Calcium-Dependent Enhancement of Myocardial Diastolic Tone and Energy Utilization Dissociates Systolic Work and Oxygen Consumption during Low Sodium Perfusion

Dale G. Renlund, Edward G. Lakatta, E. David Mellits, and Gary Gerstenblith

From the Cardiology Division, Department of Medicine, The Johns Hopkins University School of Medicine, and Cardiovascular Section, Gerontology Research Center, National Institute on Aging, Baltimore, Maryland

SUMMARY. The relationships and correlations among functional, metabolic, and ionic consequences of low sodium perfusion were studied in isovolumic, retrograde-aortic perfused working rat hearts by $^{31}$P nuclear magnetic resonance, oxygen consumption, and atomic absorption spectrometry. Reduction of perfusate sodium from 144 to 74, 51, 39, and 25 mM in four separate groups of hearts via lithium substitution for 15 minutes decreased cell sodium to mean values of 62, 51, 43, and 36 μmol/g dry weight, respectively ($P < 0.001$ vs. control of 107). There was a transient rise and then a fall in developed pressure and a decline in phosphocreatine and adenosine triphosphate, all of which were graded and correlated with perfusate sodium ($P < 0.01$ for all parameters vs. perfusate sodium). This was accompanied by a 2- to 7-fold elevation of diastolic pressure while oxygen consumption remained near control levels. All parameters except adenosine triphosphate returned toward baseline values when normal perfusate sodium was reintroduced. Although cell calcium as measured by atomic absorption spectrometry did not differ among the groups, the functional and metabolic changes did not occur if the sodium steps were performed in reduced perfusate calcium (0.08 mM). In hearts in which systolic function was obliterated by verapamil, exposure to zero sodium caused a 4-fold increase in oxygen consumption, an increase in diastolic pressure, and a reduction of high energy phosphates. In the presence of ryanodine, a specific inhibitor of sarcoplasmic reticulum calcium release, the metabolic changes did not occur, and the excess oxygen consumption in zero sodium was substantially reduced. Thus, the effect of lowered perfusate sodium in beating hearts, i.e., to dissociate oxygen consumption and systolic function, and to increase diastolic pressure and its effect in arrested hearts to increase oxygen consumption, are calcium dependent, energy consuming, and modulated by sarcoplasmic reticulum calcium cycling. (Circ Res 57: 876–888, 1985)

CELL sodium content has an important influence on calcium homeostasis and myocardial function (Reuter and Seitz, 1968; Langer, 1973; Carafoli et al., 1974; Chapman, 1974; Junct et al., 1977). Cell sodium content maintained by several mechanisms (Baker, 1972; Blaustein, 1974; Reuter, 1974; Deitmer and Ellis, 1978; Deitmer and Ellis, 1980), manipulations of which have been used therapeutically and investigationally for 200 years (Withering, 1785). Reduction of extracellular sodium (Na$_e$) induces a rapid decrease in cell sodium activity with profound effects (Chapman et al., 1970; Miller and Moisescu, 1976; Ellis, 1977), the magnitude of which clearly depends on the extent of Na$_e$ lowering (Ellis and Deitmer, 1978), the method of sodium substitution (Spring and Giebisch, 1977; Chapman et al., 1983), the status of the Na$^+$-K$^+$ pump (Ellis, 1977; Ellis and Deitmer, 1978; Li and Vasalle, 1984), the calcium concentration in the perfusate (Langer, 1982), the species, and, probably, the metabolic demands placed upon the preparation at the time of Na$_e$ lowering (Lederer and Tsien, 1976; Ellis, 1977; Ellis and Deitmer, 1978; Deitmer and Ellis, 1980; Lee et al., 1980a, 1980b; Bers and Ellis, 1982; Cohen et al., 1982; Lee and Dagastino, 1982; Gerstenblith et al., 1982; Hoerter et al., 1983; Vaughan-Jones et al., 1983). Although the electrophysiological, ionic, and force changes accompanying lowered Na$_e$ have been described (Glitsch et al., 1970; Mullins, 1979; Tillisch et al., 1979; Coeraboef et al., 1981; Ventura-Clapier and Vassort, 1981; Chapman et al., 1983; Barry and Smith, 1984; Lieberman et al., 1984), the relationships among altered ionic contents, oxygen consumption, and metabolic parameters that result from graded reductions in Na$_e$ have not been considered in the intact myocardium.

