[Na\(^+\)]\(_i\), and the driving force of the Na\(^+\)/Ca\(^{2+}\)-exchanger in heart failure

A. Baartscheer*, C.A. Schumacher, C.N.W. Belterman, R. Coronel, J.W.T. Fiolet

Experimental and Molecular Cardiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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Abstract

Objective: Diastolic calcium is increased in myocytes from failing hearts despite up-regulation of the principal calcium extruding mechanism the Na\(^+\)/Ca\(^{2+}\)-exchanger (NCX). We hypothesize that increased diastolic calcium ([Ca\(^{2+}\)]\(_d\)) is secondary to increased cytosolic sodium ([Na\(^+\)]\(_i\)) and decreased driving force of NCX (\(\Delta G_{\text{rev}}\)). Methods: The stimulation rate dependence of simultaneously measured cytosolic sodium ([Na\(^+\)]\(_i\)), calcium transients ([Ca\(^{2+}\)]\(_i\)) and action potentials were determined with SBFI, indo-1 and the perforated patch technique in midmural left ventricular myocytes isolated from rabbits with pressure and volume overload induced heart failure (HF) and in age matched controls. Dynamic changes of \(\Delta G_{\text{rev}}\) were calculated. Results: With increasing stimulation frequency, 0.2–3 Hz (all data HF versus control): [Na\(^+\)] increased (6.4 to 10.8 versus 3.8 to 6.4 mmol/l), diastolic [Ca\(^{2+}\)] increased (142 to 219 versus 47 to 98 nmol/l), calcium transient amplitude decreased in HF (300 to 250 nmol/l) but increased in control (201 to 479 nmol/l), action potential duration (APD\(_{90}\)) decreased (380 to 260 versus 325 to 205 ms) and time averaged \(\Delta G_{\text{rev}}\) decreased (6.8 to 2.8 versus 8.7 to 6.4 kJ/mol. With increasing stimulation rate the forward mode time integral of \(\Delta G_{\text{rev}}\) decreased in HF by about 30%, the reversed mode time integral increased about ninefold and the duration of reversed mode operation more than sixfold relative to control. Conclusions: [Na\(^+\)]\(_i\) is increased in HF and the driving force of NCX is decreased. NCX exerts thermodynamic control over diastolic calcium. Disturbed diastolic calcium handling in HF is due to decreased forward mode \(\Delta G_{\text{rev}}\) secondary to increased [Na\(^+\)]\(_i\) and prolongation of the action potential. Enhanced reversed mode \(\Delta G_{\text{rev}}\) may account for increased contribution of NCX to e–c coupling in HF.

Keywords: Calcium (cellular); Heart failure; Myocytes; Na/Ca-exchanger

1. Introduction

Disturbed calcium handling underlies cardiac contractile failure and arrhythmogenesis in the hypertrophic and failing heart. The electrogenic Na\(^+\)/Ca\(^{2+}\)-exchanger (NCX) is the major sarcolemmal calcium transport system regulating cytosolic calcium. Due to the electrogenic nature of NCX, carrying either inward current in forward mode (\(V_m<E_{\text{rev-NCX}}\)) or outward current in reversed mode (\(V_m>E_{\text{rev-NCX}}\)), it modulates action potential configuration and duration and plays a role in the genesis of delayed after depolarizations (DADs). Consequently, altered activity could contribute to contractile dysfunction and arrhythmogenesis.

During the cardiac cycle NCX most of the time operates in forward mode (calcium extrusion). Up-regulation of NCX on the mRNA and protein level would be expected to promote net loss of Ca\(^{2+}\) from the cell leading to decreased diastolic calcium [Ca\(^{2+}\)]\(_i\), because fluxes are basically proportional to protein expression. Indeed, acute adenovirus induced over-expression of NCX in the rat and the rabbit causes reduction of diastolic [Ca\(^{2+}\)]\(_i\), [1] and SR calcium content [2]. In human end-stage heart failure and

\(\Delta G_{\text{rev}}\) cell

\(\Delta G_{\text{rev}}\) cardiac

\(\Delta G_{\text{rev}}\) clinical
in animal models of heart failure increased expression and/or activity of NCX have been reported (reviewed in Ref. [3]). Nevertheless, increased expression of NCX in heart failure was not associated with decreased diastolic calcium, but rather with either unchanged [4] or increased diastolic calcium [5,6].

