Activity of the Vesicular Calcium Pump in the Spontaneously Failing Heart-Lung Preparation

By Edward W. Gertz, M.D., Michael L. Hess, B.S., Richard F. Lain, B.S., and F. Norman Briggs, Ph.D.

ABSTRACT

The in vitro calcium uptake activity has been examined in fragments of sarcoplasmic reticulum, i.e. vesicles, obtained from hearts spontaneously failing in the canine heart-lung preparation. The rate of uptake was found to be less than that observed for vesicles from control hearts. If the vesicles were prepared from a failed heart that had been treated in vivo with ouabain, then the rate of calcium uptake was normal. In vitro additions of ouabain increased the rate of calcium uptake only with vesicles prepared from failing hearts. This effect of ouabain on calcium uptake by vesicles appears, therefore, to be specific for the defect causing depression of calcium uptake. No evidence could be found that the depression of calcium uptake is due to uncoupling of the pump adenosine triphosphatase. Calcium uptake and adenosine triphosphatase activity were found to be proportionately depressed in the failing heart and proportionately restored by ouabain.

ADDITIONAL KEY WORDS

adenosine triphosphatase excitation-contraction coupling ouabain heart failure calcium uptake sarcoplasmic reticulum anesthetized dogs

The excitation-contraction (1, 2) coupling mechanism has long been suspected (3, 4) to be involved in heart failure. Ignorance concerning the mechanism of excitation-contraction coupling and inability to evaluate its state has, however, heretofore prevented any direct assessment of this possibility. Our present understanding of the importance of the sarcotubular calcium pump (5-8) in the operation of excitation-contraction coupling and our ability to isolate and investigate this pump has recently made it possible to determine if some abnormality of it is associated with heart failure. Briggs, Gertz and Hess, in a recent report (5), have suggested that the heart failure produced by barbiturates may be attributable to a breakdown of excitation-contraction coupling due to depression of the sarcotubular calcium pump. They found that sodium 5-ethyl-5-isoamylbarbiturate (amytal sodium) added in vitro to isolated fragments of the sarcoplasmic reticulum inhibited their ability to take up calcium. In further support of the proposed relationship between heart failure and the activity of the calcium pump, it was found that G-strophanthin (ouabain) could reverse the barbiturate depression of the calcium pump just as it can reverse barbiturate-induced heart failure (9).

The present study was undertaken to determine if acute spontaneous heart failure in the heart-lung preparation is associated with a depression of the calcium pump. This preparation was chosen because of the rapidity with which failure develops and because changes in cardiac contractility can easily be measured.
Methods

**THE HEART-LUNG PREPARATION**

Mongrel dogs of either sex weighing approximately 20 kg were anesthetized with sodium pentobarbital 30 mg/kg. The right external jugular vein was cannulated with a double lumen polyethylene catheter, the tip of which was positioned at the right atrium. One lumen of the catheter was used to measure right atrial pressure and the other to provide blood to the right atrium, hereafter called venous return. The right carotid artery was cannulated with a polyethylene catheter and the tip was positioned in the aortic arch to measure arterial pressure. The chest was then opened by a median sternotomy. The superior vena cava or precava was ligated around the venous return catheter. The right subclavian artery was ligated at its origin from the aortic arch and the azygous vein was ligated at its termination at the vena cava. The left subclavian artery was then cannulated just above its origin from the aortic arch with a stiff polyethylene catheter, whose tip was edged into the aortic arch. This outflow catheter was connected to flexible tygon tubing, which was run through a Hoffman clamp to the venous return reservoir. The pericardium was excised in all experiments. In some experiments, a fine flexible polyethylene catheter was placed in the left atrium through the aortic arch to measure arterial pressure. A reservoir of approximately 1,500 ml was filled with blood obtained from another dog anesthetized with sodium pentobarbital and given 5,000–10,000 units of heparin iv. The reservoir was maintained at 37°C. The outflow catheter was opened and the aorta clamped and ligated. Blood was permitted to flow into the reservoir until the diastolic pressure in the aorta was 25 mm Hg. At this time the inferior vena cava or postcava was ligated, and blood was pumped into the heart through the venous return catheter with a pulsatile pump placed between the reservoir and the right heart. Elastic tubing was placed between the pump and the heart to diminish the pulsatile nature of the flow to the heart. The arterial pressure was controlled with a Hoffman clamp, and respiration was maintained with a Bird respirator biased so that the end-expiratory pressure did not fall below 10 cm H_2O. Pressures were measured with Statham strain gauges connected to a Grass model 5 polygraph.