In the present study, these metabolic, functional, and ionic consequences were characterized in the isovolumic, beating, and arrested rat heart model during graded reductions in Na$_e$, and then returned to normal Na$_e$ perfusion. $^{31}$P nuclear magnetic resonance (NMR) and oxygen consumption measurements were used to define the metabolic profile, and atomic absorption spectroscopy with potassium...
ethylendiamine tetraacetate cobaltate III (K\(^{+}\),Co\(^{2+}\)-EDTA) as an extracellular marker was employed to determine cell sodium and calcium contents during the experimental protocols. Lithium replacement of sodium was used in perfusate containing no proton-donating permeant anions, thus minimizing the net intracellular calcium gain (Lehninger, 1974a, 1974b; Busseelen and Kerkhove, 1978; Ponce-Hornos and Langer, 1982) and avoiding the undesirable side effects of massive cell calcium overload (Lieberman et al., 1984; Renlund et al., in press). We have explored the mechanism of the low Na\(_i\)-induced perturbations by concomitantly altering perfusate calcium (Ca\(_{\text{a}}\)) levels in beating and verapamil-arrested hearts, and have demonstrated a dissociation between one of the "traditional" determinants of myocardial oxygen demand, developed pressure (total minus diastolic pressure), and oxygen consumption. Finally, we explored a mechanism of diastolic calcium-dependent energy utilization during these perturbations by examining the metabolic effects of ryanodine, a drug which selectively inhibits sarcoplasmic reticulum-myofilament calcium cycling and the resultant myofilament oscillations (Jenden and Lakatta, 1984).

**Methods**

**Preparation of the Heart**

Retired, male breeder Wistar rats, weighing 500–600 g, were heparinized with heparin sodium, 1000 U, ip, and anesthetized with 50–100 mg of sodium pentobarbital. After rapid excision of the heart, the aorta was cannulated and retrogradely perfused at constant flow with a Masterflex 7562-10 penstaltic pump. The hearts were paced throughout the experiments at 180 beats/min with a Grass SD-9 stimulator via a KCl wick electrode. A latex balloon attached to the end of PE 190 tubing was inserted in the left ventricle and inflated with a volume of water (usually 0.10–0.15 ml) sufficient to result in an end-diastolic pressure and EDP of 8–12 mm Hg. This was connected to a Statham P23Db transducer, and total left ventricular pressure and EDP were continuously recorded with a Brush direct writing recorder.

**\(^{31}P\) Nuclear Magnetic Resonance**

The perfused hearts were placed into the 25-mm bore of a Bruker superconducting magnet of 4.2 Tesla field strength. The phosphorus resonance was 72.8 MHz (proton decoupled). The NMR methods are described elsewhere (Moon and Richards, 1973; Garlik et al., 1977; Jacobus et al., 1977; Flaherty et al., 1982; Renlund et al., 1984). Briefly, \(^{31}P\) NMR spectra are obtained on a Bruker WH 180 spectrometer interfaced with a Nicolet 1280 computer. Pulsed, Fourier-transformed, minimally saturated spectra are obtained with a 2-second delay between pulses and a pulse duration of 25 \(\mu\)sec. Data are accumulated with a 2K table at 3.0 Hz spectral width. Changes in tissue contents of adenosine triphosphate (ATP), phosphocreatine (PCr), and inorganic phosphate (P\(_i\)) are determined by integrating areas under individual peaks with a Hewlett-Packard 9810 A digitizer. Previous studies have shown that changes in adenosine triphosphate (ATP), PCr, and P\(_i\), measured by the present NMR technique correlate well with direct biochemical determinations (Gadian et al., 1979; Morris et al., 1984). However, due to uncertainties about the region and volume of distribution of the various compounds and the size and uniformity of the sensitive region of the spectrometer, the best method for conversion of these areas into absolute concentrations is still controversial (Dawson and Wilkie, 1984). In our laboratory, however, we have measured the phosphate metabolites in five hearts after 50 minutes of control perfusion by direct chemical analysis (Hoerter et al., personal communication). After removal from the spectrometer, hearts were frozen with clamps precooled with liquid nitrogen, and the tissue was extracted with 7% perchloric acid. P\(_i\) was immediately measured in the acid extract by a calorimetric method (Berenson and Blum, 1938). ATP and PCr were measured in neutralized extract by enzymatic determination (Estabrook and Maitra, 1962). Protein was quantified by the biuret method. In these five hearts, ATP was 25.7 ± 0.8 nmol/mg protein, PCr was 33.9 ± 1.8 nmol/mg protein, and P\(_i\) was 12.9 ± 0.7 nmol/mg protein.

