Biochimica et Biophysica Acta, 729 (1983) 123–136
Elsevier Biomedical Press

BBA 71590

DEPENDENCE OF Na^+-Ca^{2+} EXCHANGE AND Ca^{2+}-Ca^{2+} EXCHANGE ON MONOVALENT CATIONS

RONALD F. LEDVORA [a,*] and CSABA HEGYVARY [a,b]

[a] *Department of Physiology and* [b] *Department of Internal Medicine, Rush University, Chicago, IL 60612 (U.S.A.)*

(Received March 30th, 1982)
(Revised manuscript received December 6th, 1982)

Key words: Na^+-Ca^{2+} exchange; Ca^{2+}-Ca^{2+} exchange; Cotransport; (Cardiac sarcolemma)

Two mechanisms of passive Ca^{2+} transport, Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange, were studied using highly-purified dog heart sarcolemmal vesicles. About 80% of the Ca^{2+} accumulated by Na^+-Ca^{2+} exchange or Ca^{2+}-Ca^{2+} exchange could be released as free Ca^{2+}, while up to 20% was probably bound. Na^+-Ca^{2+} exchange was simultaneous, coupled countertransport of Na^+ and Ca^{2+}. The movement of anions during Na^+-Ca^{2+} exchange did not limit the initial rate of Na^+-Ca^{2+} exchange. Na^+-Ca^{2+} exchange was electrogenic, with a reversal potential of about −105 mV. The apparent flux ratio of Na^+-Ca^{2+} exchange was 4 Na^+ : 1 Ca^{2+}. Coupled cation countertransport by the Na^+-Ca^{2+} exchange mechanism required a monovalent cation gradient with the following sequence of ion activation: $Na^+ \gg Li^+ > Cs^+ > K^+ > Rb^+$. In contrast to Na^+-Ca^{2+} exchange, Ca^{2+}-Ca^{2+} exchange did not require a monovalent cation gradient, but required the presence of Ca^{2+} plus a monovalent cation on both sides of the vesicle membrane. The sequence of ion activation of Ca^{2+}-Ca^{2+} exchange was: $K^+ \gg Rb^+ > Na^+ > Li^+ > Cs^+$. Na^+ inhibited Ca^{2+}-Ca^{2+} exchange when Ca^{2+}-Ca^{2+} exchange was supported by another monovalent cation. Both Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange were inhibited, but with different sensitivities, by external $MgCl_2$, quinidine, or verapamil.

Introduction

In excitable tissues, a low intracellular free-Ca^{2+} concentration is maintained by passive and active transport. Two passive Ca^{2+} transport mechanisms, Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange, have been reported in intact squid axons [1–5], in isolated sarcolemmal vesicles from mammalian hearts [6–10], and in nerve or brain synaptosomes [11,12].

Na^+-Ca^{2+} exchange is countertransport of Na^+ and Ca^{2+} driven by a gradient of Na^+. In contrast, Ca^{2+}-Ca^{2+} exchange requires the presence of a monovalent cation on both sides of the membrane, but not a concentration gradient, and it is exchange of internal Ca^{2+} for external Ca^{2+}.

One group of investigators have proposed that Na^+/Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange were mediated by the same carrier mechanism in the squid axon [5]. The main supporting observations were: (1) both exchanges had the same affinity for Ca^{2+}, (2) both exchanges were inhibited by Sr^{2+} or Mn^{2+}, and (3) the Ca^{2+} fluxes did not sum under conditions which were simultaneously opti-

* Present address (address for correspondence): Department of Biological Chemistry, University of Illinois Medical Center, 1853 West Polk Street, Chicago, IL 60612 (U.S.A.).

Abbreviations: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; Mops, 4-morpholinepropanesulfonic acid.

mal for Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange.

In this study, we first confirmed the presence of both Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange in our preparation of highly-purified dog heart sarcolemmal vesicles. We then resolved the type of transport mechanism which is Na^+-Ca^{2+} exchange, and the stoichiometry of Na^+-Ca^{2+} exchange, in the heart sarcolemma. Then, to clarify the issue of whether or not Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange are mediated by a common carrier mechanism, we compared the ion selectivity of Na^+-Ca^{2+} exchange with that of Ca^{2+}-Ca^{2+} exchange and determined the effects of several modifiers of both Ca^{2+} transport activities on the same sarcolemmal preparations.

This work was submitted in partial fulfillment of the requirements of Rush University for the degree of Doctor of Philosophy for R.F.L.

Materials

All chemicals used in these experiments were the highest grade available. Uniformly-labelled [^{3}H]ouabain, $^{86}RbCl$, and $^{45}CaCl_2$ were obtained from Amersham. ATP was obtained from Eastman. Verapamil was obtained from Knoll Pharmaceutical, Whippany, NJ. Quinidine sulfate was obtained from Sigma.

Methods

Vesicle preparation

For all experiments, tightly-sealed sarcolemmal vesicles were prepared from frozen heart ventricles of mongrel dogs.

Thawed ventricular tissue was homogenized in 3 vol. of 0.3 M sucrose, 20 mM Tris-HCl (pH 7.4), and 0.5 mM Tris-EDTA. The crude homogenate was filtered through two layers of gauze. The filtrate was resuspended in two volumes of the same sucrose solution, then centrifuged for 10 min at $8500 \times g$ at 4°C. The supernatant was collected and layered over a 22% (w/v) sucrose solution containing 0.3 M KCl, 0.1 M Tris-HCl, and 50 mM $Na_4P_2O_7$ (pH 7.4), and then centrifuged at $96000 \times g$ for 90 min and at 4°C in a Beckman T-30 rotor. The sarcolemmal membrane at the interface of the two sucrose solutions was collected and washed with three volumes of 160 mM KCl plus 20 mM Mops/Tris-HCl (pH 7.4) by resuspension and centrifugation at $96000 \times g$ for 30 min at 4°C. The sediment enriched in sarcolemmal vesicles was resuspended in an appropriate volume of 160 mM KCl plus 20 mM Mops/Tris-HCl to give a protein concentration of 3–6 mg/ml and was stored at 4°C until used. The vesicle preparations were stable for about 3 weeks, and vesicles less than 2 weeks old were used for all experiments. The discontinuous sucrose density gradient was divided into five fractions (fractions 1–5, in Table I) which were collected separately and assayed for marker enzyme activities.

Intravesicular ion and volume measurements

The intravesicular volume was estimated in several sarcolemmal vesicle preparations by measuring the distribution volumes of [^{3}H]H_2O and of [^{3}H]inulin. In these measurements, the inulin concentration was 20 mM. Inulin binding to the vesicles was minimal at this concentration. Either [^{3}H]H_2O (and 20 mM unlabelled inulin) or 20 mM [^{3}H]inulin (30000–50000 dpm of either) was added to duplicate vesicle samples (0.2 ml). These were stirred and incubated for 15 min at 23°C and then centrifuged for 30 min at about $35000 \times g$ at 4°C in a Sorvall SS-34 rotor. The counts were corrected for quenching, and the distribution volumes of [^{3}H]H_2O and [^{3}H]inulin were calculated from the dilution of the [^{3}H]H_2O dpm and [^{3}H]inulin dpm after incubation. Intravesicular volume was calculated as the difference between the [^{3}H]H_2O distribution space (total water space) and the [^{3}H]inulin distribution (or extravesicular) space.

Sodium and potassium were measured by flame photometry. Calcium was measured by atomic absorption spectroscopy. Vesicle samples were dissolved with 25% (v/v) concentrated HCl prior to ion content measurements. Ion content of vesicles and of reaction mixtures was measured after appropriate dilutions with the commercial diluents (flame photometry) or with double glass-distilled water (atomic absorption spectroscopy). Intravesicular Na^+, K^+, and Ca^{2+} concentrations were then calculated in these preparations.