Failure was achieved by permitting the heart-lung preparation to deteriorate with time. Two types of failure were produced: low output with constant arterial resistance and high output with variable arterial resistance. During the development of low-output failure, the venous return was maintained at 300 ml/min and the mean arterial pressure ranged from 90 to 160 mm Hg. During the development of high-output failure, the venous return was kept between 1,100 and 1,500 ml/min, and the mean arterial pressure between 125 and 140 mm Hg except when ventricular function curves were being run.

Five of eight of the failure experiments were of the low-output type; in these, the heart was judged to have failed when the mean right atrial pressure reached 22 to 50 mm Hg, with venous return between 310 and 640 ml per min. In the three experiments of the high-output type, the heart was judged to have failed when mean right atrial pressure reached 20 mm Hg with venous return between 640 and 2,500 ml per min. Since the rate of calcium uptake and the response to ouabain by the vesicles from the two types of preparations was the same, the data from these two groups were combined.

In all experiments in which ouabain was added in vivo, the heart failure was of the high-output type; the heart was judged to be in failure when the mean left atrial pressure was 17 to 41 mm Hg, with venous return between 960 and 1,800 ml per min. Ouabain was then added to the reservoir blood in equal doses at approximately 15-min intervals until the total dose was between 150 and 500 μg. All hearts finally developed arrhythmias, whereupon the experiment was terminated.

In the experiments in which mean left and right atrial pressures were measured simultaneously, the left atrial pressure always rose first, indicating that the left heart failed before the right heart. An example of a set of ventricular function curves obtained by varying the minute volume of the venous return and measuring concomitant mean left atrial pressures is shown in Figure 1. Stroke power was calculated with the following equation: stroke power = mean arterial pressure - left atrial pressure 100 x cardiac output, where the pressures are mean pressures in centimeters of H_2O, cardiac output is in ml x sec\(^{-1}\) and stroke power is in grams x meters x sec\(^{-1}\).

**PREPARATION OF CARDIAC VESICLES**

A modification of the methods of Carsten (10) and Briggs et al. (5) was used. The control vesicles were prepared from hearts removed from intact dogs anesthetized with sodium pentobarbital (30 mg/kg), while the vesicles from failing hearts were prepared from heart removed from the heart-lung preparation. All hearts were quickly rinsed in cold tap water and cooled for 15 min in crushed ice. The right ventricles were cut away and the left ventricles trimmed of fat, cut into small pieces and homogenized for 15...
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CALCIUM UPTAKE MEASUREMENTS

Calcium uptake was measured with the aid of 45Ca. All incubations of the vesicles were carried out at 37°C with a vesicular protein concentration of 0.15 mg/ml. The final composition of the incubation medium was 120 mM KCl, 18 mM imidazole, 5 mM MgCl₂, 5 mM ATP, 1.8 mM sodium oxalate, 0.09 or 0.18 mM CaCl₂ and 0.05 μC/ml 45Ca, at pH 7.0. The vesicular protein was initially suspended in the KCl, imidazole, oxalate portion of the above medium. When the effects of ouabain were being studied, ouabain was added to this medium at a concentration of 3 μM. The vesicles were then preincubated for 5 min at 37°C, whereupon calcium uptake was initiated by addition of the magnesium, calcium and ATP components of the incubation mixture. Calcium uptake was measured on the filtrate obtained by suction of aliquots of the incubation mixture through Millipore filters of 0.22 μm diameter pore size at 1, 2, and 3 min after addition of ATP. Radioactivity was measured with a scintillation spectrometer in a Cab-O-Sil-Dioxane (12) suspension of the filtrate.

