Myofibrillar Adenosine Triphosphatase Activity of Human Heart Tissue in Congestive Failure: Effects of Ouabain and Calcium

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Congestive heart failure of the low output type may follow prolonged pressure and volume overload. This type of failure is most frequently defined clinically in terms of the variety of congestive syndromes which it produces. While there is no simple physiologic definition of failure, it is characterized by a defect in contractility wherein less work is performed at a given fiber length.1,2 The question arises: What metabolic lesion underlies this defect in contractility?

Accumulating evidence indicates that the common types of cardiac failure are due to defects in utilization of energy, that is, to failure of the myofibril to assimilate phosphate bond energy or to shorten properly in the contractile cycle.1 Alpert and Gordon3 have reported depression in adenosine triphosphatase (ATPase) activity of myofibrils isolated from the ventricles of patients in hypertensive congestive failure. Such a finding is consistent with the constant association demonstrated between contractility and the rate of hydrolysis of adenosine triphosphate (ATP).4,5 Finally, the ultimate mechanism of action of digitalis probably lies in this same area of utilization of energy and, furthermore, is closely related to changes of ionic environment in and about the contractile apparatus.6

The present study was undertaken in an effort to answer the following questions: Will digitalis or calcium, or a combination of the two, reverse the postulated defect in myofibrillar ATPase activity? Can previous observations be extended to failure from volume overload as well as to pressure overload in both right and left ventricles? What is the effect of hypertrophy and age on myofibrillar ATPase activity? Can these areas be explored using tissue obtained during open-heart surgery?

Methods

A. SOURCES OF TISSUE

Forty-seven experiments were performed on tissue from 32 different hearts grouped as follows:

1. Postmortem tissue
   a. Normal ventricles
   b. Failing ventricles
   c. Hypertrophic nonfailing ventricles
   d. Normal papillary muscles

2. Surgical tissue
   a. Hypertrophic nonfailing pulmonary infundibulum
   b. Failing papillary muscles

1a. Postmortem normal ventricles were obtained at autopsy from nine males and one female aged 16 to 61 years; both ventricles were obtained in eight, left ventricle only in two, right in one. All were studied within 4 to 10 hours after death. Coronary atherosclerosis (on a 1 to 4 grading system) was grade 1 in eight, grade 2 in two. Heart weight was normal in relation to body weight7 and ranged from 190 to 520 g. No patient had a history of heart disease and deaths were due to violent causes, for example, automobile accident or gunshot wound, in five. In the other five, death was sudden and also "non-cardiac"; that is, asphyxia from aspiration of blood after operation for carcinoma of the tongue, suspected embolism in pulmonary carcinoma, bronchopneumonia with pulmonary edema, peritonitis, and ruptured aneurysm.

1b. Postmortem failing ventricles were obtained at autopsy from 10 males and 2 females aged 2 months to 66 years; both ventricles were included in 4, left ventricle only in 4, right in 4. All were...
studied within 3 to 11 hours after death. Coronary atherosclerosis was less than grade 2 in all cases. Every effort was made to assess clinical left and right heart failure separately. In all cases there was evidence of hypertrophy and dilatation of the ventricle under consideration, with a total increase in heart weight in relation to body weight. In the left ventricles there were five acquired and three congenital lesions; in the right ventricles, two acquired and six congenital lesions. Acquired lesions were secondary to systemic or pulmonary hypertension or rheumatic valvular disease. Congenital lesions included transposition of the great vessels, mitral atresia, endocardial cushion defects, and coarctation of the aorta.

1c. Postmortem hypertrophic nonfailing ventricles were obtained at autopsy from one female and two males, ages 3, 60, and 65 years (left ventricle in one and right ventricle in two), and were studied within 4 to 7 hours after death. Coronary atherosclerosis was less than grade 2 and clinical and pathologic evidence of hypertrophy was found in the absence of congestive heart failure.

1d. Normal papillary muscles were obtained from two of the same hearts used for normal ventricular experiments (category 1a).

2a. Surgical hypertrophic nonfailing pulmonary infundibulum was obtained from three females and two males, ages 12 to 19, undergoing primary repair of tetralogy of Fallot. Clinically, none of these patients had congestive heart failure.

2b. Surgical failing papillary muscles were obtained from two males, ages 32 and 33, undergoing valve replacement for long-standing rheumatic mitral valvular insufficiency and congestive failure.

