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

Terraciano 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 concentrations of other Ca 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]):

$$
\frac{d}{dt}[\text{Ca}^{2+}]_c = J_{\text{ret}} + I_{\text{Ca,L}} + J_{\text{int}} + J_{\text{Lig}} - J_{\text{SERCA}}
$$

$$- J_{\text{eff}} - J_{\text{Lig}}$$

(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 ($I_{\text{C.a,L}}$) and via Na$^+/Ca^{2+}$-exchange operating in Ca$^{2+}$ influx mode ($J_{\text{int}}$). [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 ($J_{\text{Lig}}$). 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 \((V_\theta - E_J)\), \(V_\theta\) is the membrane potential, and for the reversal potential for the exchanger one can write (e.g. [5])

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

At start of the action potential (AP), [Ca\(^{2+}\)]\(_i\) is low, \(E_J\) is with approximately \(-26 \text{mV}\) [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\(^{2+}\) flux depend on both fall of [Ca\(^{2+}\)], (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 \(J_{\text{eff}}\) 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 \(I_{\text{Ca}} + I_{\text{rel}} + I_{\text{inf}} = J_{\text{SERCA}} + J_{\text{serca}}\)). 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 \(\mu\text{M} \) 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 \(J_{\text{lag}}\) 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 \(\mu\text{M}\) and a \(V_{\text{max}}\) of 99 \(\mu\text{M}/\text{s}\); the Na\(^+\).Ca\(^{2+}\)-exchanger with a \(K_m\) of 0.4 \(\mu\text{M}\) and a \(V_{\text{max}}\) of 21 \(\mu\text{M}/\text{s}\). In TR myocytes \(J_{\text{SERCA}}\) has identical values, however, the Na\(^+\)/Ca\(^{2+}\)-exchanger shows a more than doubled \(V_{\text{max}}\) of 53 \(\mu\text{M}/\text{s}\) at unchanged \(K_m\) of 0.4 \(\mu\text{M}\).

In an independent set of voltage-clamp experiments [2], the authors measure the flux of releasable SR Ca\(^{2+}\) (\(J_{\text{rel}}\)) as current \(J_{\text{serca}}\) (influx of 3 Na\(^+\) ions in exchange of 1 Ca\(^{2+}\) ion). Following the suggestions of the Eisner Laboratory [10], \(J_{\text{rel}}\) 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 \(J_{\text{serca}}\). The authors estimate from the rates that the flux \(J_{\text{eff}}\) is 1.7-fold larger in TR than in non-TR myocytes. The time integral of the caffeine-induced inward current \(I_{\text{eff}}\) 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 2

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mitochondria is fast enough to equilibrate within the 12 s between the subsequent caffeine applications. However, mitochondrial Ca\(^{2+}\) uptake can also modulate the decay rate of the caffeine-induced Ca\(^{2+}\) transients, hence the quantification of the Ca\(^{2+}\) fluxes is considered with some skepticism. Together with Terraciano et al., I would like to assume that the loading indo-1-AM into compartments is similar in TR and non-TR cells. Also, one would have to postulate that the cytosolic buffering power for Ca\(^{2+}\) is the same in the two sorts of cells (see J\(_{\text{lig}}\) in Eq. (1)). The authors discuss these problems and hope to resolve them in future. Although some skepticism in regard to the quantification of the fluxes is left, the comparison between the Ca\(^{2+}\) signals in TR and non-TR myocytes on the base of relative numbers should be valid.
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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\(_{\text{Na,Ca}}\) can speed up the time course of the Ca\(^{2+}\) transients. They argue that the faster decay of [Ca\(^{2+}\)]\(_c\) would shift E\(_c\) earlier in time to the positive values at which Na\(^+\),Ca\(^{2+}\)-exchange would operate as J\(_{\text{Na,Ca}}\), feeding J\(_{\text{SERCA}}\) and thereby Ca\(^{2+}\) loading the SR [2,3]. Teracciano et al. [3] had measured that the reversal potential E\(_c\) was not different in TR and non-TR myocytes, [Na\(^+\)] \(_i\) was 9.6 and 9.6 mM, [Ca\(^{2+}\)] \(_i\) was 159 and 135 nM and E\(_{\text{ca}}\) = -27 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\(_c\) is not controlled by the local concentration [Ca\(^{2+}\)]\(_i\), (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\(_{\text{Na,Ca}}\) could reduce [Ca\(^{2+}\)]\(_{\text{SL}}\) at a rate faster to and 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\(_{\text{Na,Ca}}\) 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\textsuperscript{2+} flux via Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchange in dependence on [Ca\textsuperscript{2+}], [Na\textsuperscript{+}], and membrane potential $V_m$. The calculations used the equation from Kimura et al. [14] $I = \text{const} \cdot [\text{Na}^+]^3 \cdot [\text{Ca}^+]^2 \cdot \exp(0.5/\beta \cdot V_m) - [\text{Na}^+]^3 \cdot [\text{Ca}^+]^2 \cdot \exp(-0.5/\beta \cdot V_m)$. $\beta = \text{RT}/F$ is 39 mV. [Na\textsuperscript{+}] is 9.6 mM [3]. $V_{m}$ (light grey, right ordinate) and [Ca\textsuperscript{2+}] (dark line, left ordinate) are data from Fig. 1. Traces at the top: non-calibrated $I_{in}$, Ca influx positive. Note: this model does not incorporate effect of $I_{in}$ on other Ca\textsuperscript{2+} fluxes or on SR Ca\textsuperscript{2+} loading. 