The activity of NCX depends not only on the kinetic properties or expression level, but also on the magnitude and dynamic change of the driving force (\(\Delta G_{\text{exch}}\)) during the cardiac cycle. \(\Delta G_{\text{exch}}\) is a function of [Na\(^{+}\)], [Ca\(^{2+}\)], and trans-membrane potential (\(V_m\)); relative to [Ca\(^{2+}\)], [Na\(^{+}\)], is quantitatively of major importance, because it contributes to \(\Delta G_{\text{exch}}\) with the third power [7]. Increased [Na\(^{+}\)] has been reported in hypertrophied myocytes [8]. So far data on [Na\(^{+}\)], in heart failure are scarce [9,10]. A more detailed very recent study demonstrates increased [Na\(^{+}\)], in HF [11], which was mainly attributed to a TTX sensitive mechanism rather than to up-regulated or altered sarcolemmal Na\(^{+}\) transport mechanisms such as increased Na\(^{+}\)/H\(^{+}\)-exchange (NHE) activity [12] or decreased Na\(^{+}\)/K\(^{+}\)-ATPase activity [13]. Regardless of the responsible mechanism, increased [Na\(^{+}\)], would cause a shift of \(\Delta G_{\text{exch}}\) to lower values in forward and higher values in reversed mode. In addition, prolonged action potential duration [14] and reduced calcium transient amplitude [15] in HF would contribute to a change of the \(\Delta G_{\text{exch}}\) profile during the cardiac cycle.

This study aims to establish (1) how altered [Na\(^{+}\)], [Ca\(^{2+}\)], and action potential configuration in HF modify \(\Delta G_{\text{exch}}\) and (2) how this could explain disturbed calcium handling.

2. Methods

2.1. The rabbit model of pressure and volume overload induced heart failure and isolation of left midmural ventricular myocytes

Animal care and handling conformed to Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and the study was approved by the local ethical committee.

HF was induced in eight rabbits (New Zealand White, SPF, 3–3.5 kg) by combined volume and pressure overload as described previously [14,16]. Volume overload was produced by aortic valve rupture increasing pulse pressure by 100% and pressure overload was produced 3 weeks later by a suprarenal abdominal aortic banding to approximately 50%. After 12 weeks LVEDP was measured, animals were sacrificed, hearts were isolated. Body weight, heart and lung weight were measured and presence of ascites was documented as described previously [14,17]. Age matched untreated animals (n=6) served as controls. Midmural left ventricular myocytes were isolated as described previously [18] and stored until use at room temperature in separate vials, each containing about 10⁵ myocytes in 5 ml solution containing (mmol/l): [Na\(^{+}\)] 156, [K\(^{+}\)] 4.7, Ca\(^{2+}\) 2.6, [Mg\(^{2+}\)] 2.0, [Cl\(^{-}\)] 150.6, [HCO\(_3\)\(^{-}\)] 4.3, [HPO\(_4\)\(^{2-}\)] 1.4, [Hepes] 17, [glucose] 11 supplied with 1% fatty acid free albumin (pH 7.3). Cell dimensions of 100 randomly chosen rod shaped myocytes were determined.

2.2. Measurement of free cytosolic [Ca\(^{2+}\)], [Na\(^{+}\)], and trans-membrane potentials

Before each individual experiment, cells were loaded during 30 min with 5 \(\mu\)mol/l indo-1/AM or 120 min with 10 \(\mu\)mol/l SBFI and 0.01% pluronic, washed twice with fresh Hepes solution (without albumin), and kept for another 15 min to ensure complete de-esterification. [Ca\(^{2+}\)], and action potentials were measured simultaneously. Hardware for data recording consisted of a patch clamp amplifier (Axopatch 200B, two laboratory-made differential amplifiers for photomultiplier signals and a combined A/D and D/A board (DAS1802AO, Keithley Metrabyte) controlled by custom made software (Test-point).

