New insights into the molecular and cellular workings of the cardiac Na⁺/Ca²⁺ exchanger

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The cardiac Na⁺/Ca²⁺ exchanger (NCX1) (20, 21) is difficult to study, and its function in cardiac cells has been the subject of many controversies since its activity was first identified in squid axons (1) and in heart (25). From then until now, no work has shaped the field more importantly than the analysis by John Reeves and Calvin Hale of the exchanger stoichiometry (22). In exquisitely planned experiments, using cardiac sarcolemmal vesicles, they demonstrated that the Ca²⁺ extrusion mechanism in cardiac myocytes, although the driving force for Ca²⁺ extrusion is quite small. To explain multiple recent results, it is useful to think of the exchanger as a slow Ca²⁺ buffer that can reverse its function multiple times during the excitation-contraction cycle (ECC). An article by the group of John Reeves brings new insights to this function by analyzing the role of regulatory domains of NCX1 that mediate its activation by a rise of cytoplasmic Ca²⁺. It was demonstrated that the gating reactions are operative just in the physiological range of Ca²⁺ changes, a few fold above resting Ca²⁺ level, and that they prevent the exchanger from damping out the influence of mechanisms that transiently increase Ca²⁺ levels. Furthermore, exchangers with deleted regulatory domains are shown to reduce resting Ca²⁺ to lower levels than achieved by wild-type exchangers. A study by the group of Kenneth Philipson demonstrated that the NCX1 regulatory domain can bind and respond to Ca²⁺ changes on the time scale of the ECC in rat myocytes. At the same time, studies of transgenic mice and NCX1 knockout mice generated by the Philipson group revealed that large changes of NCX1 activity have rather modest effects on ECC. Simple simulations predict these results very well: murine cardiac ECC is very sensitive to small changes of the Na⁺ gradient, very sensitive to changes of the sarcoplasmic reticulum Ca²⁺ pump activity, and very insensitive to changes of NCX1 activity. It is speculated that the NCX1 gating reactions not only regulate coupled 3Na⁺:1Ca²⁺ exchange but also control the exchanger’s Na⁺ leak function that generates background Na⁺ influx and depolarizing current in cardiac myocytes.

excitation-contraction cycle

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Invited Review
range, has been missing all these years. It is therefore a most welcome and important contribution, and it is impressive that the Reeves group has accomplished this by manipulating innate mechanisms of Ca\(^{2+}\) homeostasis in the CHO lines employed, namely, the Ca\(^{2+}\) release, influx, and uptake mechanisms. In brief, the exchanger is activating over several seconds when free Ca\(^{2+}\) rises just above its resting level, and the exchanger inactivates over 5–15 s when Ca\(^{2+}\) decreases back toward its resting level. Thus the exchanger shuts off after it has done its job of extruding Ca\(^{2+}\), and as a result, NCX1 operation does not interfere with mechanisms that may subsequently release Ca\(^{2+}\) or otherwise tend to increase cytoplasmic Ca\(^{2+}\) within 1 or 2 s. The principle is just the same as in voltage-gated K\(^+\) channels that activate slowly upon membrane depolarization, carry out the repolarization process, and then turn off again so as not to dampen excessively subsequent depolarizing influences.

In rough approximation, the experimental results of the Reeves group confirm expectations about exchanger gating reactions from analysis of the reactions under nonphysiological conditions with higher cytoplasmic Ca\(^{2+}\) concentrations (11). Figure 1 shows the predictions, as a fraction of maximal exchange activity, using the published equations and parameters (11). Figure 1A shows the steady-state Ca\(^{2+}\) dependence of the forward exchange mode in the absence of cytoplasmic Na\(^+\), as predicted for an NCX1 system that is regulated and for an NCX1 system that is not regulated (i.e., unregulated) by the Ca\(^{2+}\)-dependent gating reactions. No clear deviations from Michaelis-Menten behavior were detected in the giant patch studies in this condition, probably because deviations are negligible when exchanger function is viewed from the perspective of its total Ca\(^{2+}\) extrusion capacity. As shown in Fig. 1B, however, major differences are indeed predicted for the steady-state activity of the regulated and unregulated exchange systems in the low free Ca\(^{2+}\) range of 0.1–0.3 μM. The regulated system activates steeply (i.e., as a power function) in dependence on cytoplasmic free Ca\(^{2+}\), and during steady-state operation, the regulatory reactions inhibit exchange activity severalfold in relation to unregulated exchangers.

