Sodium–calcium ion exchange in cardiac membrane vesicles
(exchange diffusion/sarcolemma)

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ABSTRACT Membrane vesicles isolated from rabbit ventricular tissue rapidly accumulated Ca2+ when an outwardly directed Na+ gradient was formed across the vesicle membrane. Vesicles loaded internally with K+ showed only 10% of the Ca2+ uptake activity observed with Na+-loaded vesicles. Dissipation of the Na+ gradient with the monovalent cation exchange ionophores nigericin or narucain caused a rapid decline in Ca2+ uptake activity. The Ca2+-ionophore A23187 inhibited Ca2+ uptake by Na+-loaded vesicles and enhanced the rate of Ca2+ loss from the vesicles after uptake. Efflux of preaccumulated Ca2+ from the vesicles was stimulated 30-fold by the presence of 50 mM Na+ in the external medium. Na+-dependent uptake and efflux of Ca2+ were both inhibited by La3+. The results indicate that cardiac membrane vesicles exhibit Na+-Ca2+ exchange activity. Fractionation of the vesicles by density gradient centrifugation revealed a close correspondence between Na+-Ca2+ exchange activity and specific ouabain-binding activity among the various fractions. This relationship suggests that the observed Na+-Ca2+ exchange activity derives from the sarcolemmal membranes within the vesicle preparation.

Na+-Ca2+ exchange is a process whereby transmembrane movements of Ca2+ are coupled directly to reciprocal movements of Na+. This process has been demonstrated in a variety of excitable tissues (1–3), as well as in dog erythrocytes (4). In cardiac muscle, attention has been focused on Na+-Ca2+ exchange as a possible entry mechanism for contractile Ca2+ on a beat-to-beat basis (5), as a mechanism for extrusion of Ca2+ from the cell (6), and as a mediator of the isotropic effects of cardiac glycoside administration and changes in stimulation frequency (1, 5, 7–9).

Thus far, the study of Na+-Ca2+ exchange in cardiac tissue has been based on mechanical responses (10–12) or isotopic flux data (3, 6, 9, 13) obtained with intact muscle preparations. The pioneering work of Kaback and colleagues (14, 15) showed that transport phenomena could be studied by using sealed membrane vesicles, a system in which the conditions on either side of the membrane can be manipulated at will. Vesicles derived from cardiac sarcolemma have been used to study the enzymatic activity of transport ATPases (16–19), and several investigators have recently reported that sarcolemmal membrane vesicles exhibit ATP-dependent transport of Ca2+ (20–24).

The present report shows that cardiac membrane vesicles exhibit Na+-Ca2+ exchange activity. The data demonstrate that transmembrane Ca2+ movements in either direction are markedly stimulated by generating an oppositely directed gradient of Na+. These Na+-Ca2+ interactions appear to be associated with the sarcolemmal membranes within the preparation.

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MATERIALS AND METHODS

Preparation of Membrane Vesicles. Ventricular tissue from male New Zealand rabbits (1.4–2.0 kg) was finely minced with scissors in 3–4 vol of ice-cold 0.3 M sucrose, 5 mM MgSO4/10 mM imidazole-Cl (pH 7.0). Several different homogenization procedures were used in an effort to find the optimal conditions for vesicle preparation. Our most active preparations were obtained by using five 5-sec bursts of the Brinkmann Polytron (dial setting 5) followed by two strokes with a Teflon/glass homogenizer (pestle rotation 1200 rpm). The sucrose concentration of the homogenate was adjusted to 0.6 M and the homogenate was centrifuged at 28,000 × g (max) for 30 min in a Beckman JA-20 rotor. The supernatant was diluted with 1.5 vol of 160 mM NaCl/20 mM 3-(N-morpholino)propanesulfonic acid (Mops), adjusted to pH 7.4 with Tris, and centrifuged at 43,600 × g (max) for 30 min. The pellet, which consisted of the membrane vesicles, was suspended in 160 mM NaCl/20 mM Mops/Tris (pH 7.4) at a concentration of 3–6 mg of protein per ml. The yield of membrane vesicles, starting with ventricular tissue from a single rabbit heart, was 1.5–2 mg of protein.