B. MYOFIBRILLAR PREPARATION

One to 3 g of tissue were obtained for each experiment. In some instances tissue was obtained from both ventricles. All specimens obtained during surgical procedures were placed by the surgeon directly into iced beakers in the operating room. Experiments on these specimens were begun in the laboratory within a few minutes of the time at which the tissue was removed from the patient. Two specimens obtained from surgical patients with tetralogy of Fallot were sufficiently large to permit repetition of the experiment in several hours.

The method for preparing the myofibrils is that of Perry and Grey, in which minced, homogenized tissue is differentially centrifuged with recovery of the aliquot which sediments between 300 and 600 × g. After the final centrifugation, the myofibrils were suspended in Tris buffer (11 millimolar at pH 7.1) and KCl (166 millimolar) amounting to a volume equal to 30 times the weight of the original sample, that is, in about 50 ml of solution in most cases. The concentrations of KCl and of Tris in this final diluent were such that the proper molarity for all constituents would be obtained in the experiment proper.

This final myofibrillar suspension was divided into three aliquots. One part was used in the experiment proper to measure ATPase activity, one was used to determine the protein concentration of the suspension, and one was observed microscopically for the appearance and adequacy of separation of the myofibrils. Examination by both light and electron microscopy showed that the suspensions consisted almost entirely of single myofibrils of varying length, together with a few packets of unseparated fibers. Occasional nuclear, mitochondrial, and sarcotubular remnants were seen. No difference was observable microscopically between preparations from normal and from failing hearts.

C. DESIGN OF EXPERIMENTS

Four different myofibril-ouabain* suspensions were prepared so that the concentration of ouabain was 0, 10⁻⁵, 10⁻⁶, and 10⁻⁷ M. This was done by adding one part of de-ionized distilled water, or of ouabain in concentrations of 10⁻⁴, 10⁻⁵, and 10⁻⁶ M, to nine parts of myofibrillar suspension prepared as described. The ouabain was prepared daily from a stock solution of con-
concentration 2 x 10^{-3} \text{ M}. Two milliliters of these four different myofibril-ouabain preparations were added to the 16 test tubes as referred to in table 1. The tubes were then placed in a water bath at 37.1 °C and allowed to incubate for one hour. The one-hour incubation period was chosen after preliminary experiments demonstrated (1) no deterioration in ATPase activity in myofibrils pre-incubated for five minutes to three hours in the absence of ouabain (the remarkable stability of this activity has been observed previously by several investigators)\textsuperscript{8,9} and (2) no observable effect of ouabain on this activity at 5-, 15-, 30-, and 45-minute incubation periods. While the myofibrils were incubating, five different substrate-calcium\textsuperscript{†} solutions were prepared. These solutions, and a sixth tube containing 10 ml of water, were placed in the water bath during the last five minutes of the myofibril incubation.

After one hour of myofibril incubation, the ATPase reaction was started by adding 1 ml of the five different substrate-calcium solutions to the appropriate myofibrillar preparation. In addition, 1 ml of water was added to tube 16. The reactions were stopped 15 minutes later by the addition of 12 ml of 10% trichloroacetic acid (TCA), making a final concentration of 8%. The 15-minute assay period was chosen since previous studies have demonstrated a significant difference in normal and failing myofibrillar ATPase activity at this interval.\textsuperscript{8}

Table 1 shows a summary of the different ouabain-calcium concentrations in the final experiment. All reaction vessels except 11 and 16, which were controls, had final concentrations as follows: myofibrillar protein approximately 1 mg/ml, KCl, 100 mM; Tris buffer, 10 mM; adenosine triphosphate (ATP) 5 mM; MgCl\textsubscript{2}, 1.0 mM at 37.1 °C, and pH 7.1. Tube 11 was a control of the amount of free inorganic phosphate (Pi) liberated from ATP in the absence of enzyme; tube 16 was a control of the amount of ATP present in the myofibrils that were split by the myofibrillar ATPase.

After the reaction was stopped with TCA, the test tubes containing the precipitated myofibrils were centrifuged for 20 minutes at 2,000 g. An aliquot of the supernatant was removed from these and from the controls for the determination of inorganic phosphate.

ATPase activity was expressed as micromoles of inorganic phosphate (\mu{mole Pi}) liberated per milligram of myofibrillar protein per 15 minutes and was calculated as follows: ATPase activity = \mu{mole Pi} in media containing myofibrils and ATP at any given concentration of ouabain and calcium in 15 minutes minus [Pi in media containing no myofibrils (tube 11) plus Pi in media without ATP (tube 16)] divided by protein in milligrams. Pi liberated from acid hydrolysis of ATP (tube 11) ranged from 0.1 to 0.2 \mu{moles/15 min}. Pi liberated from residual ATP or other phosphorus sources of myofibrils ranged from 0 to 0.02 \mu{moles/15 min}.

