External Na-independent Ca Extrusion in Cultured Ventricular Cells

Magnitude and Functional Significance

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ABSTRACT The relative magnitudes and functional significance of Ca extrusion by Na-Ca exchange and by an Na₉-independent mechanism were investigated in monolayer cultures of chick embryo ventricular cells. Abrupt exposure of cells in 0-Na, nominally 0-Ca, solution to 20 mM caffeine produced a large contracture (3.94 ± 0.90 μm of cell shortening) that relaxed with a tₜ₀ of 8.60 ± 1.22 s. An abrupt exposure to caffeine plus 140 mM Na resulted in a contracture that was smaller in amplitude (1.53 ± 0.50 μm) and relaxed much more rapidly (tₜ₀ = 0.77 ± 0.09 s). An abrupt exposure to caffeine in 0-Na solutions produced an increase in ⁴⁵Ca efflux that persisted for 20 s, and a net loss of Ca content, determined by atomic absorption spectroscopy (AAS), of ∼4 nmol/mg protein, within 35 s. A comparable net loss of Ca was demonstrated in the presence of 100 μM Ca₀. The abrupt exposure of cultured cells to 0 Na, in 1.8 mM Ca produced a Ca uptake, estimated with ⁴⁵Ca, of 3.2 nmol/mg protein · 15 s, but produced no increase in cell Ca content (AAS). In cells in which a 30% increase in Na was produced by 5 min exposure to 10⁻⁶ M ouabain, the abrupt exposure to 0 Na produced a Ca uptake of 6 nmol/mg protein · 15 s and an increase in Ca content (AAS) of 4 nmol/mg protein. We conclude that there is an Na₉-independent mechanism for Ca extrusion in these cells, presumably a Ca-ATPase Ca pump, with a limited Ca transport capacity of no more than 2 nmol/mg protein · 15 s. This is five times smaller than the demonstrated maximum capacity of the Na-Ca exchanger in these cells. The relaxation of twitch tension in these cells seems to be dependent primarily on sarcoplasmic reticulum uptake of Ca, with a secondary role provided by the Na-Ca exchanger. The Ca pump appears to contribute little to beat-to-beat relaxation.

INTRODUCTION

In a variety of excitable tissues, including squid giant axons (DiPolo and Beaugé, 1984), barnacle muscle fibers (Nelson and Blaustein, 1981), and frog skeletal muscle (Yamada and Schobinger, 1981), Na-Ca exchange has been implicated in the regulation of intracellular Ca concentration. Recent studies (DiPolo and Beaugé, 1984) have suggested a role for Na-Ca exchange in the regulation of intracellular Ca concentration in mammalian cardiac muscle. The relative magnitudes and functional significance of Ca extrusion by Na-Ca exchange and by an Na₉-independent mechanism were investigated in monolayer cultures of chick embryo ventricular cells.
muscle fiber (Caputo and Bolanos, 1978), net efflux of Ca$^{2+}$ against an electrochemical gradient can be accomplished by two Ca transport mechanisms. A high-capacity, Na+-dependent, ATP-independent Ca extrusion via Na-Ca exchange appears to predominate at [Ca$^{2+}$]$_{i}$ > 2 μM (Blaustein, 1977):

$$n\text{Na}_o + 1 \text{Ca}_i \rightleftharpoons n\text{Na}_i + 1 \text{Ca}_o,$$  \hspace{1cm} (Reaction 1)

where $n$ is the stoichiometric coefficient of the exchange reaction. An ATP-dependent component, presumably mediated by a low-capacity, high-affinity Ca-ATPase Ca pump, accounts for a more significant fraction of Ca$^{2+}$ efflux when [Ca$^{2+}$]$_{i}$ is <1 μM (DiPolo and Beaugé, 1984; Lederer and Nelson, 1983):

$$\text{Ca}_i + \text{ATP} \rightarrow \text{Ca}_o + \text{ADP} + \text{P}_i.$$

(Reaction 2)

In cardiac muscle as well, there is evidence for two Ca extrusion mechanisms. Reuter and Seitz (1968) noted that the removal of extracellular Na and Ca reduced $^{45}$Ca efflux in guinea pig atria to 20% of control, but did not eliminate Ca efflux entirely. The removal of Na$_o$ had a relatively minor effect on Ca efflux from cultured rat heart cells (Langer et al., 1976) or from the arterially perfused rabbit interventricular septum (Wendt and Langer, 1977). Busselen and Van Kerkhove (1978) found that residual $^{45}$Ca efflux from goldfish ventricle present in 0-Na$_o$, 0-Ca$_o$ solution was reversibly depressed by metabolic inhibitors, which suggests that an Na$_o$-independent, ATP-dependent mechanism cooperated with Na-Ca exchange in producing Ca extrusion in this tissue. Strong support for the existence of an Na$_o$-independent Ca extrusion system in cardiac muscle has also been provided by the work of Caroni and Carafoli (1980), who demonstrated an ATP-dependent Ca$^{2+}$-pumping system in canine sarcolemmal vesicles. This was confirmed by other groups (Trumble et al., 1980; Lamers and Stinis, 1981). It has been suggested that this system may be an important mechanism for Ca extrusion from myocardial cells (Caroni and Carafoli, 1981), but the relative importance of the Ca pump and Na-Ca exchange in this regard remains unestablished.

As discussed by Mullins (1979), the direction and magnitude of Ca transport via the Na-Ca exchange mechanism would be expected to depend on the stoichiometry, $n$, of the exchange process and on the phasic changes in transmembrane ion concentration gradients for Na and Ca and membrane potential that occur during the contraction-relaxation cycle. The stoichiometry of the Na-Ca exchanger can be estimated from the ratio of electrochemical gradients for Na and Ca (Sheu and Fozzard, 1982) if it is assumed that the Na-Ca exchange is at equilibrium. However, parallel pathways for Ca movement, such as a passive Ca leak or a Ca pump–mediated Ca flux, may displace the Na-Ca exchange process from equilibrium, causing an error in the measurement of the stoichiometry from electrochemical gradient ratios (Sheu and Fozzard, 1982; Axelson and Bridge, 1985). The estimation of the stoichiometry from ion movements (Pitts, 1979; Wakabayashi and Goshima, 1981; Bridge and Bassingthwaighte, 1983; Reeves and Hale, 1984) will also be distorted by the presence of parallel pathways for Ca movement. A consideration of the relative magnitude of Ca pump–mediated Ca flux in heart cells is therefore essential when estimating the
stoichiometry of the Na-Ca exchanger. It is also important for an understanding of the mechanism by which Ca is extruded from the cell in diastole, when [Ca$^{2+}$]i levels are low.

