A Simulation Study to Rescue the Na+/Ca2+ Exchanger Knockout Mice
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Abstract: The Na+/Ca2+ exchanger (NCX) is the major Ca2+ ef-flux system in cardiac myocytes, and thereby its global knockout is embryonically lethal. However, Henderson et al. (2004) found that mice with the cardiосpecific knockout of NCX1 lived to adulthood. No adaptation was detected in expression levels of other proteins except for a 50% reduction in the L-type Ca2+ current (I_{CaL}) as revealed in electrophysiological studies. To predict mechanisms of survival, we simulated cardiac myocyte activity in the absence of NCX using a mathematical model of guinea pig ventricular myocytes. The NCX knockout resulted in contracture of the model cell because of a rise in the cytoplasmic Ca2+ ([Ca2+]i). However, up-regulation of the sarcolemmal Ca2+ pump (PMCA) and/or down-regulation of I_{CaL} enables steady rhythmic contractions even if NCX is totally excluded. The simulation predicted that the steady activities are maintained by a functional up-regulation of PMCA by about 2.3 times in addition to the down-regulation of I_{CaL} to a half, as observed in the experiment. However, the model analysis predicted that the myocyte depending on PMCA for Ca2+ extrusion is unstable against any changes in ionic fluxes and energetically unfavorable in comparison with the control. The reason for the instability is that the activity of PMCA driven by the ATP hydrolysis is hardly affected by changes in [Ca2+], but NCX has a reversal potential in the middle level of the action potential and is immediately affected by the Ca2+ flux via NCX itself. The source code of the model is available at http://www.sim-bio.org/.

Key words: Na+/Ca2+ exchanger, NCX1, sarcolemmal Ca2+ pump.

Cardiac excitation-contraction coupling is initiated by the influx of Ca2+ through voltage-dependent Ca2+ channels; it subsequently triggers Ca2+ release from the sarcoplasmic reticulum (SR). To maintain stable excitation-contraction coupling, this Ca2+ influx must be balanced by Ca2+ efflux. The Na+/Ca2+ exchanger (NCX) and ATP-de-pendent Ca2+ pump (PMCA) are the two mechanisms that mediate Ca2+ efflux via the plasma membrane, and the dominant role in cardiac myocytes of NCX1, an isoform of NCX, has been well documented [1, 2]. Thus if NCX1 is removed, cardiac myocytes should become nonfunctional because of Ca2+ overload. Therefore in our current understanding, it is almost inconceivable that myocardium could survive in the absence of NCX1. In fact, four laboratories have reported that a global knockout of the NCX1 is embryonically lethal [3–6]. However, surprisingly, Henderson et al. [7] reported that mice with a cardiосpecific knockout of NCX1 lived to adulthood with only modestly reduced cardiac function. They could detect no adaptation in the expression levels of PMCA, the dihydropyridine receptor, ATP-dependent Ca2+ pump on SR (SERCA), or calsequestrin as measured by both immunoblots and microarray analyses, but demonstrated that L-type Ca2+ currents (I_{CaL}) were reduced by ~50% in the knockout mice.

These findings raise several questions. Is the functional adaptation of only Ca2+ channels sufficient for the survival of the mice? Is there any adaptation of other molecules at the functional level? For example, PMCA might be activated by phosphorylation or via a calmodulin-dependent pathway [8]. To get a testable working hypothesis for the quantitative mechanisms underlying the survival of the knockout mice, the simulation of a knockout using computer models of a cardiac cell could be useful. The minimum requirements for such models might be the establishment of the steady-state condition and the implementation of mechanisms underlying the membrane excitation, the excitation–contraction coupling, the mechanical contraction, and the ATP metabolism. Here we used the Kyoto model [9], which successfully combined the Negroni-Lascano contraction model [10] with the membrane excitation and the ATP production of the oxidative phosphorylation model [11, 12] based on the model structure created by D. Noble’s group [13].
MATERIALS AND METHODS