Electron microscopy revealed that the vesicles ranged in diameter from 0.1 to 1.0 μm; intact cellular organelles such as mitochondria were rarely seen. The intravesicular volume was determined by measuring the 3H2O space in vesicle pellets and correcting for the extravesicular space by using 14C-sucrose. Two different preparations yielded values of 5.2 ± 1.0 and 4.7 ± 0.6 μl/mg of protein (mean ± SEM of triplicate determinations). The specific activities for 5'-nucleotidase, K+-activated p-nitrophenylphosphatase, and specific ouabain binding, widely considered to be markers for sarcolemmal membranes, were found to be enriched by approximately 4-, 9-, and 6-fold, respectively, over the values determined in crude homogenates of cardiac tissue. The vesicles also exhibited (t) enhanced uptake of D-glucose relative to that of L-glucose, and (u) Na+-dependent uptake of alanine. Membranes derived from the sarcoplasmic reticulum and from mitochondria were also present in the preparation. This was indicated in the first instance by the presence of ATP-driven Ca2+ uptake and in the second by the presence of azide- and oligomycin-inhibitable Mg2+-ATPase activity.

Vesicles prepared by the above procedure were used for all the experiments reported here except for the studies involving fractionation of the vesicles by density gradient centrifugation. In this instance, the 43,600 × g pellet (see above) was thoroughly suspended in 4 ml of 1.0 M sucrose with a Teflon/glass homogenizer. The suspension was layered over 1.5 ml of 1.15 M sucrose in two centrifuge tubes and overlaid with approximately 1.5 ml of 0.3 M sucrose. All the sucrose solutions contained 5 mM MgSO4 and were buffered to pH 7.0 with 10 mM imidazole-Cl. The tubes were centrifuged for 90 min at 590

Abbreviations: Mops, 3-(N-morpholino)propanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetracetic acid.
192,000 \times g \text{ (max)} \text{ in a Beckman L2-75B centrifuge (SW 50.1 rotor). Three fractions were obtained: membranes banding at the 0.3 M/1.0 M interface (fraction 1), those banding at the 1.0 M/1.15 M interface (fraction 2), and a pellet (fraction 3).}

**Measurement of Ca^{2+} Uptake.** The vesicles were preloaded with Na^+ by incubating them in 160 mM NaCl/20 mM Mops/Tris (pH 7.4) for 20 min at 37°C. To measure Ca^{2+} uptake, 0.5-µl aliquots of the Na^+-loaded vesicles were added to a series of tubes containing 15 µl of an appropriate medium (usually 160 mM KCl, LiCl, or NaCl buffered to pH 7.4 with 20 mM Mops/Tris) plus 40 µM 45CaCl_2 (0.6-0.8 Ci/mmol, Amersham; 1 Ci = 3.7 \times 10^{10} bequerels). Ca^{2+} uptake was terminated by adding 5 ml of ice-cold 200 mM KCl/5 mM K phosphate (pH 7.4); the vesicles were harvested on glass fiber filters (Whatman GF/A), washed twice with the KCl/phosphate mixture, and assayed for radioactivity in a scintillation spectrometer. In experiments in which La^{3+} was used, 5 mM Mops/Tris (pH 7.4) was substituted for the K phosphate in the termination fluid. In most experiments, the standard deviation for the values of Ca^{2+} uptake determined by this procedure was less than 10%. Unless explicitly noted otherwise, the data represent single determinations obtained with a single vesicle preparation. Each experiment, however, has been repeated with three or more different vesicle preparations, with results similar to those presented.

**Phosphatase-Binding Assay.** Aliquots (10 µl) of vesicles in 160 mM NaCl were dispensed into 12 × 75 mm glass culture tubes at 37°C containing 0.1 ml of 5 mM Tris/1 mM MgSO_4/1 mM Tris/phosphate (pH 7.4) with or without 1 mM unlabeled ouabain. [G-3H]Ouabain (14.4 Ci/mm, New England Nuclear) was added to a final concentration of 0.12 µM. After 30 min of incubation at 37°C, the vesicles were harvested by filtration. Specific ouabain binding was calculated by subtracting the cpm obtained in the presence of 1 mM unlabeled ouabain from cpm obtained without unlabeled ouabain. This method was based on the procedure reported by Lin and Akera (25).

