}^{+}]\) influx through \([\text{Na}^{+}]\) channels or NCX, favoring more outward \(I_{\text{NCX}}\).

In control, outward \(I_{\text{NCX}}\) is only expected for a brief period (Figure 5, C and D). The rise in \([\text{Ca}^{2+}]_{\text{sa}}\) quickly favors inward \(I_{\text{NCX}}\). This would also be true in HF if \([\text{Na}^{+}]_{\text{sa}}\) were unaltered (Figure 5C, dotted line). However, the measured \([\text{Na}^{+}]\), \([\text{Na}^{+}]_{\text{sm}}\) increase in HF shifts \(I_{\text{NCX}}\) in the outward direction. Outward \(I_{\text{NCX}}\) produces calcium entry for \(\approx 150\) ms, reducing the time for NCX-mediated \([\text{Ca}^{2+}]_{\text{eflux}}\). Indeed, in human HF, it was suggested that \([\text{Ca}^{2+}]\) influx through outward \(I_{\text{NCX}}\) may occur during the AP.\(^{23}\) Three factors contribute to the increase in \([\text{Ca}^{2+}]_{\text{eflux}}\) through NCX in HF: higher \([\text{Na}^{+}]_{\text{sm}}\), prolonged AP, and smaller \([\text{Ca}^{2+}]_{\text{sm}}\) transients.\(^{19}\) Figure 6 shows the individual effect of each of these factors on the total \([\text{Ca}^{2+}]_{\text{eflux}}\) through NCX. That is, the \(I_{\text{NCX}}\) integral is less inward as \([\text{Na}^{+}]\) rises, twitch \(\Delta[\text{Ca}]\) declines, and AP duration increases. For the changes observed in this HF model (reported here and by Pogwizd et al.),\(^{4,19}\) increased \([\text{Na}^{+}]\) has the largest impact on NCX function. Indeed, the greater \([\text{Ca}^{2+}]_{\text{influx}}\) and lower \([\text{Ca}^{2+}]_{\text{efflux}}\) would tend to load the cell (and SR) with \([\text{Ca}^{2+}]\), making more available for contractile activation. Therefore, by favoring \([\text{Ca}^{2+}]_{\text{influx}}\) through NCX, higher \([\text{Na}^{+}]\), may minimize contractile dysfunction in HF. Moreover, we speculate that NCX upregulation is compensatory in allowing NCX to still extrude the greater amount of calcium that enters during the AP.

### Summary of Results

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HF</th>
</tr>
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<tbody>
<tr>
<td>([\text{Na}^{+}]), mmol/L</td>
<td>6.6±0.5 (24, 14)</td>
<td>9.7±0.7 (20, 11)</td>
</tr>
<tr>
<td>([\text{Na}^{+}]), mmol/L during stimulation,†</td>
<td>8.0±1.1 (12, 5)</td>
<td>11.3±0.9‡ (15, 6)</td>
</tr>
<tr>
<td>([\text{Na}^{+}]) through Na/K pump V(_{\text{max}}), mmol Na/L per min</td>
<td>8.0±0.8 (6, 4)</td>
<td>8.3±0.7 (10, 6)</td>
</tr>
<tr>
<td>(K_m) (Na), mmol/L</td>
<td>9.9±0.8 (6, 4)</td>
<td>9.2±1.0 (10, 6)</td>
</tr>
<tr>
<td>(n_{\text{Hill}})</td>
<td>2.7±0.4 (6, 4)</td>
<td>3.6±0.3 (10, 6)</td>
</tr>
<tr>
<td>Calculated pump efflux at resting ([\text{Na}^{+}]), mmol/L per min</td>
<td>2.0±0.8</td>
<td>4.5±1.2</td>
</tr>
</tbody>
</table>

*First number is number of cells and the second is the number of rabbits.
†Pooled data for frequencies between 0.5 and 3 Hz.
‡Significantly different between control and HF myocytes. \(\dagger P<0.05, \S P<0.01, \S P<0.001.\)