Loading the vesicles

The sarcolemmal vesicles were loaded with the

ions of choice (e.g., NaCl, RbCl, KCl, LiCl, CsCl, Tris-HCl, NaSCN, KSCN), or with a combination of ions by first sedimenting an appropriate amount of sarcolemmal membrane from the 160 mM KCl suspension by centrifugation at $35\,000 \times g$ for 30 min at 4°C in a Sorvall SS-34 rotor, and then resuspending the vesicles in 15–20 ml of 160 mM of the salt solution plus 20 mM Mops/Tris-HCl (pH 7.4). One hour incubation was sufficient to load the vesicles with either Na^+ or K^+, although for some experiments an overnight incubation was used.

$^{45}Ca^{2+}$ uptake measurements

We used two different methods to measure $^{45}Ca^{2+}$ uptake depending on whether we wanted to measure the initial rate ($^{45}Ca^{2+}$ uptake in 3 s) or the time-course of $^{45}Ca^{2+}$ uptake (up to 4 min). For the latter, 0.15 ml of sarcolemmal vesicles (3–6 mg protein/ml) were added to 2.85 ml of an isotonic reaction solution containing $^{45}CaCl_2$ (about 15 000 cpm/nmol $^{45}Ca^{2+}$), under constant stirring at 37°C. At the desired times (e.g., every 15 s for 4 min), a sample was removed from the reaction mixture, rapidly filtered through a Whatman GF/C glass fiber filter, and immediately washed with 25 ml of ice-cold 160 mM KCl + 5 mM $LaCl_3$ + 20 mM Mops/Tris-HCl (pH 7.4). The radioactivity was then counted with a Beckman scintillation spectrophotometer.

The initial rate of $^{45}Ca^{2+}$ uptake was measured by adding 0.05 ml sarcolemmal vesicles to 0.95 ml of an isotonic, stirred reaction mixture containing the $^{45}CaCl_2$. $^{45}Ca^{2+}$ uptake was stopped typically at 3 s (although $^{45}Ca^{2+}$ uptake was linear up to about 5 s) by adding 10 ml of ice-cold 160 mM KCl + 5 mM $LaCl_3$ + 20 mM Mops/Tris-HCl, rapidly filtering the mixture, and then washing the filter with an additional 10 ml of the stopping solution. La^{3+} is an inhibitor of Ca^{2+} binding and Ca^{2+} uptake by excitable tissues [18,19]. The zero-time measurement was obtained by adding 0.05 ml of the vesicles to 0.9 ml of the reaction mixture containing 5 mM $LaCl_3$, then adding 0.05 ml of $^{45}CaCl_2$. As above, 10 ml of the ice-cold stopping solution was immediately added to this mixture, and the mixture was filtered and washed. In every experiment $^{45}Ca^{2+}$ diffusion, independent of Na^+ and Ca^{2+}-binding, was distinguished from $^{45}Ca^{2+}$ uptake due to Na^+-Ca^{2+} exchange by measuring the $^{45}Ca^{2+}$ movement into the vesicles in absence of a monovalent cation gradient. This value was subtracted from the total Ca^{2+} uptake to calculate net Na^+-Ca^{2+} exchange.

To measure Ca^{2+}-Ca^{2+} exchange, the vesicles had to be loaded with Ca^{2+}. For this purpose, $CaCl_2$ (1 mM) was included in the incubation mixture containing 160 mM of the monovalent cation of choice when Ca^{2+}-loading was required for Ca^{2+}-Ca^{2+} exchange measurements. Incubation periods of at least 48 h at 4°C were best for Ca^{2+}-loading. Vesicles were sedimented at $35\,000 \times g$ at 4°C, and resuspended in a smaller volume of the loading solution, this time without Ca^{2+}, (optimally, to a final protein concentration of 3–6 mg/ml). An appropriate volume of either 0.1 M or 0.25 M Tris-EGTA was added to this vesicle suspension to a final concentration of 5 mM to remove extravesicular Ca^{2+}. These Ca^{2+}-loaded vesicles were washed two times at 4°C with 20 ml of the monovalent cations that have been present in the loading solution, but without $CaCl_2$.

Valinomycin treatment of the vesicles

In some experiments, sarcolemmal vesicles were incubated with 10 μg of valinomycin per mg protein before and during $^{45}Ca^{2+}$ uptake measurements. The valinomycin was dissolved in acetone and an equal volume of acetone was added to control vesicles and reaction mixtures. A 15-min incubation of vesicles with valinomycin at 37°C was adequate for insertion of the valinomycin into the vesicle membranes as demonstrated by linear $^{86}Rb^+$ uptake as the ^{86}Rb concentration was increased (not shown).

Chemical and marker enzyme assays

Protein concentrations were measured using the method of Miller [13]. The total activities (μmol substrate split per g tissue) and the specific activities (μmol substrate split per h per mg protein) of several marker enzymes were assayed in each membrane fraction.

Succinate dehydrogenase activity was measured by the method of Bonner [14].

Glucose-6-phosphatase was assayed by measuring the release of inorganic phosphate at 37°C in

50 mM citrate buffer (pH 6.5) from 40 mM glucose 6-phosphate [26].

We assayed 5′-nucleotidase by measuring the release of inorganic phosphate from 3 mM AMP in the presence of 10 mM $MgCl_2$ in 75 mM Tris-HCl (pH 7.5) at 37°C [27].

$(Na^+ + K^+)$-ATPase, was measured by the method of Hegyvary et al. [15]. The activity of $(Na^+ + K^+)$-ATPase is the difference between the amount of inorganic phosphate released at pH 7.5 from 3 mM ATP in a medium of 100 mM NaCl, 20 mM KCl, and 4 mM $MgCl_2$, and the amount of inorganic phosphate released in the presence of 4 mM $MgCl_2$ and 1 mM ouabain. Mitochondrial ATPases were inhibited by the addition of 5 mM NaN_3 and the Ca^{2+}-chelator, Tris-EGTA, was added to both media, in order to optimally reveal $(Na^+ + K^+)$-ATPase activity.

Ca^{2+}-ATPase activity was assayed by measuring the release of inorganic phosphate from 2 mM Tris-ATP in the presence of 30 mM Tris-HCl (pH 7.3), 100 mM KCl, 2 mM $MgCl_2$, and 50 μM $CaCl_2$, either in the presence of 5 mM NaN_3 (an assay for the azide-insensitive sarcoplasmic reticulum Ca^{2+}-ATPase), or in the absence of 5 mM NaN_3 (an assay for mitochondrial and sarcolemmal Ca^{2+}-ATPases) [16].

[^{3}H]Ouabain binding to sarcolemmal membranes was measured by the method of Matsui and Schwartz [17].

Adenine nucleotides were measured by precipitating an appropriate amount of membranes with 5% trichloroacetic acid (w/v), sedimenting the precipitate, and then measuring the absorbance of a 1 : 1000 dilution of the supernatant at 260 nM.

Results

Composition of the membranes in our vesicle preparations

We used a combination of differential and sucrose step-gradient ultracentrifugation to isolate several subcellular membrane fractions from frozen dog heart ventricles (Table I). The membranes of fraction 2, contained a specific activity of sarcolemmal $(Na^+ + K^+)$-ATPase which was enriched greater than 100-fold over the $(Na^+ + K^+)$-ATPase of the homogenate. In fraction 2, there was no azide-insensitive sarcoplasmic reticulum Ca^{2+}-ATPase activity, and only minimal activities of the following marker enzymes: mitochondrial azide-sensitive Ca^{2+}-ATPase, and succinate dehydrogenase, microsomal glucose-6-phosphatase, and endoplasmic reticulum 5′-nucleotidase. These findings, along with the distribution of marker enzymes in the other fractions suggested that fraction 2 was enriched primarily with sarcolemmal membranes. This fraction also had the highest initial rate of Na^+-Ca^{2+} exchange, so we used this fraction in our experiments. There was less than 50 nmol adenine nucleotides per mg protein in a typical fraction 2.