As shown in Fig. 1C, the Ca\(^{2+}\) extrusion activity of the regulated exchanger is predicted to be time dependent, increasing and decreasing over several seconds when cytoplasmic free Ca\(^{2+}\) increases and decreases in a step across this low range of concentrations. It is these reactions in the physiological range of free Ca\(^{2+}\) that were never resolved well using electrophysiology. It is remarkable that they are now resolved using fluorescent Ca\(^{2+}\) indicators in intact cells, and the Reeves group further demonstrated that these reactions can determine basal free Ca\(^{2+}\) in cells with Na\(^+\)/Ca\(^{2+}\) exchangers. When unregulated exchangers are expressed in CHO cells, namely, exchangers with deletion mutations in the NCX1 regulatory domain, resting free Ca\(^{2+}\) comes to an equilibrium that is significantly lower than that attained when wild-type exchangers are expressed. In other words, the gating reaction prevents Ca\(^{2+}\) from falling too low.

The kinetics of the Ca\(^{2+}\)-dependent gating reactions are important because they may determine how the exchanger modulates Ca\(^{2+}\) in myocytes on a beat-to-beat basis, over several beats, and/or over longer periods. If the kinetics are fast enough, the exchanger should activate with each ECC and inactivate between beats. The inactivation would possibly prevent Ca\(^{2+}\) from declining too far, and the time-dependent reactivation during the Ca\(^{2+}\) transient might prevent the exchanger from extruding Ca\(^{2+}\) simultaneously with Ca\(^{2+}\) entry by Ca\(^{2+}\) channels. From results of Matsuoka and colleagues (8), we know that the exchanger’s gating reactions can indeed be quite fast in membrane patches taken from the surface of freshly isolated myocytes, rather than from myocytes that have been stored for hours and whose cytoskeleton is with good certainty disrupted. Furthermore, from the group of Donald Bers (27), we know that some allosteric regulation of the exchanger can in fact be defined in intact myocytes undergoing regular ECC cycles. And finally, more recently, the group of Kenneth Philipson (19) has developed a fluorescent assay to monitor NCX1 gating. Specifically, they have expressed in cultured rat myocytes a fusion protein of the Ca\(^{2+}\)-binding regulatory domain of NCX1 with an appropriate fluorescent protein pair to generate Ca\(^{2+}\)-dependent fluorescence resonance energy transfer (FRET). The fusion protein responds fast enough to track beat-to-beat Ca\(^{2+}\) changes, and such changes can indeed be monitored in beating rat myocytes. Thus it is proved that NCX1 can in principle inactivate during the dia-

![Fig. 1](http://ajpcell.physiology.org/ajpcell)
stolic period and turn on with a delay during the immediate events of ECC.

As stimulating as these developments are for our thinking about exchanger dynamics in cardiac myocytes, still other recent results from the Philipson group bring to focus a most sobering feature of exchanger function for NCX1 “affectionados.” The conditional knockout of NCX1 in murine myocytes (10), like the overexpression of NCX1 in murine myocytes (28), has rather modest effects on cardiac ECC. At first, this result seems to contradict an important role for NCX1, but it does not. When our simplest ideas about cardiac ECC are simulated, as Denis Noble and I described 15 years ago (13), changes of Na+/Ca2+ exchange activity have very little impact on the magnitude of the cytoplasmic Ca2+ transient during continuous beating, although small changes of the Na+ gradient have very large effects on ECC by changing the exchanger’s equilibrium. This pattern is most striking for simulations in which action potentials are short and triangular, and when sarcoplasmic reticulum uptake and release dominate ECC, rather than Ca2+ influx and extrusion, and that is really the short description of ECC in murine myocytes.

The reason for this behavior goes right back to the original Reeves and Hale finding (22). The exchanger will always tend to bring cytoplasmic free Ca2+ to its thermodynamic equilibrium, which depends primarily on membrane potential and the Na+ gradient. In doing so, changes of exchanger activity change the precise timing and the momentary magnitudes of the current. However, especially in those myocytes with strong Ca2+ release and uptake function by the sarcoplasmic reticulum, the influence of NCX1 activity per se on the time course and magnitude of the Ca2+ transient is almost negligible. The exchanger tends to bring cytoplasmic Ca2+ to its equilibrium throughout the ECC cycle, and in this respect its role should be thought of as a Ca2+ buffering function, not as competition with the sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) Ca2+ pump. In this way, the exchanger tends to damp out most influences that do not affect the exchanger’s equilibrium. Changes of the Na+ pump activity can have a strong influence, whereas changes of Ca2+ channel activity have a relatively weak influence. As described subsequently, the ECC system still responds strongly to changes of the sarcoplasmic reticulum Ca2+ uptake function, and this principle reiterates the experience of the field with transgenic and knockout models relevant to cardiac ECC. An increase of SERCA Ca2+ pump activity causes a large increase of Ca2+ release in ECC (7), whereas changes of NCX activity have very minor effects. From this viewpoint, then, the exchanger’s gating reactions can serve only to fine-tune its function, whereas factors that determine the Na+ gradient set its overall role in cardiac ECC.