**Miscellaneous Assays.** Protein was measured by the method of Lowry et al. (26). Mg^{2+}-ATPase activity was measured by the method of Besch et al. (18). Azide- and oligomycin-inhibitable Mg^{2+}-ATPase activity was measured as the difference between total Mg^{2+}-ATPase activity and that measured in the presence of 5 mM Na_2Az and 0.25 µg of oligomycin per ml, respectively. Assays for 5'-nucleotidase were conducted by measuring the phosphate released from AMP, using the procedure of Chen et al. (27). K^+-activated p-nitrophenylphosphatase activity was measured in the presence of 10 mM KCl with or without 1 mM ouabain. ATP-dependent Ca^{2+} uptake was assayed according to the procedure of Jones et al. (28). ATP was measured by using the firefly-luciferase method of Stanley and Williams (29).

**Materials.** A23187, nigericin, and narasin were kindly provided by Robert L. Hamill of Lilly Research Laboratories. 45CaCl_2 was obtained from Amersham. All other chemicals were obtained from commercial sources and were either reagent grade or of the highest purity otherwise available.

**RESULTS**

**Dependence of Ca^{2+} Uptake on Intravesicular Na^+ (Na_v).** The results presented in Fig. 1 show that cardiac membrane vesicles accumulated Ca^{2+} when an outwardly directed Na^+ gradient was generated across the vesicle membrane. In this experiment, the vesicles were loaded internally with either Na^+ or K^+ and then diluted 1:30 into 160 mM LiCl containing 40 µM 45CaCl_2. As shown, the Na^+-loaded vesicles rapidly accumulated Ca^{2+} to a steady state level of 7-8 n mole/mg of protein. In contrast, the K-loaded vesicles accumulated only 1/10th as much Ca^{2+} under the same conditions. Ca^{2+} uptake was markedly inhibited by external Na^+ (Na_o). When the vesicles were diluted into 160 mM NaCl instead of LiCl, Ca^{2+} uptake was reduced to 10% for the Na^+-loaded vesicles and to 50% for the K-loaded vesicles.

No specific requirement for an external ion other than Ca^{2+} could be discerned. Ca^{2+} uptake by Na^+-loaded vesicles in various media exhibited the following sequence of relative activities: 300 mM sucrose ≈ KCl > LiCl > choline Cl ≈ NaCl (salt concentrations, 160 mM each). Thus, the driving force for Ca^{2+} uptake originated with the outwardly directed Na^+ gradient rather than an inwardly directed gradient of some other ion. It must be emphasized that ATP was not present in any of the media used in these experiments, nor could ATP be detected in the vesicle preparation itself [firefly-luciferase method (20); lower detection limit: 1 µM]. Thus, the Na^+ gradient provides the only readily apparent driving force for Ca^{2+} uptake by Na^+-loaded vesicles. If the outwardly directed Na^+ gradient drives Ca^{2+} uptake, then the rate of Ca^{2+} accumulation ought to decline when the vesicles are treated with agents that reduce the magnitude of the Na^+ gradient. This prediction is confirmed by the data shown in Fig. 2. In this experiment, Na^+-loaded vesicles were diluted into 160 mM KCl and incubated for various intervals before 45Ca^{2+} was added. As shown by the closed symbols in Fig. 2, the rate of Ca^{2+} uptake (measured over a 10-sec interval) decreased as the duration of the incubation period increased. When the same experiment was conducted in the presence of 1 µM nigericin, an ionophore that promotes monovalent cation exchange (30), the decline in the rate of Ca^{2+} uptake was markedly accelerated. Similar results were obtained with 1 µM narasin, another monovalent cation-exchange ionophore (31). Because these ionophores are very poor Ca^{2+} conductors (32), their effects on Ca^{2+} uptake must have been exerted indirectly—i.e., by promoting the dissipation of the Na^+ gradient.

**Characteristics of Na_v-Dependent Ca^{2+} Uptake.** The rate of Ca^{2+} uptake was a saturable function of the external
Ca\(^{2+}\) concentration (\(K_m = 18 \mu M\)). The inhibitory effect of external Na\(^+\) on Ca\(^{2+}\) uptake was also saturable, exhibiting half-maximal inhibition at 16 mM Na\(^+\). Ca\(^{2+}\) uptake was completely blocked by 0.1 mM ethylene glycol bis(\(\beta\)-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA).

