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A constraint on possible stoichiometries of myocardial sodium-calcium exchange

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Summary

We have examined the sodium-calcium exchange stoichiometry in Langendorff-perfused rabbit hearts using gamma-emitting tracers under conditions of sodium pump inhibition. Following a 60-min perfusion with 10^{-5} acetylthiocholine, and extracellular concentrations $[Na]_o = 70$ mM and $[Ca]_o = 300$ μ M, intracellular sodium rose to 59.2 mM. At this point an increase in extracellular calcium $[Ca]_o = 1.52$ mM caused a net efflux of sodium, but an increase in sodium $[Na]_o = 105$ mM caused no measurable change. When sodium and calcium were simultaneously increased according to the ratio $[Na]_o^n/[Ca]_o = [Na]_o'^n/[Ca]_o'$, a sodium efflux is observed when $n = 4$, but not when $n = 3$. These results are consistent with an exchange stoichiometry of 3 Na^+ for each Ca^{2+} ion, but not values of 4 or more.

Keywords

myocardial cation regulation; isolated perfused rabbit hearts; transmembrane ion transport; sodium-calcium exchange, stoichiometry

Introduction

It is hypothesized that sodium-calcium exchange is a plasma membrane process in which the transport of a calcium ion is obligatorily coupled to the counter transport of two or more sodium ions. The simplest analysis of this process allows exchange to be represented by

where i and o designate intracellular and extracellular ions, respectively, and r is a stoichiometric constant known as the exchange ratio or coupling constant. The exchange ratio has not been precisely determined in whole cardiac muscle. Although the earliest data (2,17) were compatible with a stoichiometry of 2 Na^+ to 1 Ca^{2+} , it is generally agreed that exchange is electrogenic and hence, the ratio must have a value greater than 2 (3,16). Estimates as high as 4 have recently been reported in a study of sarcolemmal vesicles (11), and theoretical arguments for a ratio of at least 4 have been advanced (12). Most recently, Reeves and Hale (15) applied changes in membrane potential and sodium concentrations to canine sarcolemmal vesicles; their measurement of calcium ion fluxes at varied transmembrane potentials indicated that the exchange ratio was 3.

We have examined the exchange stoichiometry in the intact rabbit heart using tracer methods based on the following approach. Equation (1) implies that exchange transport is governed by $\Delta\tilde{\mu}_{\text{Ca}}$ and $\Delta\tilde{\mu}_{\text{Na}}$, the transmembrane electrochemical potential differences for calcium and sodium, and the stoichiometric constant r . Thus, the driving force $\tilde{A}$, is given by

$$\tilde{A} = \Delta\tilde{\mu}_{\text{Ca}} - r\Delta\tilde{\mu}_{\text{Na}}, \quad (2)$$

and we define equilibrium to be the condition when $A = 0$ and no net ion transport occurs via exchange.

It follows from Eq. (2) that, given a system at equilibrium with extracellular activities of $[\text{Ca}]_o$ and $[\text{Na}]_o$ it should be possible to bring about a new equilibrium by appropriately changing these activities to new values, $[\text{Ca}]'_o$, and $[\text{Na}]'_o$. If these changes are made such that

$$\frac{[[\text{Na}]_o]^n}{[\text{Ca}]_o} = \frac{[[\text{Na}]'_o]^n}{[\text{Ca}]'_o} \quad (3)$$

where $n = r$, the new equilibrium may be established without changes in intracellular activity. However, if extracellular activities are changed such that $n \neq r$, the system will not remain at equilibrium and changes in intracellular ion concentrations should occur (see Appendix).

We have found that increasing extracellular sodium and calcium activities according to Eq. (3) with $n = 4$ results in a significant net efflux of sodium. No efflux occurs with $n = 3$. This suggests that the value of the stoichiometric constant for sodium-calcium exchange is less than 4. This approach did not resolve a lower bound for the stoichiometry.

Methods

Isolated heart preparation

White New Zealand rabbits were given 200 mg pentobarbital and 500 U heparin via ear vein. The hearts were rapidly excised, briefly rinsed in physiologic perfusate (see composition below), and suspended from a vertical cannula tied into the ascending aorta. The cannula was connected to the bottom end of a ca. 9.5 mm diameter “T” connecting tube which served as a bubble trap. The top end was connected to a mercury manometer.

A multi-channel Gilson Minipuls peristaltic pump was set to maintain simultaneously a constant flow of 24–25 cm³/min through each of three independent perfusate flow circuits. Control valves directed the flow from one of these circuits into the bubble trap while flow through the other circuits was recycled. Effluent from the heart was discarded. The volume of perfusate in the assembly between the valves and the heart was 4.5 cm³.

Protocol

Each experiment involved a set-up period and two or three subsequent periods, as described in Table 1.