When the heart tissue was homogenized in isotonic sucrose in the absence, or at low concentrations of chelators (0.5 mM EDTA), tightly-sealed vesicles were formed. We checked our preparations for vesicle tightness by measuring the distribution of [^{3}H]H_2O and [^{3}H]inulin in them. [^{3}H]H_2O is known to distribute freely throughout the whole water space, while [^{3}H]inulin is excluded from the intracellular space. The intravesicular volume could be estimated from the difference between the [^{3}H]H_2O and [^{3}H]inulin space. The intravesicular volume was 100–195 μl per mg protein.

Na^+-Ca^{2+} exchange

We measured $^{45}Ca^{2+}$ uptake into Na^+-loaded and K^+-loaded (not shown) sarcolemmal vesicles. Our findings confirm results of earlier experiments [6,8]. Vesicles accumulated about 6-times more $^{45}Ca^{2+}$ in the presence of an outward-directed Na^+ gradient than in the absence of an Na^+ gradient. $^{45}Ca^{2+}$ uptake in the presence of an Na^+ gradient was linear up to about 5 s and reached a maximum around 90 s. $^{45}Ca^{2+}$ uptake by Na^+-loaded vesicles in isotonic Na^+ did not change much with time. Na^+-Ca^{2+} exchange was always the difference between the $^{45}Ca^{2+}$ uptake in the presence of an Na^+ gradient and $^{45}Ca^{2+}$ uptake in the absence of an Na^+ gradient. $LaCl_3$ (2.5 mM) inhibited $^{45}Ca^{2+}$ uptake in the presence of an Na^+ gradient.

Na^+-Ca^{2+} exchange has been described as saturable Ca^2 transport process, but saturation kinetics alone could not distinguish Ca^{2+}-binding from real Ca^{2+} uptake. We used two approaches to

TABLE I

DISTRIBUTION OF MARKER ENZYMES IN SUBCELLULAR FRACTIONS

Membrane fractions	Total protein (g)	$(Na^+ + K^+)$-ATPase	Azide-insensitive Ca^{2+}-ATPase	Azide-sensitive Ca^{2+}-ATPase	Succinate dehydrogenase	Glucose-6-phosphatase	5'-Nucleotidase	Initial rate Na^+-Ca^{2+} exchange (nmol Ca^{2+}/mg per min)
Homogenate	8.46	2 [a] 16920 [b] (100)	5 42300(100)	17 143820(100)	5 42300(100)	0.64 5414(100)	0.53 4483(100)	2
8500 × *g* supernatant	0.414	25 10350 (61)	0 	93 38502 (27)	15 6210 (15)	1.78 736 (14)	0.99 409 (9)	21
8500 × *g* sediment	5.16	3 15840 (91)	8 41280 (98)	22 113520 (78)	5 25800 (61)	0.59 3044 (56)	0.56 2889 (64)	1
Step-gradient fractions								
1	0.213	4 852 (5)	0 	0 	7 1491 (4)	2.21 471 (9)	3.46 737 (16)	0
2	0.021	225 4725 (28)	0 	19 399 (0.2)	3 63 (0.1)	4.46 94 (2)	1.91 40 (0.8)	45
3	0.036	21 756 (4)	0 	10 360 (0.2)	21 756 (2)	3.27 118 (2)	3.34 120 (3)	33
4	0.056	7 392 (2)	2 112 (0.3)	73 4088 (3)	18 1008 (2)	4.10 230 (4)	3.16 177 (4)	0
5	0.088	15 1320 (8)	1 88 (0.2)	48 4224 (3)	10 880 (2)	3.68 324 (6)	1.47 129 (3)	10

[a] Specific activity in μmol product per mg per h.

[b] Total activity in μmol product per h (% recovery).

demonstrate true Ca^{2+} accumulation by Na^+-Ca^{2+} exchange (or Ca^{2+}-Ca^{2+} exchange), as opposed Ca^{2+} binding.

First: we reversed the direction of the Na^+ gradient during Na^+-Ca^{2+} exchange (Fig. 1) to see what fraction of the accumulated Ca^{2+} could be released. Na^+-loaded vesicles in the presence of an Na^+ gradient accumulated about 4-times as much $^{45}Ca^{2+}$ in one minute as in the absence of an Na^+ gradient. At this time, the direction of the Na^+ gradient during Na^+-Ca^{2+} exchange was reversed by adding external Na^+. The vesicles lost about 80% of the accumulated Ca^{2+} in 30 s. The amount of $^{45}Ca^{2+}$ remaining in these vesicles after Na^+ gradient reversal was about the same amount as was measured in vesicles in the absence of an Na^+ gradient.

Second: we disrupted the vesicles during Na^+-Ca^{2+} exchange (or Ca^{2+}-Ca^{2+} exchange) by detergent lysis or by osmotic shock with distilled water (not shown). Again, about 80% of the accumulated Ca^{2+} was lost, but this time it was after the integrity of the vesicles was disrupted. The amount of $^{45}Ca^{2+}$ remaining after disruption of the vesicles was the same amount as was measured in vesicles in the absence of an Na^+ gradient (or in the absence of Ca^{2+}-Ca^{2+} exchange).

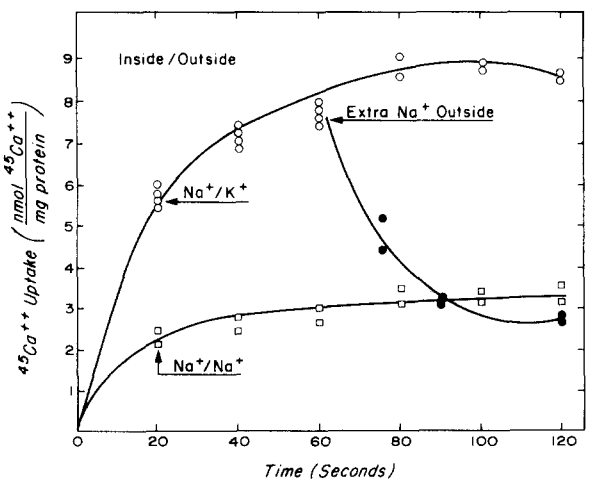

Fig. 1. Reversal of $^{45}Ca^{2+}$ movement across sarcolemmal vesicles by reversing the Na^+ gradient. Dog heart sarcolemmal vesicles were loaded with 60 mM NaCl/100 mM KCl as described in Methods. $^{45}Ca^{2+}$ uptake at 37°C and pH 7.4 was initiated by adding 0.15 ml of vesicles to 2.85 ml of one of the following reaction mixtures: 60 mM NaCl/100 mM KCl/50 μM $^{45}CaCl_2$ (□), or 160 mM KCl/50 μM $^{45}CaCl_2$ (○). At the times indicated on the graph, 0.6-ml samples were filtered, washed, and the radioactivity counted. At 60 s, an equal volume of 180 mM NaCl/50 μM $^{45}CaCl_2$ was added to some reaction mixtures (●), reversing the Na^+ gradient, and the $^{45}Ca^{2+}$ in these vesicles was measured at the times indicated.

Ion movements across excitable membranes are often accompanied by, or are associated with other bioelectric membrane events. We wanted to explore the possibility that Na^+-Ca^{2+} exchange, also, was influenced by the membrane potential, in which case, Na^+-Ca^{2+} exchange would be electrogenic.

We first determined whether or not the movement of a negative charge along with the positive charge of Na^+ or Ca^{2+} was rate-limiting. We measured the initial rate of Na^+-Ca^{2+} exchange using either sodium and potassium salts of a lipid-permeable anion, thiocyanate, or sodium and potassium salts of the less lipid-permeable anion, chloride. The initial rate was 37 ± 5 nmol $^{45}Ca^{2+}$/mg per min in both cases, for this vesicle preparation.