The complex anatomy and large extracellular space of intact cardiac muscle make it difficult to measure transsarcolemmal Ca movement. In previous studies (Barry and Smith, 1982, 1984), we have used monolayer cultures of chick embryo ventricle, a preparation of lesser anatomic complexity, to investigate Ca extrusion. Our initial results suggested that upon abrupt exposure to solution that contained no Na, efflux of Ca was maintained or increased. This finding is consistent with the presence of an Na$_o$-independent Ca extrusion mechanism, presumably an ATP-dependent Ca pump, in these cells. However, this result might also be explained by enhanced Ca-Ca exchange (Barry and Smith, 1984; Eisner and Lederer, 1985), since this process could affect the magnitude of unidirectional efflux measurements made using $^{45}$Ca as a tracer (Blaustein, 1977; Bartschat and Lindenmayer, 1980).

In the experiments to be described, we used both $^{45}$Ca flux methods and atomic absorption spectroscopy to measure the extent to which cultured myocardial cells extrude Ca via Na-Ca exchange and via Na$_o$-independent mechanisms. We have also attempted to assess the relative importance of these processes in myocardial cell relaxation. Our results demonstrate the presence of both Na$_o$-dependent and Na$_o$-independent transsarcolemmal Ca transport. Na-Ca exchange is a more rapid Ca extrusion mechanism, which appears to be able to contribute to a slow phase of twitch relaxation. The Ca pump in these cells is a relatively low-capacity, slow Ca extrusion system, which appears to be incapable of participating in a quantitatively significant fashion in beat-to-beat relaxation.

**METHODS**

**Tissue Culture**

Spontaneously contracting monolayers of chick embryo ventricular cells were prepared as described previously (Barry et al., 1975). Briefly, ventricles from 10-d-old embryos were minced and placed in Ca-Mg-free Hanks' solution. The tissue was trypsinized in 10 ml 0.025% trypsin in Ca-Mg-free Hanks' solution at 37°C for four cycles of 7 min each. The supernatant suspensions containing dissociated cells were placed in 20 ml of cold trypsin inhibitor medium and centrifuged at 2,000 rpm for 10 min. The supernatant was discarded and the cells were resuspended in culture medium consisting of 6% heat-inactivated fetal calf serum, 40% medium 199 (Gibco Laboratories, Grand Island, NY), 0.1% penicillin-streptomycin antibiotic solution, and 54% balanced salt solution containing (in mM): 116 NaCl, 1.0 Na$_2$HPO$_4$, 0.8 MgSO$_4$, 1.18 KCl, 26.2 NaHCO$_3$, and 5 glucose. The cell suspension was diluted to 4 x 10$^6$ cells/ml and placed in plastic petri dishes containing 25-mm circular glass coverslips. Cultures were incubated in a 5% CO$_2$ atmosphere for 3 d at 37°C. All studies were performed on cells after 3 d of culture.

**Measurement of Cell Motion**

A glass coverslip with an attached cell layer was placed in a chamber in which a small segment of the culture was continuously superfused with a layer of fluid 0.5 mm deep. The chamber was placed on the stage of a Nikon Diaphot inverted phase-contrast
microscope (S & M Microscopes, Colorado Springs, CO), enclosed in a Lucite box heated to 37°C. The inlet to the perfusion chamber was connected by a manifold and polyethylene tubing to five Sage syringe pumps (Orion Research Inc., Cambridge, MA) so that a culture segment could be sequentially superfused with different media. At a flow rate of 2 ml/min, the medium bathing a cell in the center of the superfused segment could be exchanged, with a time constant of 2 s.

The optical apparatus was supported by an air table (Barry Wright Corp., Watertown, MA) to damp vibration, and the cells were magnified using a 40× objective. Plastic microspheres 2–3 μm in diameter (3M, New Brighton, MA) were added to the cultures on day 2 of culture; they became attached to the cell surfaces and provided an improved image for contraction measurement. The image was monitored with a low-light-level TV camera (Dage 650 SSX, Dage-MTI, Inc., Michigan City, IN) attached to the microscope observation port. Motion along a selected raster line segment was quantitated using a modification of the system previously described (Barry et al., 1980). The video signal was processed using a Processor 604, a Sync Stripper 302-2, and a Video Motion Analyzer 633 (Colorado Video, Boulder, CO). The analog output of the motion analyzer was recorded as an analog signal on a strip-chart recorder (custom built) and on magnetic tape (3968A, Hewlett-Packard Co., Palo Alto, CA).

Membrane Voltage Recording

Intracellular potentials were measured using 80–90-MΩ glass capillary microelectrodes (1.2 mm o.d., extruded fiber 30-31-1; Frederick Haer & Co., Brunswick, ME). Electrodes were pulled on an Industrial Sciences Associates (Ridgewood, NJ) model M1 puller. Cell penetration was achieved with a Burleigh Instruments (Fishers, NY) Inchworm Controller (PZ577) mounted on a conventional micromanipulator. Potentials were obtained from the same cell from which motion was recorded or from an immediately adjacent cell. The zero level of potential was established before cell penetration; recordings in which this potential changed by >5 mV after intracellular recordings were made were discarded.

Ion Fluxes and Contents

For these measurements, both isotopic tracer and atomic absorption techniques were employed.

**⁴⁰Ca Tracer Methods.** In ⁴⁰Ca tracer experiments, cells were first exposed for 24 h to medium containing [²H]leucine (0.2 μCi/ml, [leucine] = 28 mg/liter). The incorporation of [²H]leucine into cell protein permitted the subsequent normalization of ⁴⁰Ca counts relative to milligrams of cell protein (Lowry et al., 1951), using the measured ²H counts per milligram cell protein for each culture. For Ca uptake experiments, individual coverslips were immersed in uptake medium containing ⁴⁰Ca (5 μCi/ml) for periods ranging from 3 to 90 s. Coverslips were subsequently removed and washed for 30 s in ice-cold balanced salt solution containing 0 Ca and 0.1 mM K-EGTA. The monolayer was then scraped off the coverslip, and the cells were placed in 2 ml of a solution containing 1% sodium dodecyl sulfate and 10 mM Na borate for 2 h. A 1.6-ml aliquot of the dissolved cell mixture was placed in 15 ml Aquasol liquid scintillation fluid (New England Nuclear, Boston, MA) and counted on a liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, IL). From the measured counts per minute per Ca concentration in the uptake medium, uptake data were calculated as nanomoles of Ca per milligram of cell protein.