As shown in Fig. 3, La\(^{3+}\) was a potent inhibitor of Ca\(^{2+}\) uptake. In KCl, half-maximal inhibition was observed at 10 \(\mu M\) La\(^{3+}\); the low levels of Ca\(^{2+}\) uptake observed in NaCl were about 1/10th as sensitive to La\(^{3+}\) inhibition. The data in the inset of Fig. 3 show that the addition of 1 mM La\(^{3+}\) to the vesicles after 4 min of Ca\(^{2+}\) uptake in 160 mM KCl displaced less than 20% of the preaccumulated Ca\(^{2+}\). In contrast, more than 50% of the Ca\(^{2+}\) taken up in NaCl was displaced by the added La\(^{3+}\). La\(^{3+}\) interacts strongly with Ca\(^{2+}\) binding sites in biological systems (33, 34) and rapidly displaces a portion of the superfluously bound Ca\(^{2+}\) in intact cardiac tissue (35) and in cultured heart cells (36). The ineffectiveness of La\(^{3+}\) in displacing the Ca\(^{2+}\) taken up by the vesicles in KCl suggests that the bulk of the accumulated Ca\(^{2+}\) had been sequestered in the vesicle interior and hence was not accessible to La\(^{3+}\). In support of this conclusion, it was found that 85% of the Ca\(^{2+}\) taken up in KCl was released within 10 sec when the vesicles were diluted into a hypotonic medium in the presence of 0.1 mM EGTA; in the absence of osmotic shock, EGTA released only 10–15% of the preaccumulated Ca\(^{2+}\) (cf. Fig. 5).

Is Ca\(^{2+}\) accumulated against a concentration gradient? Equality of internal and external Ca\(^{2+}\) concentrations (40 \(\mu M\)) would yield a value for Ca\(^{2+}\) uptake of 0.2 nmol/mg of protein. Although this value is considerably less than the levels of Ca\(^{2+}\) uptake observed in these studies, it does not take into account the possibility of internal Ca\(^{2+}\) binding or the influence of the transmembrane potential. Indirect evidence supporting the accumulation of Ca\(^{2+}\) against a gradient is presented in Fig. 4. As shown, treating the vesicles with the divalent cation ionophore A23187 (37, 38) reduced the maximal level of Ca\(^{2+}\) accumulation to approximately 65%. More importantly, however, the subsequent loss of Ca\(^{2+}\) by the ionophore-treated vesicles was considerably enhanced relative to that of the control vesicles. The results suggest that Ca\(^{2+}\) had accumulated within the vesicle interior against a concentration gradient and that the ionophore increased the rate of dissipation of this gradient.

Na\(^+-\)Dependent Ca\(^{2+}\) Efflux. The effect of external Na\(^+\) on the rate of Ca\(^{2+}\) efflux from the vesicles is shown in Fig. 5. In this experiment, Na\(^+-\)-loaded vesicles were allowed to accumulate 45Ca\(^{2+}\) for 5 min in 160 mM KCl following the usual procedure. The vesicles were then diluted 1:6 with either 160 mM LiCl or 110 mM KCl/10 mM NaCl. Both solutions contained 0.1 mM EGTA to block further Ca\(^{2+}\) uptake and were buffered to pH 7.4 with 20 mM Mops/Tris. In 160 mM KCl, there was an initial rapid loss of 10–15% of the total vesicular Ca\(^{2+}\) (Fig. 5A). The amount of rapidly lost Ca\(^{2+}\) is similar in magnitude to that displaced by La\(^{3+}\) (cf. above) and probably represents the Ca\(^{2+}\) bound to external sites on the vesicle membrane. The remaining Ca\(^{2+}\) was lost with a half-time of 9.4 min, as calculated from the slope of the first-order plot in Fig. 5B. A similarly slow rate of efflux was observed when the dilution medium contained 160 mM LiCl instead of KCl (data not shown). When the dilution medium consisted of 110 mM KCl/50 mM NaCl, the rate of Ca\(^{2+}\) efflux was dramatically increased. The initial slope of the first-order plot in Fig. 5B showed a half-time of 0.51 min. Thus, the presence of 50 mM external Na\(^+\) stimulated the initial rate of Ca\(^{2+}\) efflux approximately 30-fold. This enhancement in the rate of Ca\(^{2+}\) efflux was blocked by the inclusion of 1 mM LaCl\(_3\) in the dilution medium (data not shown).