Early experiments with internally-dialyzed squid axons [20] or with sarcolemmal vesicles [6,9,21] indicated that Na^+-Ca^{2+} exchange was electrogenic, and therefore, influenced by the membrane potential. Since it is not possible to measure or control the membrane potential in a vesicle preparation with microelectrodes, we used an indirect approach to measure the effect of the membrane potential on Na^+-Ca^{2+} exchange.

Membranes become permeable primarily to K^+ when valinomycin inserts across the lipid bilayer. Threfore, the equilibrium potential across a valinomycin-treated membrane becomes a function of the ratio of internal and external K^+ concentrations. We varied internal and external K^+ concentrations, and used Tris-HCl to balance tonicity. The equilibrium potentials of the valinomycin-treated vesicles were calculated from the Nernst equation:

$$E_{K^+} = \frac{2.3R \cdot T}{z \cdot F} \cdot \log_{10} \frac{[K^+]_{out}}{[K^+]_{in}} \quad (1)$$

The physical constants R, T, and F have their usual meanings, and z, the charge per ion is $+1$ for K^+. The initial rate of Na^+-Ca^{2+} exchange increased roughly exponentially as the membrane was depolarized (Fig. 2). The intercept of the Na^+-

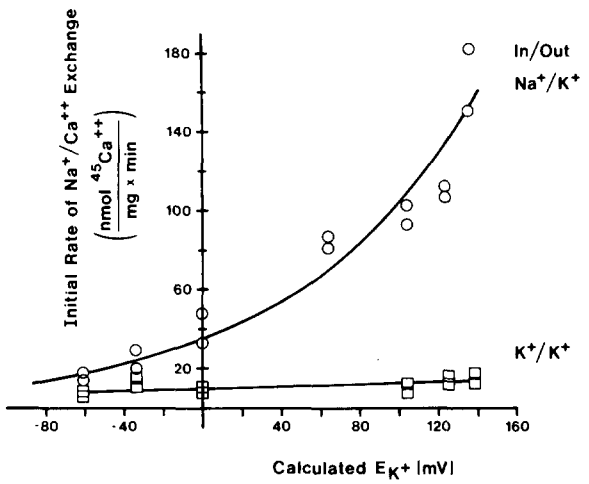

Fig. 2. Dependence of the initial rate of Na^+-Ca^{2+} exchange on membrane potential. Valinomycin-treated (10 μg/mg protein) sarcolemmal vesicles were loaded with either 159 mM NaCl/1 mM KCl or 150 mM NaCl/10 mM KCl as described in Methods. The initial rate of Na^+-Ca^{2+} exchange was measured in one of the following reaction mixtures (all contained 50 μM $^{45}CaCl_2$): 150 mM KCl/10 mM Tris-HCl, 100 mM KCl/60 mM Tris-HCl, 50 mM KCl/110 mM Tris-HCl, 10 mM KCl/150 mM Tris-HCl, 2.5 mM KCl/157.5 mM Tris-HCl, or 1 mM KCl/159 mM Tris-HCl. The Ca^{2+} leak was measured using valinomycin-treated vesicles loaded with 10 mM KCl/150 mM Tris-HCl or 1 mM KCl/159 mM Tris-HCl and the above KCl/Tris-HCl plus $^{45}CaCl_2$ solutions. The equilibrium potentials were calculated from the Nernst equation: $E_K = 61.6 \cdot \log([K]_{out}/[K]_{in})$. The curve drawn through the open circles is a single exponential fit to the data using a computer program. The r^2 for this curve was 0.9550.

Ca^{2+} exchange curve at the line for $^{45}Ca^{2+}$ uptake in the absence of an Na^+ gradient is the reversal potential for Na^+-Ca^{2+} exchange. From the data on the figure, the reversal potential of Na^+-Ca^{2+} exchange is -105 mV.

There is a second way to determine not only the reversal potential, but also the apparent stoichiometry of Na^+-Ca^{2+} exchange. Na^+-Ca^{2+} exchange can be macroscopically defined by the reaction:

$$x\,Na^+_{in} + y\,Ca^{2+}_{out} \leftrightharpoons x\,Na^+_{out} + y\,Ca^{2+}_{in}$$

in which x is the number of sodium ions transported for y number of calcium ions. At equilibrium, the Na^+-Ca^{2+} exchange reaction in terms of electrochemical energy is:

$$\left(xRT\ln a_{Na^+_{in}} + xFV_{in}\right) + \left(yRT\ln a_{Ca^{2+}_{out}} + 2yFV_{out}\right) = \left(xRT\ln a_{Na^+_{out}} + xFV_{out}\right) + \left(yRT\ln a_{Ca^{2+}_{in}} + 2yFV_{in}\right) \quad (2)$$

The physical constants R, T, and F have their usual meanings, V_{in} and V_{out} are the voltages at the inside and outside of the membrane, respectively, and x and y are defined above. The concentrations of Na^+ and Ca^{2+} can be substituted for the activities (a_{Na^+}, $a_{Ca^{2+}}$) in Equation 2, if the ions are truly dissolved in solution or if the fraction dissolved in solution is known. If the membrane potential, E_M, is $V_{in} - V_{out}$, and the concentrations of Na^+ and Ca^{2+} are substituted for the respective activities, then Equation 2 can be rearranged to:

$$\frac{R \cdot T}{(2y - x)F} \cdot \left(\ln\left(\frac{[Na^+]_{in}}{[Na^+]_{out}} \right)^x + \ln\left(\frac{[Ca^{2+}]_{out}}{[Ca^{2+}]_{in}} \right)^y \right) = E_M \quad (3)$$

At equilibrium, there is no net current and E_M is then the reversal potential for Na^+-Ca^{2+} exchange. The reversal potential of Na^+-Ca^{2+} exchange is a function of both the internal and external Na^+ and Ca^{2+} concentrations and the $Na^+ : Ca^{2+}$ flux ratio ($x : y$). We estimated two reversal potentials for the flux ratios of 3 Na^+ : 1 Ca^{2+} and 4 Na^+ : 1 Ca^{2+} by measuring internal and external Na^+ and Ca^{2+} concentrations. Internal Na^+ and Ca^{2+} concentrations were 150 mM and 54 μM, respectively, and the external Na^+ and Ca^{2+} concentrations were 10 mM and 50 μM, respectively. The calculated reversal potential for a flux ratio of 3 Na^+ : 1 Ca^{2+} was -215 mV, while the calculated reversal potential for a flux ratio of 4 Na^+ : 1 Ca^{2+} was -139 mV. The calculated reversal potential for a flux ratio of 4 Na^+ : 1 Ca^{2+}, therefore, was in better agreement with the measured reversal potential in Fig. 2.

Most investigators have suggested that Na^+-Ca^{2+} exchange occurs via a carrier mechanism, rather than a channel mechanism. We examined this issue next by measuring the saturation kinetics and substrate affinities of this transport process.

First, we measured the saturation kinetics of an internal Na^+-binding site by loading the vesicles with increasing concentrations of Na^+, and then measuring the initial rate of Na^+-Ca^{2+} exchange at one external $^{45}CaCl_2$ concentration. We con-

firmed that initial rate of Na^+-Ca^{2+} exchange by native and valinomycin-treated (not shown) sarcolemmal vesicles increased as the internal Na^+ concentration was raised. The K_m for Na^+ in our preparations were: 75 mM for native sarcolemmal vesicles and 60 mM for valinomycin-treated sarcolemmal vesicles. These values are similar to those reported by Pitts [7].