For Ca efflux measurements, cells were labeled to equilibrium for 2 h in ⁴⁰Ca. Individual coverslips were then washed for 15 s at 37°C to remove the extracellular tracer. They were then immersed for 5 s serially in seven 2-ml volumes of efflux solution at 37°C.
Thus, efflux was measured over a 35-s period. $^{45}$Ca counts were determined in each 2-ml volume. Then we measured the total $^{45}$Ca remaining in the cells on each individual coverslip after the efflux was measured. Next, we calculated the fractional efflux for each 5-s period as the fraction of total $^{45}$Ca counts per minute lost during the entire efflux period and counts per minute remaining in the cells after completion of the 35-s efflux.

**Atomic Absorption Spectroscopy.** Atomic absorption methods were also used to detect changes in cellular contents of Ca. Glass coverslips with an adherent monolayer of cells were washed for 30 s in 4°C 0.1 mM K-EGTA and 140 mM choline Cl buffered with HEPES, pH 7.35. The monolayer was scraped from the glass coverslip with a Teflon scraper that had been carefully washed in deionized water. Small (5 ml) Teflon sample vials were carefully washed in deionized water, and 0.75 ml of 33% nitric acid extract solutions was added to the vials. The cells were washed off the Teflon scraper into this extract media, where they were then digested. The nitric acid (GFS Chemicals, Columbus, OH) was double-distilled in Vycor glass and was spectrophotometrically pure. Prewashed plastic pipettes were used and the acid was stored in a contaminant-free Teflon bottle.

Samples of acid-digested cells were analyzed for Ca using a Varian AA1275 atomic absorption spectrophotometer in conjunction with a Varian GTA 95 microprocessor-controlled graphite furnace and a programmable auto-sampler (Varian Associates, Palo Alto, CA). Because the amount of Ca contained in a single monolayer of cells is small, contamination by Ca posed a serious problem. For this reason, solution vials for the auto-sampler were machined out of Teflon, which can be washed free of containing Ca. All Teflon containers used in these analyses were refluxed for several days in 50% HCl. They were then refluxed for several more days in deionized H$_2$O. Sample and standard vials were stored in deionized water (in a Teflon jar). Before an analysis, sample vials were carefully rinsed, filled with blank solution, and analyzed for contaminating Ca.

Standards were diluted from certified atomic absorption standards (Fisher Scientific, Springfield, NJ). Only prewashed plastic volumetric flasks and prewashed plastic Eppendorf pipette tips were used in the preparation. These standards were made up in 0.5% spectrophotometrically pure nitric acid. A blank was made from 0.5% nitric acid.

Standard curves were constructed using the microprocessor-controlled programmable auto-sampler and graphite furnace. Ca concentrations were plotted against absorbance. Each point was the mean of three readings. Ca was measured at a wavelength of 422.7 nm. Sample concentrations were obtained by interpolation, which was performed automatically by the microprocessor. The standard curve was corrected every eight samples by the reslope capability of this instrument.

Sample and standard analysis was accomplished with uncoated graphite tubes. Although these tubes are less sensitive and produce a broader absorbance profile than pyrolytically coated tubes, they produce results with high precision. They also exhibit slow deterioration with repeated firing and little incandescence. After the injection of either sample or standard into a graphite tube, the tube was subjected to a series of temperature changes designed to dry, ash, and atomize the analyte. The sequence and time course of the temperature changes were determined empirically and then programmed. After atomization, the graphite furnace was subject to a programmed series of tube-cleaning firings to 2,800°C. This eliminated all contaminating memory from previous analyses. Ca was atomized at 2,600°C. Ca phosphate can interfere with Ca atomization. However, at 2,600°C, Ca phosphate is completely dissociated, so that La is not necessary as an interference suppressant. In all determinations, the blank readings were at most a few percent of the sample value.

The standard Ca solutions were made up in 0.5% HNO$_3$, whereas the samples were digested in 33% HNO$_3$. We found that the acid in the samples was the source of matrix
interference that suppressed the Ca absorbance signal. The origin of this interference appears to reside in the different manner in which high- and low-acid samples are distributed in the graphite tube after injection. Since this interference was not recognized until a considerable number of samples had been analyzed, we measured the percent interference produced in five standard samples and corrected the previous measurements.

Protein was measured in the acid digest by the Lowry technique (Lowry et al., 1951) after the analysis. The results are expressed as nanomoles of Ca per milligram of protein.

**Solutions**

All experiments were done using HEPES-buffered solutions (pH 7.35), as described previously (Barry et al., 1978). For 0-Na, experiments, choline Cl was substituted for NaCl on a molar basis. In nominally 0-Ca, experiments, no Ca was added to the solutions, and the total Ca concentration ranged from 1 to 5 μM, as measured with a Ca-sensitive electrode or by atomic absorption spectroscopy. Unless otherwise specified, the Ca concentration in all other experiments was 1.8 mM.

**Statistical Analysis**

Paired or nonpaired Student’s t tests were used in comparing two groups. For multiple comparisons, a one-way analysis of variance with repeated measures was used.

**RESULTS**

**Effects of Caffeine and Na on Cell Motion**

Previous studies in our laboratory (Rasmussen and Barry, 1983) have shown that the abrupt exposure of cultured ventricular cells to caffeine results in a transient positive inotropic effect followed by a sustained negative inotropic effect, which is associated with an increase in \(^{45}\)Ca efflux and a decrease in exchangeable Ca content. These results are consistent with a caffeine-induced release of Ca from the sarcoplasmic reticulum (SR) (Thorpe, 1973). We reasoned that abrupt exposure to caffeine might provide a means of suddenly elevating [Ca\(^{2+}\)] at the sarcolemmal sites of Ca extrusion, and thus allow us to examine the relative rates of Ca extrusion via Na-Ca exchange and via other mechanisms. First, we performed the experiment shown in Fig. 1. Cells were exposed to 0-Na, nominally 0-Ca solution. They exhibited oscillatory mechanical activity, which is consistent with phasic uptake and release of Ca from the SR, as occurs in myocardial cells during Ca overload (Allen et al., 1984; Lakatta et al., 1985). Exposure to caffeine in the absence of extracellular Na produced a large contracture that subsequently relaxed slowly. If the same cell was abruptly exposed to caffeine plus 140 mM Na, the magnitude and duration of the contracture were markedly reduced. Table 1 shows the results from seven paired experiments comparing the amplitude and duration of caffeine contracture in the presence and absence of Na in the same cell. The table also shows the effects of 1 min exposure to 1 mM La, which rapidly inhibited Na-Ca exchange in these cells, but which had little effect initially on Na\(_c\)-independent Ca efflux (Barry and Smith, 1982). In seven different cells, La increased the amplitude and prolonged the duration of the contracture observed in caffeine plus 140 mM Na.

