Editorial

How can overexpression of Na\(^+\)/Ca\(^{2+}\)-exchanger compensate the negative inotropic effects of downregulated SERCA?

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See article by Terracciano et al. [2] (pages 38–47) in this issue.

1. Importance of the Na\(^+\)/Ca\(^{2+}\)-exchanger for the Ca\(^{2+}\) distribution in the failing human heart

The failing human ventricle suffers from two major problems: (1) During diastole, relaxation is retarded and remains eventually incomplete. (2) During systole, the force-frequency relation is blunted, i.e. an increase from 60 to 120 beats-per-min does not increase the contractile force as it is typical in non-failing tissue. Both problems have been linked to reduced expression and function of sarcoplasmic reticulum (SR) Ca\(^{2+}\) ATPase (SERCA) proteins. Studies in isolated human ventricular trabeculae [1] have shown that incomplete Ca\(^{2+}\) reuptake by SERCA can cause (1) a diastolic accumulation of Ca\(^{2+}\) ions in the cytosol which impairs diastolic relaxation, and (2) a reduction of releasable SR Ca\(^{2+}\) with the consequence of a reduced systolic Ca\(^{2+}\) activation of force and a blunted force-frequency relation. Since failing human myocardium was shown to overexpress the Na\(^+\)/Ca\(^{2+}\)-exchanger (mRNA and protein [1]), enhanced Ca\(^{2+}\) efflux by Na\(^+\)/Ca\(^{2+}\)-exchange has been suggested to partially compensate impaired diastolic Ca\(^{2+}\) removal. The paper of Terracciano et al. ([2] in this issue) confirms this view. In addition, it introduces a new idea: the enhanced expression and function of the Na\(^+\)/Ca\(^{2+}\)-exchanger may facilitate Ca\(^{2+}\) reuptake by SERCA and thereby compensate for the impaired SR Ca\(^{2+}\) load.

2. Transgenic mice with overexpressed Na\(^+\)/Ca\(^{2+}\)-exchanger as a model

Terracciano et al. [2,3] compared protein concentrations and functions between ventricular myocytes from transgenic mice (TR) that overexpress the Na\(^+\)/Ca\(^{2+}\)-exchanger and non-transgenic (non-TR) wild-type littermates. They find that the protein levels of the Na\(^+\)/Ca\(^{2+}\)-exchanger are approximately 2.4-fold elevated [3,4] whilst the concentration of Ca\(^{2+}\) handling proteins such as SERCA, calsequestrin and phospholambam were not different. With this background, the authors can evaluate the consequences of the overexpression of a single protein species for the Ca\(^{2+}\) fluxes mediated by Na\(^+\)/Ca\(^{2+}\)-exchange.

3. The balance of Ca\(^{2+}\) fluxes

Terraciano et al. [2] evaluated the Ca\(^{2+}\) fluxes from experiments that measured time-dependent changes (d/dt) of the concentration of Ca\(^{2+}\) ionized in the cytosol ([Ca\(^{2+}\)\(_c\)]) by means of the fluorescence indicator Indo-1. There are several fluxes that increase and decrease [Ca\(^{2+}\)\(_c\)] during the contractile cycle (from [5]):

\[
\frac{d}{dt}[Ca^{2+}]_c = J_{\text{ret}} + I_{\text{Ca,L}} + J_{\text{inf}} + J_{\text{Lig}} - J_{\text{SERCA}} - J_{\text{eff}} - J_{\text{Lig}} \quad (1)
\]

The SR Ca\(^{2+}\) release flux (J\(_{\text{ret}}\)) contributes most (ca. 80%) of the Ca\(^{2+}\) when the transient rises to its peak, it is complemented by Ca\(^{2+}\) influx through L-type channels (I\(_{\text{Ca,L}}\)) and via Na\(^+\)/Ca\(^{2+}\)-exchange operating in Ca\(^{2+}\) influx mode (J\(_{\text{inf}}\)). [Ca\(^{2+}\)\(_c\)] is decreased (negative sign) by SR Ca\(^{2+}\) re-uptake (J\(_{\text{SERCA}}\)) and by Ca\(^{2+}\) efflux via Na\(^+\)/Ca\(^{2+}\)-exchange (J\(_{\text{eff}}\)). Last not least, ionized Ca\(^{2+}\) binds to (-J\(_{\text{Lig}}\)) and unbinds from (+J\(_{\text{Lig}}\)) numerous ligands such as troponin C. When the cellular Ca\(^{2+}\) load is steady,
i.e. when the frequency is constant and no pharmacological interventions are done, the sum of the positive and negative fluxes has to be in balance.