We then varied the external $^{45}Ca^{2+}$ concentration and measured the initial rate of Na^+-Ca^{2+} exchange by vesicles loaded with a saturating internal Na^+ concentrations (> 150 mM). The initial rate of Na^+-Ca^{2+} exchange by native sarcolemmal vesicles and valinomycin-treated sarcolemmal vesicles (not shown) increased as the external $^{45}CaCl_2$ concentration increased. The K_m for Ca^{2+} was about 34 μM for native and valinomycin-treated sarcolemmal vesicles.

Coupled ion translocation across membranes can occur in either a simultaneous or a sequential manner. The kinetic scheme which fits a transport mechanism can be resolved by measuring the saturation kinetics of both substrates at the internal and external surfaces of the membrane [22]. There is good evidence that Na^+-Ca^{2+} exchange is simultaneous countertransport of Na^+ and Ca^{2+} in the squid axon [5], so we approached this issue to see if Na^+-Ca^{2+} exchange was also simultaneous countertransport in the heart. We measured the affinity of Na^+-Ca^{2+} exchange for external $^{45}Ca^{2+}$ (K_m for Ca^{2+}) at internal Na^+ concentrations which were not saturating (25 and 50 mM) and at a saturating concentration of Na^+ (160 mM). The K_m for external Ca^{2+} did not depend on the internal Na^+ concentration and remained 30 μM (Fig. 3).

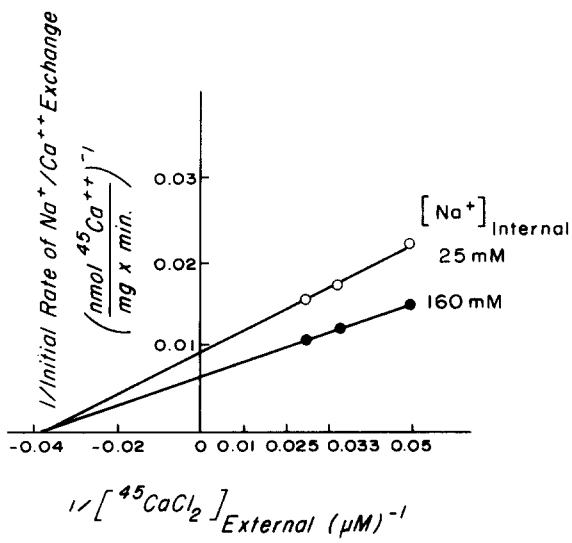

Fig. 3. Affinity of Na^+/Ca^{2+} exchange for external Ca^{2+} at two intravesicular Na^+ concentrations. Sarcolemmal vesicles were loaded with either 25 mM NaCl/135 mM Tris-HCl (○) or 160 mM NaCl (●), and the initial rate of Na^+-Ca^{2+} exchange was measured as described in Methods at the following external $^{45}CaCl_2$ concentrations: 20, 30, and 40 μM.

Ca^{2+}-Ca^{2+} exchange

Ca^{2+}-Ca^{2+} exchange has been described along with Na^+-Ca^{2+} exchange in the squid axon [5]. We have also found Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange activities to be enriched simultaneously during our preparations of sarcolemmal membrane vesicles. Our preliminary experiments showed that vesicles loaded with a monovalent cation plus Ca^{2+} accumulated more $^{45}Ca^{2+}$ from an isotonic reaction mixture containing that monovalent cation and 50 μM $^{45}CaCl_2$ than vesicles loaded only with the monovalent cation. Vesicles loaded with sucrose plus Ca^{2+} did not accumulate $^{45}Ca^{2+}$ from an isotonic sucrose plus $^{45}CaCl_2$ reaction mixture. Ca^{2+}-Ca^{2+} exchange was, by definition, the $^{45}Ca^{2+}$ uptake by vesicles loaded with a monovalent cation plus Ca^{2+} minus the $^{45}Ca^{2+}$ uptake by vesicles loaded only with the monovalent cation. It is important to note that during Ca^{2+}-Ca^{2+} exchange, there is no monovalent cation gradient (which is required for Na^+-Ca^{2+} exchange), but the same monovalent cation is added to both sides of the vesicle in the same concentration.

We measured the time-course of Ca^{2+}-Ca^{2+} exchange by vesicles loaded with Ca^{2+} plus one of the following monovalent cations: K^+, Rb^+, Na^+, Cs^+, and Li^+. The greatest enhancement of Ca^{2+}-Ca^{2+} exchange was in the presence of K^+ (not shown). Rb^+, Na^+, Cs^+, and Li^+ were not as effective as K^+ in enhancing Ca^{2+}-Ca^{2+} exchange.

To characterize the kinetics of Ca^{2+}-Ca^{2+} exchange, we measured the initial rate of Ca^{2+}-Ca^{2+} exchange in the presence of K^+ on both sides of the membrane at increasing external $^{45}Ca^{2+}$ concentrations. The initial rate of Ca^{2+}-Ca^{2+} exchange increased as the external $^{45}Ca^{2+}$ concentra-

tion was raised (not shown). The K_m for Ca^{2+} was 34 μM.

We obtained the kinetic constants, K_m values for Na^+ and Ca^{2+}, for Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange from experiments with the same sarcolemmal preparation. The K_m for Ca^{2+} was the same (34 μM) for Ca^{2+}-Ca^{2+} exchange and Na^+-Ca^{2+} exchange for native or valinomycin-treated vesicles.

Monovalent cation selectivities of Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange in the same vesicle preparations

Since Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange depended on the presence of some monovalent cations, we examined the effects of different monovalent cations on the initial rates of Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange on the same preparation. Our experiments showed that the order of effectiveness of monovalent cations in enhancing the initial rate of $^{45}Ca^{2+}$ uptake was different for Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange (Table II). For Na^+-Ca^{2+} exchange, an Na^+ gradient was the most effective monovalent cation gradient in driving $^{45}Ca^{2+}$ uptake. Li^+ was a weak substitute for Na^+, but Cs^+ and K^+ gradients supported less than 2% of the maximal initial rate of $^{45}Ca^{2+}$ uptake. A Rb^+ gradient did not drive any $^{45}Ca^{2+}$ uptake. When vesicles contained internal Ca^{2+} and in the absence of a monovalent cation gradient, K^+ maximally enhanced Ca^{2+}-Ca^{2+} exchange. Rb^+ was a weak substitute for K^+ in Ca^{2+}-Ca^{2+} exchange, while Na^+, Li^+, and Cs^+ had decreasing effects in that order. The maximum initial rate for $^{45}Ca^{2+}$ uptake was about the same for Na^+-Ca^{2+} exchange (154 nmol $^{45}Ca^{2+}$/mg per min) and Ca^{2+}-Ca^{2+} exchange (149 nmol $^{45}Ca^{2+}$/mg per min). Extravesicular NaCl (25 mM) in the Ca^{2+}-Ca^{2+} exchange reaction mixture inhibited 75% of the Ca^{2+}-Ca^{2+} exchange activity measured in the absence of any external NaCl (not shown).

Effects of modifiers of Ca^{2+} uptake on Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange

To clarify the issue of whether or not Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange were activities of the same molecule, we examined the effects of modifiers of Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange activities, Mn^{2+} and Mg^{2+} [5,10], on Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange in the same membrane preparations. We also measured the effects of quinidine on Na^+-Ca^{2+}

TABLE II

THE EFFECTIVENESS OF VARIOUS MONOVALENT CATIONS IN ENHANCING Ca^{2+} UPTAKE BY TWO TRANSPORT MECHANISMS

X^+-Ca^{2+} exchange was the countertransport of a monovalent cation and Ca^{2+} in the presence of a monovalent cation concentration gradient (e.g. Na^+-Ca^{2+} exchange). The initial rate of X^+-Ca^{2+} exchange by vesicles loaded with the listed monovalent cations was measured in a reaction mixture of 160 mM KCl/50 μM $^{45}CaCl_2$ (160 mM Tris-HCl was used for the K^+-loaded vesicles), as described in Methods. X^+,Ca^{2+}-X^+,Ca^{2+} exchange was the exchange of internal Ca^{2+} for external Ca^{2+} in the absence of a monovalent cation gradient. The initial rate of X^+,Ca^{2+}-X^+,Ca^{2+} exchange was measured as described in Methods.