PMCA, which might functionally replace the NCX1 after its knockout, has not been included in the Kyoto model (the guinea pig ventricular cell model), which has a hypothetical SERCA model. Since we are concerned with the amplitude of carrier-mediated Ca\(^{2+}\) flux, but not with detailed Ca\(^{2+}/H^+\) exchange or ATP dependency of PMCA, we used essentially the same mathematical equations for PMCA as SERCA and replaced the intra-SR Ca\(^{2+}\) with the extracellular Ca\(^{2+}\). The Michaelis constant for intracellular Ca\(^{2+}\) (\(K_{mCa}\)) was taken from experimental data [14]. Thus the total PMCA current \(I_{PMCA}\) is given by the equations below, where \(E_1\) represents the carrier with its ion binding site on the extracellular space, and \(E_2\) with the intracellular ion binding site.

\[
\begin{align*}
\frac{d[E_1Ca]}{dt} &= 1/(1 + K_{mCa}/[Ca^{2+}]) \\
\frac{d[E_2Ca]}{dt} &= 1/(1 + K_{mCa}/[Ca^{2+}]) \\
\frac{d[E_1]}{dt} &= 1 - \left(\frac{[Ca^{2+}]}{K_{mCa}}\right) \\
\frac{d[E_2]}{dt} &= 1 - \left(\frac{[Ca^{2+}]}{K_{mCa}}\right)
\end{align*}
\]

We reduced the four-state model into a simple two-state model shown in the right part of Scheme 1.

\[
I_{PMCA} = I_{max} - \frac{K_{p} p(E_{2Ca})(1-y) - k_{1} p(E_{1Ca}) y}{(1 + K_{mCa}/[Ca^{2+}])}
\]

where concentrations are given in mM, \(I_{max}\) represents the maximum amplitude (\(A/F\)), \(C_m\) the membrane capacitance (\(pF\)), \(F\) Faraday constant (coulomb/mM), and \(v_i\) the osmotically active cell volume (\(\mu\)m\(^3\)). To convert the ionic current to molar flux, a stoichiometry of 1 Ca\(^{2+}\) for 1 H\(^+\) by 1 ATP was assumed. Although every electronic charge movement across the cell membrane must equal the amounts of ion movements to establish the steady state of the cardiac cell model [15], the current Kyoto model did not consider H\(^+\) ion movements. Thus we calculated only the Ca\(^{2+}\) flux and the ATP consumption of PMCA, neglecting changes in the intracellular H\(^+\) concentration.

The addition of this hypothetical PMCA to the ventricular cell model resulted in a decrease of cytoplasmic Ca\(^{2+}\) and Na\(^+\) concentration ([Ca\(^{2+}\)], [Na\(^+\)], respectively). Thus the activities of NCX and the Na\(^+\)/K\(^+\) pump were adjusted to reach a steady state comparable to the original Kyoto model, that is, 4.9 mM [Na\(^+\)], and 1.3 \(\mu\)M of a peak Ca\(^{2+}\) transient. The rate of the \(Ca^+\) extrusion by PMCA is set at 30% of that by NCX on average [16], and \(I_{max}\) of NCX is set at 4.8 \(A/F\), and the Na\(^+\)/K\(^+\) pump at 20.5 \(A/F\).

In simulation, we examined the effects of systematically changing the magnitude of NCX-mediated current (\(I_{NaCa}\), \(I_{CaL}\), and \(I_{PMCA}\) by scaling the amplitude parameters in the model by a factor \((F_{NCX}, F_{CaL}, F_{PMCA})\), respectively) without changing the kinetic parameters. For example, \(I_{CaL}\) in the control model \((I_{CaL}^c)\) is multiplied by an appropriate value of \(F_{CaL}(0 < F_{CaL})\).

\[
I_{CaL} = F_{CaL} \cdot I_{CaL}^c
\]