Myocytes were placed in a thermally controlled (37 °C) cell chamber on the stage of an inverted fluorescence microscope (Nikon Diaphot) and superfused with the same Hepes buffered solution as above (without albumin) at a rate of 1–2 ml/min. A quiescent rod-shaped myocyte was selected, stimulation was started and a rectangular diaphragm was used to restrict the fluorescence measuring area to the myocyte surface. Dual wavelength emission of Indo-1 was recorded (410/516 nm, excitation at 340 nm) and cellular free [Ca\(^{2+}\)], was calculated and calibrated as described previously [19]. Fig. 1 shows representative examples of raw data and processing of signals. Signals were corrected for background recorded from indo-1 free myocytes (about 10% of raw signals). A second correction (allowing for mitochondrial compartmentalization of indo-1 (37%, see Ref. [19]) and stimulation rate dependence of mitochondrial calcium [20] was applied, in order to obtain cytosolic free [Ca\(^{2+}\)], as described previously [19]. In parallel experiments dual wavelength emission of SBFI was recorded (516/590 nm, excitation at 340 nm), signals were corrected for background fluorescence recorded from SBFI free myocytes and [Na\(^{+}\)], was calculated and calibrated as described previously [21]. Action potentials were recorded using the perforated patch-clamp technique with a pipette solution containing (mmol/l): [Na\(^{+}\)] 6 or 10 (in control or HF myocytes, respectively), [K\(^{+}\)] 140, [Mg\(^{2+}\)] 1.0, [Cl\(^{-}\)] 153.6, [HPO\(_4\)\(^{2-}\)] 1.4, [Hepes] 17, [glucose] 11 [Ca\(^{2+}\)] 2.6 and 0.2 mg/ml amphotericin B (pH 7.1). Pipette resistance was 3–5 MΩ. The potential to bath solution was adjusted to zero. Capacitance and the pipette series resistance were compensated to about 80%. Access resistance to the cell decreased within 10 min after...
Fig. 1. Representative examples in a HF myocyte stimulated at 2 Hz of measured raw fluorescence signals, ratio data, cellular \([Ca^{2+}]\) and calculated 'true' cytoplasmic \([Ca^{2+}]\). Respective background fluorescence signals were 0.15 at 410 nm and 0.10 at 516 nm. \(R_{max}, R_{min}, \beta\) and \(k_a\) were 4.5, 0.55, 2.1 and 250 nM, respectively. Mitochondrial compartmentation 37% (see Ref. [19]).

Seal formation. Fluorescence and action potential signals were digitized at 1 kHz.

Stimulation rate dependence (0 to 3 Hz) of \([Na^+]\), relative to bodyweight and LVEDP were significantly increased. Ventricles were hypertrophied and dilated. Ascites was found in three out of eight HF rabbits. The average maximal myocyte length and width (largest cross sectional diameter) were significantly increased in HF myocytes (t-test).

2.3. Formulation of \(\Delta G_{exch}\)

\[
\Delta G_{exch} = 3\Delta G_Na - \Delta G_{Ca}
\]

\[
= RT \ln \left( \frac{[Na]^o}{[Na]^i} \right)^3 \frac{[Ca^{2+}]_i}{[Ca^{2+}]_o} = F V_m \text{ (kJ/mol)}
\]

in which \(V_m\) is membrane potential, \(F\) the Faraday constant and \(R\) and \(T\) have their usual meaning. In this study positive values of \(\Delta G_{exch}\) correspond to driving force in forward mode (inward sodium transport) and negative values to reversed mode (outward sodium transport), respectively.

2.4. Statistics

Data are expressed as mean\(\pm\)S.E.M. for six control rabbits (18 myocytes) and eight HF rabbits (24 myocytes), three myocytes per heart. Values for myocytes of individual hearts were averaged. Two-way analysis of variance (ANOVA; with a post hoc test according to Student–Newman–Keuls) or Student’s t-test was used to test for statistical significance where appropriate at a level of significance of \(P<0.05\).