The hearts were perfused at room temperature (21–22 °C), using an ordinary physiological perfusate without choline or isotopic tracer (Solution 1, Table 1). The hearts typically beat about 30 times per minute. The perfusion pressure was typically 30–40 mm Hg corresponding to a specific perfusion rate between 3 and 4 cm³ g⁻¹ min⁻¹.

Perfusates used in the equilibration period contained Na^+ 70 mM, choline⁺ 75 mM, Ca^{2+} 0.3 mM, and the gamma-emitting tracers ^{22}Na and $^{58}\text{CoEDTA}$. This 30-min period allowed tissue sodium specific activity to equilibrate and $^{58}\text{CoEDTA}$ to distribute uniformly throughout the extracellular space (see Discussion). At the beginning of the accumulation period, 10^{-5} mol/l of acetylcholine (ACh) was added to the perfusate. After 1 h the hearts were either analyzed as described below for control values, or subjected to a 10-min intervention period and then analyzed.

Solutions

A stock solution prepared weekly, consisted (in mM) of NaHCO_3 24, NaCl 5.6, NaH_2PO_4 0.4, MgCl_2 1.0, KCl 5.3, and KCoEDTA 0.1 in distilled water. All additional components were added immediately prior to each experiment. Solutions were bubbled with 95 % O_2 /5 % CO_2 bringing the pH to 7.35 ± 0.05 during preparation and throughout each experiment. The “physiological” perfusate used during set up was prepared by adding dextrose 1.0 g/l, NaCl 115 mmol/l, and CaCl_2 1.8 mmol/l to the stock solution.

The following method for preparing other perfusates was used in order to achieve, as nearly as possible, constant specific activities of ^{22}Na and $^{58}\text{CoEDTA}$ in the various perfusates throughout each experiment. Dextrose, 2.8 g, was added to 2800 ml stock along with sufficient $^{58}\text{CoEDTA}$ to yield 1000–3000 cpm per cm^3 . For the periods of equilibration and accumulation, 2400 ml of the above was measured into a flask and solutes were added to yield the following final concentrations: NaCl 70 mM, Choline Cl 75 mM, and CaCl_2 0.3 mM. The remaining 400 ml was made up to NaCl 105 mM, Choline Cl 40 mM, for use during the intervention period. The experiment type dictated the amount of CaCl_2 to be added to this portion. Since the final sodium concentration was 70 mM in the 2400 ml portion, and 105 mM in the 400 ml portion, the ratio of the total molar quantity of sodium in each portion was four to one. Accordingly, four volumes of a $^{22}\text{NaCl}$ solution was added by pipette into the 2400 ml portion, and one volume into the 400 ml portion to yield between 1000–3000 cpm of ^{22}Na in each milliliter of perfusate. The molar quantity of NaCl added in this step was negligible.

The sodium ion activity in the 400 ml portion was confirmed to be 1.5 times that of the 2400 ml portion using a sodium ion selective electrode (Orion Research). Calcium ion activity proved to be directly proportional to concentration in these solutions using a calcium ion selective electrode (Orion Research). The ^{22}Na activity in each perfusate was measured for each experiment and the calculated specific activities were never found to differ by more than 1.5 %. Osmolarity differences were always less than 10 mOsm as measured with an Osmette A (Precision Scientific).

Sodium-22 and Cobalt-58 were obtained from New England Nuclear, Boston. Cobalt-57, used in the intervention period on several occasions, was obtained from Amersham. $^{58}\text{CoEDTA}$ and $^{57}\text{CoEDTA}$ were prepared as by Bridge et al. (5). Acetylcholine (ACh) was prepared as by Bridge et al. (6), and dissolved in undenatured ethanol to yield a 10^{-2} M solution. 1 ml of this was added to 11 of perfusate when needed to yield 10^{-5} M ACh in the perfusate.

Analysis

At the termination of an experiment, the hearts were removed from the cannula and the base cut off. The right ventricular wall was cut from the septum and the remaining left ventricular tissue was divided longitudinally into three parts of roughly equal size. All four pieces (including the RV wall) were then lightly blotted and individually weighed to the nearest 0.1 mg in a small aluminum foil boat of known weight. These were dried for 24 h at 100 °C and

reweighed to determine the tissue water content. Drying of these samples for an additional 10 h caused less than 0.2 % additional weight loss. Isotope activities were measured in a well-type NaI crystal and analyzed with a Tracor-Northern NS-700 pulse height analyzer to an accuracy of ± 0.5 % for ^{22}Na and ± 1.0 % for ^{58}Co and ^{57}Co . 1-ml aliquots of each perfusate and pure samples of each isotope were counted with each set of tissue pieces.