Adenosine triphosphatase activity was determined from the rate at which inorganic phosphate was liberated during the incubation. Samples of the filtrates used for 45Ca measurements were used for determination of the inorganic phosphate by the method of King (13). The ATP hydrolysis associated with calcium uptake (extra ATPase) was calculated as the difference between the ATPase activity of the vesicles in the absence and presence of substrate quantities of calcium and is thus equivalent to Hasselbach's (14) extra ATPase (14).

ATP was obtained from Pabst Laboratories, and ouabain from Nutritional Biochemicals. All other chemicals were Mallinckrodt analytical grade reagents. Water was distilled and then passed through a deionizing cartridge.

Results

Figure 2 compares the rates of calcium uptake by vesicles prepared from control hearts, hearts allowed to fail spontaneously in the heart-lung preparation, and hearts treated with ouabain after spontaneous failure. Statistical analysis of the data showed that the uptake rate by the vesicles from failed hearts is less than the rate found in the vesicles from control hearts (P < 0.05 for the single-tailed t test). The addition of ouabain to failing hearts (Fig. 2) appeared to completely reverse the depression of calcium uptake by the vesicles from these hearts. Since this effect of oua-
Effect of cardiac failure and ouabain on the uptake of calcium by cardiac vesicles. The bars represent the rate of calcium uptake in μmoles of calcium per milligrams of vesicular protein per min. The standard errors of these rates are given.

Ouabain persisted through the isolation and washing procedures, one must assume that it is strongly bound at its site of action. This appears to agree with the observation (15) that about 5% of a tracer dose of digitoxin is bound to the microsome fraction of a cardiac muscle homogenate. This is a significant degree of binding, since in our experience the microsome fraction represents only about 0.5% of the cardiac mass.

Figure 2 also shows the effects of the in vitro addition of ouabain to the vesicular preparations. The drug was preincubated with the vesicles for 5 min at 37° before calcium uptake rates were measured. Although ouabain may have produced a slight inhibition of calcium uptake in the vesicles from control hearts and hearts treated with ouabain in vivo, the effect was not statistically significant. The rate of calcium uptake by the vesicles from failing hearts, however, returned to control levels. Thus the effect of ouabain in these studies appears to be quite specific for the defect causing depression of uptake.

All in vitro tests with ouabain were carried out at 3 μM as indicated in Figure 2, except for a study on one failed heart from a prepara-

![Figure 2](image1)

**FIGURE 2**
Effect of cardiac failure and ouabain on the uptake of calcium by cardiac vesicles. The bars represent the rate of calcium uptake in μmoles of calcium per milligrams of vesicular protein per min. The standard errors of these rates are given.

![Figure 3](image2)

**FIGURE 3**
Influence of ouabain concentration on calcium uptake by vesicles prepared from a failed heart. Ouabain concentrations are in μM.

![Figure 4](image3)

**FIGURE 4**
In vitro effect of ouabain on the rate of calcium uptake by cardiac vesicles: a, 8 control hearts, b, 8 failed hearts from heart-lung preparations, c, 5 failed hearts from heart-lung preparations treated with ouabain in vivo. Solid black bars indicate standard errors.
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**TABLE 1**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>(\text{amole P}_1) mg(^{-1}) min(^{-1})</th>
<th>(\text{amole Ca}^{2+}) mg(^{-1}) min(^{-1})</th>
<th>(\text{amole P}_1) (\text{amole Ca}^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>.494</td>
<td>.239</td>
<td>2.08</td>
</tr>
<tr>
<td>+ Ouabain (in vitro)</td>
<td>.447</td>
<td>.209</td>
<td>1.4</td>
</tr>
<tr>
<td>Control</td>
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<td>.308</td>
<td>2.28</td>
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<tr>
<td>+ Ouabain (in vitro)</td>
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<td>.297</td>
<td>2.34</td>
</tr>
<tr>
<td>Failed</td>
<td>.434</td>
<td>.236</td>
<td>1.83</td>
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<tr>
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<td>.282</td>
<td>2.46</td>
</tr>
<tr>
<td>Failed</td>
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<td>.109</td>
<td>2.41</td>
</tr>
<tr>
<td>+ Ouabain (in vitro)</td>
<td>.347</td>
<td>.224</td>
<td>1.55</td>
</tr>
<tr>
<td>Failed + Ouabain (in vivo)</td>
<td>.594</td>
<td>.290</td>
<td>2.04</td>
</tr>
<tr>
<td>+ Ouabain (in vitro)</td>
<td>.420</td>
<td>.271</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Extra ATPase was calculated from the difference in the rate of ATP splitting in the presence or absence of substrate calcium; this is expressed as the formation of inorganic phosphate per milligram of vesicular protein per minute. The rate of calcium uptake is expressed in terms of micromoles of calcium per milligram of vesicular protein per minute. The in vitro concentration of ouabain was \(3 \mu M\). There were two experiments with control hearts, two with failed hearts from heart-lung preparations and one with a failed heart from a heart-lung preparation treated with ouabain.