Origin of Na\(^+-\)Dependent Ca\(^{2+}\) Uptake. The vesicles used in these experiments were a heterogeneous mixture of membranes derived from sarcolemma, sarcoplasmic reticulum,
Fig. 4. Effect of A23187 on Ca$^{2+}$ uptake by cardiac membrane vesicles. Vesicles were loaded with Na$^+$ and then treated with either 0.1 mM A23187 (18 nmol/mg of protein) (△, ○) or 1% dimethyl sulfoxide (-, △), the solvent used to solubilize the ionophore. Ca$^{2+}$ uptake was then monitored in either 160 mM KCl (closed symbols) or 160 mM NaCl (open symbols). The increased uptake of Ca$^{2+}$ by the ionophore-treated vesicles in NaCl may reflect the entry of Ca$^{2+}$ into an intravesicular space that was inaccessible in the absence of the ionophore or direct binding of Ca$^{2+}$ by the ionophore itself.

mitochondria, and perhaps other cellular organelles. In an attempt to specify the origin of the Na$^+$–Ca$^{2+}$ interactions described above, the vesicles were fractionated by centrifugation in a discontinuous sucrose gradient. As shown in Table 1, there was a close correspondence among the various fractions in the relative values for Na$^+$-dependent Ca$^{2+}$ uptake and for specific ouabain-binding activity, a marker for sarclemmal membranes. Both activities decreased in the order of increasing density: fraction 1 > fraction 2 > fraction 3. The distribution of total vesicle protein, as well as that of the specific activity for azide-inhibitable Mg-ATPase, a marker for mitochondrial membranes, showed a different pattern, increasing in the order fraction 1 < fraction 2 < fraction 3 (data not presented). In our hands, ATP-dependent Ca$^{2+}$ uptake in the presence of oxalate, a marker for sarcoplasmic reticulum membranes, did not survive the fractionation procedure and so the distribution pattern for this marker could not be ascertained. The results in Table 1 suggest that the sarclemmal membranes in the preparation are the source of the observed Na$^+$-dependent Ca$^{2+}$ uptake.

**DISCUSSION**

The results presented here demonstrate that cardiac membrane vesicles take up Ca$^{2+}$ rapidly when an outwardly directed Na$^+$ gradient is generated across the vesicle membrane. Dissipation of the Na$^+$ gradient by passive diffusion or with the aid of monovalent cation-exchange ionophores leads to a corresponding decline in the rate of Ca$^{2+}$ uptake. External Na$^+$ blocks the uptake of external Ca$^{2+}$ and enhances manifold the rate of efflux of internal Ca$^{2+}$. These results provide strong support for the operation of a Na$^+$–Ca$^{2+}$ exchange mechanism in the membrane vesicles. Thus, Ca$^{2+}$ accumulates within the vesicles only when it can exchange for intravesicular Na$^+$. The same process operating in reverse accounts for the stimulatory effect of external Na$^+$ on Ca$^{2+}$ efflux. The mechanism by which external Na$^+$ blocks Na$^+$-dependent Ca$^{2+}$ uptake most likely involves a direct competition between external Na$^+$ and Ca$^{2+}$ for binding sites on the exchange carrier (5, 6).

An alternative interpretation of the data is that the outwardly directed Na$^+$ gradient generates a diffusion potential, negative inside, with which Ca$^{2+}$ equilibrates passively, resulting in increased Ca$^{2+}$ uptake. However, it is difficult to explain on this basis why a similarly directed gradient of K$^+$, to which sarclemmal membranes are presumably more permeable than to Na$^+$, does not produce an equal or greater accumulation of Ca$^{2+}$ (cf. Fig. 1). Indeed, K$^+$-loaded vesicles did not accumulate Ca$^{2+}$ even when their permeability to K$^+$ was increased by treatment with valinomycin, a K$^+$-specific ionophore (unpublished observations). Thus, the generation of a negative diffusion potential is not sufficient in itself to cause Ca$^{2+}$ uptake by the vesicles.