4. Na\(^+\)/Ca\(^{2+}\)-exchange provides both Ca\(^{2+}\) efflux and Ca\(^{2+}\) influx

Our conventional understanding of how the Na\(^+\)/Ca\(^{2+}\)-exchanger contributes to the Ca\(^{2+}\) transient is dominated by the interpretation of the positive inotropy caused by cardioactive glycosides [6]. According to the “Na\(^+\)-lag hypothesis” [7] ouabain inhibits the Na\(^+\)/K\(^+\)-ATPase, the increase in the cytosolic sodium concentration [Na\(^+\)], reduces J\(_{\text{SERCA}}\), and a correspondingly larger part of Ca\(^{2+}\) ions is sequestered by J\(_{\text{SERCA}}\). Amplitude and direction of Ca\(^{2+}\) flux via Na\(^+\)/Ca\(^{2+}\)-exchange are determined by the difference (V\(_{m}\) - E\(_{j}\)). V\(_{m}\) is the membrane potential, and for the reversal potential for the exchanger one can write (e.g. [5])

\[
E_j = 3 E_{Na} - 2 E_{Ca} = 3 \cdot 61 \text{ mV} \cdot \log([Na^+]_i/[Na^+]_o) - 2 \cdot 30.5 \text{ mV} \\
\cdot \log([Ca^{2+}]_i/[Ca^{2+}]_o) \tag{2}
\]

At start of the action potential (AP), [Ca\(^{2+}\)]\(_i\) is low, E\(_j\) is with approximately 26 mV [3] negative to V\(_{m}\) (+30 mV), and the Na\(^+\)/Ca\(^{2+}\)-exchanger operates in the Ca\(^{2+}\) influx mode. When the Ca\(^{2+}\) transient peaks, E\(_j\) increases beyond V\(_{m}\) and the exchanger changes into the Ca\(^{2+}\) efflux mode (J\(_{\text{SERCA}}\), positive in Fig. 2). During the following time, direction and amplitude of the Ca\(^{2+}\) influx depend on both fall of [Ca\(^{2+}\)]\(_i\), (more negative E\(_j\)) and AP repolarization (V\(_{m}\)), usually the amplitude fades away but the direction does remains in the Ca\(^{2+}\) efflux mode (Fig. 2). Increments in [Na\(^+\)], e.g. due to ouabain shift E\(_j\) to more negative potentials, and the reduced driving force attenuates J\(_{\text{SERCA}}\) with the result that more Ca\(^{2+}\) is sequestered by J\(_{\text{SERCA}}\) (compare [8]).

5. The Ca\(^{2+}\) transients in myocytes from transgenic mice

Terracciano et al. compare the Ca\(^{2+}\) transients between field-stimulated TR and non-TR myocytes. The results suggest (see Fig. 1 in [2]):

1. The Ca\(^{2+}\) transients peak earlier (time to peak, TTP, 100 instead of 146 ms) and last shorter in TR than in non-TR myocytes (234 instead of 332 ms for TTP+T50=time to 50% decay). The faster time course is expected in a cell where J\(_{\text{SERCA}}\) is enhanced whilst the other Ca\(^{2+}\) fluxes are non-modified.
2. The amplitude of the Ca\(^{2+}\) transients is not significantly different between TR (126 nM) and non-TR myocytes (133 nM). Result (2) is somewhat unexpected; augmented J\(_{\text{SERCA}}\) (overexpression) should have reduced the peak Ca\(^{2+}\) by earlier cutting off its rising phase (the Ca\(^{2+}\) transient peaks when \(I_{Ca} + I_{rel} + I_{inf} = J_{\text{SERCA}} + J_{\text{SERCA}}\)). By competition with J\(_{\text{SERCA}}\), augmented J\(_{\text{SERCA}}\) should have diminished the SR Ca\(^{2+}\) load, as a consequence a smaller J\(_{\text{rel}}\) should have caused a smaller peak [Ca\(^{2+}\)].