Discussion

There is clearly a contradiction between our hypothesis that depression of the vesicular calcium pump is associated with cardiac failure in which varying concentrations of ouabain were used. The effect of varying the in vitro concentration of ouabain on calcium uptake by vesicles from this one heart is shown in Figure 3. This figure indicates that 0.1 and 0.3 \(\mu M\) ouabain produced no effect, but concentrations of 1 and 3 \(\mu M\) produced progressively larger increases in uptake rates. The effect of ouabain thus appears to be dose dependent.

So far, the only evidence that the vesicles from the failed heart are different from those of control heart is based on the 27\% difference in uptake rates between the two groups. Since this difference could occur as a result of contamination of the vesicle preparation from the failed heart with nonvesicular protein, additional evidence that the two groups are different is needed. This is provided by the results obtained with in vitro additions of ouabain. As shown in Figure 4, only the vesicles obtained from the failed heart responded to in vitro ouabain \((P < .0025)\). This response cannot be accounted for on the basis of contamination but must represent a real difference in the nature of the vesicles.

The response of the failed vesicles to in vitro ouabain was similar to that observed by Briggs et al. in vesicles inhibited by barbiturates \((5)\). This suggested that the mechanism leading to acute spontaneous heart failure may be very similar to that caused by barbiturates. In the barbiturate studies it was established that the depression of calcium uptake rate was due to an uncoupling of the calcium pump. Whether the calcium uptake rate in failing vesicles is depressed because of uncoupling of the pump was examined in a number of hearts. The results are presented in Table 1. There is no indication in these data that the pump is uncoupled. It shows, rather, that ATPase and calcium uptake are depressed in parallel; there is no apparent difference between the control and failed hearts in the efficiency of calcium uptake as estimated by the ratio of moles of ATP split to the moles of calcium taken up. Thus the defect in the calcium pump of the failed heart may not be like that produced by barbiturates; the pump may not be uncoupled but simply depressed in parallel with the associated ATPase activity.

Discussion

There is clearly a contradiction between our hypothesis that depression of the vesicular calcium pump is associated with cardiac failure...
and the hypothesis proposed by Lee (16) that depression of the vesicular pump, for example by ouabain, leads to a positive inotropic effect. Our major objection to Lee’s hypothesis is that it fails to consider what effects inhibiting the pump will have on the ultimate distribution of cellular calcium. For a few heart beats, Lee’s hypothesis would be correct, because, as he suggests, a depression of the calcium pump would tend to prevent reaccumulation of calcium into the sarcoplasmic reticulum, thereby increasing the amount of calcium available to activate contraction. However, what is not taken into account is the fact that the sarcolemmal calcium pump, as well as other possible sites of calcium loss, would be put into a more competitive position in regard to the distribution of calcium which would lead to a loss of calcium from the sarcoplasmic reticulum. As this compartment becomes increasingly depleted of calcium there would be less calcium available for coupling of excitation to contraction. Evidence that such a sequence of events can follow inhibition of the calcium pump is suggested by the experiments of Foulks and Perry (17). These investigators found that sodium pentobarbital, a drug known to inhibit the calcium pump of skeletal muscle (18, 19), produces an initial increase in contractility followed by an inhibition of contractility.

