Control of the Na-Ca Exchanger in Isolated Heart Cells

I. Induction of Na-Na Exchange in Sodium-Loaded Cells by Intracellular Calcium

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The Na-Ca exchanger is an active component of the sarcolemma in heart and nerve. Although the participation of the exchanger in excitation–contraction coupling in heart has long been postulated, its role has remained controversial. There is mounting evidence that the exchanger does have a major role in calcium efflux and perhaps also in calcium influx during excitation. To elucidate this role it is important that the properties of the exchanger in heart be well understood. To date, most of the detailed studies on Na-Ca exchange in intact tissue have been done with squid axon, where both internal and external solutes can be controlled. Two significant properties revealed by these studies are that the exchanger is controlled by ATP and by intracellular calcium. The exchanger in heart is likewise controlled by ATP and by intracellular calcium. In squid axon, Na-Na exchange activated by intracellular calcium has also been demonstrated. These authors showed that Na-Na exchange had a similar affinity for activation by intracellular calcium and concluded that the Na-Na exchange was a mode of operation of the Na-Ca exchanger. We show here that a similar Na-Na exchange mode exists for the Na-Ca exchanger in heart and that this activity can be induced by calcium that enters the cell through calcium channels. We propose that this Na-Na exchange activity is indicative of the extent of activation of the Na-Ca exchanger by intracellular calcium. The significance of this result goes beyond a comparison between heart and nerve. In the following article we demonstrate that in normal cells at rest there is a near absence of intracellular calcium–dependent Na-Na exchange activity induced by electrical stimulation. On the basis of our conclusions here, this implies that, at rest, the Na-Ca exchanger is essentially inactive, and it becomes activated during excitation.

Materials and Methods

Cell Isolation

Heart cells were isolated from female retired breeder rats according to our original method, as recently modified. The modification used was condition 5 in Table 2 of that article: the perfusion buffers contained 25 mM HEPES, adjusted to pH 7.4 with NaOH, in place of bicarbonate, plus basal Eagle
medium amino acids. Calcium (1 mM) was restored to the recirculating perfusate 15 minutes after en-
zyme addition. This method gave a high yield of cells
with a high percentage (74.3±6.0) of rod-shaped cells
in the presence of 1 mM calcium.

Experimental Medium

Cells were suspended (16–22 mg protein/ml) in a
medium containing (mM) NaCl 118, KCl 4.8, HEPES 25, KH2PO4 1.2, MgSO4 1.2, CaCl2 1.0,
pyruvic acid 5, glucose 11, and (µM) insulin 1,
adjusted to pH 7.4 with NaOH. Suspensions were
maintained aerobic by equilibration with air in a
shaking incubator at 37°C.

Isotope Flux Measurements

Rates of 22Na uptake and efflux were measured
using a dilution protocol designed to facilitate the
accurate measurement of the rate of exchange of
low levels of intracellular sodium. The method is an
extension of that previously used in our laboratory
for measuring solute fluxes. The new feature of
the method is that concentrated cells are labeled
with 22Na to a high specific activity and then diluted
10-fold into unlabeled medium before centrifuga-
tion. The dilution results in a 10-fold higher specific
activity of 22Na inside the cell compared with 22Na
outside and hence allows a 10-fold enhancement of
the sensitivity of measurement of intracellular so-
dium compared with that of our basic method. This
is illustrated in Figure 1 for measuring the 22Na
uptake rate. Panel A was done without dilution.
Cells in experimental medium plus 2 mM carrier
succrose plus tritiated water (1 µCi/ml) were given
[^14]C)sucrose or 22Na (0.1 µCi/ml) at time zero. At
the times shown, aliquots (0.5 ml) of cell suspension
were removed and centrifuged through bromo-
dodecane (0.5 ml) into perchloric acid (0.1 ml). The ratio
of [^14]C to tritium or 22Na to tritium in aliquots of
the perchloric acid and of the supernatant was mea-
sured in a liquid scintillation counter. The pellet
ratio (Rp) was then expressed as a percentage of the
supernatant ratio (Rs). Thus

\[ \% \text{Permeation} = \frac{R_p}{R_s} \times 100 \]