Calculations

Intracellular sodium concentration $[\text{Na}]_i$ was calculated using the known perfusate sodium concentration, $[\text{Na}]_o$, and the following equations:

$$[\text{Na}]_i = \frac{N_{\text{IC}}}{H_2O_{\text{IC}}} = \frac{N_{\text{T}} - N_{\text{EC}}}{H_2O_{\text{T}} - H_2O_{\text{EC}}}$$

$$N_{\text{T}} = \text{total tissue sodium (mol g}^{-1} \text{ tissue)} = \frac{^{22}\text{Na (cpm g}^{-1} \text{ tissue)}}{^{22}\text{Na (cpm l}^{-1} \text{ perfusate)}} \cdot [\text{Na}]_o$$

$$N_{\text{EC}} = \text{extracellular sodium (mol g}^{-1} \text{ tissue)} = H_2O_{\text{EC}} \cdot [\text{Na}]_o$$

$$H_2O_{\text{EC}} = \text{extracellular water (l g}^{-1} \text{ tissue)} = \frac{^{58}\text{Co (cpm g}^{-1} \text{ tissue)}}{^{58}\text{Co (cpm l}^{-1} \text{ perfusate)}}$$

$$H_2O_{\text{T}} = \text{tissue water (l g}^{-1} \text{ tissue)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \cdot \frac{1}{\rho}$$

where $[\]$ designates moles l^{-1} and ρ is the density of water, $10^3 \text{ g} \cdot \text{l}^{-1}$.

The data from each set of left ventricular pieces were weighted according to their contribution to the total wet weight and averaged. The overall mean and standard deviation were calculated from the individual weighted means and subjected to statistical analysis using Cochran's method (20) for independent samples with unequal variances. Results are listed as mean $\pm$ one standard deviation.

Electrophysiological measurements

Conventional glass microelectrodes were drawn from 1.2 mm microfilament glass (A & M Systems Inc., Everett, WA) using a Narishige Pipette Puller. These were filled with 3 M KCl and mounted in a commercial AG-AgCl half cell (A & M Systems Inc., Everett, WA). The half cell was directly connected to the head stage of a differential input electrometer amplifier with an input resistance $\approx 10^{14} \Omega$. The reference input was connected to the bath by means of a chloridized silver wire, and to ground. Electrodes used had tip resistances $\approx 10 \text{ M}\Omega$ and tip potentials less than 5 mV.

Recordings were initially made from the surface of a perfused heart using microelectrode tips suspended from the end of a coiled 0.127 mm silver wire. Although accurate-appearing action potentials were readily obtained for long periods, it was not possible to obtain adequate recordings following exposure to AcS. These measurements were obtainable with lower calcium concentrations (4). Therefore, papillary muscles were removed from right ventricles and pinned in a well. Perfusate flowed around the muscle at a rate which replaced the well volume four times each minute.

Results

Intracellular sodium accumulation during perfusion with AcS

During the equilibration period (30 min) all hearts were perfused with 70 mM Na^+ and 0.3 mM Ca^{2+} . The beginning of the accumulation period was marked by the addition of AcS to the perfusate, and is designated as time $t = 0$. Tissue concentrations were measured at $t = 20$, 50 and 60 min. These data show an accumulation of intracellular sodium following the addition of AcS (Table 2). In accordance with much previously reported experience with sodium pump inhibitors, intracellular water content and total tissue water content did not

change. From the data given in Tables 1 and 2, one can calculate the fraction of total tissue water in either the intracellular or the extracellular compartment. Noting however, that experimental uncertainties are amplified severalfold in such calculations, we found no evidence for shifts in water content between the intracellular and extracellular compartments during the course of an experiment.

Although no differences in total tissue water were measured between ventricles, intracellular water content was consistently lower, and intracellular sodium concentration was consistently higher in right ventricles. Furthermore, data on intracellular $[Na]_i$ and water in RV exhibited much greater specimen to specimen variation than LV.

Effects of single ion concentration changes

Changes in perfusate composition were made after 60 min of perfusion with AcS. Results obtained after these interventions are shown in Table 3. Values prior to intervention are included as experiment type A for reference.

An increase in perfusate $[Na]_o$ from 70 mM to 105 mM (experiment type E) caused no significant change in $[Na]_i$ concentration over the 10-min intervention period. In contrast, an increased $[Ca]_o$ over this same interval caused a substantial sodium efflux (experiment types B and C). The magnitude of this change was similar regardless of whether $[Ca]_o$ was increased 2.3-fold or 5.1-fold, although only experiments of the latter type were repeated enough times for statistical validation ($p < 0.025$). The efflux was demonstrable in both left and right ventricular myocardium, and no concomitant changes in water contents were seen. A 5.1 fold increase in $[Mg]_o$ failed to cause a similar efflux of sodium (experiment type D).

Effects of simultaneous changes in sodium and calcium concentrations

Simultaneous step increases in both calcium and sodium were made according to the manner described in Methods. In all such experiments, sodium concentration was increased from 70 mM to 105 mM. Increases in calcium concentration were made as indicated in Table 3 (experiment types F-I).