3. The Ca\(^{2+}\) transients of TR and non-TR myocytes superimpose after TTP and TTP+T50 of TR myocytes has been prolonged by inhibiting J\(_{\text{SERCA}}\) of TR myocytes by 200 \(\mu\)M thapsigargin.

6. In myocytes from transgenic mice, SR Ca\(^{2+}\) load is augmented

As an explanation for the constant amplitude of the Ca transient in TR and non-TR myocytes, Terracciano et al. [2] suggest that overexpression of Na\(^+\)/Ca\(^{2+}\)-exchanger facilitates the SR Ca\(^{2+}\) load. The idea was tested by experiments in Ca\(^{2+}\)- and Na\(^+\)-free extracellular solution where the Na\(^+\)/Ca\(^{2+}\)-exchanger does not operate. With J\(_{\text{SERCA}}\) = 0, the decay of the Ca\(^{2+}\) transient can quantify J\(_{\text{SERCA}}\). The amplitudes of the caffeine induced Ca\(^{2+}\) transients were 1556 nM in TR and 880 nM in non-TR myocytes, suggesting that the TR had a larger SR Ca\(^{2+}\) content than non-TR myocytes. J\(_{\text{SERCA}}\) and J\(_{\text{SERCA}}\) cannot be directly extracted from the Ca\(^{2+}\) transient (since the decay rate depends also on J\(_{\text{SERCA}}\) that varies with [Ca\(^{2+}\)], [9]). Instead, the authors estimate J\(_{\text{SERCA}}\) and J\(_{\text{SERCA}}\) and plot them as a function of pCa. Their fit with sigmoidal functions yields the following flux parameter: in non-TR myocytes, SERCA operates with a K\(_{m}\) of 0.4 \(\mu\)M and a V\(_{max}\) of 99 \(\mu\)M/s, the Na\(^+\)/Ca\(^{2+}\)-exchanger with a K\(_{m}\) 0.4 \(\mu\)M and a V\(_{max}\) of 21 \(\mu\)M/s. In TR myocytes J\(_{\text{SERCA}}\) has identical values, however, the Na\(^+\)/Ca\(^{2+}\)-exchanger shows a more than doubled V\(_{max}\) = 53 \(\mu\)M/s at unchanged K\(_{m}\) = 0.4 \(\mu\)M.

In an independent set of voltage-clamp experiments [2], the authors measure the flux of releasable SR Ca\(^{2+}\) (J\(_{rel}\)) as current J\(_{rel}\) (influx of 3 Na\(^+\) ions in exchange of 1 Ca\(^{2+}\) ion). Following the suggestions of the Eisner Laboratory [10], J\(_{rel}\) was activated by rapid application of 10 mM caffeine for 12 s. In continuous presence of caffeine, the SR release channels do not close and J\(_{\text{SERCA}}\) is ineffective, hence, all released Ca\(^{2+}\) ions are extruded via J\(_{\text{rel}}\). The authors estimate from the rates that the flux J\(_{\text{rel}}\) is 1.7-fold larger in TR than in non-TR myocytes. The time integral of the caffeine-induced inward current I\(_{\text{rel}}\) reflects the amount of the caffeine-releasable SR Ca\(^{2+}\) [9]. The authors estimate that the SR of TR is loaded with significantly (32%) more Ca\(^{2+}\) than the SR of non-TR myocytes, and that this difference disappears after SERCA inhibition by thapsigargin.
7. Problems in quantification of the Ca\(^{2+}\) flux