D. CHEMICAL METHODS

Phosphate was determined by the method of Fiske and SubbaRow.\textsuperscript{10} Protein was determined by the Weichselbaum-Kirk biuret method as modified by Beisenherz,\textsuperscript{11} and standardized against bovine plasma albumin* solutions containing 2.25 and 4.5 mg of protein treated similarly.

Results

A. POSTMORTEM TISSUE

1. Normal and Failing Ventricles

a. Effect of Ouabain on Calcium Chloride Activated ATPase Activity. The data show that concentrations of calcium chloride up to 2.5 mM accelerate ATPase activity. Greater increase in calcium does not augment further the enzyme response. It is clear that the addition of digitalis does not affect the system. This was found in the left and right ventricles of both normal and failing groups. The effect of varying concentrations of ouabain at any given concentration of calcium chloride was tested by means of unpaired t tests and in no case did the populations differ if the concentration of calcium remained the same (figs. 1 and 2). The mean values for ATPase activity at all ouabain concentrations fell well within two standard deviations of the mean value for ATPase activity when no ouabain was present. This was true at each concentration of calcium chloride.

b. Comparison of Left-to-Right Ventricular ATPase Activity. No difference of statistical significance was found between left and right normal ventricles and between left and right failing ventricles when tested by unpaired t-tests at each of the five concentrations of calcium chloride. For some of the following analyses, therefore, all normal values were considered as one population and all values for failing hearts as a second population, without respect to the side of the heart from which they were obtained.

\textsuperscript{*Crystallized bovine plasma albumin was obtained from the Armour Pharmaceutical Company, lot no. X75508.}
c. Comparison of ATPase Activities of Normal and of Failing Hearts.
In comparing normal left ventricles to failing left ventricles a highly significant depression of ATPase activity was found in the failing group at all five different concentrations of calcium chloride as evaluated by unpaired t-tests (fig. 1).

A comparison of normal right ventricles to failing right ventricles showed that no significant difference of ATPase activity existed between these groups at the five different concentrations of calcium chloride as evaluated by unpaired t-tests (fig. 2).

d. ATPase Activity and Age of the Patient.
No correlation was found between ATPase activity and age of the patient (fig. 3). This was tested at each concentration of calcium chloride. Left ventricular and right ventricular controls and combined left and right ventricles of the failure group were tested separately by determining the correlation co-

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TABLE 2

| Mean values for ATPase activity* with calcium concentration of: |
|-------------------|----------------|---------|---------|---------|---------|
|                   | 0 mM           | 0.5 mM  | 1.0 mM  | 2.5 mM  | 5.0 mM  |
| **Postmortem:**   |                |         |         |         |         |
| Hypertrophic      | Mean (n = 3)   | .87     | .98     | 1.17    | 1.16    |
| nonfailing left   |                |         |         |         |         |
| ventricle†        |                |         |         |         |         |
| **Postmortem:**   | Mean (n = 2)   | .97     | 1.08    | 1.21    | 1.16    |
| normal left       |                |         |         |         |         |
| papillary muscle†|                |         |         |         |         |
| **Surgical:**     | Mean (n = 3)   | .90     | 1.08    | 1.12    | 1.07    |
| **hypertrophic**  |                |         |         |         |         |
| nonfailing        | Mean (n = 3)   | 1.05    | 1.04    | ±.06    | ±.07    |
| pulmonary         |                |         |         |         |         |
| infundibulum      | Standard error | ±.05    | ±.05    | ±.04    | ±.06    |
|                   | P value        | .001    | .001    | .001    | .02     |
| **Surgical:**     | Mean (n = 2)   | .54     | .80     | .77     | .97     |
| failing left       |                |         |         |         |         |
| papillary muscle†|                |         |         |         | .89     |

*ATPase activity expressed as μmoles Pi liberated/mg protein/15 min.
†For ease of comparison a single mean value for ATPase activity at the same calcium concentration in each experiment is shown. The small number of samples, however, does not permit statistical analysis of the effect of ouabain on the system or comparison of normal to failing tissue.
‡Difference in ATPase activity between surgical specimens of hypertrophic nonfailing pulmonary infundibulum and postmortem specimens of combined left and right failing ventricle.