**Perfusates**

All hearts underwent a stabilization period wherein they were perfused for 30 minutes with oxygenated solution (at 36°C, pH 7.4) containing, in mmoles: sodium, 144; potassium, 6; calcium, 2.5; HEPES, 4; cobalt EDTA (used as an extracellular marker, vide infra), 1; magnesium, 1.2; chloride, 152.4; and glucose, 16. Reductions in Na\(_i\) were made by equimolar substitution with lithium. No ion was substituted for calcium when its concentration was reduced.

**Oxygen Consumption**

Myocardial oxygen consumption was calculated by the method described by Neely et al. (1967) and modified by Deckere and Hoor (1976). The oxygen tension of the perfusate and of the effluent from the cannulated pulmonary artery were measured on a Radiometer MK-2 blood gas analyzer, and the following formula was employed:

\[
MVO_2 = (PAO_2 - PVO_2)(Q_d KO)/g
\]

where MVO\(_2\) = myocardial oxygen consumption in ml O\(_2\)/g dry weight, PAO\(_2\) = perfusate oxygen tension in mm Hg, PVO\(_2\) = oxygen tension of pulmonary artery effluent in mm Hg, Q\(_d\) = coronary flow (which is constant at 15 ml/min), K = solubility of oxygen in crystalloid (3.25 × 10\(^{-2}\) ml O\(_2\)/ml solution/mm Hg O\(_2\)), Umbreit et al., 1947), and g = weight in grams of hearts after drying for 48 hours at 110°C.

**Cell Ionic Determinations**

The perfusate for all hearts contained 1 mm K\(^{+}\),Co\(^{2+}\)-EDTA as an extracellular marker (Bridge et al., 1981, 1982; Ruano-Arroyo et al., 1984), prepared in pure crystalline form by the method of Dwyer et al. (1965). After completion of the experiments, heart wet weights were obtained immediately and dry weights after 48 hours at 110°C. The dried tissue then was digested with nitric and perchloric acids and total tissue calcium, cobalt, and sodium were ascertained by flame atomic absorption spectrophotometry on a Perkin Elmer 5000 Atomic Absorption spectrophotometer. The extracellular space was calculated by the following formula:

\[
ECS = CO_2/CO_2/H_2O
\]
where ECS is the extracellular space, $C_0$ is the total tissue cobalt, $C_00$ is the perfusate cobalt concentration, and $H_2O$ is total tissue water in ml obtained from the dry weight assuming 1 ml $H_2O = 1$ g.

The value of ECS was then used to calculate cell ionic contents as follows:

$$Q = C_1 - (ECS)(H_2O)(C_p)$$

where $Q$ is the cellular ion content, $d$ is the total tissue ion content, and $C_p$ is the perfusate concentration of the ion. The cell ionic contents are expressed as micromole/gram dry weight.

**Protocol**

All hearts initially underwent a stabilization period of 30 minutes and then were perfused with control solution (vide supra), and baseline measurements were obtained. In the first series of experiments, $Na_e$ was lowered to 74, 51, 39, or 25 mM in four separate groups of hearts, and the metabolic and functional parameters were monitored for 15 minutes. $Na_e$ was restored to 144 mM in some hearts from each group for another 30 minutes, and in the remainder the experiment was terminated for the determination of ionic contents. A second series of experiments evaluated the effect of perfusion with 0.08 mM calcium on the metabolic and functional changes accompanying the same graded reductions in $Na_e$. In a third series of experiments, oxygen consumption was measured in two groups of hearts in which developed pressure (DP) was decreased to a similar extent by two different methods. In the first, this was achieved by perfusion with 39 mM $Na_e$ and normal $Ca_2+$ of 2.5 mM, and in the second by perfusion with 0.2 mM calcium and normal $Na_e$ of 144 mM. In a fourth series of experiments, hearts were first treated with 25 $\mu$M verapamil to cause cessation of all systolic activity. A $Na_e$ step from 144 to 0 mM was then performed in the presence and absence of 10 $\mu$M ryanodine, a plant-derived blocker of sarcoplasmic reticulum calcium cycling (Marban and Weir, 1985) kindly supplied by John Sutko.

**Statistical Analyses**

Data are presented as mean ± SEM and groups are compared by unpaired t-test or the repeated-measures analysis of variance where appropriate (Winer, 1982).