Monovalent cations (X^+)	Initial rate (nmol $^{45}Ca^{2+}$ per mg per min)	
	X^+-$^{45}Ca^{2+}$ exchange	X^+,Ca^{2+}-X^+,$^{45}Ca^{2+}$ exchange
Na^+	154	8
Li^+	35	6
Cs^+	3	1
Rb^+	0	32
K^+	1	149

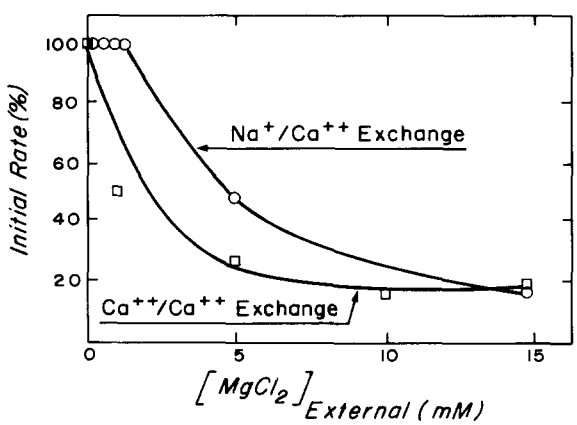

Fig. 4. Inhibition of Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange in sarcolemmal vesicles by extravesicular $MgCl_2$. The initial rate of Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange was measured in the absence of $MgCl_2$, and in the presence of the extravesicular $MgCl_2$ concentrations shown above. Na^+-Ca^{2+} exchange (○), Ca^{2+}-Ca^{2+} exchange (□).

exchange and Ca^{2+}-Ca^{2+} exchange. Quinidine blocks the voltage-sensitive Na^+ channel at low concentrations [33–36]. We also measured the effects of the Ca^{2+} channel blocker, verapamil [36,37], on the steady-state level of Na^+-Ca^{2+} exchange ($^{45}Ca^{2+}$ uptake after a 90 s incubation).

Our preliminary results showed that either $MnCl_2$ or $MgCl_2$ at an external concentration of 10 mM was an effective inhibitor of both Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange. We titrated Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange with increasing external concentrations of $MgCl_2$ (Fig. 4). The initial rate of Na^+-Ca^{2+} exchange was inhibited 50% at an external $MgCl_2$ concentration of 5 mM, while the initial rate of Ca^{2+}-Ca^{2+} exchange was inhibited 50% at an external $MgCl_2$ of 1 mM. Maximum inhibition of both transport processes by external Mg^{2+} (in the concentration range of 0.25–15 mM $MgCl_2$) was about 80%.

The antiarrhythmic agent, quinidine, exerted different effects on Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange (Fig. 5). The concentration range of quinidine used in these experiments was 5–150 μM (the therapeutic concentration of quinidine in man is about 10μM). Na^+-Ca^{2+} exchange was enhanced up to 200% by low concentrations of external quinidine (10 μM), but was inhibited by quinidine concentrations greater than 50 μM. At 150 μM external quinidine, 50% of the Na^+-Ca^{2+} exchange activity remained. Ca^{2+}-Ca^{2+} exchange was inhibited by quinidine concentrations greater than 5 μM and Ca^{2+}-Ca^{2+} exchange was inhibited 100% at 150 μM external quinidine. Qualitatively similar results were obtained whether we used native sarcolemmal vesicles or valinomycin-treated vesicles. The steady-state level of Na^+-Ca^{2+} exchange activity was decreased up to 65% by concentrations of quinidine greater than 0.1 mM, and up to 80% by concentrations of verapamil greater than 0.1 mM.

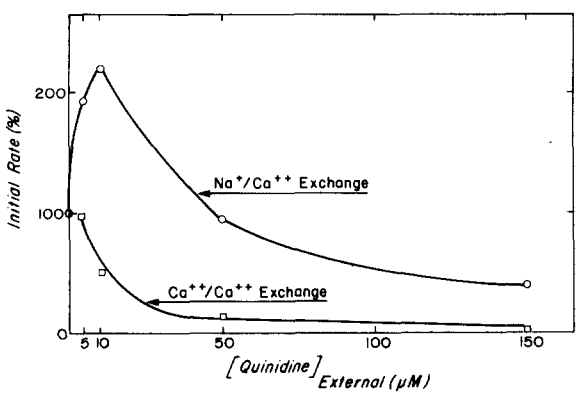

Fig. 5. Effect of quinidine on Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange by valinomycin-treated sarcolemmal vesicles. The initial rate of Na^+-Ca^{2+} exchange (○) and Ca^{2+}-Ca^{2+} exchange (□) by valinomycin-treated (10 μg/mg protein) sarcolemmal vesicles was measured as described in Methods, in the absence of quinidine and in the presence of 5, 10, 50, and 150 μM quinidine.

Discussion

Localization and characterization of Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange

We were able to isolate the membranes containing the passive Ca^{2+} transport mechanisms, Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange from cardiac muscle cells. Na^+-Ca^{2+} exchange has been described previously in the intact squid axon [1–5], in which it was possible to demonstrate that two passive Ca^{2+} transport mechanisms were indeed located in the cell membrane. Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange have recently been demonstrated in broken-cell preparations from the heart and it has been proposed that these two transport mechanisms reside in the sarcolemmal membrane [6,8,10].

We have examined the marker enzyme profile of several membrane fractions obtained by our methods (Table I). We could reasonably conclude that Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange are activities of transport mechanisms which are localized in the sarcolemmal membrane, since both Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange activities were co-purified in parallel with the activity of (Na^+ + K^+)-ATPase, a marker enzyme for sarcolemmal membranes.

Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange were passive Ca^{2+} transport since both activities were present even though the concentration of endogenous ATP was too low to support an active Ca^{2+} pump.

We have demonstrated that passive Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange have a binding and a transport component. Reversing the direction of the Na^+ gradient or disruption of the

integrity of the vesicles removed 80% of the accumulated $^{45}Ca^{2+}$. Therefore, it appears that about 20% of Ca^{2+} taken up may not be freely-exchangeable across the sarcolemmal membrane.

Electrogenicity of Na^+-Ca^{2+} exchange

Our results support earlier findings that Na^+-Ca^{2+} exchange in sarcolemmal membranes is coupled countertransport of Na^+ and Ca^{2+} [6], as it is in the squid axon [3]. As coupled countertransport, Na^+-Ca^{2+} exchange displaces the positive electrical charges associated with Na^+ and Ca^{2+} in opposite directions across the sarcolemmal membrane. If the charge associated with the Na^+ flux is greater than the charge associated with the Ca^{2+} flux, then Na^+-Ca^{2+} exchange would be electrogenic. The activity of an electrogenic Na^+-Ca^{2+} exchange mechanism would influence the membrane potential, and therefore, the excitability of the tissue in which it is found. Conversely, the electrical potential of the membrane will necessarily influence the rate of transport if this transport is electrogenic.

Na^+-Ca^{2+} exchange in the squid axon was potential-sensitive [20], and recent reports indicated that there was net charge movement during Na^+-Ca^{2+} exchange by sarcolemmal vesicles [9,21]. However, it is possible that during Na^+-Ca^{2+} exchange, there is also a flux of anions. Restricting the flux of anions would then change the activity of Na^+-Ca^{2+} exchange. Previous reports did not account for this possibility, although there are several known transport mechanisms which couple the movement of anions with cations [23]. We found that the initial rate of Na^+-Ca^{2+} exchange was not dependent on the movement of anions across the sarcolemma.