Figure 2 illustrates the simplest possible simulations that reconstruct these ECC principles and are relevant to murine myocytes. Briefly, we have developed over the last 15 years numerous simulations of cardiac ECC, including many different formulations of Ca2+ release, cardiac ion channels, SERCA function, background Ca2+ fluxes, and NCX1 function. The

Fig. 2. Function of a simplistic model of murine excitation-contraction cycle (ECC) functioning at 3 Hz in steady state. At the start of each cycle, it was assumed that membrane potential jumps to 40 mV, that a constant fraction (0.4) of total sarcoplasmic reticulum Ca2+ is released, and that 20 μmol of Ca2+ per liter of cell water enter the cytoplasm. See text for more details. A: predicted Ca2+ transients for the control simulation, for a simulation with NCX1 activity decreased by a factor of 10 (NCX1 ÷ 10), and for simulation with an increase of the sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) pump activity by a factor of 2 (SERCA × 2). Note that Ca2+ transients are increased more by a small increase of SERCA than by a large decrease of NCX1. B: sensitivity of the peak Ca2+ level to changes of the indicated model parameters. The γ-axis gives the fractional change of steady-state peak cytoplasmic Ca2+ when the indicated parameter is changed by a fraction of its initial value (Δpeak Ca2+ /ΔX). Na+, cytoplasmic Na+; Ca2+, cytoplasmic Ca2+; CaSR, sarcoplasmic reticulum. C: simulated ECC functions for the 3 conditions given in A: S, SERCA × 2; N, NCX1 ÷ 10; and C, control. Records shown are as follows: Emem, membrane potential; Ik, K+ current; Ca2+, sarcoplasmic reticulum Ca2+ load; Ina,Ca, Na+/Ca2+ exchange current; total Ca2+, total cytoplasmic Ca2+ concentration; and Ca2+, extracellular Ca2+ concentration, which gives the integral of transsarcolemmal Ca2+ flux with the assumption of an extracellular space of 50% of total tissue volume and an extracellular Ca2+ concentration of 1.5 mM.
principles outlined apply in our experience to all models with strong SERCA function and relatively weak Ca\(^{2+}\) channel contribution to ECC. For results shown in Fig. 2, the ECC is assumed to start instantly with three events: 1) membrane potential moves suddenly to +40 mV, 2) a constant fraction of Ca\(^{2+}\) in the sarcoplasmic reticulum is released to the cytoplasm, and 3) a constant amount of Ca\(^{2+}\) enters the cytoplasm as a result of Ca\(^{2+}\) channel activity (here, 10 \(\mu\)mol per liter of cell water). Cytoplasmic Ca\(^{2+}\) is buffered at a constant ratio of 50:1, Ca\(^{2+}\) is pumped back into the sarcoplasmic reticulum at a constant rate proportional to free Ca\(^{2+}\), the K\(^{+}\) conductance of the membrane is constant [i.e., K\(^{+}\) current is given by \(G_K*(E_m - E_K)\), and NCX function is represented by the simple biexponential equation suggested by DiFrancesco and Noble (5). In short, all the complexities of NCX1 function are ignored, no further conductances are simulated, ion concentrations besides Ca\(^{2+}\) are constant, and the cytoplasmic Na\(^{+}\) concentration is 8 mM.