These results suggest that during the [Ca\(^{2+}\)] transient produced by caffeine, Na-Ca exchange can extrude sufficient Ca to influence the amplitude and time...
FIGURE 1. Tracings showing the effects of an abrupt exposure to caffeine in the presence and absence of Na. Control cell motion is shown on the left, in 1.8 mM Ca, 140 mM Na. Cells were then sequentially exposed to nominally 0 Ca ([Ca] = 1–5 μM) and nominally 0-Ca, 0-Na solutions for 1 min each. In 0-Na, 0-Ca solution, the cell developed oscillatory mechanical activity (3 Hz) indicative of Ca overload of the SR. An abrupt exposure to 0 Na, 0 Ca with 20 mM caffeine (first vertical arrow) produced a transient contracture, with a loss of oscillatory activity. The return to normal Na and Ca solution resulted in a return of spontaneous contractile activity within 5 min (middle panel, left tracing). Exposure to 0 Na, 0 Ca again produced mechanical oscillations, and a subsequent abrupt exposure to a solution containing 20 mM caffeine, 140 mM Na (second vertical arrow) caused only a small brief contracture. The right panel shows a repeat of the initial protocol, which demonstrates that the response of this cell to caffeine in the absence of Na is essentially unchanged over this time interval.

The course of the resulting contracture. The amplitude of the contracture in cells exposed simultaneously to both Na and caffeine was less than half that achieved in cells exposed to caffeine alone. The rate of relaxation in Na was 10 times as fast as in its absence. These effects of Na on caffeine contracture could be nearly completely blocked by exposure to an inhibitor of Na-Ca exchange.

Effects of Caffeine and Na on \(^{45}\)Ca Flux and Ca Content

We were particularly interested in the relaxation of cells after a caffeine contracture even in the continued absence of extracellular Na. This suggested the existence of an alternative pathway for Ca extrusion from the cells and/or a reduction in \([\text{Ca}^{2+}]\), by intracellular Ca buffering (Robertson et al., 1981). To investigate these possibilities, we measured \(^{45}\)Ca efflux. The \(^{45}\)Ca efflux experiments corresponding to the conditions shown in Fig. 1 and Table I are depicted in Fig. 2. Caffeine produced an increase in \(^{45}\)Ca efflux in the presence and

| TABLE I |
| --- |
| Effects of Na and La on Caffeine Contracture |

| CaExtrusion in Cultured Ventricular Cells | 399 |
| --- |
| 0 Na, 0 Ca | 3.94±0.90 | 8.60±1.22 |
| 149 mM Na, 0 Ca | 1.53±0.50 | 0.77±0.09 |
| 140 mM Na, 0 Ca, 1 mM La | 2.28±0.33 | 6.51±2.18 |

\( n = 7. P \) values shown indicate a paired t test comparison of the 0 Na, 0 Ca vs. 140 mM Na, 0 Ca groups. By nonpaired t test analysis, there was no significant difference between the 0 Na, 0 Ca and the 140 mM Na, 0 Ca, 1 mM La groups.
FIGURE 2. (A) Effect of an abrupt exposure to caffeine in the absence of Na on fractional Ca efflux. Cells were labeled with $^{45}$Ca in normal medium for 2 h, in 0 Ca, 140 mM Na for 1 min (just $^{45}$Ca tracer present), and in 0 Ca, 0 Na for 1 min. Cells were then washed for 15 s in unlabeled 0 Na, 0 Ca to remove extracellular tracer, and efflux was measured at 5-s intervals. The bottom curve (squares) shows the efflux of $^{45}$Ca in 0-Na, 0-Ca solution for the entire time period. The upper curve (circles) shows efflux in 0 Na, 0 Ca for the first 5-s period, with exposure to 20 mM caffeine (vertical arrow) for subsequent efflux intervals. Each point represents the mean ± SEM for 19 coverslips. This efflux curve corresponds to the experimental conditions associated with the responses to caffeine shown in the left and right panels of Fig. 1. By ANOVA, differences between the 10-, 15-, and 20-s time points were significant at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively. (B) Effect of an abrupt exposure to caffeine in 140 mM Na on fractional Ca efflux. Cells were labeled as in A. The first 5 s of efflux for both curves was in 0 Na, 0 Ca. At the vertical arrow, the cells represented by the squares were exposed to 140 mM Na. The cells represented by the circles were exposed to 140 mM Na plus 20 mM caffeine; this curve corresponds to the experimental conditions associated with the response to caffeine and Na shown in the middle panel of Fig. 1. The differences between the 10-, 15-, and 20-s time points were significant at $P < 0.001$, $P < 0.05$, and $P < 0.01$, respectively.
absence of Na. However, the efflux of $^{45}$Ca released by caffeine occurred somewhat more rapidly when Na was applied at the same time as caffeine, a finding consistent with the effects of Na on the contracture relaxation rate. As pointed out by Eisner and Lederer (1985), an increase in $^{45}$Ca efflux in 0 Na could be due to Ca-Ca exchange. Ca-Ca exchange will contribute to unidirectional Ca fluxes but will not produce changes in total Ca content. We were therefore concerned that a component of the increase in $^{45}$Ca efflux after exposure to caffeine (even in nominally 0 Ca) might be due to Ca-Ca exchange. To demonstrate net Na-independent Ca movement against an electrochemical gradient, we measured total Ca content after exposure to caffeine in the absence of Na. The results are shown in Fig. 3. Ca content determined by atomic absorption spectroscopy declined more significantly over a 35-s period in 0 Na, 0 Ca plus caffeine than in 0 Na, 0 Ca alone. This finding indicates that Ca-Ca exchange cannot account entirely for the increase in $^{45}$Ca efflux occurring after exposure to caffeine in the absence of extracellular Na. This supports the hypothesis that there is a process in these cells capable of extruding Ca against an electrochemical gradient in the absence of Na (Barry and Smith, 1984). However, since the true transsarcolemmal Ca$^{2+}$ gradient and the membrane potential are unknown, the electrochemical gradient for Ca$^{2+}$ is unknown. It is possible that the loss of Ca from these cells after abrupt exposure to caffeine occurs by electrodiffusion. To examine this possibility, we measured membrane potential during and after the exposure of cells to caffeine in the absence of Na. Fig. 4 shows recordings obtained in a typical experiment. The membrane potential in this cell averaged about $-50$ mV during and for 35 s after caffeine exposure.