The data in Table 3 show that a significant net sodium efflux occurs when external sodium is raised from 70 mM to 105 mM and calcium is simultaneously raised from 0.3 mM to 1.52 mM (experiment type I, $p < 0.001$). This efflux is suggested in the right ventricular data as well; however, the differences are not statistically significant. No significant change in intracellular sodium concentration is evident when calcium is raised to lesser values. This is in contrast with experiments as described above in which sodium concentration was not increased, yet raising the external calcium concentration to 0.68 mM was apparently sufficient to cause a sodium efflux.

Changes in total tissue water content or in the distribution of water between intracellular and extracellular compartments were not significant. Attempts to reproduce these results using a substitute for sodium chloride other than choline chloride were unsuccessful. Both the use of sucrose (for constant osmolarity) and the use of magnesium chloride/sucrose combination (for constant osmolarity and chloride concentration) caused large changes in intracellular water content and a tremendous degree of data scatter, despite correction of activity coefficients for changes in ionic strength.

Accessibility of the extracellular space after exposure to AcS

$^{57}\text{CoEDTA}$ was added only to perfusates used for intervention periods in 11 experiments of various types. The distribution of this tracer compared to the distribution of $^{58}\text{CoEDTA}$ at the end of each experiment thereby reveals the relative accessibility of the extracellular

space during intervention period (10 min) versus accumulation periods (60 min). ^{57}Co distribution – ^{58}Co distribution $\times 100\%$.

$^{57}\text{CoEDTA}$ distributed throughout $86.0 \pm 7.1\%$ of the $^{58}\text{CoEDTA}$ space after 10 min in left ventricles in which choline was used as a sodium substitute. This distribution did not increase with the duration of the intervention, being 83.7% at 15 min (one experiment) and $83.9 \pm 6.4\%$ at 20 min (three experiments). In contrast, the distributions of $^{57}\text{CoEDTA}$ and $^{58}\text{CoEDTA}$ in right ventricles were comparable in all cases, as were the distributions for both ventricles in all sucrose experiments. Thus the restricted access of the extracellular space is a source of error when calculating $[\text{Na}]_i$ in data from left ventricular tissue in which choline partially substitutes for sodium. Curiously, the distributions of both tracers were nearly equal when sucrose was used as a sodium substitute instead of choline. This correlates with a very flaccid condition of the heart, whereas choline-substituted hearts have considerable tone. This experiment demonstrated the ability of $^{57}\text{CoEDTA}$ to fully distribute throughout left ventricular extracellular space under some conditions, but we have not investigated the phenomenon further to explain precisely the reason for the difference between sucrose and choline effects.

If we define the $^{58}\text{CoEDTA}$ space as the extracellular space of the tissue, and assume that sodium concentration remains 70 mM in the portion of extracellular space that is inaccessible to $^{57}\text{CoEDTA}$, a worst case error introduced into the calculation of intracellular sodium may be estimated. When perfusate sodium concentration is raised from 70 mM to 105 mM, inaccessibility of $14.0 \pm 7.1\%$ of the extracellular space leads to an overestimate of the average extracellular sodium concentration by $14.0 \pm 7.1\% \times (105 - 70 \text{ mM}) = 4.9 \pm 2.5 \text{ mM}$. The calculated underestimation of intracellular sodium concentration is therefore about $2.7 \pm 1.4 \text{ mM}$ since intracellular water content is typically 1.8-fold larger than extracellular water content in these preparations.

By implication, a maximal value of $2.7 \pm 1.4 \text{ mM}$ should therefore be added to all left ventricular data of Table 3 in which sodium concentration was increased in the intervention period (experiment types E-I). Such a correction appears to have no material effect on the experimental results, however. In experiments in which Na_o is increased, for example, adding the value 2.7 mM to the result, yields a statistically insignificant suggestion of net sodium influx (experiment type E). In experiments where $[\text{Na}_o]$ is increased to 105 mM and $[\text{Ca}_o]$ is increased to 0.45, 0.68, or 1.01 mM, the correction has the effect of negating any suggestion of net sodium efflux during the intervention period. The net loss of sodium which occurred when $[\text{Ca}_o]$ was increased to 1.52 mM remains significant at the $p < 0.01$ level even when a value of $50.7 \pm 2.4 \text{ mM}$ is used as a corrected concentration ($48.0 \pm$ added to 2.7 ± 1.4).

A second source of error is recognized if we assume that the net sodium losses observed arise from only 86 % of the total intracellular space. Correction of such an error, however, serves only to amplify the measured sodium losses and partially offset the error discussed above. Since our conclusions are not materially affected by even a worst case treatment of the first error, no further consideration of this second error is required.