**A**: Ca\textsuperscript{2+} transients due to 80 ms clamp steps, data from Fig. 1. After a spiky Ca\textsuperscript{2+} influx, the Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger operates in Ca\textsuperscript{2+} efflux. The dotted line (top) suggest that cellular Ca\textsuperscript{2+} load would progressively fall when $I_{in}$ were absent. 

**B**: Action potential (AP) induced Ca\textsuperscript{2+} transients (bottom) are fast and of large amplitude (own unpublished experiments). AP repolarization at high [Ca\textsuperscript{2+}], induces large Ca\textsuperscript{2+} efflux that decays with diastolic fall of [Ca\textsuperscript{2+}], to a steady value. Cellular Ca\textsuperscript{2+} load would fall (dotted line). 

**C**: In the small volume of the fuzzy space [Ca\textsuperscript{2+}]_{sl} (dotted line) could fall faster and to lower concentrations than global [Ca\textsuperscript{2+}], (solid line), turning Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchange into Ca\textsuperscript{2+} influx mode. Diastolic Ca\textsuperscript{2+} influx, however, is based on the unlikely assumption that [Ca\textsuperscript{2+}]_{sl} would stay below [Ca\textsuperscript{2+}]. 

**D**: Long APs keep $V_m$ positive to $E_C$, thereby promoting Ca\textsuperscript{2+} influx and cellular Ca\textsuperscript{2+} load.
8.2. Faster accumulation of $[Na^+]_{sl}$

$J_{eff}$ should increase $[Na^+]_{sl}$ along a time course that is faster in TR than in non-TR myocytes, and $E_C$ could reach potentials where $Na^+$/Ca$^{2+}$-exchange would run as $J_{inf}$ at earlier times. However, the effect should be transient since $J_{inf}$ (and and Na$^-$/K$^+$-ATPase operating in parallel) would restore $[Na^+]_{sl}$ more rapidly in TR than in non-TR myocytes. As discussed above for Ca$^{2+}$ accumulation, faster Na$^+$ accumulation could change the time course of the decay in activator Ca$^{2+}$. To not cellular Ca$^{2+}$ load, however, $[Na^+]_c$ should accumulate independent of the Na$^-$/Ca$^{2+}$-exchanger, for example due to inhibition of the Na$^-$/K$^+$-ATPase with ouabain [8].

8.3. Longer action potential (AP)

The inward current generated by Ca$^{2+}$ influx prolongs the plateau of the AP. Even at low $[Ca^{2+}]_c$, the Na$^+$/Ca$^{2+}$-exchanger can operate in the 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 Ca$^{2+}$ stores in TR myocytes with increased activity of Na$^+$/Ca$^{2+}$-exchange.

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

In TR cells (elevated $J_x$), inhibition of $J_{SERCA}$ with thapsigargin prolongs the duration of the 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 Ca$^{2+}$-transients and twitches in TR myocytes (2.4-fold increased Na$^+$/Ca$^{2+}$-exchange activity) correspond to those from non-TR controls when 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 Na$^+$/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). Na$^+$/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 Terracciano et al. [2], i.e. the increase in Na$^+$/Ca$^{2+}$-exchange activity (2.4-fold) can compensate the disturbed Ca$^{2+}$ redistribution caused by a modest (28%) inhibition SERCA.

The second major conclusion of Terracciano et al. [2] was that the increased activity of Na$^+$/Ca$^{2+}$-exchange increases via $J_{inf}$ the SR Ca$^{2+}$ load and thereby the amount activator 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 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 Ca$^{2+}$ load, as if the overexpressed Na$^+$/Ca$^{2+}$-exchanger had increased the activity of $J_{eff}$ and not of $J_{inf}$ [1]. Obviously, quantification of $J_{SERCA}$, $J_{inf}$ and $J_{eff}$ from Ca$^{2+}$ transients in mice or human preparations is a difficult task that needs knowledge not only of $[Ca^{2+}]_c$, but also of the cytosolic 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 Na$^+$/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, Terracciano 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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References

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