3. Results

3.1. Animal characteristics

Table 1 summarizes the parameters relevant to HF rabbits and age matched controls. Bodyweight was not different in HF and control. In HF, heart and lung weights relative to bodyweight and LVEDP were significantly increased. Ventricles were hypertrophied and dilated. Ascites was found in three out of eight HF rabbits. The average maximal myocyte length and width (largest cross sectional diameter) were significantly increased in HF myocytes (t-test).

3.2. \([Na^+]\), \([Ca^{2+}]\) and trans-membrane potentials

\([Na^+]\) did not show cycle related fluctuations in either group of animals. Fig. 2 summarizes the average \([Na^+]\) as a function of stimulation frequency (0 to 3 Hz). At all stimulation rates \([Na^+]\) was significantly higher in HF than in control (ANOVA). \([Na^+]\) increased with stimulation rate from 6.4 to 10.8 mmol/l in HF and from 3.8 to 6.1 mmol/l in control. The difference between \([Na^+]\) at 0 Hz and 3 Hz was significantly larger in HF than in control (t-test \(P<0.0001\)).

Fig. 3 shows the stimulation rate dependence of diastolic \([Ca^{2+}]\), (left panel) and calcium transient amplitude (right panel). At all stimulation rates diastolic \([Ca^{2+}]\) was significantly higher in HF than in control (ANOVA). With increasing stimulation rate diastolic \([Ca^{2+}]\) increased.

Table 1

<table>
<thead>
<tr>
<th>Animal characteristics</th>
<th>Control (n=6)</th>
<th>HF (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodyweight (kg)</td>
<td>4.3±0.18</td>
<td>4.4±0.15</td>
</tr>
<tr>
<td>Relative heart weight (ww/kg 10^-7)</td>
<td>2.5±0.11</td>
<td>4.4±0.23*</td>
</tr>
<tr>
<td>Relative lung weight (ww/kg 10^-7)</td>
<td>2.6±0.14</td>
<td>3.9±0.15*</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4±0.6</td>
<td>21±2.6*</td>
</tr>
<tr>
<td>Ascites</td>
<td>0</td>
<td>3*</td>
</tr>
<tr>
<td>Myocyte width (µm)</td>
<td>29±0.9</td>
<td>37±1.0*</td>
</tr>
<tr>
<td>Myocyte length (µm)</td>
<td>142±4.8</td>
<td>201±3.9*</td>
</tr>
</tbody>
</table>

ww=Wet weight; LVEDP=left ventricular end-diastolic pressure.

data: mean±S.E.M.; n=number of animals; * \(P<0.05\) versus control (t-test).
Fig. 4 shows representative examples of simultaneously measured action potentials and calcium transients. In HF action potential duration was prolonged, diastolic [Ca\textsuperscript{2+}], was higher, calcium transient amplitude was smaller and relaxation rate was slowed compared to control.

Fig. 5 shows that action potential duration at 90% repolarization (APD\textsubscript{90}) decreased with increasing stimulation frequency in HF and control. APD\textsubscript{90} was significantly prolonged at any frequency (ANOVA) in HF relative to control by about 50 ms.

3.3. The driving force of the Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger (\(\Delta G_{\text{exch}}\))

The dynamic change of \(\Delta G_{\text{exch}}\) during the cardiac cycle was calculated from [Na\textsuperscript{+}], [Ca\textsuperscript{2+}], and the trans-membrane potential in each individual myocyte. Fig. 6 shows representative examples of \(\Delta G_{\text{exch}}\) profiles in a steady state 2 Hz stimulated control (left panel) and HF (middle panel) myocyte. In HF forward mode \(\Delta G_{\text{exch}}\) was shifted to less positive values and duration was reduced, reversed mode \(\Delta G_{\text{exch}}\) was shifted to more negative values (dotted area) and duration was substantially prolonged relative to control. In control myocytes an overshoot was observed in forward mode \(\Delta G_{\text{exch}}\) upon repolarisation, due to the difference between action potential and calcium transient duration. In HF, where action potential and calcium transient duration approached each other, the overshoot was greatly reduced. The right panel summarizes the stimulation rate dependence of time averaged \(\Delta G_{\text{exch}}\) (averaged over the entire cardiac cycle; solid lines in the two left panels). Time averaged \(\Delta G_{\text{exch}}\) was significantly