In the Kyoto model, the ryanodine-receptor channel on SR (RyR) is activated by both the [Ca\(^{2+}\)], and the single channel current of the L-type Ca\(^{2+}\) channel \((i_{CaL})\). The influence of varying the \(i_{CaL}\) amplitude on the excitation–contraction coupling was described by scaling the average amplitude of single channel current \((i_{CaL}:p(openCaL))\) with the same scaling factor \(F_{CaL}\). Namely, the rate constant \(k_1\) of RyR (Eq. 2 in Table 8 in Matsuoka et al. [9]) is modified as

\[
k_1 = 288000 \cdot [Ca^{2+}]^2 - 150 \cdot F_{CaL} \cdot i_{CaL}^c \cdot p(openCaL)
\]

The original equations for describing the other current systems are the same as used in Matsuoka et al. [9] for Figs. 1 to 3, and the Kyoto model with the mitochondrial oxidative phosphorylation model [12] is used for Fig. 4. It should be noted that the current amplitude during the action potential is not necessarily proportional to the scaling factor if compared with the control because of secondary changes in the action potential shape and other currents.

The ventricular myocyte was stimulated every 400 ms by a 2 ms current pulse of 4 nA in magnitude. A stretch of simulation under each condition was continued for at least 1,500 beats (10 min) to establish a new steady state, as confirmed by the fact that the electrical and mechanical activities as well as the intracellular ion concentrations obtained after 1,500 beats with a new parameter set were almost the same as those obtained after 100 beats. All calculations were conducted using the Runge-Kutta method with the adaptive time step by the ordinary differential equation solver, simBio [17]. The source code of the model, which can reproduce every result, is available at http://www.sim-bio.org/.
RESULTS

Simulation of the NCX knockout

A complete removal of NCX ($F_{NCX}$ is set at 0 at time 0 s) from the ventricular cell model induced a steep rise in amplitudes of both the Ca$^{2+}$ transient and the twitch shortening of the half-sarcomere length (hSL), as shown in Fig. 1. After 7 beats from NCX removal, however, the Ca$^{2+}$ transient was rapidly abolished, and hSL did not recover, but remained shortened progressively because a certain fraction of the RyR channels became continuously open as a result of the rise in $[\text{Ca}^{2+}]_i$, and the Ca$^{2+}$-content of SR was reduced (data not shown). Thereafter, $[\text{Ca}^{2+}]_i$ increased in a cumulative way with each membrane excitation, because of pulsatile Ca$^{2+}$ influx via $I_{\text{CaL}}$ without sufficient Ca$^{2+}$ extrusion. The transient vertical deflection of $I_{\text{PMCA}}$, observed with the initial 11 beats, is triggered by the sudden rising of $[\text{Ca}^{2+}]_i$ transient and quickly subsides after redistribution of the four states of PMCA in Scheme 1. The increase in the diastolic $[\text{Ca}^{2+}]_i$, far beyond the half-saturating concentration of 64 nM nearly fixed $p(E_{\text{Ca}})$; thus $I_{\text{PMCA}}$ lost rhythmic oscillation and became flat. The lethality of the NCX knockout is evident in this simulation unless adaptation occurs in other current systems.

To address the question of what is the critical level of NCX deletion for maintaining steady myocyte contraction, we decreased $F_{NCX}$ with a step size of 0.1. We found that the minimum requirement of $F_{NCX}$ is 0.3 to keep a stable Ca$^{2+}$ transient. The amplitudes of the Ca$^{2+}$ transient and the twitch shortening of the sarcomere with $F_{NCX}$ 0.3 is much larger compared to the control at a steady-state as shown in Fig. 2, A and B. The decrease of inward currents, which is generated by NCX during the early phase of action potential plateau (see $I_{\text{NaCa}}$ in Fig. 2A), slightly shortens the action potential duration ($V_m$ in Fig. 2B). The increase in the amplitude of $I_{\text{CaL}}$ after the NCX knockout is secondary to the increase in the driving force for Ca$^{2+}$ influx caused by the negative shift of the plateau potential. $I_{\text{PMCA}}$ also slightly increases during diastole because its substrate concentration, $[\text{Ca}^{2+}]_i$ is increased. The change in the time course of $I_{\text{NaCa}}$ is mainly due to the increase in the Ca$^{2+}$ transient. It should be noted that these secondary modifications occur automatically when a single parameter $F_{NCX}$ is changed.