This number reflects the extent to which the cell
was permeated by the labeled solute. For sucrose, a
low percent permeation was found, as previously, which
diffused quickly across the sarcolemma of intact cells. The
space permeated by sucrose is related to the fraction
of dead cells in the preparation. The percent
permeation by sodium at equilibrium was not much
greater than that of sucrose (Figure 1A). This is
because, even though sodium can cross the sarco-
lemma, the sodium pump continuously removes it,
maintaining a low intracellular concentration. The
difference between the sucrose and sodium values
thus is a measure of intracellular sodium. The small
size of this difference in comparison to the extracel-
lar sodium makes it difficult to measure accurately.
Panel B was done with dilution. Concentrated cells
plus tritiated water were given [^14]C)sucrose or 22Na
at time zero. At the times shown, 0.07-ml aliquots were
removed and diluted into 0.63 ml unlabeled medium.
Aliquots (0.5 ml) of the mixture were removed
immediately and centrifuged within 15 seconds of
dilution. The ratios of activities in pellets and super-
nants were calculated as before, but with values
measured on the diluted suspension. The rate of
equilibration of tritiated water on dilution is faster
than we can measure. This allows us to use the rate
of change of percent permeation as a measure of the
rate of change of uptake or efflux of labeled solute.
For sucrose, a low percent permeation was again
found, which changed little with time (Figure 1B).
This again is consistent with the inability of sucrose
to cross the sarcolemma: on dilution the extracellu-
lar label would immediately be diluted; any sucrose
that has entered the cell, on the other hand, would
retain its original specific activity on dilution, and any
permeation with time would appear as a positive
slope 10-fold greater than that in Figure 1A. It is
indeed evident from Figure 1B that there is a signif-
icient positive slope, of 0.41%/min best fit value,
showing that there was a small but finite rate of
sucrose entry of 0.041%/min in the units of Figure
1A. For 22Na, with the dilution method, the cellular
sodium uptake was also magnified by a factor of 10,
and the rate of uptake of label was readily measured
(Figure 1B). The best-fit asymptote for intracellular
sodium corresponds to 17.0 nmol/mg, which, if all
were free in the intracellular space, is equivalent to
14 mM (using 2.45 µl/mg and the intercept of Figure
1B). Intracellular sodium activity in resting rat ven-
tricle has been measured with sodium-specific elec-
trodes, the most recent value being 12.7 mM, in fair
agreement with this result. To measure efflux rates,
concentrated cells were first equilibrated with 22Na
for 20 minutes and then diluted into unlabeled

![Figure 1. Measurement of intracellular sodium by the dilution method. Panel A: No dilution. Panel B: With dilution. Experimental procedure is described in the text.](http://circres.ahajournals.org/)

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**Haworth et al. Activation of the Na-Ca Exchanger by Intracellular Calcium**

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Dr. Haworth et al. describe a method for measuring the intracellular sodium concentration using a dilution technique. Cells were suspended in a medium containing calcium, glucose, insulin, and other components, and then diluted into unlabeled medium. Isotope flux measurements were used to determine the rate of exchange of low levels of intracellular sodium. The method was validated by comparing the permeation of sucrose and sodium, demonstrating the ability of sodium to cross the sarcolemma versus the inability of sucrose to do so. The technique allowed for the measurement of sodium uptake and efflux rates, providing insights into intracellular sodium dynamics.
medium at time zero. Aliquots (0.5 ml) were then removed at time intervals and centrifuged. Results are expressed as percent permeation as defined above, that is, relative to the specific activities of solute and tritiated water in the diluted suspension. It should be borne in mind that the bottom 40% or so of this is extracellular (Figure 1B, sucrose intercept), while the intracellular label on this scale is magnified by a factor of 10. We have not attempted to correct for extracellular medium in every experiment shown; having verified with $[^{14}C]$sucrose that the extent of cell disruption under the experimental conditions used here was minimal (data not shown), the contribution from the extracellular medium for each experiment is expected to remain a constant.

**Sodium-Loaded Cells**

Concentrated cells with tritiated water were loaded with sodium in experimental medium without calcium and plus 0.25 mM EGTA by incubation for 30 minutes at 37°C with ouabain (1 mM). To measure $^{22}$Na efflux, $^{22}$Na was added with the ouabain, and cells were diluted after the 30-minute incubation, at time zero in the figures, into the media described in the text under "Results." Aliquots were removed at time intervals and centrifuged, as above. To measure rates of $^{22}$Na uptake, $^{22}$Na was added to the concentrated cells after the 30-minute incubation. Aliquots were removed at time intervals, diluted, and centrifuged immediately (see above).