![Figure 3: Effect of an abrupt exposure to caffeine on Ca content.](image)

**Figure 3.** Effect of an abrupt exposure to caffeine on Ca content. In this experiment, cells were treated as described in Figs. 1 and 2 with 0 Na and 0 Ca, and Ca content was determined by atomic absorption spectroscopy. In coverslips of cells from the same cultures, cells were allowed to remain in 0-Na, nominally 0-Ca solution for 35 s in the absence (open circles) and presence (solid circle) of caffeine. Points plotted are the means ± SEM for 24 coverslips from four different cultures. The total Ca content is significantly smaller after 35 s in the presence of caffeine than in 0 Na alone ($P < 0.01$).
exposure. Similar measurements were performed in four different cells, and comparable results were obtained. Assuming a peak \([Ca^{2+}]_i\) during the caffeine contracture of not more than \(10^{-5}\) M, and an external Ca concentration of \(10^{-6}\) M in nominally 0-Ca solution (see Methods), the electrochemical gradient for \(Ca^{2+}\) would remain inwardly directed. Simple diffusion of Ca out of the cell after exposure to caffeine would not, therefore, be expected. Nevertheless, in view of the uncertainty as to the exact level of intracellular \(Ca^{2+}\) attained during the contracture, we repeated the experiment in the presence of 0.1 mM Ca. Control Ca content was 10.0 ± 0.56 nmol/mg protein. This declined to 7.38 ± 0.36 (n = 20) after 35 s in 0 Na, 0.1 mM Ca, and to 6.05 ± 0.25 (n = 20) after 35 s in 0 Na, 0.1 mM Ca, and 20 mM caffeine (\(P < 0.001\) vs. control). Thus, we conclude that the non-Na-dependent Ca extrusion system in these cells is capable of functioning against a very considerable electrochemical gradient, and the loss of Ca in the absence of Na after exposure to caffeine cannot be explained by simple diffusion.

**Capacity of Na-independent Ca Extrusion**

An inspection of Fig. 3 and the above data shows that the Na-independent extrusion system in these cells, presumably the sarcolemmal \(Ca\)-ATPase Ca pump, can extrude \(\sim 4\) nmol Ca/mg protein within 35 s. To determine whether this is the maximum capacity of the pump, we performed additional experiments in which the influx of Ca into the cell was abruptly increased by exposure to 0 Na, and changes in total Ca content were measured.

We initially exposed cells that had been incubated in normal Na and Ca
medium to solution containing 0 Na (choline substitution) and 1.8 mM Ca. As previously reported (Barry and Smith, 1984), this causes a transient contracture. As shown in Fig. 5, exposure to 0 Naₐ produced an increase in Ca uptake to ~3.20 nmol/mg protein · 15 s. We have shown (Biedert et al., 1979) that exposure of these cells to an inotropic concentration of cardiac glycoside, 10⁻⁶ M ouabain, elevates cell Na by ~30% and markedly increases the magnitude of ⁴⁵Ca influx that occurs on exposure to 0 Naₐ (Barry et al., 1985). As shown in Fig. 5, in

![Figure 5](image_url)

**Figure 5.** Effect of abrupt exposure to 0 Naₐ on Ca uptake. Coverslips of cells previously incubated in normal HEPES-buffered balanced salt solution were placed in ⁴⁵Ca uptake medium containing 140 mM Na, 1.8 mM Ca (lower curve, solid circles) or 0 Naₐ, 1.8 mM Ca (middle curve, open circles). Uptake of Ca was also measured in cells previously treated with 10⁻⁶ M ouabain for 5 min, and then immersed in 0 Naₐ, 1.8 mM Ca (upper curve, squares). Ca uptake was determined at the indicated time points (means ± SEM, n = 18–24), and calculated as nanomoles per milligram of protein, based on the specific activity of ⁴⁵Ca in the uptake medium.

ouabain-treated cells, Ca influx on exposure to 0 Naₐ increased to ~6 nmol/mg protein · 15 s. Thus, exposure to 0 Naₐ with different levels of Naₐ produced a considerable range of increased Ca uptake.

Fig. 6 shows the changes in total cell Ca content determined by atomic absorption spectroscopy under similar conditions (cells previously incubated in normal medium and then exposed to 0 Naₐ, or cells treated for 5 min with 10⁻⁶ M ouabain and then exposed to 0 Naₐ). There was no change in cell Ca content
upon abrupt exposure to 0 Na, when cells were not Na-loaded. Assuming negligible Ca-Ca exchange, this indicates a potential capacity of the Ca pump of 3.2 nmol/mg protein·15 s (Figs. 5 and 6). In ouabain-treated cells, 0-Na exposure produced a gain in cell Ca content of ~4 nmol/mg protein within 15 s, which then gradually declined over the subsequent 75 s (Fig. 6). Since uptake of Ca on abrupt exposure to 0 Na in Na-loaded, ouabain-treated cells was 6.0 nmol/mg protein·15 s, but the content of Ca increased by only 4.0 nmol/mg protein during an identical time period, the Na-independent extrusion mechanism appears to have a maximum capacity of ~2 nmol Ca/mg protein·15 s. If there were significant Ca-Ca exchange contributing to the observed ⁴⁵Ca uptake, this value could be an overestimation of the Ca pump capacity. However, it is in reasonable agreement with the capacity estimated from the data shown in Fig. 3, in which Ca-Ca exchange cannot be a factor. This value is also consistent with the fractional efflux results shown in Fig. 2, in which ~12% of total ⁴⁵Ca counts per minute are lost in the first 5 s of immersion in 0 Na, 0 Ca. As the exchangeable Ca content of these cells under these conditions is ~5 nmol/mg protein, this corresponds to an initial rate of Ca loss of 0.6 nmol Ca/mg protein·5 s.

Taken together, these data suggest that most of the Ca uptake measured with
4^5Ca on exposure to 0 Naio is due to Na-Ca exchange rather than to Ca-Ca exchange. Additional evidence for a relatively small component of Ca-Ca exchange in these cells is shown in Fig. 7. The fractional efflux of 4^5Ca from cells treated with 10^{-6} M ouabain was measured in normal Caio, normal Naio, in 0 Caio, normal Naio, and in 0 Caio, 0 Naio. In this experiment, 0.1 mM EGTA was present in the 0-Ca solutions to reduce [Ca^{2+}] below 10^{-9} M. Efflux of 4^5Ca was reduced in 0 Caio, normal Naio, as compared with normal Naio, normal Caio, by ~3% of total exchangeable Ca in the first 5 s, or ~0.15 nmol Ca/mg protein. A larger component of initial efflux can be seen to be Naio dependent. Thus, Ca-Ca exchange, although demonstrable, does not appear to account for more than 10–20% of total transsarcolemmal Ca fluxes under these conditions.