Membrane potentials, E_m

Sodium efflux in the preceding experiments could conceivably occur via Na/Ca exchange if the exchange is electrogenic and the interventions caused shifts of the membrane potential to relatively more positive values. We cannot, however, contrive any plausible physiological events which could shift the membrane potential in a positive direction in only those experiments in which net sodium efflux was observed.

We nevertheless attempted to measure membrane potentials in superfused papillary muscles during an intervention. We found that continuous impalements were virtually impossible to maintain with $[Ca]_o$ of 0.3 mM in the presence of AcS (these measurements were somewhat easier in 0.05 mM $[Ca]_o$, (4)).

Figure 1 represents the only recording obtained that we found useful. In a papillary muscle from which resting membrane potentials had been measured at -78 mV, we measured an E_m of -52 mV after 17 min exposure to AcS. Continuous impalement was maintained as simultaneous increases were made in the perfusate concentrations of sodium (70 mM to 105 mM) and calcium (0.3 mM to 1.52 mM). After 10 min, the measured E_m had increased to -57 mV. A step increase in the concentration of potassium (5.4 mM to 8.0 mM) resulted in depolarization to -50 mV. Following return to 5.4 mM potassium, the membrane repolarized to -60 mV.

In other experiments, we observed membrane potentials for lesser periods following intervention and found both slight increases as well as slight decreases in membrane potential, but in no case was there a change greater than 5 mV.

In two experiments, we observed E_m following an increase in calcium alone from 0.3 mM to 1.52 mM. In the first case, there was a 4 mV hyperpolarization after 10 min, and in the second case, a 1 mV depolarization after 5 min.

We conclude that this preparation could not be adequately studied electrophysiologically, but that no evidence could be obtained to suggest that sodium efflux in our experiments could be explained by changes in membrane potential. Neither could any change be detected which might be attributed to stimulation of electrogenic exchange.

Discussion

The preparation

The arterially perfused mammalian heart offers several advantages in experiments of the foregoing type. Firstly, it is free of artifacts resulting from crushed tissue or cut edges. Secondly, there is rapid exchange of perfusate components between the intravascular and interstitial compartments due to high capillary permeabilities and short diffusion paths. Thirdly, the high surface area to volume ratio enables membrane transport systems to have easily detectable effects on intracellular ion content. Finally, there exists no ambiguity regarding orientation of the membrane.

Nevertheless, we have shown that under conditions of sodium pump inhibition and subsequent rigor, access to the extracellular compartment may be somewhat compromised. It was found to be advantageous to process and analyze right and left ventricular tissue separately since there exist substantial differences in composition as well as reproducibility. Having done this, we found that $^{57}\text{CoEDTA}$ distributes through only 86 % of the extracellular space in left ventricular myocardium.

This incomplete equilibration of $^{57}\text{CoEDTA}$ is not explained by postulating that $^{58}\text{CoEDTA}$ leaked into the intracellular compartment, since water contents estimated with extracellular $^{58}\text{CoEDTA}$ remained constant throughout the experiments. Furthermore, in right ventricles, distribution of $^{58}\text{CoEDTA}$ was equivalent to that of $^{57}\text{CoEDTA}$. Finally, experience with $^{58}\text{CoEDTA}$ as an extracellular marker has shown that when unchelated ^{58}Co is detectable in perfusates by thin layer chromatography, distortion of the extracellular space measurements is quite large and obvious (Bridge, unpublished data). The portion of the extracellular space which is inaccessible to $^{57}\text{CoEDTA}$ does not change

between 10 and 20 min. This, and the right ventricular data, indicate that distribution of this second extracellular marker was not limited by diffusion.

The most likely explanation for our findings is that myocardial rigor compromises perfusion of the left ventricular subendocardium. This conclusion is supported by data from hearts in which sucrose instead of choline was used as a sodium and osmotic substitute. In these experiments, the myocardium remained flaccid, and the marker distributions were similar in both right and left ventricles. These difficulties notwithstanding, we have shown that errors arising from this problem are partially offsetting. Moreover, our conclusions are supported by a worst case treatment of the data.

Assumptions

The model of sodium-calcium exchange under examination in these experiments is described by the following assumptions:

1. Exchange transport is a sarcolemmal process specific for sodium and calcium ions.
2. Net transport of either Na^+ or Ca^{2+} is obligatorily coupled to net transport of the other ion species in the opposite direction.
3. The direction of transport is governed exclusively by the relative magnitudes of the transmembrane electrochemical potential differences for these ions.
4. Exchange proceeds according to a fixed stoichiometry denoting the scalar ratio of sodium and calcium fluxes via exchange.

These conditions lead to Eq. (2) in the Introduction and are discussed in reviews of the subject (e.g. [16]). In addition, our analysis requires the assumption that exchange is at equilibrium prior to the beginning of the interventions. We offer the following justification for this assumption.