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**Fig. 1.** Changes in membrane potential ($V_m$ in mV), individual current components ($I_{\text{PMCA}}$ and $I_{\text{CaL}}$ in pA), $[\text{Ca}^{2+}]_i$ in µM, and half sarcomere length (hSL in µm) after $F_{NCX}$ set 0 at time 0 s. The horizontal axis is time in s.

**Fig. 2.** Wave forms of $V_m$ (mV), $I_{\text{PMCA}}$, $I_{\text{NaCa}}$, and $I_{\text{CaL}}$ (pA), $[\text{Ca}^{2+}]_i$ (µM), and hSL (µm). The horizontal axis is time (ms). A: The control wave forms after introduction of $I_{\text{PMCA}}$ into the original Kyoto model [9]. B: With a 70% inhibition of $I_{\text{NaCa}}$ ($F_{NCX}$ 0.3). C: With the complete block of NCX ($F_{NCX}$ 0) and 50% inhibition of $I_{\text{CaL}}$ ($F_{CaL}$ 0.5). D: With $F_{NCX}$ 0 and $F_{CaL}$ 0.2. E: With $F_{NCX}$ 0 and $F_{PMCA}$ 4.4. F: With $F_{NCX}$ 0, $F_{CaL}$ 0.5, $F_{PMCA}$ 2.3.
Rescue of the NCX-knockout myocyte by down-regulating $I_{CaL}$ and/or by up-regulating $I_{PMCA}$

The 50% reduction in $I_{CaL}$, observed experimentally in surviving NCX1 knockout myocytes by Henderson et al. [7] should be helpful in maintaining the Ca$^{2+}$ homeostasis. However, our simulation showed that the 50% inhibition of $I_{CaL}$ ($F_{CaL} = 0.5$) alone is not enough to rescue the NCX knockout myocyte from the Ca$^{2+}$ overload (Fig. 2C), though the time course of developing cell contracture is slower than in the case of complete knockout. When $F_{CaL}$ is further decreased to 0.2, the amplitude of $I_{CaL}$ during the action potential is decreased only to 54% of the control because the increase in the driving force for $I_{CaL}$ compensates the effect of decreasing $F_{CaL}$, and we can observe stable oscillation in $[Ca^{2+}]_{i}$, $I_{PMCA}$, hSL, and $[Ca^{2+}]_{i}$. The waveforms of these parameters are similar to those in the control cell (Fig. 2A). Under these conditions of depressed Ca$^{2+}$ influx, SERCA performs a strong buffering action to maintain the Ca$^{2+}$ store in SR. With a further decrease in $F_{CaL}$ to 0.1 (the amplitude of $I_{CaL}$ decreases to 39%), the peak [Ca$^{2+}$], becomes too small (5% of the control) to induce the contraction in myocytes (not shown).

Alternatively, a stable cycle of excitation–contraction coupling could be established in the knockout myocyte model by increasing $I_{PMCA}$ over 4.4 times control without modifying $I_{CaL}$ (Fig. 2E), although the control level of $I_{PMCA}$ was set rather arbitrarily. The peaks of the Ca$^{2+}$ transient as well as the twitch contraction are comparable to those in the control (Fig. 2A). If the down-regulation of $I_{CaL}$ is fixed at 50% as observed in the experiment of Henderson et al. [7], a less up-regulation of $I_{PMCA}$ by $F_{PMCA} = 2.3$ is enough to recover the stable repetitive contractions (Fig. 2F).