![Graph showing stimulation rate dependence of cytosolic [Na\textsuperscript{+}]. Control (open circles) and HF (closed circles).](image1)

![Graph showing stimulation rate dependence of diastolic [Ca\textsuperscript{2+}]. Control (open circles) and HF (closed circles).](image2)

![Graph showing calcium transient amplitude. Control (open circles) and HF (closed circles).](image3)
Table 2
Calcium transient kinetic characteristics

<table>
<thead>
<tr>
<th>Stimulation rate (Hz)</th>
<th>Control (n = 6)</th>
<th>HF (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time to peak (ms)</td>
<td>80% recovery (ms)</td>
</tr>
<tr>
<td>0.2</td>
<td>80 ± 6.0</td>
<td>453 ± 35.6</td>
</tr>
<tr>
<td>0.5</td>
<td>60 ± 6.1</td>
<td>359 ± 19.8</td>
</tr>
<tr>
<td>1</td>
<td>54 ± 3.5</td>
<td>295 ± 9.6</td>
</tr>
<tr>
<td>2</td>
<td>48 ± 2.0</td>
<td>247 ± 8.8</td>
</tr>
<tr>
<td>3</td>
<td>46 ± 2.7</td>
<td>205 ± 10.7</td>
</tr>
</tbody>
</table>

Data: mean ± S.E.M.; n = number of animals; * P < 0.05 HF versus control (ANOVA).

For the calcium transient amplitudes see Fig. 3 (right panel).

Fig. 4. Representative examples of action potentials (left panel) and calcium transients (right panel) measured simultaneously in a control (solid lines) and a HF (dotted lines) myocyte stimulated at 2 Hz.

Fig. 5. Stimulation rate dependence of action potential duration measured at 90% repolarization (APD$_{90}$). At any frequency APD$_{90}$ was significantly larger in HF than in control (P < 0.03).

smaller in HF than in control at all frequencies (ANOVA) and decreased more (t-test, P < 0.0001). Forward and reversed mode both contribute to time averaged $\Delta G_{\text{exch}}$. The respective surface areas are representative measures of these contributions. Fig. 7 shows the stimulation rate dependence of the forward mode (top left) and reversed mode (top right) surface areas. At all frequencies the forward mode surface area in HF was less and the reversed mode surface area was larger than in control. The bottom panels show the change of the respective surface areas in HF relative to control; forward mode was reduced frequency dependently from 0.85 to 0.45, and reversed mode was enhanced about nine fold over the entire range of frequencies. Consequently, calcium efflux becomes reduced, and calcium influx during the action potential becomes increased in HF relative to control.

$[\text{Na}^+]_i$, $[\text{Ca}^{2+}]_i$ and action potential configuration all contribute to the difference in time averaged $\Delta G_{\text{exch}}$ between HF and control ($\Delta G_{\text{exch-HF}} = \Delta G_{\text{exch-ctrl}}$). Fig. 8 shows the frequency dependence of the individual contributions of $[\text{Na}^+]_i$, $[\text{Ca}^{2+}]_i$, and $V_m$. Increased of $[\text{Na}^+]_i$...
Fig. 6. The dynamic change of $\Delta G_{\text{exh}}$ and the time averaged $\Delta G_{\text{exh}}$ during one cardiac cycle. Dynamic change of $\Delta G_{\text{exh}}$ in a 2 Hz stimulated control (left panel) and HF (middle panel) myocyte. The dotted area corresponds to reversed mode operation of NCX. The solid lines represent $\Delta G_{\text{exh}}$ time averaged over one cardiac cycle. The stimulation rate dependence of the time averaged $\Delta G_{\text{exh}}$ (right panel). Control (open circles) and HF (closed circles).

contrasts most, but is hardly stimulation rate dependent. Altered action potential contributes particularly at the higher stimulation rates, but much less than $[\text{Na}^+]_i$. Altered diastolic $[\text{Ca}^{2+}]_i$ and calcium transient configuration partly compensate for the effects of increased $[\text{Na}^+]_i$ and action potential, but more at the lower stimulation rates.