Different to the caffeine-induced Ca\(^{2+}\) transients, Ca\(^{2+}\) transients induced by action potentials (field stimulation) were of low amplitude (approximately 130 nM, Fig. 1 [2]). The low amplitude and the long duration (234 and 332 ms, in non-TR and TR myocytes, respectively) of the Ca\(^{2+}\) transients are in conflict with the duration of contraction that was 128 (TR) and 164 ms (non-TR) as well as with the literature on Ca\(^{2+}\) transients where the myocytes were loaded with the acid form of Indo-1 instead of the acetoxymethylester (AM). An example for isolated mice ventricular myocytes is shown in Fig. 1: [Ca\(^{2+}\)]\(_c\) starts from diastolic 100 nM, rises after a 10 ms delay, peaks to 1100 nM 36 ms after start of the clamp step, and completely relaxes within 200 ms. Thus, the 4 Hz stimulation does not induce a diastolic Ca\(^{2+}\) accumulation or incomplete relaxation. Similar fast and large Ca\(^{2+}\) transients have been measured from mouse trabeculae loaded with the acid form of Indo-1 [11] We interpret that the low amplitude and the slow kinetics of the Ca transients in Fig. 1 [2] were caused by Indo-1 that has been loaded as AM into the cell, i.e. the Na\(^+\),Ca\(^{2+}\)-exchanger must operate in Ca influx nearly all time (see Fig. 2A and B). To solve this dilemma, one may assume that \(E_x\) is not controlled by the global concentration [Ca\(^{2+}\)]\(_c\), (measured by the photomultiplier from the whole cell) but by the local concentrations [3] in the approximately 15 nm narrow subsarcolemmal space (index SL, synonymous “fuzzy space” [12,13]). In this very small volume, augmented \(J_{x,\text{eff}}\) could reduce [Ca\(^{2+}\)]\(_{\text{SL}}\) at a rate faster and to concentrations lower than those indicated by [Ca\(^{2+}\)]\(_c\). If [Ca\(^{2+}\)]\(_{\text{SL}}\) would be as low as e.g. 60 nM, the Na\(^+\),Ca\(^{2+}\)-exchanger would operate in Ca\(^{2+}\) influx mode most of the time (Fig. 2C). However, in order to reduce [Ca\(^{2+}\)]\(_{\text{SL}}\) below [Ca\(^{2+}\)]\(_c\), there must be a net Ca\(^{2+}\) efflux from the cell, i.e. the Na\(^+\),Ca\(^{2+}\)-exchanger must operate in Ca\(^{2+}\) efflux mode and could not operate as \(J_{x,\text{inf}}\) feeding \(J_{\text{SR}}\). Thus, without an additional Ca\(^{2+}\) efflux mechanism (plasmalemmal Ca\(^{2+}\) ATPase?) the above explanation seems to be unlikely.

8. Mechanisms by which overexpressed Na\(^+\)/Ca\(^{2+}\)-exchanger could augment SR Ca\(^{2+}\) filling

How can a Ca\(^{2+}\) efflux increase the SR Ca\(^{2+}\) load by 30% when it operates at a faster rate in TR than in non-TR myocytes?

8.1. Faster decay of [Ca\(^{2+}\)]\(_c\)

The authors have shown that augmented \(J_{x,\text{eff}}\) can speed up the time course of the Ca\(^{2+}\) transients. They argue that the faster decay of [Ca\(^{2+}\)]\(_c\) would shift \(E_x\) earlier in time to the positive values at which Na\(^+\),Ca\(^{2+}\)-exchange would operate as \(J_{x,\text{inf}}\), feeding \(J_{\text{SERCA}}\) and thereby Ca\(^{2+}\) loading the SR [2,3]. Teracciano et al. [3] had measured that the reversal potential \(E_x\) was not different in TR and non-TR myocytes, [Na\(^+\)]\(_c\) was 9.6 and 9.6 mM, [Ca\(^{2+}\)]\(_c\) was 159 and 135 nM and \(E_x\) was −27 and −24 mV, respectively, and model calculations suggest that the Na\(^+\)/Ca\(^{2+}\)-exchanger would operate in Ca\(^{2+}\) efflux nearly all time (see Fig. 2A and B). To solve this dilemma, one may assume that \(E_x\) is not controlled by the global concentration [Ca\(^{2+}\)]\(_c\), (measured by the photomultiplier from the whole cell) but by the local concentrations [3] in the approximately 15 nm narrow subsarcolemmal space (index SL, synonymous “fuzzy space” [12,13]). In this very small volume, augmented \(J_{x,\text{eff}}\) could reduce [Ca\(^{2+}\)]\(_{\text{SL}}\) at a rate faster and to concentrations lower than those indicated by [Ca\(^{2+}\)]\(_c\). If [Ca\(^{2+}\)]\(_{\text{SL}}\) would be as low as e.g. 60 nM, the Na\(^+\),Ca\(^{2+}\)-exchanger would operate in Ca\(^{2+}\) influx mode most of the time (Fig. 2C). However, in order to reduce [Ca\(^{2+}\)]\(_{\text{SL}}\) below [Ca\(^{2+}\)]\(_c\), there must be a net Ca\(^{2+}\) efflux from the cell, i.e. the Na\(^+\),Ca\(^{2+}\)-exchanger must operate in Ca\(^{2+}\) efflux mode and could not operate as \(J_{x,\text{inf}}\) feeding \(J_{\text{SR}}\). Thus, without an additional Ca\(^{2+}\) efflux mechanism (plasmalemmal Ca\(^{2+}\) ATPase?) the above explanation seems to be unlikely.
Fig. 2. Modelled Ca$^{2+}$ flux via Na$^{+}$/Ca$^{2+}$-exchange in dependence on [Ca$^{2+}$], [Na$^{+}$], and membrane potential $V_m$. The calculations used the equation