The inhibition of Na$^+$–Ca$^{2+}$ exchange activity by La$^{3+}$ is not in accord with the results of Katzung et al. (39), who found that Na$^+$-dependent Ca$^{2+}$ efflux from guinea pig atria was unaffected by 0.2 mM La$^{3+}$ [Langer and Frank (36), however, have reported that 0.5 mM La$^{3+}$ markedly inhibits Ca$^{2+}$ fluxes in cultured heart cells.] Possible explanations for the discrepancy between our results and those of Katzung et al. include differences in the species and/or tissues used, changes in the surface structure or orientation ("sidedness") in the vesicle prep-

**Table 1. Ouabain binding and Ca$^{2+}$ uptake by separated vesicle fractions**

<table>
<thead>
<tr>
<th>Vesicle fraction</th>
<th>Specific ouabain binding, pmol/mg protein</th>
<th>Ca$^{2+}$ uptake, nmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated vesicles</td>
<td>2.46 ± 0.04</td>
<td>7.5 ± 0.9</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>5.43 ± 0.23</td>
<td>21.1 ± 1.7</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>1.86 ± 0.04</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>0.62 ± 0.04</td>
<td>2.1 ± 0.3</td>
</tr>
</tbody>
</table>

The values given are the means (±SEM) of triplicate determinations obtained with a single preparation. Qualitatively similar results have been obtained with three other preparations.
arations, and the possibility that vesicular Na\(^+\)-Ca\(^{2+}\) exchange reflects the activity of intracellular rather than sarcolemmal membranes (cf. below). The resolution of this discrepancy is of considerable importance because La\(^3+\) blocks excitation-concentration coupling in cardiac tissue at concentrations similar to those that inhibit Na\(^+\)-Ca\(^{2+}\) exchange in the vesicles (35, 40). Sanborn and Langer (35) have attributed this effect of La\(^3+\) to the displacement of a specific pool of bound Ca\(^{2+}\) from the outer surface of myocardiak cells. Our results raise the question whether the inhibition of Na\(^+\)-Ca\(^{2+}\) exchange might also occur in some fashion in the uncoupling action of La\(^3+\).

The sarcolemmal membranes within the vesicle preparation appear to be the source of the observed Na\(^+\)-Ca\(^{2+}\) exchange activity. This is supported by the correlation between Na\(^+\)-dependent Ca\(^{2+}\) uptake and specific ouabain-binding activities in vesicle fractions separated by density gradient centrifugation (Table 1). The most likely alternate source of Na\(^+\)-Ca\(^{2+}\) exchange activity is mitochondrial. Crompton et al. (41, 42) have reported that Na\(^+\) and Li\(^+\) stimulate Ca\(^{2+}\) efflux in heart mitochondria, presumably through the operation of a Na\(^+\)-Ca\(^{2+}\) exchange system. Two observations, however, suggest that mitochondrial membranes do not contribute to a major way to the results reported here. First, there was an inverse correlation between a mitochondrial membrane marker (N\(_2\)-sensitive Mg\(^{2+}\)-ATPase) and Na\(^+\)-dependent Ca\(^{2+}\) uptake among vesicle fractions separated by density gradient centrifugation. Second, 160 mM Li\(^+\) neither blocks Ca\(^{2+}\) uptake nor stimulates Ca\(^{2+}\) efflux in the vesicle system, although Li\(^+\) markedly influences the Na\(^+-\)Li\(^+\) countertransport in heart mitochondria (41, 42). The results of studies with more highly enriched preparations of sarcolemmal mitochondrial vesicles, combined with extensive marker enzyme analyses, should provide additional evidence as to the origin of Na\(^+\)-Ca\(^{2+}\) exchange activity within the vesicle preparations.

Membrane vesicles offer several experimentally advantageous features for the study of Na\(^+\)-Ca\(^{2+}\) exchange. Among these are the minimization of compartmentation effects, the ability to manipulate the ionic compositions on either side of the membrane, and the ease with which the effects of possible energy sources, inhibitors, or regulatory agents can be examined. Thus, questions concerning the stoichiometry, kinetics, and electrogenicity of Na\(^+\)-Ca\(^{2+}\) exchange and the possible role of ATP in this process (43) are all amenable to the vesicle approach. We feel that the use of membrane vesicles will greatly facilitate the characterization of Na\(^+\)-Ca\(^{2+}\) exchange activity in the heart.

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