**Results**

The baseline data obtained in a group of six control hearts following a 30-minute stabilization period were: total pressure (TP) 147 ± 7 mm Hg, EDP 9 ± 1 mm Hg, PCr 0.78 ± 0.03 in2, ATP 0.44 ± 0.02 in2, P 0.08 ± 0.03 in2, and oxygen consumption 0.523 ± 0.047 ml O2/g per min. During an additional 45 minutes of control perfusion, the longest time period of any of these experiments, these parameters showed no significant change. At 45 minutes, TP was 5 ± 1% less, EDP 8 ± 4% more, PCr 8 ± 4% less, ATP 10 ± 4% less, P 10 ± 4% more, and oxygen consumption 2 ± 1% more than control, indicating that this isovolumic working rat heart preparation is both mechanically and metabolically stable for at least 45 minutes. In the experimental groups described below, the control values...
of these parameters are not significantly different from those in the six control hearts.

Figure 1 shows the functional and metabolic alterations caused by 15 minutes of perfusion with low Na (25 mM) in one heart. An initial increase in systolic pressure is followed by a rapid decline. Despite this marked decline in systolic contractile function, oxygen consumption is relatively constant for the duration of the experiment. Note, however, the gradual and relatively marked rise in EDP, the increase in P, and the decrease in ATP and PCr.

The effect of 15 minutes of perfusion with graded Na (74, 51, 39, and 25 mM) on developed pressure (DP), EDP, ATP, PCr, and P, is shown in Figure 2. There is an initial increase in DP followed by a rapid decline, whereas ATP and PCr decline and P, and EDP rise throughout most of the exposure period. These metabolic and functional changes are graded with the decrease in Na, lowering.

Figure 3 shows the ATP, PCr, oxygen consumption, TP, DP, and ATP after 15 minutes of low Na, perfusion. Note that although DP, one of the "traditional" determinants of myocardial oxygen demand, shows a progressive decline with increased reductions in Na, oxygen consumption does not. At Na = 74 mM, there is a decline in DP but no significant change in oxygen consumption. At Na = 51 mM, there is only a small decline in oxygen consumption despite a substantial decrease in DP. At Na = 25 mM, DP falls to 2% of control, whereas oxygen consumption remains at the same level as when Na = 51 mM, i.e., 78% of control. Oxygen consumption is thus dissociated from systolic contractile function at low Na, concentrations. TP correlates better with oxygen consumption and shows less dissociation, compared with DP alone, because it includes EDP.

When Na is returned to 144 mM for 30 minutes after 15 minutes of perfusion with lowered Na, recovery of PCr ranges between 73 ± 15 and 88 ± 7%, ATP between 42 ± 12 and 67 ± 15%, P, between 188 ± 39 and 173 ± 60%, DP between 112 ± 20 and 119 ± 6 mm Hg, and EDP between 26 ± 8 and 10 ± 2 mm Hg in the 25 mM Na, and 74 mM Na, groups, respectively. In all groups there is a substantial recovery in all parameters except ATP (compare Fig. 2).

The cell sodium and calcium contents at 15 minutes after 15 minutes of perfusion with lowered Na, are not significantly different from those in the six control hearts. The results of the Na, step to 25 mM in the presence of these two calcium concentrations are presented in Figure 3. It can be seen that PCr and ATP show no significant change from control during the Na, step in the presence of lowered Ca, = 0.08 mM, whereas they do exhibit a marked decline when the Na, step is performed in the presence of normal Ca, (P < 0.001 for both PCr and ATP). The marked rise in EDP is also prevented when Na, is reduced in the presence of Ca, = 0.08 mM (P < .001). The experiment depicted in Figure 4 was also carried out at Na, 74, 51, and 39 mM. These experiments likewise show no significant change from control of PCr, ATP, and diastolic pressure in the presence of Ca, = 0.08 mM.

Since the data in Figure 3 compare a group of beating hearts in lowered Na, and normal Ca, with a group in markedly lowered Ca, which are not beating, we sought to control for systolic function in order to examine further the effect of low Na, on the relationship between systolic function and oxygen consumption. Therefore, another series of experiments was performed in which oxygen consumption was measured in two groups of hearts with similarly decreased systolic function. In one group, the decrease in DP, to a mean of 31 ± 5% of control, was achieved by lowering Na, to 39 mM with normal Ca,. In the other group, DP was reduced to a mean of 32 ± 5% of control by lowering Na, to 0.2 mM in the presence of normal Na. Figure 5 shows that, although oxygen consumption and DP do not differ under control conditions in the two groups (see legend), and that DP falls similarly in both groups, this decline is accompanied by a fall in oxygen consumption only in the low Ca, group, whereas, in the low Na, group, oxygen consumption is not significantly changed. Despite similar reductions in DP, oxygen consumption is 0.420 ± 0.041 ml O₂/min per g in the low Na, group compared with 0.189 ± 0.049 ml O₂/min per g in the low Ca, group (P < 0.001). Since DP and heart rate are equal in both groups, the greater than 2-fold higher oxygen consumption in the group perfused with low Na, cannot be attributed to altered systolic determinants of myocardial oxygen demand.