Although the Naio-independent Ca extrusion process, presumably the Ca pump, can apparently eliminate Ca from these cells at a rate of ~2 nmol/mg protein·15 s, several observations suggest that this rate is inadequate to produce complete relaxation in 0 Naio, 1.8 mM Caio in a brief period of time. First, as shown in Fig. 6, only about half of the 4 nmol/mg protein Ca gained in the first 15 s in 0 Na was extruded from the cell over the subsequent 75 s. In addition, we frequently observed that on abrupt exposure to 0 Naio, normal Caio in 10^{-6} M ouabain-treated cells (Fig. 8), complete relaxation and loss of mechanical oscillatory activity did not occur over a 2-min period unless external Na was resupplied. It is also apparent in Figs. 1 and 4 that the mechanical oscillatory behavior in 0 Naio, 0 Caio, which indicates Ca overload of the SR and/or elevations in [Ca^{2+}]i, can persist for some time in 0 Naio. After 2–3 min, however, this activity disappears, presumably because of the action of the Ca pump in lowering [Ca^{2+}]i.
FIGURE 8. Effects of abrupt exposure to 0 Na, 1.8 mM Ca, on the motion of cultured cells previously treated with 10^-5 M ouabain. This figure shows the response to 0 Na (first vertical arrow) of three different cells previously exposed for 5 min to 10^-5 M ouabain. The cell shown in the upper trace relaxed almost completely in 0 Na, and only a slight further relaxation on the return to superfusion with solution containing a normal Na concentration (second vertical arrow). The middle trace shows a cell that did not relax at all in 0 Na, but did relax fully in 140 mM Na. The bottom trace shows a cell that had an intermediate response. All cells showed mechanical oscillations in 0 Na after the initial contracture development; the oscillations were most prominent in the cell shown in the bottom trace.

DISCUSSION

Presence of a Ca Pump in Cultured Heart Cells

A number of investigators have studied the effects of a sudden reduction in Na, which simultaneously produces an increased influx of Ca via Na-Ca exchange and a reduction in the contribution of Na-Ca exchange to Ca extrusion. Bers and Ellis (1982) noted that a reduction of Na, from 140 to 14 mM produced only a slight or negligible increase in [Ca^2+]; in sheep Purkinje fibers, and suggested that this could be due to the presence of a sarcolemmal Ca pump or to buffering of [Ca^2+], by the cell cytosol. Allen et al. (1983) noted that a reduction of Na, to zero by KCl substitution in ferret ventricular muscle injected with aequorin produced a tension increase and an increase in [Ca^2+], that then returned toward resting levels. They found that the relaxation and fall in [Ca^2+], were energy-requiring processes, and probably reflected either sequestration of Ca by intracellular organelles or an ATP-dependent sarcolemmal transport.
process. Subsequently, experiments with caffeine and FCCP (Eisner et al., 1984) suggested that Ca\(^{2+}\) buffering by the SR and mitochondria could not account entirely for tension relaxation in 0 Na\(_o\), and provided further support for a functionally significant sarcolemmal Ca pump. We reported (Barry and Smith, 1984) that the abrupt exposure of cultured chick embryo ventricular cells to 0 Na\(_o\) increased \(^{45}\)Ca influx and produced a transient contracture, but did not increase the exchangeable Ca content in cells with a normal [Na\(^+\)], possibly because of an increased \(^{45}\)Ca efflux in 0 Na\(_o\). We interpreted this finding as providing further support for the existence of a functionally important sarcolemmal Ca pump. However, because the degree to which Ca-Ca exchange contributed to the apparent increase in \(^{45}\)Ca influx and efflux in these experiments was uncertain, we were unable to reach any firm conclusions about the capacity of the Ca pump.

The experiments reported here extend these results and provide further support for the presence of a Ca pump in these cells. The demonstration that caffeine induces a net loss of Ca from the cells in the absence of Na\(_o\), against a considerable Ca\(^{2+}\) electrochemical concentration gradient, is evidence for the presence of an Na\(_o\)-independent Ca transport system. This result is consistent with the finding of Busse and Van Kerhove (1978) that caffeine increased the efflux of \(^{45}\)Ca from goldfish ventricle in the absence of Na\(_o\). However, Jundt et al. (1975) noted that in guinea pig atria, caffeine in the absence of extracellular Na produced a contracture, without increasing \(^{45}\)Ca efflux. Thus, the relative importance of the Na\(_o\)-independent Ca extrusion system in cardiac tissue may vary from preparation to preparation.

Functional Significance of the Ca Pump

The above evidence supports the existence of an Na\(_o\)-independent Ca extrusion system in these cultured heart cells. We will now consider its possible functional significance. As shown in Fig. 1 and Table I, relaxation of a caffeine contracture in the presence of Na\(_o\) occurred with a \(t_\text{rel} \) of 0.77 s, more than 10 times as rapidly as in the absence of Na\(_o\). Vassort et al. (1978) reported that the relaxation rate of frog ventricle after a voltage-clamp repolarization was decreased 10-fold in Na-free solution. Thus, in frog myocardial cells that do not have a functional SR, relaxation of tension, presumably because of extrusion of Ca across the sarcolemma, also appears to occur 10 times faster by Na-Ca exchange than by an Na\(_o\)-independent mechanism. The effect of La on the relaxation rate in caffeine plus Na\(_o\) in our experiments suggests that La inhibits Na\(_o\)-dependent Ca efflux in these cells, as it does Na\(_o\)-dependent Ca influx (Barry and Smith, 1982). Although La has been reported not to inhibit Na\(_o\)-dependent Ca efflux in guinea pig atria (Katzung et al., 1973) or in barnacle muscle cells (Lederer and Nelson, 1983), La does inhibit Na\(_o\)-induced Ca efflux from sarcolemmal vesicles (Reeves and Sutko, 1979; Trosper and Philipson, 1983).