Performing tissue homogenates and might reflect changes in nonmyocytes. Moreover, such measurements cannot differentiate between the internalized versus the sarcolemmal Na/K pumps\(^ {26}\) nor between functional and inactive pumps. It is therefore possible that whereas the total pool size of immunoreactive subunits decreases, the density of the Na/K pump in the sarcolemma is relatively unchanged. There are few functional Na/K pump studies in HF myocytes, with somewhat contradictory results. Decreased \(V_{\text{max}}\) and unchanged \([\text{Na}^{+}]\) affinity have been reported in rats with HF after myocardial infarction.\(^ {13}\) On the contrary, unaltered \(V_{\text{max}}\) and \([\text{Na}^{+}]\) affinity have been found in myocytes from dogs with chronic atrioventricular block and hypertrophy.\(^ {8}\) Reduced \(I_{\text{p}}\) has been reported in a hypertrophic rat model with increased SR \([\text{Ca}^{2+}]\) content.\(^ {10}\)

The \(V_{\text{max}}\) values here at 37°C are approximately twice what we measured at 23°C in rabbit ventricular myocytes.\(^ {18}\) The \(K_m\) found here compares well with values derived from Na/K pump current measurements in cardiac cells in the presence of intracellular K\(^ {−}\) or Cs\(^ {−}\).\(^ {20,27,28}\) The most important result regarding the Na/K pump is that under physiological conditions (i.e., appropriate resting \([\text{Na}^{+}]\)), the rate of \([\text{Na}^{+}]\) extrusion by the pump is higher in HF (Table and Figure 2C). Higher resting Na/K pump current (≈2-fold) has also been reported for dogs with chronic atrioventricular block.\(^ {8}\)

Higher \([\text{Na}^{+}]\), \(\text{in HF Is Due to Enhanced} \,<br>\text{Na}^{+}\), \text{Influx}\)

Higher \([\text{Na}^{+}]_{\text{eflux}}\) in HF must be balanced by enhanced \([\text{Na}^{+}]_{\text{influx}}\) to maintain steady-state \([\text{Na}^{+}]\) balance. Our results confirm that resting \([\text{Na}^{+}]_{\text{influx}}\) in HF is twice as high as in control (Table and Figure 4B). Most of the excess resting \([\text{Na}^{+}]_{\text{influx}}\) in HF myocytes occurs through a TTX-sensitive pathway. This suggests that a larger number of \([\text{Na}^{+}]\) channels are open in quiescent HF myocytes versus control (perhaps like a window current). Resting HF cells were not more depolarized (not shown), ruling out one simple explanation. Another explanation might be functional alteration or expression of \([\text{Na}^{+}]\) channels in HF. There are indications that the density of a slow inactivating, persistent, TTX-sensitive \([\text{Na}^{+}]\) current is increased in
HF depends especially on the elevated \([\text{Na}^+]_i\) and cytosolic volume were used (6 A/F control). Dashed vertical lines represent HF values used in Figure 5. A, \([\text{Na}^+]_i\) was varied from control to 100 ms prolongation. In each case, only the indicated parameter was modified (others were as for control). Dashed vertical lines represent HF values used in Figure 5. Control \(V_{\text{maxNCX}}\) and cytosolic volume were used (6 A/F and 27 pL). Rise in \([\text{Na}^+]_i\) in HF produces the largest reduction in net Ca\(^{2+}\) extraction through NCX (55%).

Figure 6. Simulations show that outward \(\text{Na}^+\) is more favored during HF. For each panel, the integrated Ca\(^{2+}\) flux through NCX (\(\int_{\text{NCX}}\)) during an AP plus diastole (0.5 second) was based on simulations, as in Figure 5. A, \([\text{Na}^+]_i\) was varied from control value of 8.0 mmol/L to 12 mmol/L. B, Ca\(^{2+}\) transient amplitude was varied from control value to 50% of control. C, AP duration was varied from control to 100 ms prolongation. In each case, only the indicated parameter was modified (others were as for control). Dashed vertical lines represent HF values used in Figure 5. Control \(V_{\text{maxNCX}}\) and cytosolic volume were used (6 A/F and 27 pL). Rise in \([\text{Na}^+]_i\) in HF produces the largest reduction in net Ca\(^{2+}\) extraction through NCX (55%).

In summary, \([\text{Na}^+]_i\) is higher in HF as the result of an elevation of diastolic Na\(^+\) influx with unaltered Na/K pump characteristics. Along with decreased Ca\(^{2+}\) transients and longer AP duration, the increased \([\text{Na}^+]_i\) contributes to greater Ca\(^{2+}\) influx through NCX during the AP in HF. This would increase cellular and SR calcium content. This increased Ca\(^{2+}\) influx in HF depends especially on the elevated \([\text{Na}^+]_i\), and may be functionally important in limiting the extent of contractile dysfunction in HF.

Acknowledgments

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