Figure 2A shows the ECCs that occur in the steady state of the simulation at a frequency of 3 Hz. In the control simulation, free cytoplasmic Ca\(^{2+}\) reaches 2.6 \(\mu\)M, total Ca\(^{2+}\) reaches 130 \(\mu\)M, and during the course of the 330-ms cycle, the same amount of Ca\(^{2+}\) leaves the cell via NCX as entered at the upstroke. When the control simulation is compared with a simulation with 10 times less NCX activity, the peak Ca\(^{2+}\) level is increased by only 65%. When the control simulation is compared with a simulation with a twofold higher Ca\(^{2+}\) uptake rate by the sarcoplasmic reticulum, the peak Ca\(^{2+}\) level is more than doubled. Figure 2B shows the effect that small changes of the major ECC simulation parameters have on the peak Ca\(^{2+}\) level achieved on activation. From all of the model parameters, small changes of cytoplasmic Na\(^{+}\) (from 8 to 8.8 mM in the simulation) have the largest influence on peak Ca\(^{2+}\). The relationship of cytoplasmic Na\(^{+}\) to peak cytoplasmic Ca\(^{2+}\) is minimally a second-power relationship. Changes of extracellular Ca\(^{2+}\) result in proportional changes of peak Ca\(^{2+}\) because extracellular Ca\(^{2+}\) is also a simple determinant of the exchanger equilibrium. Changes of the magnitude of Ca\(^{2+}\) influx have a much smaller influence because of the buffering function of the exchanger over many beats. For example, a 50% increase of Ca\(^{+}\) influx results in only a 15% increase of the peak Ca\(^{2+}\). Similarly, changes of the Ca\(^{2+}\) release fraction, NCX activity, and the sarcoplasmic reticulum Ca\(^{2+}\) leak function all have very small influences. As already noted, the surprise is that changes of SERCA activity result in changes of peak Ca\(^{2+}\) that are fractionally even somewhat greater than the fractional change of pump activity.

Shown in Fig. 2C are the action potentials, exchange currents, K\(^{+}\) currents, total cell Ca\(^{2+}\), sarcoplasmic reticulum Ca\(^{2+}\) load, and the integral of the transmembrane Ca\(^{2+}\) movements [which would correspond to the extracellular Ca\(^{2+}\) transient in a multicellular cardiac tissue (13)]. As noted at the beginning of this article, the overall function of NCX1 in murine myocytes may usually be trimodal. Here, an initial small phase of Ca\(^{2+}\) influx at the action potential upstroke is absent because the Ca\(^{2+}\) release function is simplified to be instantaneous. Thereafter, the exchanger extrudes Ca\(^{2+}\) when cytoplasmic Ca\(^{2+}\) is high, but it shifts to import Ca\(^{2+}\) slowly in early diastole. This import function requires that Ca\(^{2+}\) can be reduced transiently by the SERCA pump to a value that is less than the equilibrium value of NCX1, and the obvious possibility is that this occurs in domains where internal and surface membranes are in close contact.

The peak inward exchange current in the control situation is only −30 pA, which is very small compared with the peak K\(^{+}\) current of 250 pA that brings about repolarization. Thus, in this ECC setting, the exchanger’s influence on the action potential is negligible, but the NCX1 activity is still enough to overshoot its Ca\(^{2+}\) extrusion function. More Ca\(^{2+}\) is extruded than entered the myocyte at the upstroke. As the sarcoplasmic reticulum proceeds to take up Ca\(^{2+}\) between beats, the exchanger maintains a supply of cellular Ca\(^{2+}\) (see the extracellular Ca\(^{2+}\) simulation). When the exchanger activity is reduced by a factor of 10, the overshoot of exchanger function is much reduced, but this change has almost no influence. When SERCA activity is increased, the overshoot becomes enhanced as the sarcoplasmic reticulum Ca\(^{2+}\) loading increases. These basic results are not fundamentally changed by adding Ca\(^{2+}\) leaks in either the surface or sarcoplasmic reticulum membranes. The take-home message is that NCX1 and SERCA need not be operating as competitors but can operate in parallel to load the sarcoplasmic reticulum between beats in the murine myocyte.

When included in these simple simulations, the exchanger gating reactions controlled by cytoplasmic Ca\(^{2+}\) frankly have rather little influence on ECC, and experimental results from the Bers group (27) with murine myocytes are consistent with this conclusion. The fact that murine myocytes can display nearly normal ECC in the absence of NCX1 (10) strongly supports the idea that NCX1 is functioning effectively as a Ca\(^{2+}\) buffer with little influence on the steady-state Ca\(^{2+}\) transients. Are the exchanger gating reactions really irrelevant in mice? What might be the role of the second exchanger gating reaction, the strong Na\(^{+}\)-dependent inactivation reaction (12)? The answers to these questions are not obvious from any experimental or theoretical results outlined up to now.