In cultured chick ventricular cells, two components of relaxation can be detected by analysis of cell motion. An initial rapid phase with a time constant of 30–40 ms presumably reflects Ca uptake by the SR. A second, slower phase, with a time constant of several hundred milliseconds, may be due to Ca extrusion
across the sarcolemma (Miura et al., 1981; Clusin, 1981). The present results (see also Clusin et al., 1983) indicate that the slow second phase of relaxation in spontaneously contracting cultured cells might well be due to Na-Ca exchange, as previously suggested (Miura et al., 1981; Clusin, 1981). It is too rapid, however, to be accounted for by the Ca pump. It should be noted that the relaxation rate of a caffeine contracture in 0 Na+ may also be influenced by intracellular Ca2+ binding (Robertson et al., 1981). Thus, the relaxation rate that can be attributed to the Ca pump alone may actually be slower than that observed in 0 Na+.

Evidence for a limited capacity of the Na+-independent Ca extrusion system is also apparent in mechanical records showing Ca overload (mechanical oscillatory activity, incomplete relaxation; Figs. 1, 4, and 8) in 0 Na+, which continued for several minutes. This may reflect the fact that some Ca influx continues via Na-Ca exchange in 0 Na+ until cell Na is depleted (see Fig. 5), thus preventing the Ca pump from restoring normal [Ca2+]i. The inability of the Ca pump to effectively lower [Ca2+]i levels during these conditions is also suggested by the observation by Eisner et al. (1984) that [Ca2+]i in aequorin-injected ferret papillary muscle did not return entirely to normal in 0-Na+ solution. Furthermore, Ca content did not return to normal, in 0 Na+, over a 90-s period (Fig. 6). If the Ca pump were the primary system for elimination of Ca2+ from the cell, these results would not be anticipated.

In this regard, it is of interest to consider the relative maximal capacities of the Na-Ca exchanger and the Ca pump in these cells. As discussed in the Results, the maximum capacity of the Na+-independent Ca extrusion system in these cells appears to be ~2 nmol/mg protein·15 s. However, the maximum rate of Ca transport by Na-Ca exchange in these cells, defined as initial rate of Ca influx in 0 Na+, 140 mM KCl in Na+ loaded cells, is ~10 nmol/mg protein·15 s (Ishida, H., J. H. B. Bridge, and W. H. Barry, unpublished data), or five times greater. There may be some asymmetry of the Na-Ca exchanger with respect to its affinities for Na (Philipson and Nishimoto, 1982), which makes a quantitative comparison of this sort difficult. However, this result is consistent with the study of Caroni and Carafoli (1981) in dog heart sarcolemmal vesicles, in which the Km for the Ca-ATPase Ca transport system was 0.3 ± 0.2 μM, with a Vmax of 0.5 nmol Ca/mg·s, whereas for the Na-Ca exchanger, Km = 1.5 ± 0.2 μM, with Vmax = 15 nmol Ca/mg·s. Thus, although the Km for the Ca-ATPase is lower than that for the exchanger, the maximal rate of Ca transport by the pump in sarcolemmal vesicles is 30 times slower than that for the Na-Ca exchanger.

In summary, our results provide evidence supporting the existence of a relatively low-capacity, Na+-independent Ca transport system in cultured chick embryo ventricular cells. The Na-Ca exchanger appears to be able to account for a slow phase of relaxation of tension. However, as suggested by its low Km of 0.3 μM in vesicle studies, the Na+-independent Ca transport system does not seem to be able to contribute in a significant fashion to the relaxation of twitch tension at normal beating rates. It may, however, provide a relatively continuous slow extrusion of Ca2+, especially at low [Ca2+]i levels during diastole, as proposed by Caroni and Carafoli (1981). Such a Ca extrusion, summed over many beats, could indeed be physiologically significant.
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REFERENCES

Allen, D. G., D. A. Eisner, M. J. Lab, and C. H. Orchard. 1983. The effects of low sodium solutions on intracellular calcium concentration and tension in ferret ventricular muscle. Journal of Physiology. 345:391-407.

Allen, D. G., D. A. Eisner, and C. H. Orchard. 1984. Characterization of oscillations of intracellular calcium concentrations in ferret ventricular muscle. Journal of Physiology. 352:113-128.

Axelson, P. H., and J. H. B. Bridge. 1985. Electrochemical ion gradients and the Na/Ca exchange stoichiometry. Measurements of these gradients are thermodynamically consistent with a stoichiometry coefficient $\geq$. Journal of General Physiology. 85:471-475.

Barry, W. H., D. Goldminz, T. Kimball, and J. W. Fitzgerald. 1978. Influence of cell dissociation and culture of chick embryo ventricle on inotropic response to calcium and lanthanum. Journal of Molecular and Cellular Cardiology. 10:967-979.

Barry, W. H., Y. Hasin, and T. W. Smith. 1985. Sodium pump inhibition, enhanced calcium influx via sodium-calcium exchange, and positive inotropic responses in cultured heart cells. Circulation Research. 56:231-241.

Barry, W. H., R. Pitzen, K. Protas, and D. C. Harrison. 1975. Inotropic effects of different calcium ion concentrations on embryonic chick ventricle: comparison of single cultured cells and intact muscle strips. Circulation Research. 36:727-734.

Barry, W. H., J. Pober, J. D. Marsh, S. R. Frankel, and T. W. Smith. 1980. Effects of graded hypoxia on contraction of cultured chick embryo ventricular cells. American Journal of Physiology. 239:H651-H657.

Barry, W. H., and T. W. Smith. 1982. Mechanisms of transmembrane calcium movements in cultured chick embryo ventricular cells. Journal of Physiology. 325:243-260.

Barry, W. H., and T. W. Smith. 1984. Movement of Ca$^{2+}$ across the sarcolemma: effects of abrupt exposure to zero external Na concentration. Journal of Molecular and Cellular Cardiology. 16:155-164.

Bartschat, D. K., and G. E. Lindenmayer. 1980. Calcium movements promoted by vesicles in a highly enriched sarcolemmal preparation from canine ventricle. Journal of Biological Chemistry. 255:9626-9634.

Bers, D. M., and D. Ellis. 1982. Intracellular calcium and sodium activity in sheep heart Purkinje fibers: effect of changes of external sodium and intracellular pH. Pflügers Archiv. 393:171-178.

Biedert, S., W. H. Barry, and T. W. Smith. 1979. Inotropic effects and changes in sodium and calcium contents associated with inhibition of monovalent cation transport by ouabain in cultured myocardial cells. Journal of General Physiology. 74:479-494.

Blaustein, M. P. 1977. Effects of internal and external cations and ATP on sodium-calcium and calcium-calcium exchange in squid axons. Biophysical Journal. 20:79-111.

Bridge, J. H. B., and J. B. Bassingthwaighte. 1983. Uphill sodium transport driven by an inward calcium gradient in heart muscle. Science. 219:178-179.