Differences among various combinations of $I_{CaL}$ and $I_{NaCa}$ in maintaining stable activity

As indicated so far, the rhythmic contractions of the model cell can be maintained by various combinations of $I_{CaL}$, $I_{NaCa}$, and $I_{PMCA}$. We examined the effects of systematically changing both $F_{NCX}$ and $F_{CaL}$ on [Ca$^{2+}$]$_{i}$ at the peak of Ca$^{2+}$ transient (upper panel in Fig. 3A) and at the end-diastole (lower panel). The standard [Ca$^{2+}$]$_{i}$ is given at the point (1,1) in the ($F_{NCX}$, $F_{CaL}$) matrix and is expressed with green in the 2-dimension contour plot. It is obvious that both peak and end-diastolic [Ca$^{2+}$]$_{i}$ increase with decreasing $F_{NCX}$ along the vertical axis, or with increasing $F_{CaL}$ along the horizontal axis. The green area, indicating [Ca$^{2+}$]$_{i}$ near the standard value, stretches from the lower left to the upper right in both matrices in Fig. 3A, indicating that almost a normal amplitude of the Ca$^{2+}$ transient is obtained by varying $F_{NCX}$ and $F_{CaL}$ in propor-

![Fig. 3. Peak and end-diastolic [Ca$^{2+}$] measured at the 1500th beat in each simulation, or at the time when [Ca$^{2+}$] exceeded 10 μM. The range of the color bar is set 0.5 to 1.5 times the control. Each square in the 2-dimensional contour plot represents one simulation result. A: The dependency on both $F_{CaL}$ (horizontal axis) and $F_{NCX}$ (vertical axis) is scanned from 0 to 2 in 0.1 step. The point (1,0) represents the NCX knockout condition with intact $I_{CaL}$. B: The [Ca$^{2+}$]$_{i}$ dependency on both $F_{PMCA}$ (0 to 4.6) and $F_{NCX}$ (0 to 2) are measured. C: The dependency on both $F_{PMCA}$ (1 to 4.6) and $F_{CaL}$ (0.1 to 1) are measured with $F_{NCX}$.](image-url)
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The width of this green belt increases with an increasing amplitude of both parameters, suggesting that the system becomes more stable as the [Ca\textsuperscript{2+}] homeostasis is achieved with greater amplitudes of the influx and efflux.

The blue belt near the right lower corner represents that the generation of cyclic Ca\textsuperscript{2+} transient is interfered with by the continuous activation of the RyR channel when the diastolic [Ca\textsuperscript{2+}] is increased, as indicated in the lower panel (see also the trace of [Ca\textsuperscript{2+}] in Fig. 1).

**Substitution of \( I_{\text{PMCA}} \) for \( I_{\text{NaCa}} \)**

Although the ion species coupled with the Ca\textsuperscript{2+} extrusion are totally different between PMCA (coupled with H\textsuperscript{+} influx) and NCX (coupled with Na\textsuperscript{+} influx), PMCA can replace the role of NCX in extruding Ca\textsuperscript{2+}. The H\textsuperscript{+} influx via PMCA is finally compensated with the Na\textsuperscript{+}/K\textsuperscript{+} pump through the intermediate Na\textsuperscript{+}/H\textsuperscript{+} exchange, like the compensation of Na\textsuperscript{+} influx via NCX by the Na\textsuperscript{+}/K\textsuperscript{+} pump. Figure 3B shows the effects of systematically changing \( F_{\text{NCX}} \) and \( F_{\text{PMCA}} \) on the peak as well as on the end-diastolic [Ca\textsuperscript{2+}] levels. The green band, indicating almost normal [Ca\textsuperscript{2+}], extends obliquely in the matrices, indicating the decrease in \( F_{\text{NCX}} \) is compensated by increasing \( F_{\text{PMCA}} \). However, it should be noted that the [Ca\textsuperscript{2+}] regulation is more stable in the NCX-dominant system than in the PMCA-dominant system, as revealed by the wider width of the green belt as \( F_{\text{PMCA}} \) gets smaller.