$$I_c = \text{const} \cdot \left( [\text{Na}^+] \cdot \text{exp}(0.5/\beta V_m) - [\text{Ca}^+] \cdot \text{exp}(-0.5/\beta V_m) \right).$$

$\beta =$ RT/F is 39 mV, [Na$^+$] is 9.6 mM [3]. $V_m$ (light grey, right ordinate) and [Ca$^{2+}$] (dark line, left ordinate) are data from Fig. 1. Traces at the top: non-calibrated $I_c$, Ca$^{2+}$ influx positive. Note: this model does not incorporate effect of $I_c$ on other Ca$^{2+}$ fluxes or on SR Ca$^{2+}$ loading. A: Ca$^{2+}$ transients due to 80 ms clamp steps, data from Fig. 1. After a spiky Ca$^{2+}$ influx, the Na$^{+}$/Ca$^{2+}$-exchanger operates in Ca$^{2+}$ efflux. The dotted line (top) suggest that cellular Ca$^{2+}$ load would progressively fall when $I_{calc}$ were absent. B: Action potential (AP) induced Ca$^{2+}$ transients (bottom) are fast and of large amplitude (own unpublished experiments). AP repolarization at high [Ca$^{2+}$], induces large Ca$^{2+}$ efflux that decays with diastolic fall of [Ca$^{2+}$], to a steady value. Cellular Ca$^{2+}$ load would fall (dotted line). C: In the small volume of the fuzzy space [Ca$^{2+}$]$_{SL}$ (dotted line) could fall faster and to lower concentrations than global [Ca$^{2+}$] (solid line), turning Na$^{+}$/Ca$^{2+}$-exchange into Ca$^{2+}$ influx mode. Diastolic Ca$^{2+}$ influx, however, is based on the unlikely assumption that [Ca$^{2+}$]$_{SL}$ would stay below [Ca$^{2+}$]. D: Long APs keep $V_m$ positive to $E_c$, thereby promoting Ca$^{2+}$ influx and cellular Ca$^{2+}$ load.
8.2. Faster accumulation of $[\text{Na}^+]_{\text{SL}}$

$J_{\text{inf}}$ should increase $[\text{Na}^+]_{\text{SL}}$ along a time course that is faster in TR than in non-TR myocytes, and $E_c$ could reach potentials where $\text{Na}^+/\text{Ca}^{2+}$-exchange would run as $J_{\text{inf}}$ at earlier times. However, the effect should be transient since $J_{\text{inf}}$ (and $\text{Na}^+/\text{K}^+$-ATPase operating in parallel) would restore $[\text{Na}^+]_{\text{SL}}$ more rapidly in TR than in non-TR myocytes. As discussed above for $\text{Ca}^{2+}$ accumulation, faster $\text{Na}^+$ accumulation could change the time course of the decay in activator $\text{Ca}^{2+}$. To net cellular $\text{Ca}^{2+}$ load, however, $[\text{Na}^+]_{\text{c}}$ should accumulate independent of the $\text{Na}^+/\text{Ca}^{2+}$-exchanger, for example due to inhibition of the $\text{Na}^+/\text{K}^+$-ATPase with ouabain [8].