Busselen, P., and E. Van Kerkhove. 1978. The effect of sodium, calcium, and metabolic inhibitors on calcium efflux from goldfish heart ventricles. Journal of Physiology. 282:263-283.
Caputo, C., and P. Bolanos. 1978. Effect of external sodium and calcium on calcium efflux in frog striated muscle. *Journal of Membrane Biology.* 41:1-14.

Caroni, P., and E. Carafoli. 1980. An ATP-dependent Ca\(^{2+}\) pumping system in dog heart sarcolemma. *Nature.* 283:765-767.

Caroni, P., and E. Carafoli. 1981. The Ca\(^{2+}\) pumping ATPase of heart sarcolemma. Characterization, calmodulin dependence, and partial purification. *Journal of Biological Chemistry.* 256:3263-3270.

Clusin, W. T. 1981. The mechanical activity of chick embryonic myocardial cell aggregates. *Journal of Physiology.* 320:149-174.

Clusin, W. T., R. Fischmeister, and R. L. DeHaan. 1983. Caffeine-induced current in embryonic heart cells: time course and voltage dependence. *American Journal of Physiology.* 245:H528-H532.

DiPolo, R., and L. Beaugé. 1984. Interactions of physiological ligands with the Ca pump and Na/Ca exchanger in squid axons. *Journal of General Physiology.* 84:895-914.

Eisner, D. A., and W. J. Lederer. 1985. Na-Ca exchange: stoichiometry and electrogenicity. *American Journal of Physiology.* 248:C189-C202.

Eisner, D. A., C. H. Orchard, and D. G. Allen. 1984. Control of intracellular ionized calcium concentration by sarcolemmal and intracellular mechanisms. *Journal of Molecular and Cellular Cardiology.* 16:137-146.

Jundt, H., H. Porzig, H. Reuter, and J. W. Stucki. 1975. The effect of substances releasing intracellular calcium ions on sodium-dependent calcium efflux from guinea-pig auricles. *Journal of Physiology.* 246:229-253.

Katzung, B. G., H. Reuter, and H. Porzig. 1973. Lanthanum inhibits Ca inward current but not Na-Ca exchange in cardiac muscle. *Experientia.* 29:1073-1075.

Lakatta, E. G., M. C. Capogrossi, A. A. Kort, and M. D. Stern. 1985. Spontaneous myocardial calcium oscillations: overview with emphasis on ryanodine and caffeine. *Federation Proceedings.* 44:2977-2983.

Lamers, J. M. J., and J. T. Stinis. 1981. An electrogenic Na\(^+\)/Ca\(^{2+}\) antiporter in addition to the Ca\(^{2+}\) pump in cardiac sarcolemma. *Biochimica et Biophysica Acta.* 640:521-534.

Langer, G. A., L. M. Nudd, and N. Y. Ricchiuti. 1976. The effect of sodium deficient perfusion on calcium exchange in cardiac tissue culture. *Journal of Molecular and Cellular Cardiology.* 8:321-328.

Lederer, W. J., and M. T. Nelson. 1983. Effects of extracellular sodium on calcium efflux and membrane current in single muscle cells from barnacles. *Journal of Physiology.* 341:325-339.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry.* 193:265-275.

Mullins, L. J. 1979. The generation of electric current in cardiac fibers by a Na-Ca exchange. *American Journal of Physiology.* 236:C103-C110.

Nelson, M. T., and M. P. Blaustein. 1981. Effects of ATP and vanadate on calcium efflux from barnacle muscle fibres. *Nature.* 289:314-316.

Philipson, K. D., and A. Y. Nishimoto. 1982. Na-Ca exchange in inside-out cardiac sarcolemmal vesicles. *Journal of Biological Chemistry.* 257:5111-5117.

Pitts, B. J. 1979. Stoichiometry of sodium-calcium exchange in cardiac sarcolemmal vesicles. *Journal of Biological Chemistry.* 254:6232-6235.

Rasmussen, C. A. F., Jr., and W. H. Barry. 1983. Effects of caffeine on contractility and calcium fluxes in cultured chick embryo ventricular cells. *Circulation.* 68:672. (Abstr.)
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Reeves, J. P., and C. C. Hale. 1984. Stoichiometry of the cardiac Na-Ca exchange system.  *Journal of Biological Chemistry*. 259:7733–7739.

Reeves, J. P., and J. L. Sutko. 1979. Sodium-calcium ion exchange in cardiac membrane vesicles.  *Proceedings of the National Academy of Sciences*. 76:590–594.

Reuter, H., and N. Seitz. 1968. The dependence of calcium efflux from cardiac muscle on temperature and external ion composition.  *Journal of Physiology*. 195:451–470.

Robertson, S. P., J. D. Johnson, and J. D. Potter. 1981. The time course of Ca2+ exchange with calmodulin, tropomycin, parvalbumin, and myosin in response to transient increases in Ca2+.  *Biophysical Journal*. 34:559–569.

Sheu, S. S., and H. A. Fozzard. 1982. Transmembrane Na+ and Ca2+ electrochemical gradients in cardiac muscle and their relationship to force development.  *Journal of General Physiology*. 80:325–351.

Thorpe, W. R. 1973. Some effects of caffeine and quinidine on sarcoplasmic reticulum of skeletal and cardiac muscle.  *Canadian Journal of Physiology and Pharmacology*. 51:499–503.

Trosper, T. L., and K. D. Philipson. 1983. Effects of divalent and trivalent cations on Na+-Ca2+ exchanges in cardiac sarcolemmal vesicles.  *Biochimica et Biophysica Acta*. 731:63–68.

Trumble, W. R., J. L. Sutko, and J. P. Reeves. 1980. ATP-dependent calcium transport in cardiac sarcolemmal membrane vesicles.  *Life Sciences*. 27:207–214.

Vassort, G. M., M. J. Roulet, K. G. Mongo, and R. F. Ventura-Clapier. 1978. Control of the frog heart relaxation by Na-Ca exchange.  *European Journal of Cardiology*. 7(Suppl.):17–25.

Wakabayashi, S., and K. Goshima. 1981. Kinetic studies of sodium-dependent calcium uptake by myocardial cells and neuroblastoma cells in culture.  *Biochimica et Biophysica Acta*. 642:158–172.

Wendt, I. R., and G. A. Langer. 1977. The sodium-calcium relationship in mammalian myocardium. Effect of sodium deficient perfusion on calcium fluxes.  *Journal of Molecular and Cellular Cardiology*. 9:551–564.