**Data Analysis**

Values shown are the mean±SD of at least three experiments. Efflux and uptake data were fit by nonlinear least squares to the functions

$$\%P = (a-b) + b[\exp(-kt) - 1]$$

for efflux and

$$\%P = a + b[1 - \exp(-kt)]$$

for uptake, where a, b, k are constants, %P is percent permeation, and t is time. The slower curves (without calcium, for example) were used to optimize a and k for an estimated value of b. The faster curves were then used to optimize b and their own k by using the value of a found with the first curves. This process was then repeated to convergence of common values for a and b for fast and slow curves and individual values of k. Lines in figures are these best-fit curves, unless otherwise indicated. In some instances, where no difference between two treatments was observed, data were combined for the best fit. Initial rates of efflux and uptake were calculated from the product of b and k. Values in percent per minute were converted to nanomoles per milligram per minute by multiplication by the factor $PV \times SC/(100 \times DF)$, where $PV$ is pellet volume (in $\mu$l/mg; we use a value of 2.45, based on pellet tritium values), $SC$ is solute concentration (in mM; here 140 mM for sodium), and $DF$ is the dilution factor (here 10).

**Results**

Concentrated cells in experimental medium without calcium and with EGTA (0.25 mM) were loaded with sodium and $^{22}$Na by incubation with ouabain (1 mM) for 30 minutes at 37°C. The cells were then diluted into nine volumes of unlabeled medium containing either calcium (0.45 mM, plus 0.25 mM EGTA) or no calcium plus EGTA (0.25 mM). All the dilution media also contained ouabain (1 mM). First of all, we found that the sodium-loading protocol had increased the level of intracellular sodium approximately 10-fold (Figure 2A), as reflected by the time zero intercept of the ordinate (Figure 2A) and the asymptotic value for full equilibration of label (Figure 2B, compare with Figure 1B). This is consistent with our earlier observations of sodium loading under these conditions. Cells showed a much faster rate of $^{22}$Na efflux when diluted into the calcium-containing medium than when diluted into the EGTA medium (Figure 2A). There was also no stimulation of $^{22}$Na efflux when manganese was substituted for calcium in the dilution medium (data not shown). Because calcium entry by Na-Ca exchange is expected under the conditions of Figure 2A, a calcium-dependent stimulation of $^{22}$Na efflux is perhaps not surprising. However, the absolute magnitude of the initial rate of calcium-dependent $^{22}$Na efflux under these conditions was very rapid: 46.44 nmol/min/mg from the difference between the best-fit curves with and without calcium in Figure 2A. On the other hand, the initial rate of calcium uptake measured with $^{45}$Ca (not shown) was just 1 nmol/min/mg. Clearly, only a tiny fraction of the $^{22}$Na efflux could be in exchange for calcium, unless the calcium immediately exchanged back out for sodium. In support of this possibility, calcium did also stimulate $^{22}$Na entry (Figure 2B). In this experiment, concentrated cells were loaded with sodium as before, and then at time zero $^{22}$Na with or without 0.2 mM excess free calcium was added. At the times shown, the cells were diluted.
into medium plus EGTA (0.25 mM) and centrifuged immediately. It is clear (Figure 2B) that calcium stimulated the rate of $^{22}$Na uptake to an extent similar to its effect on efflux (Figure 2A). Other experiments showed no detectable effect of calcium on total cellular sodium under these conditions (data not shown). Also, no effect of tetrodotoxin (25 μM) on the sodium flux rates could be demonstrated (data not shown).

The preventive action of verapamil on calcium-induced Na-Na exchange was also studied in the experiment shown in Figure 2A. For the final 1 minute of incubation before dilution, half of the cells were given verapamil (10 μM). These cells were diluted into media containing calcium plus verapamil or no calcium plus EGTA plus verapamil, each with ouabain. The calcium-induced increase in rate of $^{22}$Na efflux was prevented when verapamil was present (Figure 2A). Visual inspection of cells by light microscopy 5 minutes after calcium addition showed that control cells had rounded up, while cells with verapamil were still rod shaped. Verapamil (10 μM) did not, however, prevent the calcium-induced increase in rate of $^{22}$Na efflux when the level of extracellular calcium was 1 mM (data not shown). The rate of $^{22}$Na efflux in the absence of calcium was not sensitive to verapamil (Figure 2A).