**Pinpoint combination of \( I_{\text{CaL}} \) and \( I_{\text{PMCA}} \) to rescue the NCX-knockout myocyte**

In the complete absence of NCX, the adjustment of [Ca\textsuperscript{2+}], can be achieved only by varying \( F_{\text{CaL}} \) and \( F_{\text{PMCA}} \). Thus independently from the experimental amplitude of \( F_{\text{CaL}} \), the matrix analysis was carried out for both \( F_{\text{PMCA}} \) (0 to 4.6) and \( F_{\text{NCX}} \) (0 to 2). The simulation was terminated when [Ca\textsuperscript{2+}] exceeded 10 \( \mu \)M before the 1500th beat. The range of the color bar is set 0 to 2 times the control for PMCA (A) and 0.9 to 1.1 for the rest (B and C).

**Energetics**

In the Ca\textsuperscript{2+} extrusion, PMCA consumes 1 ATP for 1 Ca\textsuperscript{2+}, and NCX also consumes 1 ATP for 1 Ca\textsuperscript{2+} through the Na\textsuperscript{+}/K\textsuperscript{+} pump. However, PMCA is driven directly by ATP, but NCX has feedback mechanisms through \( V_{\text{m}} \) and [Ca\textsuperscript{2+}]. Thus they may have different profiles of ATP consumption. We measured the average rate of ATP consumption calculated during 10 min simulation (dATP in Fig. 4) by PMCA (Fig. 4A), the Na\textsuperscript{+}/K\textsuperscript{+} pump (Fig. 4B), and by both PMCA and the Na\textsuperscript{+}/K\textsuperscript{+} pump (Fig. 4C) at the same combination of \( F_{\text{NCX}} \) and \( F_{\text{PMCA}} \) as in Fig. 3B. dATP by PMCA is more dependent on \( F_{\text{PMCA}} \) than [Ca\textsuperscript{2+}] (Fig. 4A), but dATP by the pump increases with the \( F_{\text{NCX}} \) decrease (0 < \( F_{\text{NCX}} < 1 \) in Fig. 4B) because the turnover rate of NCX is enhanced via increased [Ca\textsuperscript{2+}], regardless of the decrease in \( F_{\text{NCX}} \).

To examine the energetic efficiency, the sum of dATP by both PMCA and the pump (Fig. 4C) was used for maintaining the physiological [Ca\textsuperscript{2+}] was compared along the green belt in Fig. 3B: 7.0 mM/min with \( F_{\text{NCX}} \) 1.3 and \( F_{\text{PMCA}} \) 0 (corresponding to the original Kyoto model [12]), 7.2 with \( F_{\text{NCX}} \) 1 and \( F_{\text{PMCA}} \) 1, and 7.9 with \( F_{\text{NCX}} \) 0 and \( F_{\text{PMCA}} \) 4.4. It is obvious that the system becomes energetically unfavorable as the role of NCX is replaced by PMCA.


**DISCUSSION**

The analysis of the cardiac cell activity using the comprehensive cardiac cell model successfully demonstrated that NCX takes the major role in extruding the intracellular Ca$^{2+}$ and that its global knockout is lethal. Namely, the removal of NCX induced a fatal Ca$^{2+}$-overload followed by a cessation of the Ca$^{2+}$ transient and contracture of the myocyte model. Although the Kyoto model is based on the guinea pig ventricular cells, the conclusion of the lethality of NCX knockout might be applicable to other mammalian cardiac cells, like the mouse ventricular cells used in the experiments of Henderson et al. [7], as far as the cytoplasmic Ca$^{2+}$ is balanced mainly by effluxes through $I_{\text{NaCa}}$ and $I_{\text{PMCA}}$ and influx via $I_{\text{Cal}}$.