Any competing sodium flux due to the sodium pump has been minimized by concentrations of AcS used in these experiments (10). In earlier work (4) it was shown that stimulation of sodium efflux via exchange is followed by calcium rather than potassium accumulation. Moreover, the accumulation of calcium which occurred upon such stimulation was dramatic. Deitmer and Ellis (10) and Chapman, Coray and McGuigan (8) also showed that in special circumstances raising Ca_o , the extracellular calcium activity, could result in a diminution of Na_i , intracellular sodium activity. However, the obligatory nature of the coupling and the degree of fixity of the ratio, n , of Na^+ ions to Ca^{2+} ions remained in doubt. While sodium-calcium exchange appears to be the predominating regulator of both intracellular calcium and sodium under these conditions, the situation *in perfuso* is not as simple as for the vesicle studies of Reeves and Hale (15). Chapman (9) discusses stoichiometry varying from 3–4. There are at least two processes which probably prevent complete equilibration, and may affect the apparent stoichiometry.

Firstly, a sarcolemmal calcium pump may exist in this preparation as has been demonstrated in sarcolemmal vesicles from dog myocardium (7). However, we note that a small calcium pump flux would result in immeasurably small differences between the measured and equilibrium intracellular sodium concentrations due to the minute quantities of calcium involved.

Secondly, passive sodium influx is undoubtedly occurring at the point of intervention. The magnitude of this flux is likely to be diminished by partial depolarization of the membrane and elevation of intracellular sodium. These two processes, acting either alone or in concert, may be expected to bring about conditions in which $\Delta\tilde{\mu}_{\text{Ca}} > r\Delta\tilde{\mu}_{\text{Na}}$, thus causing exchange

to operate in a sodium efflux mode. Dietmer and Ellis (10) have suggested that exchange operates in this manner to reduce N_i in a strophanthidin-poisoned Purkinje fiber system. Similar phenomena, the consequences of our equilibrium assumption, and its potential errors, are discussed below.

Interpretation of results

Step increases in extracellular calcium activity were seen to cause a net efflux of sodium against an electrochemical gradient. This is expressible as an increase in the $\Delta\tilde{\mu}_{Ca}$ term of Eq. (2). These results confirm those of Bridge and Bassingthwaighte (4) who used atomic absorption methodology and 40-fold larger step increases in calcium concentration.

Step increases in perfusate $[Na]$ may be expressed as an increase in the $\Delta\tilde{\mu}_{Na}$ term of Eq. (2), which yields a driving force in the direction of sodium influx. Chapman (9) saw increases in Na_i in ferrets in which the NaKATPase was not blocked. However, further increases in intracellular sodium were not demonstrated in our experiments, in which the NaKATPase was blocked and in which Na_i was already exceedingly high at the time of the intervention. We suspect that further intracellular sodium accumulation is limited by the low concentration of intracellular free calcium limiting the amounts available for exchange, and further, that re-establishment of equilibrium might be accomplished by exchanging such small amounts of extracellular sodium for intracellular calcium that the increased Na_i could not be measured.

The effects of choline used in these experiments must be considered since choline has been shown to facilitate some exchange related phenomena in other preparations (13,19). In our experiments, choline is reduced from 75 mM to 40 mM whenever sodium is increased from 70 mM to 105 mM. Yet, this intervention alone does not result in detectable sodium flux (experiments of type E, Table 3) nor any change in membrane potential (Fig. 1). Therefore we consider it highly unlikely that the use of choline interferes with the conclusions we draw from our data.

When performing experiments with simultaneous increases in both $[Na]_o$ and $[Ca]_o$, final concentrations were selected so as to test specific stoichiometries according to the analysis below. It is seen that a calcium increase from 0.3 mM to 0.68 mM while also raising $[Na]_o$ from 70 to 105 mM (experiment type G, Table 3) does not cause any significant sodium efflux, in contrast to experiments in which only the calcium concentration was changed (type B). Neither could a sodium efflux be demonstrated by raising calcium to 1.01 mM (type H) while raising $[Na]_o$. Thus, we cannot draw specific conclusions about the exchange stoichiometry from these particular experiments.

In contrast, a dramatic efflux of sodium is observed when calcium is raised to 1.52 mM (experiment type I). We must consider two possible explanations for this. First, if the equilibrium assumption discussed above is valid, it can be shown for experiments wherein a sodium efflux is demonstrated that the log ratio of the calcium and sodium increments are related to r by

$$\frac{\log ([Ca]'_o/[Ca]_o)}{\log ([Na]'_o/[Na]_o)} > r \quad (4)$$

where primes indicate values following intervention (see Appendix for derivation). These data therefore indicate that $r < 4$. This analysis does not apply to experiments in which $[Ca]_o$ is increased to 1.01 mM or less since net sodium fluxes were not observed in these experiments.