One interpretation of Figures 4 and 5 is that exposure to low Na, results in a calcium-dependent stimulation of respiration which is unrelated to any systolic function. If this is true, low Na, in the presence of normal Ca, would induce an increase in oxygen consumption and diastolic pressure even in the absence of systolic function. Therefore, two groups of hearts were perfused with sufficient verapamil, 25 μM, to abolish systolic function. One group then was exposed sodium-free perfusate, to abolish systolic function. One group was exposed sodium-free perfusate, to abolish systolic function.
changes occur in $Na_\text{c}$ of 144 mM, oxygen consumption increases 4-fold in sodium-free perfusate, and that this is accompanied by an increase in diastolic pressure (panels A and B, respectively) and a significant decline in PCR and ATP (panel C). Note that these changes occur in the complete absence of systolic function and that, in these arrested hearts, TP = diastolic pressure.

The above results suggest that the metabolic and functional changes accompanying exposure to lowered $Na_\text{c}$ are due in part to a calcium-dependent increase in energy expenditure which is unrelated to systolic function, but is related to diastolic pressure. To investigate a role for sarcoplasmic reticulum modulation of these phenomena, e.g., via spontaneous oscillatory calcium release (Stern et al., 1983), the protocol in Figure 6 was repeated in the presence of ryanodine, a drug known to block diastolic calcium oscillations by interfering with sarcoplasmic reticulum function (Sutko and Kenyon, 1983).

When a group of verapamil-arrested hearts, in $Na_\text{c} = 144$ mM, is treated with ryanodine, 10 $\mu$M, there is no effect on PCR, ATP, and oxygen consumption, but there is a 30% increase in diastolic pressure ($P < 0.01$) when compared with the group of verapamil-arrested hearts in the same $Na_\text{c}$ depicted in Figure 6 which is not exposed to ryanodine (Table 2). Thus, although ryanodine has no effect on the metabolic parameters under the conditions of this experimental protocol when $Na_\text{c} = 144$ mM,
TABLE 1

<table>
<thead>
<tr>
<th>Na₆</th>
<th>n</th>
<th>Naᵢ</th>
<th>Caᵢ</th>
</tr>
</thead>
<tbody>
<tr>
<td>144</td>
<td>6</td>
<td>107 ± 1.9</td>
<td>2.0 ± 0.21</td>
</tr>
<tr>
<td>74</td>
<td>5</td>
<td>65 ± 5.0</td>
<td>2.3 ± 0.38</td>
</tr>
<tr>
<td>51</td>
<td>6</td>
<td>51 ± 4.5</td>
<td>2.0 ± 0.38</td>
</tr>
<tr>
<td>39</td>
<td>5</td>
<td>43 ± 5.9</td>
<td>1.8 ± 0.25</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>36 ± 8.4</td>
<td>1.8 ± 0.40</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Na₆ = perfusate sodium concentration, Naᵢ = cell sodium content, Caᵢ = cell calcium content.

**Discussion**

The results of this study demonstrate profound graded metabolic and functional changes caused by graded reductions in Na₆ which are, to a large extent, reversible. In beating hearts, these changes include declines in systolic contractile function, i.e., DP, a rise in EDP, and a dissociation between systolic function and oxygen consumption with maintenance of the latter at near normal levels despite marked falls in DP. TP correlates better than DP, since it also reflects EDP. In arrested hearts, these changes consist of a rise in diastolic pressure over control levels. The relative increase in oxygen consumption is, in both cases, accompanied by a decrease in average levels of ATP and PCr and an increase in Pi. Although these changes are not accompanied by a measurable rise in total cell calcium, they are calcium dependent in that they are markedly attenuated or absent when Caᵢ is reduced to 0.08 mM. Furthermore, they are prevented when sarcoplasmic reticulum-dependent calcium cycling is inhibited by ryanodine. These inhibition of sarcoplasmic reticulum function does increase diastolic pressure.