Fig. 7. Stimulation rate dependence of forward and reversed mode contributions to time averaged $\Delta G_{\text{exh}}$. Forward mode surface area during the cardiac cycle (upper left). Reversed mode surface area during the cardiac cycle (upper right). Control (open circles) and HF (closed circles). The respective surface areas in HF relative to control for forward mode (bottom left) for reversed mode (bottom right). Note the different y-axis scaling for control and HF in the upper right panel.
4. Discussion

This study quantifies the impact of altered sodium homeostasis in left ventricular myocytes from hearts of rabbits with pressure and volume overload-induced heart failure on dynamic changes of the driving force of NCX ($\Delta G_{\text{exch}}$) and calcium handling. We demonstrate that in HF frequency dependently $[\text{Na}^+]$, and diastolic $[\text{Ca}^{2+}]$, are increased, action potential is prolonged and that $\Delta G_{\text{exch}}$ is shifted to less positive values during diastole and to more negative values during systole. These changes are stimulation rate dependent. Besides prolongation of the action potential, increased $[\text{Na}^+]$, contributes most to this shift, while increased diastolic $[\text{Ca}^{2+}]$, and altered calcium transient configuration have a compensatory effect. We conclude from the data presented that, despite up-regulation of NCX in heart failure (for a review, see Ref. [3]), the decreased driving force, secondary to increased $[\text{Na}^+]$, is causal to increased diastolic $[\text{Ca}^{2+}]$, and associated disturbed diastolic function.

We used a well-established model of combined volume and pressure overload-induced heart failure in rabbits as described previously [14,16]. This model of heart failure has been widely and successfully used over the last decade. Advantages of the model are (1) the reproducible development of failure within 2 to 3 months with all features also clinically observed in humans (hypertrophy, dilatation, dyspnoea, decreased diastolic function and arrhythmogenesis) [17,22] and (2) the greater similarity in action potential characteristics and excitation–contraction coupling between human and rabbit than other models of heart failure such as, e.g., a rat model.

4.1. $[\text{Na}^+]$, diastolic $[\text{Ca}^{2+}]$, and action potential prolongation in HF

Increased $[\text{Na}^+]$, in hypertrophy has been reported previously [8], but so far data on $[\text{Na}^+]$, in heart failure are scarce [9,10]. Very recently, a more detailed study in a similar model of HF reported increased $[\text{Na}^+]$, which was attributed mainly to a TTX sensitive mechanism, presumably Na-channel activity, but little contribution of Na+/H+ exchange (NHE) and no involvement of Na+/K+-ATPase [11]. Quite intriguingly, these data were obtained in quiescent myocytes where both Na-channel and NHE activity are supposed to be minimal. Our $[\text{Na}^+]$, data at rest are about 50% less than those of Despa et al. [11] both in control and HF. Our previous data [9,10] and unpublished observations (submitted) rather suggest a key role for NHE and no involvement of altered Na-channel dependent sodium influx. This would agree with data on up-regulated activity but unchanged expression of NHE [12,23] and on unchanged sodium channel kinetics in HF [24], although calcium overload induced calmodulin dependent modulation of slow inactivation of the Na-channel in HF can not be excluded [25]. Also reduced sodium efflux secondary to depressed Na+/K+-pump activity should be considered [13].

Prolongation of the action potential is commonly found in heart failure (for a review, see Ref. [26]). We measured prolongation of action potential duration, which was rather independent of stimulation rate (Fig. 4), which contrasts with other studies reporting convergence with increasing stimulation rate [14,26,27]. It may be speculated that methodological differences underlie the discrepant results. We used pipette sodium concentrations corresponding to those found in HF and control in the perforated patch configuration have a compensatory effect. We conclude from the data presented that, despite up-regulation of NCX in heart failure (for a review, see Ref. [3]), the decreased driving force, secondary to increased $[\text{Na}^+]$, is causal to increased diastolic $[\text{Ca}^{2+}]$, and associated disturbed diastolic function.