Implicit in the above discussion is the idea of competitive pathways for the metabolism of calcium. Although the number of such pathways may not have been completely identified, it is clear that one such pathway is across the sarcolemma. It has been reported by Winegrad and Shanes (20) that there is a flux of about 0.15 μmoles of calcium in both directions across this membrane per beat per kilogram of heart muscle when calcium in the extracellular fluid is 1.25 mM. Since there is a large calcium concentration gradient between the intra- and extracellular fluid, one must postulate that the efflux is dependent on a calcium pump. Figure 5 shows in diagrammatic form the relationship between these calcium pumps and the distribution of calcium. If most of the calcium involved in coupling contraction to excitation came across the sarcolemma, then depression of the sarcotubular calcium pump would be unimportant. On the other hand, if it comes from the sarcoplasmic reticulum, as appears to be the case, then the pump is important. The evidence for this assertion is based on the following data and calculations. There are about 54 g of myofibrillar protein per kilogram of cardiac muscle (21, 22); it takes 1 to 2 μmoles of calcium to fully activate contraction in 1 g of myofibrils (23); therefore, 54 to 108 μmoles of calcium are required to fully activate tension development in 1 kg of heart muscle. Under physiological conditions of calcium concentration (1.25 mM) and temperature (37°), cardiac contractility is about one-sixth of its maximal value (24, 25); therefore, 9 to 18 μmoles of calcium are involved in excitation-contraction coupling. Of this amount, 0.15 μmoles come from the extracellular fluid, that is about 2% of the total amount of calcium involved in coupling. The calcium stores of the sarcoplasmic reticulum are thus of considerable importance. However, if one were to suddenly raise the extracellular fluid calcium to 100 mM, then the amount of calcium coming into the heart from the extracellular fluid, if calcium
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permeability remained unchanged, would increase to 12.0 μmoles/kg of heart muscle, an amount which would make a significant contribution to coupling and, therefore, augment contractility as described by Weidman (26).

According to our hypothesis, inhibition of the rate of calcium uptake by the sarcoplasmic reticulum should lead to a decreased rate of muscular relaxation, for relaxation depends on the withdrawal of calcium from the myofibrils by the reticulum. Thus, the theory predicts that failure, whether spontaneous or caused by barbiturates, should be accompanied by a decrease in the rate of cardiac relaxation. This indeed has been observed by Covell et al. (27), who found — dp/dt to be depressed under these conditions. Foulks and Perry (17) have also noted that a few minutes after the exposure of frog skeletal muscle to barbiturates the rate of muscle relaxation begins to decline. Thus this prediction appears to be borne out by existing experimental data.

Although the present report is not directly concerned with the question of how positive inotropic effects are produced, one agent possessing such an activity, ouabain, was investigated. Our data can only be interpreted as indicating that ouabain reverses whatever defect is causing a depression of the calcium pump in the spontaneously failing heart. The data give no indication as to how ouabain produces its positive inotropic effect in the normal heart, for in it the drug appeared to have little effect on, or to slightly depress, the vesicular calcium pump.

In view of the recent report by Sonnenblick (28) that ouabain increases contractility and that by Edman and Nilsson (29) that ouabain increases the intensity and shortens the duration of the active state in the nonfailing heart, the lack of an effect of ouabain on vesicles from nonfailing hearts was unexpected. It appeared in theory that ouabain should increase the rate of calcium uptake in vesicles from nonfailing hearts, for such an action seems to explain how the drug could effect the active state in this way. At present we have no explanation for this. However, we can propose three possibilities: (1) the non-failing hearts that have been studied may not have been comparable to ours; (2) we may not yet have discovered the appropriate conditions to demonstrate a positive effect of ouabain on a nonfailing heart; and (3) the site of action of ouabain in the nonfailing heart is not the vesicular calcium pump.

References


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