Another perspective on NCX1 function arises from recent findings that the exchanger stoichiometry is not entirely fixed. Although the reversal potential for Ca\(^{2+}\) influx lies close to that predicted for a 3:1 exchanger, just as measured originally by Reeves and Hale (22), the reversal potential for current is shifted toward that expected for a 4:1 exchanger (9, 15, 17). In some measurements, the current reversal is just a little bit closer (14), in some roughly halfway closer (9, 15), and in some nearly at the 4:1 potential (17). These deviations of the flux ratios from 3:1 appear to come about because the exchanger occasionally moves an extra Na\(^{+}\) across the membrane with Ca\(^{2+}\) (15). That the exchanger can bind Na\(^{+}\) and Ca\(^{2+}\) together is again a fundamental property of the exchanger associated with John Reeves, this time together with John Sutko (24). The consequence for cardiac physiology is that the exchanger can contribute a significant part of background Na\(^{+}\) influx and background inward current that determines resting free Na\(^{+}\) in myocytes and that supports some forms of cardiac pacemaking, respectively. Possibly, this Na\(^{+}\) leak function is relevant to an understanding of the role of the gating reactions in cardiac physiology.

Figure 3 outlines the significance of the exchanger Na\(^{+}\) leak function for cardiac pacemaking by the sinus node atrial cells. This simulation contains just the minimum mechanisms needed to allow pacemaking and to maintain the homeostasis of Ca\(^{2+}\), Na\(^{+}\), and K\(^{+}\). Sarcoplasmic reticulum function is left out.
Also, the hyperpolarization-activated cation channels are left out. These channels are expected to prevent excessive hyperpolarization, thereby keeping the pacemaker system robust, and to mediate faster pacemaking with a rise of cyclic AMP (26), but their presence is not necessary to generate basal pacemaking. The simulation includes a simple L-type Ca\(^{2+}\) channel that, for simplicity, has instantaneous activation. It includes a simple HERG-type K\(^+\) conductance that, for simplicity, has instantaneous inactivation. It includes a simple model of Na\(^+\) pump current function that predicts accurately Na\(^+\) pump current-voltage relations and that assumes a free energy of ATP hydrolysis of 16 kcal/mol. And finally, it includes the NCX1 system, formulated exactly as published (15) and as needed to describe accurately NCX1 ion flux coupling and current-voltage relations.

The cytoplasmic concentrations of the three cations simulated are in complete steady state in the model function presented in Fig. 3. Action potentials are generated at a spontaneous frequency of just 2 Hz, whereby the upstroke is brought about by Ca\(^{2+}\) channels. Ca\(^{2+}\) extrusion is brought about by NCX1, coupled to Na\(^+\) influx and thereby to depolarizing current. To extrude the Ca\(^{2+}\) that entered the myocyte via Ca\(^{2+}\) channels, inward exchange current must be very significant in the early part of the diastolic period (4). Naturally, the exchange current will drive depolarization faster when Ca\(^{2+}\) influx and, consequently, Ca\(^{2+}\) extrusion are increased with catecholamines.

The current generated by a perfect 3Na\(^+\):1Ca\(^{2+}\) exchange process naturally decays as Ca\(^{2+}\) is extruded, and it therefore would presumably become irrelevant late in the diastolic period. What are the depolarizing influences during late diastole of the sinus node cells? This is an old and still unresolved question. It could be the hyperpolarization-activated cation current, or it could be a novel nifedipine-sensitive voltage-activated Na\(^+\) current (3). However, it may also be the contribution of NCX1 that may carry inward current late into the diastolic period by two means. A new proposal is that Ca\(^{2+}\) is released by sarcoplasmic reticulum spontaneously in sinus node cells with a certain delay after the previous action potential (18). The rise of cytoplasmic Ca\(^{2+}\) causes an increased Ca\(^{2+}\) extrusion by Na\(^+\)/Ca\(^{2+}\) exchange in the diastole period and with that a “delayed” Na\(^+\)/Ca\(^{2+}\) exchange current that promotes generation of the next action potential. Quantitatively, the possible contribution of this mechanism, if present, cannot be of large magnitude, because inhibition of sarcoplasmic reticulum Ca\(^{2+}\) recycling by ryanodine has only modest negative chronotropic effects, ∼19%, in true pacemaker cells (16). The second possibility, then, concerns the exchanger’s Na\(^+\) leak function, which is predicted to continue unabated through the diastolic period (15). This has been illustrated in Fig. 3 by plotting the total NCX1 current and the current generated specifically by 3:1 Na\(^+\)/Ca\(^{2+}\) exchange. Although the real Na\(^+\)/Ca\(^{2+}\) exchange ratio under maximized transport conditions is only ∼5% different from 3:1, the extra Na\(^+\) current generated is about the same magnitude when the exchanger is operating close to equilibrium. It is therefore a very significant component of background Na\(^+\) influx, and the corresponding inward current is completely appropriate to support diastolic depolarization up to the onset of the next action potential. The Ca\(^{2+}\)-dependent gating reaction of the exchanger would naturally control this background current as follows: When the average free Ca\(^{2+}\) is increased in the sinoatrial node, as will be the case with catecholamines, the number of active exchangers will naturally increase, and the diastolic depolarization rate will naturally be increased via both the increased Ca\(^{2+}\) transport current and the Na\(^+\) leak function of the exchanger.