We then investigated the ability of verapamil and EGTA to reverse the calcium-induced increase in rate of $^{22}$Na efflux. Cells were exposed to calcium (0.45 mM total, 0.2 mM free) during the final 2 minutes of sodium loading. A quarter of the cells was treated with verapamil 1 minute after the calcium, and another quarter was treated with EGTA (0.45 mM) 1 minute after the calcium. However, those treated with EGTA were incubated for an extra 10 minutes before dilution. Cells pretreated with calcium showed a fast rate of $^{22}$Na efflux on dilution into a calcium-containing medium (Figure 3, filled circles), perhaps even faster than that of cells without calcium diluted into a calcium-containing medium (Figure 2A). The calcium-sensitive rate of $^{22}$Na efflux was 86.06 nmol/mg/min from Figure 3 from the difference between cells activated with calcium and those reversed by incubation with EGTA. This shows that the accelerated rate of $^{22}$Na efflux is not transient under these conditions but is maintained and increases even more the disparity between sodium efflux and calcium uptake. When verapamil was added to calcium-treated cells, however, essentially no reversal of the rate of $^{22}$Na efflux was observed (Figure 3, open triangles). The accelerated rate of $^{22}$Na efflux was still observed initially on dilution of calcium-treated cells into an EGTA medium, although some inhibition was apparent at longer times (Figure 3, open circles). This rules out the possibility that the increased sodium fluxes can be explained by calcium cycling back and forth across the membrane in exchange for sodium, since the extracellular EGTA would not allow any cellular calcium uptake. These data could not be fit by a simple exponential because of the time-dependent decline of sodium efflux rate. Complete reversal was observed in the sample incubated for 10 minutes with EGTA before dilution (Figure 3, filled circles). This shows that under these conditions the calcium-dependent increase in $^{22}$Na efflux was fully reversible, but it took some time.

Next, the extracellular sodium requirement of the calcium-induced $^{22}$Na efflux was evaluated. To show a sodium requirement, extracellular sodium must be removed, and if much extracellular calcium is present, a massive calcium loading occurs. To avoid this complication, we used the ability of sodium-loaded cells activated with calcium to maintain accelerated rates of $^{22}$Na efflux, at least for a short while, when diluted into a medium without calcium (Figure 3). Concentrated cells were loaded as before with sodium plus $^{22}$Na by incubation with ouabain (1 mM) plus EGTA (0.25 mM) and treated (or not) with calcium (0.45 mM) for the final 2 minutes. They were then diluted into calcium-free medium (no EGTA) either with normal sodium or with sodium replaced by lithium. We found that, although cells activated with calcium showed an accelerated efflux of $^{22}$Na into the sodium medium (Figure 4A), the accelerated rate was abolished when extracellular sodium was replaced by lithium (Figure 4B). This shows that the accelerated rate of $^{22}$Na efflux induced by intracellular calcium occurs via Na-Na exchange and is specific for sodium over lithium. The efflux from activated cells into the sodium medium under these conditions could not be fit by a simple exponential, rather like the efflux into a sodium medium with EGTA (Figure 2A).

Then, the intracellular monovalent cation specificity of the increased efflux induced by calcium was investigated. We tested whether calcium could cause a stimulation of efflux of $^{86}$Rb. Concentrated cells without calcium were allowed to accumulate trace
levels of $^{86}$Rb for 30 minutes before being sodium loaded by incubation with ouabain as before. Cells were exposed to calcium (or not) for the final 2 minutes, as before. They were then diluted into medium with ouabain containing either 1 mM calcium or 1 mM manganese final concentration. Manganese was substituted for calcium to prevent a stimulation of $^{86}$Rb efflux by calcium removal, a manifestation of the increased permeability of the sarcolemma to sodium and potassium that occurs with calcium removal. No difference in rate of $^{86}$Rb efflux was observed, whether or not the concentrated cells had been exposed to calcium or whether or not they were diluted into a calcium-containing medium (Figure 5). This is in striking contrast to the acceleration of $^{22}$Na efflux (Figures 2A, 3, and 4).

The ability of an inhibitor of the Na-Ca exchanger to prevent and reverse the calcium-induced sodium fluxes was also investigated. The best-known inhibitor of the Na-Ca exchanger is probably dichlorobenzamil. To test it, the experimental design was identical to that of Figures 2A and 3 in which verapamil was tested, except that dichlorobenzamil (100 μM) was used in place of verapamil. Dichlorobenzamil prevented the induction of accelerated Na-Na exchange observed when cells in EGTA were diluted into a calcium-containing medium (Figure 6A). Dichlorobenzamil had no effect on the calcium-independent component of $^{22}$Na efflux (Figure 6A), suggesting that this component did not pass through the Na-Ca exchanger. The action of dichlorobenzamil as a preventer of calcium-induced sodium efflux was similar to that observed with verapamil (Figure 2A). However, dichlorobenzamil also reversed the accelerated rate of Na-Na exchange in calcium-treated cells (Figure 6B), an action that was not seen with verapamil (Figure 3).