Two groups of investigators have monitored indirectly the net charge movement during Na^+-Ca^{2+} exchange with lipophilic cations [9,21]. They found net movement of the lipophilic cation into the vesicle membrane of Na^+-loaded vesicles during Na^+-Ca^{2+} exchange. However, we used a different approach to address the issue of the whether or not Na^+-Ca^{2+} exchange was electrogenic. We measured the initial rate of Na^+-Ca^{2+} exchange at several single potentials using valinomycin-treated vesicles. Our results were in agreement with the qualitative results reported by Reeves and Sutko [9] and Caroni et al. [21]. Depolarization of the Na^+-loaded vesicle membranes greatly enhanced the initial rate of Na^+-Ca^{2+} exchange (Fig. 2). This indicates that the net charge movement is probably carried by the Na^+ flux, since making the vesicle interior more positive would enhance the exit of Na^+.

We have also obtained additional information about Na^+-Ca^{2+} exchange from an extrapolation of the data in Fig. 2. We found the value for the reversal potential of Na^+-Ca^{2+} exchange (-105 mV), at which there is no net charge movement by Na^+-Ca^{2+} exchange. However, since the potential of cardiac cells is always less negative in vivo than this potential, this implies that Na^+-Ca^{2+} exchange can contribute to the cardiac membrane potential. Na^+-Ca^{2+} exchange in vivo, however, is operating in the opposite direction as in our vesicle preparations, since the Na^+ gradient in vivo is directed towards the cell interior. Ca^{2+} would be transported outward by Na^+/Ca^{2+} exchange, as proposed by Reuter [24], but the rate of Ca^{2+} efflux would decrease as the cell is depolarized. Depolarization, then, would impede Ca^{2+} transport out of the cell and repolarization would enhance Ca^{2+} transport out of the cell. The Na^+-Ca^{2+} exchange mechanism can provide the cardiac cell with another way of maintaining the low intracellular free-Ca^{2+} concentration. As such, the homeostatic activity of electrogenic Na^+-Ca^{2+} exchange would be synchronous with the Ca^{2+} movements into the cell through the voltage-sensitive Ca^{2+} channels during the cardiac action potential.

The Na^+ : Ca^{2+} flux ratio of electrogenic Na^+-Ca^{2+} exchange

Flux ratios of 3 Na^+ : 1 Ca^{2+} and 4 Na^+ : 1 Ca^{2+} have been proposed for Na^+-Ca^{2+} exchange by the cardiac sarcolemma [6,26]. Pitts proposed a 3 Na^+ : 1 Ca^{2+} flux ratio from an extrapolation of rate measurements of Na^+-Ca^{2+} exchange. Mullins and Brinley [20] have measured the sensitivity of Na^+-Ca^{2+} exchange in the squid axon to changes in the membrane potential. They provided good evidence that Na^+-Ca^{2+} exchange was more sensitive to a given change in potential than would be predicted from a 3 Na^+ : 1 Ca^{2+} flux ratio and that this sensitivity to a given change in membrane potential was dependent on the Na^+

and Ca^{2+} concentrations [20]. To account for his data, Mullins [26] derived a kinetic model for Na^+-Ca^{2+} exchange which required the binding of 4 Na^+ on one side of a carrier for each Ca^{2+} bound to the other side of the carrier. He has extended his model for the mechanism of Na^+-Ca^{2+} exchange by the squid axon as a model for the mechanism of Na^+-Ca^{2+} exchange by the cardiac sarcolemma [26].

We have estimated the $Na^+ : Ca^{2+}$ flux ratio by measuring the reversal potential using the values of the initial rate (Fig. 2) and by measuring the internal and external ion concentrations, then applying the thermodynamic argument of Eqns. 1–3. The assumption of 4 Na^+ : 1 Ca^{2+} in Eqn. 3 yielded a value for the reversal potential of -139 mV, which was fairly close to the measured reversal potential of -105 mV. Though there are several Ca^{2+} homeostatic mechanisms in an intact cardiac cell, a flux ratio of 4 Na^+ : 1 Ca^{2+} theoretically could maintain an intracellular Ca^{2+} concentration which would be 80-times less than Na^+-Ca^{2+} exchange operating at a 3 Na^+ : 1 Ca^{2+} flux ratio [20].

The nature of the countertransport of Na^+ and Ca^{2+} during Na^+-Ca^{2+} exchange

Coupled ion translocation across cellular membranes can occur in either a simultaneous or sequential manner. The actual manner of ion translocation can be predicted from its saturation kinetics [22]. For example, if Na^+-Ca^{2+} exchange is a sequential process, then saturation of an Na^+-binding site on the membrane component on one side of the membrane must precede the formation of the Ca^{2+}-binding site on the opposite side of the membrane. The affinity (K_m) of the Ca^{2+}-binding component at different external Ca^{2+} concentrations for sequential Na^+-Ca^{2+} exchange would be dependent on the internal Na^+ concentration. For simultaneous Na^+-Ca^{2+} exchange, the loading of the two cation binding sites on opposite sides of the membrane would not determine the maximum rate of Na^+-Ca^{2+} exchange, and the affinity of the membrane component for Ca^{2+} at the external binding site would not be dependent on the degree of saturation of the internal Na^+-binding site. Using the criteria of Heinz [22], Blaustein [5] has described Na^+-Ca^{2+} exchange as simultaneous Na^+ and Ca^{2+} translocation in the squid axon.

We have found evidence by the criteria of Heinz, that Na^+-Ca^{2+} exchange in the cardiac sarcolemma is also simultaneous countertransport of Na^+ and Ca^{2+}. The K_m for Ca^{2+} at the external binding site was independent of the internal Na^+ concentration (Fig. 3). The molecular organization of passive Na^+-Ca^{2+} exchange may be significantly different from other well-characterized, sequential active transport mechanisms.

Ion selectivity sequence of Na^+-Ca^{2+} exchange

A common finding among investigations of ion movements through biological membranes, natural or artificial, is that there are relatively few selectivity sequences for the alkali cations, Na^+, K^+, Cs^+, Li^+, and Rb^+ [27].

We have found that the Na^+-Ca^{2+} exchange mechanism discriminates between the alkali cations. The ion sequence for Na^+-Ca^{2+} exchange was $Na^+ \gg Li^+ > Cs^+ > K^+ \gg Rb^+$. With exception of Cs^+, this cation sequence has been described for other biological ion transport processes [23].

The ion selectivity sequence of Na^+-Ca^{2+} exchange was similar to the sequence of ion activation of ($Na^+ + K^+$)-ATPase [28–30]. The cation selectivity sequence of ($Na^+ + K^+$)-ATPase is an important factor in determining the asymmetric activity of the $Na^+ + K^+$ pump in vivo. Since the Na^+ gradient in vivo is always directed towards the cell interior, the enhancement of the Na^+-Ca^{2+} exchange mechanism primarily by Na^+ would direct the activities of Na^+-Ca^{2+} exchange asymmetrically across the sarcolemmal membrane. It is possible that the major activation of different transport mechanisms by a common, single ion is an indication of an important physiological regulatory property which determines the orientation of the transport mechanisms in vivo.

The sequence of ion activation of Na^+-Ca^{2+} exchange is very different from the sequence of ion activation of Ca^{2+}-ATPase of cardiac membranes [16]. It is not likely that this passive mechanism of Ca^{2+} transport is an activity of the active Ca^{2+} pump in the sarcolemma.

The relationship of Na^+-Ca^{2+} exchange to Ca^{2+}-Ca^{2+} exchange

Both Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange were described together in squid axon [5]. Both processes transport freely-exchangeable Ca^{2+} across the membrane, and are probably carrier-mediated. Blaustein argued that, in the squid giant axon, Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange were mediated by a common exchange mechanism. His main criteria were: (1) both exchanges had the same affinity for Ca^{2+}, (2) both exchanges were inhibited by Sr^{2+} or Mn^{2+}, and (3) the Ca^{2+} fluxes did not sum under conditions which were simultaneously optimal for Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange. The experiments we have performed provide less convincing data that Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange are mediated by the same carrier mechanism in the cardiac sarcolemma.