During exposure to sodium-free perfusate, even in the absence of systolic function, the fall in PCr and ATP noted in Figure 6 is prevented by ryanodine (Fig. 7, panel A). Ryanodine also decreases energy utilization, as measured by oxygen consumption (Fig. 7, panel B). Ryanodine causes a higher peak in diastolic pressure (75 ± 5 mm Hg vs. 44 ± 5 mm Hg, P < 0.01) at 2 minutes, which is followed by partial normalization in both groups after 30 minutes (Fig. 7, panel C; 26 ± 5 mm Hg in the ryanodine group and 36 ± 7 mm Hg in the absence of ryanodine, P = NS).
RESULTS AND DISCUSSION

The results indicate therefore that, in the isovolumic rat heart model at 36°C, using lithium as a sodium substitute, the transsarcolemmal sodium gradient and cell sodium content have important roles, likely mediated by calcium, in the regulation of myocardial metabolism and contractile function.

Although the importance of calcium in controlling myocardial function and metabolism has been recognized for over 100 years (Ringer, 1883), the role of sodium modulation of cell calcium has been described relatively recently (Langer, 1973). In 1970, Glitsch et al. showed that calcium can enter the cell via sodium/calcium exchange. In some preparations, e.g., isolated cardiac cells, as much as 80% of calcium influx occurs via this mechanism (Barry and Smith, 1982). Sodium/calcium exchange is necessary for loading the sarcoplasmic reticulum as well (Weir and Isenberg, 1982), and calcium efflux is also sensitive to Na⁺ (Reuter and Seitz, 1968).

Perfusion with lowered Na⁺ has been shown to affect calcium homeostasis and contractile function in a predictable manner. There is an initial electrogenic sodium efflux coupled with calcium influx which then diminishes as the sodium gradient lessens. Allen et al. (1983) showed that, in ferret ventricular muscle, much of the rise in intracellular calcium activity dissipates even in the absence of sodium. Mullins (1979) has also reported that replacement of Na⁺ with lithium transiently loads the myoplasm with calcium. Barry and Smith (1984) reported that in cultured chick ventricular cells, the absence of Na⁺ increases calcium influx and efflux with only a transient increase in measurable total cell calcium. This occurs even in the presence of partial Na⁺-K⁺ pump inhibition. They found that the extrusion mechanism can be overwhelmed, though, by total Na⁺-K⁺ pump inhibition and metabolic blockade with cyanide. In addition, no calcium gain has been noted in goldfish ventricle exposed to zero sodium (Busselen and van Kerkhove, 1978). The degree of calcium gain is also dependent on the type of cation substituted, with lithium substitution resulting in the least calcium gain (Allen et al., 1983; Chapman et al., 1983), which may in part be explained by a direct effect of lithium on the Na⁺/Ca²⁺ exchanger (Ponce-Hornos and Langer, 1980).

In other protocols, the presence of a permeant proton-donating anion in the perfusate influences cellular and mitochondrial calcium accumulation (Lehniger, 1974a, 1974b; Ponce-Hornos and Langer, 1982). The lack of a detectable calcium gain in the present study is therefore probably related to the use of lithium substitution, the lack of a permeant proton-donating anion, and the absence of Na⁺/K⁺ blockade and metabolic inhibition (Lehniger, 1974a; Lee et al., 1980; Marban et al., 1980; Bers and Ellis, 1982; Sheu and Fozzard, 1982; Allen et al., 1983; Bridge and Bassingthwaighte, 1983; Orchard et al., 1983; Vaughan-Jones et al., 1983; Wier et al., 1983). Alternatively, the magnitude of the calcium gain may not be detectable by the atomic absorption methodology employed.

With transient calcium loading in the absence of Na⁺, there is a transient increase in resting force which relaxes despite continued zero sodium perfusion (Coraboef et al., 1981; Allen et al., 1983; Barry and Smith, 1984; Walford et al., 1984). However, the enhanced resting force, when carefully observed in ventricular myocardium, does not completely relax, and the resting tension remains elevated at a level which is inversely graded with gradations in Na⁺ (Walford et al., 1984). Similarly, a reduction of cellular sodium activity is accompanied by an apparent steady state increase in myoplasmic calcium activity as measured by calcium electrodes (Sheu and Fozzard, 1982) and by the luminescence of the photoprotein aequorin (Allen et al., 1983). It has also been observed that cell calcium loading results in calcium oscillations that cause localized myofilament motion which is manifest as intensity fluctuations in laser light scattered from the preparation (Stern et al., 1983). Upon exposure to low Na⁺, these intensity fluctuations are markedly enhanced (Lakatta and Lappe, 1981; Stern et al., 1983; Kort and Lakatta, 1984) and then decline with time in low Na⁺ to a level which is above that observed.
prior to exposure to lowered Na⁺ (Stern et al., 1983), a pattern similar to that which occurs in resting tension (Walford et al., 1984). Thus, calcium electrode measurements, aequorin luminescence, scattered light intensity fluctuations, and resting force all indicate that average myoplasmic calcium remains elevated in the steady state in lowered Na⁺, and likely has an oscillatory component as well.