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The decreased electrochemical potential of $[\text{Na}^+]$ across the sarcolemma caused by the increased $[\text{Na}^+]$, reduces the forward driving force of NCX, and causes increased steady state diastolic $[\text{Ca}^{2+}]$, which is confirmed by the inter-dependence of $[\text{Na}^+]$, and diastolic $[\text{Ca}^{2+}]$, shown in Fig. 3. Increased diastolic $[\text{Ca}^{2+}]$, in HF in combination with down regulation of the SR Ca$^{2+}$-ATPase (SERCA) [28] interferes with SR calcium handling. On the one hand, down regulation of SERCA may underlie decreased SR calcium loading in particular at the higher stimulation rates explaining at least in part the negative (or flat) relationship between the calcium transient amplitude and stimulation rate (Fig. 3) [29]. On the other hand, increased diastolic $[\text{Ca}^{2+}]$, contributes to this by affecting open probability of
Limitations of the study

We took maximal cell length and width as a measure of the degree of cellular hypertrophy. Obviously such an approach precludes estimation of changes of cell volume. In addition we can not entirely exclude that a small fraction of the myocyte population consisted of still side to side coupled cell pairs. However, it seems reasonable to assume that there would be no difference between HF and control in this respect.

In vivo resting heart rate of rabbits is about 2.5–3 Hz, which may increase to well above 4 Hz with exercise and/or stress. It is common experience that in isolated control rabbit myocytes the maximal stimulation rate is around 4 Hz. In HF myocytes, with prolonged action potential and refractory period, maximal stimulation rate is even less. Absence of adrenergic regulation in isolated myocytes might explain the difference with the in vivo situation. Indeed, application of noradrenalin to myocytes enhances maximal stimulation rates. Although some HF myocytes could be stimulated up to 3.5 Hz, the majority of HF cells failed to respond to such a rate. Therefore, this study can not provide information on sodium and calcium handling at higher, more physiological stimulation rates.

We used bulk cytosolic [Na\(^+\)] and [Ca\(^{2+}\)] to calculate \(\Delta G_{\text{exch}}\). It may be argued that the actual driving force of NCX could deviate from calculated \(\Delta G_{\text{exch}}\) due to the existence of sub-sarcolemmal gradients of these ions. So far, there is few data on the actual magnitude of sub-sarcolemmal sodium and the quantitative importance is disputed. The existence of sub-sarcolemmal calcium gradients has been demonstrated using optical [38] and electrophysiological [39] techniques; estimates for maximal systolic sub-sarcolemmal [Ca\(^{2+}\)] were up to four-times bulk values [39]. Although ‘fuzzy’ space effects would thus affect our calculations quantitatively, this is not necessarily so qualitatively.

Some recent studies suggest a that the Na:Ca stoichiometry for NCX might be higher than 3:1 used in our calculations of \(\Delta G_{\text{exch}}\) [40,41]. This would not only implicate that all our \(\Delta G_{\text{exch}}\) data should be scaled up by at least 6 kJ/mol, but also that NCX would never operate in reversed mode due to less positive values in sub-sarcolemmal sodium and the quantitative importance is disputed. The existence of sub-sarcolemmal calcium gradients has been demonstrated using optical [38] and electrophysiological [39] techniques; estimates for maximal systolic sub-sarcolemmal [Ca\(^{2+}\)], were up to four-times bulk values [39]. Although ‘fuzzy’ space effects would thus affect our calculations quantitatively, this is not necessarily so qualitatively.

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5. Conclusions

Increased [Na\(^+\)] and prolonged duration of action potential cause a decrease of \(\Delta G_{\text{exch}}\) in HF and increased duration the Na\(^+\)/Ca\(^{2+}\)-exchanger is in reversed mode. This results in an increase of diastolic calcium despite an...
up-regulated Na\(^+\)/Ca\(^{2+}\)-exchanger and might be a prerequisite for arrhythmogenesis in HF.

Acknowledgements

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