The data from this study which support a model of a single carrier mechanism for both transport processes come from the measurements of the steady-state kinetics. Both Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange had the same affinity for Ca^{2+} (34 μM).

There was evidence, however, which indicates that Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange may not simply be activities of a single carrier mechanism. The mode of Na^+-Ca^{2+} exchange is clearly different from Ca^{2+}-Ca^{2+} exchange. Na^+-Ca^{2+} exchange requires an Na^+ concentration gradient, and is coupled, electrogenic countertransport of Na^+ and Ca^{2+}. Since it is electrogenic countertransport, Na^+-Ca^{2+} exchange may contribute to the membrane potential of the cardiac muscle cell, although the actual contribution of Na^+-Ca^{2+} exchange in vivo has not yet been quantitated. In contrast, Ca^{2+}-Ca^{2+} exchange does not require a monovalent cation gradient, but does require the presence of Ca^{2+} plus a specific monovalent cation on both sides of the membrane. Therefore, Ca^{2+}-Ca^{2+} exchange may be symmetrical cotransport of a monovalent cation and Ca^{2+}, resulting in an exchange of both Ca^{2+} and K^+. Valinomycin-treatment of the vesicles did not change the initial rate of Ca^{2+}-Ca^{2+} exchange. Presumably, no cation gradient is built by Ca^{2+}-Ca^{2+} exchange, so Ca^{2+}-Ca^{2+} exchange is probably electroneutral.

The order of effectiveness of the monvalent cations in enhancing Na^+-Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange were also different, though the maximal rates of Ca^{2+} movements by each process were about the same (Table II). Na^+-Ca^{2+} exchange required an Na^+ gradient; for Ca^{2+}-Ca^{2+} exchange, there was an absolute requirement for the presence of K^+ on both sides of the membrane, but not a concentration gradient. External Na^+ (25 mM) decreased the initial rate of Ca^{2+}-Ca^{2+} exchange (not shown). If both of these processes were catalyzed by a single carrier transport mechanism, it is doubtful that the ionic milieu in vivo would allow simultaneous activation of both processes. The high extracellular Na^+ concentration would probably inhibit Ca^{2+}-Ca^{2+} exchange, but would activate Na^+-Ca^{2+} exchange.

Finally, both processes were inhibited by Mn^{2+}, Mg^{2+}, or quinidine, but each agent modified the activity of Na^+-Ca^{2+} exchange differently from Ca^{2+}-Ca^{2+} exchange, indicating that there may be different conformations of the same carrier, or different carriers for each exchange process. The titration curves for the effects of Mg^{2+} (Fig. 4) and quinidine (Fig. 5) on both processes clearly show that Ca^{2+}-Ca^{2+} exchange was more sensitive to the inhibitory effects of either agent than Na^+-Ca^{2+} exchange. Na^+-Ca^{2+} exchange was enhanced by low concentrations of quinidine (5–50 μM), while Ca^{2+}-Ca^{2+} exchange was inhibited up to 90% in this concentration range. Ca^{2+}-Ca^{2+} exchange was inhibited 50% by 1 mM $MgCl_2$, while Na^+-Ca^{2+} exchange was unaffected at this concentration. The effect of low concentrations of quinidine on Na^+-Ca^{2+} exchange could possibly implicate that Na^+-Ca^{2+} exchange has a role in the mode of action of quinidine on the heart.

Acknowledgements

This work was supported by the National Research Service Award HL-07320, grant 1R01HL-16611 from the National Heart, Lung, and Blood Institute of Health, United States Public Health Service, and grant C81-A from the Chicago Heart Association.

References

1 Baker, P.F., Blaustein, M.P., Manil, J. and Steinhardt, R.A. (1967) J. Physiol. 11, 100P–102P
2 Baker, P.F. and Blaustein, M.P. (1968) Biochim. Biophys. Acta 150, 167–170
3 Baker, P.F., Blaustein, M.F., Hodgkin, A.L. and Steinhardt, R.A. (1969) J. Physiol. 200, 431–458
4 Blaustein, M.P. (1974) Rev. Physiol. Biochem. Pharm. 70, 33–82
5 Blaustein, M.P. (1977) Biophys. J. 20, 79–111
6 Pitts, B.J.R. (1979) J. Biol. Chem. 254, 6232–6235
7 Pitts, B.J.R. and Okhuysen, C.H. (1980) Ann. N.Y. Acad. Sci. 358, 357–358
8 Reeves, J.P. and Sutko, J.L. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 590–594
9 Reeves, J.P. and Sutko, J.L. (1980) Science 208, 1461–1464
10 Bartschat, D.K. and Lindenmayer, G.E. (1980) J. Biol. Chem. 255, 9626–9634
11 Blaustein, H.P. and Weissman, W.P. (1970) Proc. Natl. Acad. Sci. U.S.A. 66, 664–671
12 Stahl, W.D. and Swanson, P.D. (1970) J. Neurochem. 19, 2395–2407
13 Miller, G.L. (1959) Anal. Chem. 31, 964
14 Bonner, W.D. (1955) in Methods of Enzymology (Colowick, S.P. and Kaplan, N.O., eds.), Vol. 1, pp. 722–724, Academic Press, New York
15 Hegyvary, C., Kang, K. and Bandi, Z. (1979) Anal. Biochem. 94, 397–401
16 Jones, L.R., Besch, H.R. and Watanabe, A.M. (1977) J. Biol. Chem. 252, 3315–3323
17 Matsui, H. and Schwartz, A. (1968) Biochim. Biophys. Acta 151, 655–663
18 Krasnow, N. (1972) Biochim. Biophys. Acta 282, 187–194
19 Weiss, G.B. and Wheeler, E.S. (1978) Arch. Int. Pharmacodyn. 233, 4–20
20 Mullins, L.J. and Brinley, F.J., Jr. (1975) J. Gen. Physiol. 65, 135–152
21 Caroni, P., Reinlib, L. and Carafoli, E. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6354–6358
22 Heinz, E., Geck, P. and Wilbrandt, W. (1972) Biochim. Biophys. Acta 255, 442–461
23 Stekhoven, F.S. and Bonting, S.L. (1980) Physiol. Rev. 61, 1–76
24 Reuter, H. and Seitz, N. (1968) J. Physiol. 195, 451–471
25 Mullins, L.J. (1977) J. Gen. Physiol. 70, 681–695
26 Mullins, L.J. (1979) Am. J. Physiol. 236, C103–C110
27 Diamond, J.M. and Wright, E.M. (1969) Annu. Rev. Physiol. 31, 581–646
28 Skou, J.C. (1960) Biochim. Biophys. Acta 42, 6–23
29 Glynn, I.M. and Karlish, S.J.D. (1975) Annu. Rev. Physiol. 37, 13–55
30 Skou, J.C. (1965) Physiol. Rev. 45, 596–617
31 Barman, T.E. (1969) in Enzyme Handbook, Vol. 2, pp. 530–531, Springer-Verlag, New York
32 Heppel, L.A. and Hilmore, R.J. (1955) in Methods of Enzymology (Colowick, S.P. and Kaplan, N.O., eds.), Volume 2, pp. 547–555, Academic Press, New York
33 Harrow, J.C. and Dhalla, N.S. (1976) Biochem. Pharm. 25, 897–902
34 Hauswirth, O. and Singh, B.N. (1979) Pharmacol. Rev. 30, 5–67
35 Ducouret, P. (1976) Br. J. Pharmacol. 57, 163–184
36 Fleckenstein, A. (1977) Annu. Rev. Pharmacol. Toxicol. 17, 149–166
37 Wit, A.L. and Cranefield, P.F. (1974) Circ. Res. 35, 413–425