Despite the lack of a gain in total cell calcium contents as measured by the present technique, the

![Figure 6](image)

**TABLE 2**

The Effect of Ryanodine on Metabolic Parameters and Diastolic Pressure in Verapamil-Arrested Hearts in 144 mM Na⁺

<table>
<thead>
<tr>
<th></th>
<th>ATP (μM)</th>
<th>PCr (μM)</th>
<th>O₂ CON (ml O₂/min per g)</th>
<th>Diastolic pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>86 ± 4</td>
<td>103 ± 5</td>
<td>0.094 ± 0.018</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>Ryanodine = 10 μM</td>
<td>86 ± 4</td>
<td>100 ± 3</td>
<td>0.082 ± 0.014</td>
<td>22 ± 3*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (% of baseline for ATP and PCr), n = 4 for each group. Na⁺ = perfusate sodium concentration, O₂ CON = oxygen consumption expressed as ml O₂/min per g.

*P < 0.01.
The effect of ryanodine in the absence of Na+, in verapamil-arrested hearts. ATP (squares, panel A), PCR (circles, panel A), oxygen consumption (circles, panel B), and diastolic pressure (circles, panel C), in verapamil-treated hearts devoid of systolic function during zero Na+, with (closed, n = 5) and without (open, n = 6, same hearts as depicted in Fig. 6) 10 micromolar ryanodine. Note that ryanodine prevents the fall in high energy phosphates and the increased oxygen consumption caused by zero Na+ perfusion. The overall test of difference between the groups has a significance level of P < 0.02 for ATP, P < 0.007 for PCR, and P < 0.002 for oxygen consumption. There is an initial rise in diastolic pressure which peaks at about 2 minutes. At that time, diastolic pressure is higher in the ryanodine treated hearts (75 ± 10 mm Hg) than in those not treated with ryanodine (44 ± 5 mm Hg, P < 0.01). Diastolic pressure then falls and, although the average values are less in the ryanodine-treated hearts, the differences do not reach statistical significance.

 marked decline in high energy phosphate levels is calcium dependent, since it does not occur in the presence of CaO = 0.08 mm. Several calcium-dependent energy-consuming ATP pumps are stimulated in the presence of elevated myoplasmic calcium and may be influenced by altered cellular sodium (DiPolo and Beauge, 1979; Caroni and Carafoli, 1980; Trumble et al., 1980; Caroni and Carafoli, 1981; Lamers and Stinis, 1981; Marcos, 1981). These are associated with the myofibrils, sarcolemma, and sarcoplasmic reticulum. Increased energy utilization during the calcium loading may be related to increased activity of myofilibril adenosine triphosphatase (ATPase) associated with the early increase in contractility and the subsequent and persistent rise in diastolic pressure and oxygen consumption. In these isovolumetric rat heart preparations, the cavity size did not change during the manipulations. It is unlikely that ventricular wall thickness was significantly altered either. Therefore, the predominant factor influencing wall stress in these experiments is intracavitary or diastolic pressure. In addition, the sarcolemmal ATPase would be stimulated to dissipate the initial calcium load that occurs when Na+ is decreased (Barry and Smith, 1984).

The experiments conducted with verapamil, in the absence of systolic function, provide further insight into the mechanism for altered metabolism, since they allow an examination of the low Na+ effects on diastolic phenomena per se. These studies show that even in the absence of systolic contractile activity, zero Na+ perfusion results in a rise in diastolic pressure and a decline in high energy phosphates in the presence of increased oxygen consumption. The fact that these effects are eliminated by ryanodine, a drug which selectively inhibits sarcoplasmic reticulum-dependent calcium release (Sutko et al., 1974), strongly suggests that the decrease in ATP and PCR and relative increase in oxygen consumption are due to increased energy utilization which is abolished when spontaneous sarcoplasmic reticulum-generated diastolic calcium oscillations are blocked. That oscillations can be increased over the control level in the absence of Na+ is not inconsistent with the absence of a net gain of calcium large enough to be detected by the method employed, since small changes in myoplasmic calcium cause a substantial increase in the spontaneous oscillation frequency (Cheisi et al., 1981; Capogrossi and Lakatta, 1985). These spontaneous calcium oscillations result in transient increases in calcium above the mean myoplasmic level that is regulated by sarcolemmal function and other cell calcium sinks. The localized myoplasmic calcium levels achieved during these spontaneous oscillations have been estimated to be 5–40 μM (Wier et al., 1984; Orchard et al., 1984), i.e., as high or higher than that achieved during systole. The fact that ryanodine causes an increase in diastolic pressure in the absence of Na+ is greater than that observed in the absence of ryanodine (Fig. 7) might be due to ryanodine-induced trapping of calcium in the sarcoplasmic reticulum which, via mass action, may inhibit the sarcoplasmic reticulum pump and thereby diminish its effectiveness as a calcium buffer. Alternatively, the higher diastolic pressure may be explained by the nonlinear relationship between force and calcium; under conditions in which cellular calcium is relatively high, e.g., initially following the removal of Na+, theoretical modeling in cardiac muscle predicts that for a relatively high average myoplasmic calcium, force
as measured across the ends of the preparation will be less in the presence of oscillations, and thus abolition of the oscillations will increase tonic force. Conversely, the model predicts that the presence of oscillations when cellular calcium is low, i.e., as it becomes with time in low Na⁺, will increase force (Stern et al., 1983). It is important to note, however, that the course of the cellular calcium transients which occurs under these conditions, i.e., exposure to zero Na⁺ in the presence of ryanodine, is still incompletely understood.