**Discussion**

Although the exposure of sodium-loaded cells to extracellular calcium resulted in a stimulation in the rate of $^{22}$Na efflux and uptake (Figure 2), two observations taken together strongly suggest that the action of the calcium was at an intracellular site. First, the effect of calcium was not immediately reversed on the removal of calcium from the extracellular space but was reversed after incubation (Figure 3). This suggests that it was the calcium that entered the cells that induced the increased sodium fluxes. Second, the action of the calcium was prevented but not reversed by verapamil, an agent that is expected to block cellular calcium entry through calcium channels. Verapamil would not be expected to reverse the action of calcium if the site of action was inside the cell, and calcium was already accumulated.

By what route do the calcium-dependent sodium fluxes pass? Their insensitivity to tetrodotoxin rules out their possible mediation by sodium channels. A case could be made that the $^{22}$Na fluxes observed on calcium addition occur through calcium channels.
Calcium channels will pass sodium,\textsuperscript{31,32} and they are blocked by verapamil.\textsuperscript{33} However, three properties of the calcium channel, and one observation here, call this interpretation into question. First, sodium flux through the calcium channel is known to be increased by the removal of extracellular calcium.\textsuperscript{33} This trend is the opposite of our observation that the addition of extracellular calcium increases the rate of \textsuperscript{22}Na efflux (Figure 2). Second, intracellular calcium is known to exert an inhibitory effect on calcium channels.\textsuperscript{34,35} If the calcium-induced efflux of \textsuperscript{22}Na is caused by a rise in intracellular calcium, as our evidence indicates, then this is opposite to known calcium channel behavior. Third, if the calcium-induced \textsuperscript{22}Na efflux from sodium-loaded cells occurred through calcium channels, we would not expect that there would be any requirement for extracellular sodium or that lithium would fail to substitute in any such requirement (Figure 4). Even sodium channels pass lithium.\textsuperscript{36,37} Fourth, we have found that, although verapamil blocks the activation of calcium-dependent \textsuperscript{22}Na efflux, it does not reverse the activation in experimental situations while levels of intracellular calcium are expected to remain high (Figure 3). If the \textsuperscript{22}Na were passing directly through calcium channels, we would expect verapamil not only to prevent but also to reverse the increased \textsuperscript{22}Na flux observed on addition of calcium. This observation indicates rather that the calcium channel involvement is indirect.

It is, perhaps, surprising that calcium channel activity could be a factor in the calcium-induced \textsuperscript{22}Na efflux observed here, since the cells are not stimulated. During the sodium-loading process, however, about half of the cell potassium is lost (data not shown), so there will be a significant degree of depolarization and hence the possibility of some activation of calcium channels.

Two systems in the sarcolemma are known to be activated by intracellular calcium: a nonspecific cation channel observed in neonatal heart cells\textsuperscript{38} and the Na-Ca exchanger.\textsuperscript{18,20,21} Three observations favor the Na-Ca exchanger as the site of calcium-induced \textsuperscript{22}Na translocation in sodium-loaded cells.

First, the calcium-induced \textsuperscript{22}Na efflux in sodium-loaded cells was not the result of a nonspecific increase in cation permeability.\textsuperscript{22}Na efflux required extracellular sodium (Figure 4), and intracellular calcium did not promote rubidium efflux in such cells (Figure 5). Such selectivity is not expected of a nonspecific cation channel, but the Na-Ca exchanger is known to be specific for sodium over lithium or potassium.\textsuperscript{3,39} The failure of lithium to substitute for sodium also serves to distinguish the Na-Ca exchanger from the Na-H exchanger, since the latter will carry lithium.\textsuperscript{40,41} The observation with \textsuperscript{86}Rb also, incidentally, provides no evidence here for an intracellular calcium–activated potassium channel in these cells.\textsuperscript{42}

The second observation in favor of the Na-Ca exchanger is that the induction of increased sodium fluxes in sodium-loaded cells by calcium not only was inhibited by an inhibitor of the Na-Ca exchanger, but also was reversed by it (Figure 6B). Dichelobenzamid is a known inhibitor of the Na-Ca exchanger in heart,\textsuperscript{30} although it does also inhibit calcium channels.\textsuperscript{43} Its action on calcium channels may in fact be a sufficient explanation for its ability to prevent activation of \textsuperscript{22}Na efflux on the addition of calcium (Figure 6A), since verapamil also was able to do this (Figure 2A). However, the ability of dichelobenzamid to block \textsuperscript{22}Na efflux after activation by intracellular calcium contrasts with the inability of verapamil to do this (Figure 3) and provides strong evidence that the Na-Ca exchanger was the actual route of the calcium-dependent sodium fluxes under these conditions.