8.3. Longer action potential (AP)

The inward current generated by $\text{Ca}^{2+}$ influx prolongs the plateau of the AP. Even at low $[\text{Ca}^{2+}]_{\text{c}}$, the $\text{Na}^+/\text{Ca}^{2+}$-exchanger can operate in the $\text{Ca}^{2+}$ influx mode when the membrane potential is positive to $E_c$ (see Fig. 2D). Unfortunately, the authors do not provide information whether the AP in TR is longer than non-TR myocytes.

In summary, we are still waiting for the definite answer which mechanism is facilitating the filling of the SR $\text{Ca}^{2+}$ stores in TR myocytes with increased activity of $\text{Na}^+/\text{Ca}^{2+}$-exchange.

9. The overexpressed $\text{Na}^+/\text{Ca}^{2+}$-exchanger can compensate for suppressed SERCA activity

In TR cells (elevated $J_v$), inhibition of $J_{\text{SERCA}}$ with thapsigargin prolongs the duration of the $\text{Ca}^{2+}$ transient and the duration of the twitch. The authors plot these values as a function of exposure time to thapsigargin and compare them with those from non-TR myocytes (no thapsigargin). The comparison indicates that $\text{Ca}^{2+}$-transients and twitches in TR myocytes (2.4-fold increased $\text{Na}^+/\text{Ca}^{2+}$-exchange activity) correspond to those from non-TR controls where SERCA activity is inhibited by 28%. The authors extrapolate to the failing heart: a 28% reduced SERCA function can be compensated by a 2.4-fold increase in $\text{Na}^+/\text{Ca}^{2+}$-exchange activity.

10. From the transgenic mice back to the failing human heart

Human heart failure has been classified in three groups of increased severity [1]. When compared with non-failing hearts, the reduction of SERCA protein was significant in group III (48% reduction) but not in groups II or I (42 and 27% reduction). $\text{Na}^+/\text{Ca}^{2+}$-exchanger protein was unchanged in group III but increased by 80% in group I, this overexpression correlated inversely with the impaired diastolic relaxation [1]. Speculating that reduction of SERCA in failing human hearts of group I could become significant when more data could have been analyzed, the interpretation of the group I failure in human hearts would be in analogy to the first conclusion of Terraciano et al. [2], i.e. the increase in $\text{Na}^+/\text{Ca}^{2+}$-exchange activity (2.4-fold) can compensate the disturbed $\text{Ca}^{2+}$ redistribution caused by a modest (28%) inhibition SERCA.

The second major conclusion of Terraciano et al. [2] was that the increased activity of $\text{Na}^+/\text{Ca}^{2+}$-exchange increases via $J_{\text{inf}}$ the SR $\text{Ca}^{2+}$ load and thereby the amount activator $\text{Ca}^{2+}$. I am not yet ready to accept this conclusion in general terms, or to extrapolate it to the failing human heart. For example, the amplitudes of the physiological systolic $\text{Ca}^{2+}$ transients of TR, TR thapsigargin-treated and non-TR myocytes were not significantly different (Fig. 1 [2]). Further, experiments on trabeculae from failing human hearts indicated a reduced SR $\text{Ca}^{2+}$ load, as if the overexpressed $\text{Na}^+/\text{Ca}^{2+}$-exchanger had increased the activity of $J_{\text{eff}}$ and not of $J_{\text{inf}}$ [1]. Obviously, quantification of $J_{\text{SERCA}}, J_{\text{inf}}$ and $J_{\text{eff}}$ from $\text{Ca}^{2+}$ transients in mice or human preparations is a difficult task that needs knowledge not only of $[\text{Ca}^{2+}]_{\text{c}}$, but also of the cytosolic $\text{Ca}^{2+}$ buffering power, the volume fraction of the SR etc., numbers whose extrapolation from rat ventricular myocytes is questionable. In addition to the changed expression of SERCA and $\text{Na}^+/\text{Ca}^{2+}$-exchanger proteins, additional influences such as cell hypertrophy, metabolism etc. are likely to be involved in development of cardiac failure. In the transgenic mouse model, Terraciano et al. [2–4] have analysed the isolated effects of two key proteins, and obtained results that are necessary and important for the further understanding of the complex interactions during development of cardiac failure.

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