What about the rest of the myocardium? A speculative possibility is that the exchanger plays a significant role in control of the myocyte Na\(^+\) gradient by mediating background Na\(^+\) influx, independent of Ca\(^{2+}\) extrusion. This Na\(^+\)-promoting influence would be controlled and countered by the exchanger’s Na\(^+\)-dependent inactivation reaction, which depends on complete loading of the exchangers transport sites and therefore has a third-power dependence on cytoplasmic Na\(^+\) (12). In short, activation of Na\(^+\)/Ca\(^{2+}\) exchangers by any relevant regulatory influence would tend to increase cytoplasmic Na\(^+\), and this Na\(^+\)-loading function of NCX1 would be opposed by the exchanger’s Na\(^+\)-dependent inactivation reaction. One would expect NCX1 overexpression to cause a rise in cytoplasmic Na\(^+\). It must be admitted that this was not detected in initial studies (28), but more detailed analysis seems justified. Up to now, no clear explanation for the Na\(^+\)-dependent inactivation function of NCX1 has been offered that makes cellular sense, that can tested in experiments, and that can be analyzed in simulations of ECC.

In summary, we are now learning a lot about how the Na\(^+\)/Ca\(^{2+}\) exchanger gating reactions are working in live cells,

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**Fig. 3. Steady-state cardiac sinus node pacemaking simulated with the minimum mechanisms needed to maintain homeostasis of Ca\(^{2+}\), Na\(^+\), and K\(^+\). Records shown are \(E_m\), \(I_{Ca}\), \(I_K\), \(I_pump\), \(I_{NaCa(transport)}\), \(I_{NaCa(total)}\) being the sum of the current specifically associated with Ca\(^{2+}\) transport \([I_{NaCa(transport)}]\) and the Na\(^+\) leak function of the exchanger; and Ca\(^{2+}\), free cytoplasmic Ca\(^{2+}\) concentration.**
reactions that have remained very enigmatic up to now. The Reeves group (2) has provided new insights into the function and kinetics of the Ca\(^{2+}\)-dependent activation function by using physiological mechanisms to change Ca\(^{2+}\) in a cell line. These insights could not have been accomplished with electrophysiology. The Philipson group (19) has provided clear new information about the kinetics of the actual Ca\(^{2+}\)-sensing domain of the exchanger in intact myocytes, with the outcome that the Ca\(^{2+}\)-dependent regulation may be fast enough to modulate NCX1 function in the time course of each ECC. The Philipson group (10) has also generated transgenic and knock-out models that challenge our understanding of NCX1 function in cardiac ECC. The simplest possible models of cardiac ECC are entirely consistent with the outcome that substantial changes of NCX1 activity have little influence on cardiac ECC. Yet, small changes of the Na\(^+\) gradient can have the largest effect of any cellular parameter on cardiac ECC via modulation of the NCX1 function. It seems possible that important control mechanisms for cytoplasmic Na\(^+\) exist in heart and are still waiting to be discovered. One possibility is that the exchanger’s Na\(^+\)- and Ca\(^{2+}\)-dependent gating reactions are fine-tuning the Na\(^+\) gradient by controlling background Na\(^+\) influx. Clearly, we still have a long way to go to understand many important details of NCX1 function and Na\(^+\) homeostasis in heart. The recent studies of NCX1 outlined here provide new and important clues, and they invite future NCX1 research along multiple fruitful paths.

ACKNOWLEDGMENTS

I thank Dr. Kenneth Philipson (University of California, Los Angeles, CA) for discussions and criticisms. The simulation programs were written in Matlab 6.5, and the corresponding M-files are available on request from D. Hilgemann.

GRANTS

This work was supported by NIH-HL051323.

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