It is noteworthy that the ability of verapamil to block the activation of \textsuperscript{22}Na efflux on calcium addition to sodium-loaded cells is only good at relatively low levels of added calcium. Two factors could contribute to this effect. First, the inhibitory action of calcium antagonists such as verapamil on calcium channels is antagonized by increases in extracellular calcium, both through a decrease in the fraction of inhibited channels and through an increase in calcium flux through uninhibited channels.\textsuperscript{33} With this level of calcium and verapamil, we have previously found from measurements of \textsuperscript{54}Mn uptake that calcium channels are almost completely blocked.\textsuperscript{44} On the other hand, the relation between verapamil inhibition and extracellular calcium seen in normal cells (see next article) suggests that not much calcium entry through calcium channels is needed to activate the exchanger. Second, it is possible that a limited amount of calcium influx can occur through the Na-Ca exchanger in the absence of activation by intracellular calcium if the driving force for calcium entry is high enough, resulting in self-catalysis of the exchange reaction.

Our data further indicate that under the conditions used here the mode of \textsuperscript{22}Na movement through the exchanger could be by Na-Na exchange directly, rather than by Na-Ca exchange. We observed that the \textsuperscript{22}Na efflux induced by intracellular calcium did not require extracellular calcium (Figure 3) but did require extracellular sodium (Figure 4). There is thus no requirement here for even a catalytic amount of calcium to cycle across the membrane: the sodium fluxes cannot be accounted for by Na-Ca exchange followed by Ca-Na exchange. More precisely, Na-Ca exchange cycling could be occurring, but if it is, then Na-Na exchange cycling substitutes in full when the extracellular calcium is removed.

A regulatory role for intracellular calcium has already been recognized for the Na-Ca exchanger of heart\textsuperscript{16–21} as well as squid axon where it was first observed.\textsuperscript{9,10,17} In squid axon, intracellular calcium is needed to move extracellular calcium into the cell by the exchanger.\textsuperscript{9,17} Also, Na-Na exchange activated by intracellular calcium has been observed in this tissue, along with a calcium-independent Na-Na exchange.\textsuperscript{10} We also observed both an intracellular calcium–dependent component and an intracellular calcium–
independent component of $^{22}\text{Na}$ efflux in heart cells (Figure 2). The level of intracellular calcium needed to half-activate Na-Ca exchange in squid axon was similar to that needed to half-activate Na-Na exchange. Thus, intracellular calcium appears to similarly regulate both Na$_i$-Ca$_o$ exchange and Na$_o$-Na$_i$ exchange. DiPolo and Beauge concluded that the intracellular calcium–dependent Na-Na exchange was a mode of operation of the Na-Ca exchanger, and we concur, based on the evidence presented. However, we would also like to propose that the magnitude of intracellular calcium–dependent Na-Na exchange reflects the state of intrinsic activity of the exchanger, for any given values of intracellular and extracellular sodium and calcium. By activity we mean the ability of the exchanger to carry ions, whether sodium or calcium. Whether the carried ions would be sodium or calcium would depend on their intracellular and extracellular concentrations and the membrane potential, but we emphasize that the regulatory site for intracellular calcium appears to control whether the exchanger can carry ions at all, that is, whether it is active.

In the following article we show that the magnitude of intracellular calcium–dependent Na-Na exchange is very low in cells at rest with normal levels of sodium and that large calcium-dependent sodium fluxes, apparently mediated by the Na-Ca exchanger, are induced by electrical stimulation. If the magnitude of the basal calcium-dependent exchange does indeed reflect the degree of activation of the Na-Ca exchanger, then this would imply that the Na-Ca exchanger in these cells is inactive until the cell is stimulated to beat. This postulate about the significance of intracellular calcium–dependent Na-Na exchange thus has important implications for excitation–contraction coupling.

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