Alternatively, if the equilibrium assumption is not valid, exchange can be expected to be operating in a sodium efflux mode prior to intervention. An intervention resulting in an observable sodium efflux therefore may have simply acted to unmask or accelerate this process. We have argued above that the system must be initially close to equilibrium in view of the large capacity and transport rate of the exchange in comparison to competing fluxes. Acceleration of the exchange process must necessarily bring the system still closer to equilibrium. It seems very unlikely that such a process could account for an efflux of approximately 20 % of total intracellular sodium.

The constraint suggested by our data, namely that $r < 4$, is in complete agreement with the stoichiometry of 3 reported for cardiac sarcolemmal vesicles by Reeves and Hale (15), Caroni and Carafoli (7), and Pitts (14). Moreover, a test of 3:1 stoichiometry in our system (experimental type H) fails to contradict their result. Several features critically distinguish the work of these authors from the experiments reported herein. Most importantly, our experiments were performed in perfused intact heart. In addition, sodium movements were measured in response to sodium and calcium concentration changes. Finally, we used choline instead of lithium as a sodium substitute and added AcS to inhibit the sodium pump.

The direct measurements of intracellular sodium and calcium activities by Sheu and Fozzard (18) also weigh against a 4:1 stoichiometry. Although their measurements are consistent with values of 3 or more (1), the demonstration in the resting fiber that the sodium and calcium gradients are related by a factor of ≈ 2.5 reduces the theoretical requirements for a 4:1 stoichiometry advanced by Mullins (12).

The further evidence we offer here against a 4:1 stoichiometry is admittedly very model dependent, yet the assumptions and model used appear to yield satisfactorily consistent thermodynamic information whether applied to vesicles, ion selective electrodes, or whole heart.

Appendix

As defined by Guggenheim (1929), the electrochemical potential for an ions is given by $\tilde{\mu} = \mu^\circ + RT \ln a + ZF\psi$, where μ° is a standard state chemical potential, a is the ionic activity, z is the valence, ψ is the electrostatic potential, and R , T , and F have their usual meanings. From Eq. (1), we may write the equilibrium relationship as follows:

$$r\tilde{\mu}_i^{\text{Na}} + \tilde{\mu}_o^{\text{Ca}} = r\tilde{\mu}_o^{\text{Na}} + \tilde{\mu}_i^{\text{Ca}} \quad (7)$$

Defining the transmembrane electrochemical potential difference to be $\Delta\tilde{\mu} = \Delta\tilde{\mu}_i - \Delta\tilde{\mu}_o$, Eq. (7) condenses to:

$$\Delta\tilde{\mu}^{\text{Ca}} = r\Delta\tilde{\mu}^{\text{Na}} \quad (8)$$

Since a driving force in the direction of sodium efflux implies that

$$\Delta\tilde{\mu}^{\text{Ca}} > r\Delta\tilde{\mu}^{\text{Na}} \quad (9)$$

an intervention applied to a system at equilibrium which causes sodium efflux, must bring about changes in the $\Delta\tilde{\mu}$ terms such that

$$\Delta\tilde{\mu}'^{\text{Ca}} - \Delta\tilde{\mu}^{\text{Ca}} > r(\Delta\tilde{\mu}'^{\text{Na}} - \Delta\tilde{\mu}^{\text{Na}}) \quad (10)$$

where primes indicate values at the point of intervention.

Equation (10) may be simplified by assuming that the intervention is accomplished instantaneously, and applying it at a point prior to any secondary changes in intracellular concentrations or membrane potential. Accordingly, the term $\Delta\tilde{\mu}' - \Delta\tilde{\mu}$ for each ion simplifies to $RT \ln ([A']_o/[A]_o)$. Equation (10) may then be written, after rearrangement, as

$$\frac{\ln ([Ca']_o/[Ca]_o)}{\ln ([Na']_o/[Na]_o)} > \mathbf{r} \quad (11)$$

showing that a least upper bound for $\mathbf{r}$ may be found by finding, for a given $\Delta[Na]_o$, the least $\Delta[Ca]_o$ causing net sodium efflux.

In the event that the equilibrium assumption is not valid prior to intervention, Eq. (8) becomes

$$\Delta\tilde{\mu}^{Ca} > \mathbf{r} \Delta\tilde{\mu}^{Na}. \quad (12)$$

Applying this through the preceding analysis one sees that the least $\Delta[Ca]_o$ causing a net sodium efflux is artificially high using the equilibrium assumption. Consequently, the least upper bound for $\mathbf{r}$ would be lower.

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Figure 1.

Recording of intracellular potential in left ventricular myocardium perfused with Krebs-Ringer bicarbonate, with solution changed to include 10^{-5} M acetylcholine 3 minutes before the start of the figure. Changes of the Ca and Na concentrations caused minor potential shifts but raising K_o caused depolarization by 10 mV to -50 mV.