Although the findings that oxygen consumption is increased in the nonbeating preparation when Na⁺ is lowered and also in the beating preparation when DP is controlled suggest increased energy utilization, a component of calcium-dependent uncoupling of oxidative phosphorylation (Rossi and Lehninger, 1964; Carafoli and Lehninger, 1971) in reduced Na⁺ cannot be excluded. The fact that oxygen consumption falls at all in some of the protocols (see Fig. 3) while high energy phosphates are declining suggests that some of the low Na⁺ effect is related to a direct decrease in the ability to utilize oxygen to maintain high energy phosphates.

In summary, there are many factors that influence the functional and metabolic changes which occur when Na⁺ is lowered in the isolated heart preparation. These include, but are probably not limited to, the substituted cation, the calcium concentration in the perfusate, the presence of a proton-donating permeant anion, the degree to which the sodium is lowered, the species, and the amount of work being performed by the heart during the substitution period. It is clear, however, that under the conditions employed, graded reductions in Na⁺ in beating rat hearts are accompanied by graded reductions in developed pressure and increases in diastolic pressure. However, oxygen consumption remains relatively stable and is thus dissociated from systolic contractile function. When the same decline in developed pressure is produced by lowering Cao to 0.2 mM or Na⁺ to 39 mM, oxygen consumption is twice as high as in the low Na⁺ model. In hearts in which systolic function is abolished by verapamil, diastolic pressure and oxygen consumption increase on exposure to zero Na⁺. ATP and PCr decrease in both beating and nonbeating hearts exposed to zero Na⁺, and this, coupled with an increase in oxygen consumption, appears to be due largely to increased energy utilization. The increase in oxygen consumption, marked increase in diastolic pressure, and the decline in high energy phosphates are calcium dependent, as they do not occur when Cao is lowered to 0.08 mM. Furthermore, even in the presence of normal perfusate calcium, these metabolic alterations are abolished by ryanodine, suggesting that sarcoplasmic reticulum-myofilament calcium oscillations modulate the calcium-dependent metabolic and functional effects of lowered Na⁺.

Circulation Research / Vol. 57, No. 6, December 1985

We wish to thank Kathryn A. May for technical assistance in the performance of the experiments and Rosemary Baumgarden for assistance with the statistical analyses. We also wish to thank Spring Metail for assistance in the preparation of this manuscript.

These results were presented in part at the 57th Scientific Sessions of the American Heart Association, Miami Beach, Florida, November 1984.

This study was supported in part by National Heart, Lung, and Blood Institute Specialized Center of Research Grant P-50-HL765508, Bethesda, Maryland, and Coronary Heart Disease Research, a program of the American Health Assistance Foundation. Computational assistance was received from Grant 5 M01RR35-20 from the National Institutes of Health.

Address for reprints: Dale G. Renlund, M.D., Carnegie 591, The Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, Maryland 21205.

Accepted February 2, 1985; accepted for publication September 12, 1985.

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INDEX TERMS: Low sodium perfusion • Sodium/calcium exchange • Ryanodine • Calcium oscillations • Myocardial energetics

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Calcium-dependent enhancement of myocardial diastolic tone and energy utilization
dissociates systolic work and oxygen consumption during low sodium perfusion.
D G Renlund, E G Lakatta, E D Mellits and G Gerstenblith

Circ Res. 1985;57:876-888
doi: 10.1161/01.RES.57.6.876
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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