The systematic search in the parameter space (Figs. 2 and 3) indicated that the disturbance of the Ca$^{2+}$ homeostasis induced by experimental interventions, such as knockout of NCX, or by a variety of pathological mechanisms can be recovered by modulating other components involved directly or indirectly in the Ca$^{2+}$ transport. Our model predicts that myocytes could survive even after eliminating 70% of NCX. Under this condition, however, the remaining NCX molecules are highly enhanced in their turnover rate by elevated [Ca$^{2+}$], as seen in Fig. 2B. Furthermore, turnover of PMCA is also fully accelerated by the increase in [Ca$^{2+}$]. Moreover, close inspection revealed that [Ca$^{2+}$], increases by 2.5 times the control, and the amount of Ca$^{2+}$ stored in SR is also increased (data not shown). This 70% inhibition of NCX might cause a failure of the real cell after a long run of activities because of the secondary effects of a continuous rise in the resting [Ca$^{2+}$], which might cause the activation of Ca$^{2+}$-dependent proteases. Also in the acute phase of modulating the Ca$^{2+}$ flux, for example, the enlargement of $I_{\text{Cal}}$ by β-adrenergic stimulation might easily destroy the narrow balance between the influx via $I_{\text{Cal}}$ and the efflux via $I_{\text{PMCA}}$ shown in Fig. 3C, resulting in the Ca$^{2+}$ overload. Similarly, if PMCA is activated by protein kinase A through increased Ca$^{2+}$ affinity and increased turnover rate, [Ca$^{2+}$], may decrease resulting in a cessation of the repetitive Ca$^{2+}$ transient.

Under the condition with a 50% depression of $I_{\text{Cal}}$, as detected in experimental NCX1 knockout [7], our simulation predicted that a 2.3-fold increase in PMCA activity is enough to restore the beating. Henderson et al. [7] reported no up-regulation of PMCA in expression level in the knockout mice. However, the pump can be functionally activated. In fact it is reported that many factors can modulate the PMCA activity [8]. For example, Ca$^{2+}$/calmodulin interacts with the pump and induces an increase in Ca$^{2+}$ affinity and $I_{\text{max}}$. It is also known that phosphorylation by protein kinase A activates the pump. Thus we postulate a functional up-regulation of PMCA in the NCX1 knockout mice.

The model analysis in the present study demonstrated several feedback mechanisms. The decrease of $F_{\text{CaL}}$, which represents a decreased number of channels expressed on the cell membrane, failed to directly decrease the amplitude of $I_{\text{Cal}}$ during the action potential. For example, the amplitude of $I_{\text{Cal}}$ during the action potential is decreased only to 54% of the control, but $F_{\text{CaL}}$ decreased to 20% (Fig. 2D) because the driving force of membrane potential was increased through a decrease in the plateau potential. The magnitude of $I_{\text{NaCa}}$ did not change in proportion to the increase or decrease in $F_{\text{NCX}}$ because of its substrate, the intracellular Ca$^{2+}$, decreased or increased, respectively. In other words, the reversal potential of the exchanger easily shifts to more positive when [Ca$^{2+}$], increased by the decrease in NCX activity; thus the driving force for Ca$^{2+}$ extrusion increases. Thus NCX is efficient in achieving the Ca$^{2+}$ homeostasis, but the reversal potential of PMCA is kept far more negative than the physiological potential range by the hydrolysis energy of ATP, and the affinity of PMCA to Ca$^{2+}$ is higher than that to NCX. Therefore the magnitude of $F_{\text{PMCA}}$ is almost linearly dependent on $F_{\text{PMCA}}$ as demonstrated in Fig. 4A, and thereby only a slight change in $I_{\text{Cal}}$, caused by the neural and/or hormonal regulation, results in a sharp accumulation or depletion in [Ca$^{2+}$], in the PMCA-dominant system (Fig. 3C). H$^+$ exchanged by PMCA may be extruded via Na$^+$/H$^+$ exchanger so that the Na$^+$/K$^+$ pump will consume additional ATP. So the NCX-dominant system might be more favorable in the energetic aspect. Although our model of PMCA is still preliminary, the advantage of using NCX in place of PMCA will hold true, even when a more precise model of $I_{\text{PMCA}}$ will be introduced in the model analysis.

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