Table 1

	Stock soln.	Period			
		Set up		Equilibration	Accumulation Intervention
Duration	–	3–5 min	30 min	60 min	10 min
AcS	No	No	No	Yes	Yes
Tracers	No	No	Yes	Yes	Yes
Na ⁺	30.0	145.0		70.0	105.0
Choline ⁺	0	0		75.0	40.0
K ⁺	5.4	5.4		5.4	5.4
Ca ⁺⁺	0	1.8		0.3	0.3–1.52
Mg ⁺⁺	1.0	1.0		1.0	
Cl [–]	12.9	131.5		128.5	128.5**
HCO ₃ [–]	24.0	24.0		24.0	24.0
H ₂ PO ₄ [–]	0.4	0.4		0.4	0.4
CoEDTA [–]	0.1	0.1		0.1	0.1
Dextrose	0	5.5		5.5	5.5

AcS = Acetylstrophanthidin;

** changes slightly with added calcium

Table 2

Tissue water and sodium content in the presence of AcS.

Time (min)	n	Tissue water (% wet weight)		Intracellular water (mg/mg dry weight)		[Na] _i (mM)	
		LV	RV	LV	RV	LV	RV
0	2	83.9 ± 1.2	84.4 ± 0.2	2.92 ± 0.03	2.45 ± 0.27	22.6 ± 2.12	24.9 ± 5.0
20	1	81.8	83.3	2.95	2.39	42.0	55.3
50	2	82.8 ± 0.2	82.7 ± 0.1	3.06 ± 0.06	2.24 ± 0.23	51.8 ± 1.1	60.1 ± 12.5
60	6	82.3 ± 0.9	81.9 ± 2.0	2.94 ± 0.19	2.04 ± 0.54	59.2 ± 3.0	67.4 ± 9.4

n = number of preparations; LV = left ventricle, RV = right ventricle

Table 3

Tissue water and sodium contents following 10-min interventions.

Type	n	Intervention conditions		Time(min)	$\Delta\tilde{\mu}_{\text{Cs}}:\Delta\tilde{\mu}_{\text{Na}}$	Tissue water (% wet weight)		Intracellular water (mg/mg dry wet)		[Na] _i (mM)	
		[Ca]mM	[Na]mM			LV	RV	LV	RV	LV	RV
A	6	0.3	70	0	-	82.3 ± 0.9	81.9 ± 2.0	2.94 ± 0.19	2.04 ± 0.54	59.2 ± 3.0	67.4 ± 9.4
B	2	0.68	70	10	-	81.8 ± 0.8	80.4 ± 1.1	3.12 ± 0.01	2.22 ± 0.19	52.7 ± 2.7	54.2 ± 3.4
C	4	1.52	70	10	-	82.5 ± 0.5	82.5 ± 0.7	3.07 ± 0.19	2.31 ± 0.38	52.6 ± 2.6 ²⁾	49.1 ± 3.7 ³⁾
D	2	0.3 [*]	70	10	-	83.5 ± 0.1	83.7 ± 1.3	3.17 ± 0.01	2.24 ± 0.14	64.1 ± 7.8	66.4 ± 3.2
E	4	0.3	105	10	-	82.9 ± 0.6	84.2 ± 0.8	2.96 ± 0.18	2.38 ± 0.46	58.8 ± 2.7	67.1 ± 6.8
F	4	0.45	105	10	1:1	83.3 ± 0.1	84.3 ± 0.5	3.19 ± 0.10	2.77 ± 0.20	56.8 ± 4.3	68.4 ± 6.6
G	4	0.68	105	10	2:1	82.6 ± 0.3	83.0 ± 0.8	3.04 ± 0.11	2.49 ± 0.14	56.8 ± 4.1	61.6 ± 3.6
H	6	1.01	105	10	3:1	82.5 ± 1.1	82.9 ± 2.1	2.98 ± 0.33	2.32 ± 0.38	56.1 ± 6.5	72.4 ± 13.7
I	4	1.52	105	10	4:1	81.8 ± 0.5	81.8 ± 1.4	2.89 ± 0.05	2.41 ± 0.13	48.0 ± 1.0 ⁴⁾	57.9 ± 4.1 ¹⁾

* [Mg]_o increased from 1.0 mM to 5.1 mM

1) p < 0.1 different from type A result

2) p = .p25

3) p < .025

4) p < .001

Table 4Cobalt 57 distribution ^{*}.

Na substitute	N	LV	RV
Choline	7	86.0 ± 7.1%	101.1 ± 6.7%
Sucrose	4	106.4 ± 7.5%	108.5 ± 6.1%

^{*} after 10-min intervention, expressed as a percentage of ⁵⁸Co distribution