2. Hypertrophic Nonfailing Ventricles and Normal Papillary Muscles

Although the limited number of experiments does not permit statistical analysis, certain tentative observations seem reasonable.

a. Effect of Ouabain and Calcium Chloride Activated ATPase Activity. In both groups, calcium chloride accelerates the reaction to a maximum reached at a calcium level of 2.5 mM. Further increase of calcium to 5 mM does not result in an appreciable change in activity. Ouabain has no apparent effect on either group. For this reason, and to facilitate comparison to other data, single mean values for ATPase activity at the same concentration of calcium in each experiment are shown in table 2.

b. Comparison of ATPase Activities of Hypertrophic Nonfailing Ventricles, Normal Papillary Muscles and Normal Ventricles. Both normal papillary muscle and hypertrophic ventricles had ATPase activities that were strikingly similar to normal ventricular ATPase activity. This can be readily appreciated by comparing the values at each calcium chloride concentration shown in table 2 with those in figures 1 and 2.

B. SURGICAL TISSUE

1. Hypertrophic Nonfailing Pulmonary Infundibula

a. Effect of Ouabain on Calcium Chloride Activated ATPase Activity. Calcium chloride up to a concentration of 2.5 mM increases ATPase activity while ATPase activity is unaffected by ouabain. The effect of varying concentrations of ouabain at any given concentration of calcium chloride was tested by means of unpaired t-tests and in no case were the populations different at the same concentration of calcium chloride. Since ouabain did not affect ATPase activity, all samples at the same calcium concentration in any one infundibular specimen were considered as duplicates. Single mean values for each calcium concentration are shown in table 2.

b. Comparison of Hypertrophic Nonfailing Pulmonary Infundibulum to Normal and Failing Postmortem Ventricles. A significant difference in ATPase activity was found between surgical hypertrophic pulmonary infundibular tissue and postmortem failing left and right ventricles at all five concentrations of calcium chloride as evaluated by unpaired t-tests. The ATPase activity of failing tissue was lower in all cases. On the other hand, there was no difference when compared to normal ventricular ATPase activity. The absence of a
change in ATPase activity in infundibular tissue assayed at 0 time and up to 4 hours later would seem to justify such a comparison.

2. Failing Papillary Muscles

In the case of the failing papillary muscles, the data are inadequate for statistical analyses but it may be observed tentatively that ouabain has no apparent effect on ATPase activity. For this reason and again to facilitate comparison with other data, single mean values for ATPase activity at the same calcium concentration in each experiment are shown in table 2.

The mean ATPase activity of the limited number of surgical samples is clearly in the range of the mean values for this enzyme of failing left and right ventricles obtained at necropsy. A statistical comparison is not possible with the limited data (table 2, fig. 4).

Discussion

A decrease of contractility has been a constant finding in the several studies of muscle models (actomyosin systems) prepared from failing hearts. Because of the intimate relationship between contractility and the rate of hydrolysis of ATP, one might expect a corresponding decrease of ATPase activity in congestive failure. Alpert and Gordon have demonstrated such a depression in the ATPase activity of myofibrils extracted from the left ventricles of patients in hypertensive congestive failure. This finding is supported by the report of Aras and Hass. Miyahara, along with others, using an ionic environment and substrate concentration similar to that of Alpert and Gordon, also demonstrated a depression in ATPase activity in actomyosin extracted from failing volume-overloaded right ventricles of dogs. On the basis of our present study we may now extend our earlier observations to include volume-overloaded as well as pressure-overloaded left ventricles of human beings. The data also demonstrate that the depression in myofibrillar ATPase activity in failing hearts is not a function of the age of the patient.

The mechanism underlying the depression of ATPase activity in failure may reside in an alteration of the contractile proteins, actin and myosin, especially since H meromyosin is the ATPase. Olson stated that he had not been able to confirm his earlier high estimates of the molecular weight of failing canine myosin. It may be, however, that an aberration in the contractile proteins in failure is not reflected by a change in molecular weight. Alterations in myocardial protein synthesis have been reported in the failure of experimental pressure overload.

The data show clearly that the depression of ATPase activity in failing right ventricular muscle is not statistically significant when compared to that of normal right ventricles. The question may then be asked: Are the right ventricular failures in any way different from the left ventricular failures?

It is notable that in our study six of the eight specimens from failing right ventricles, but only three of the eight from failing left ventricles, were obtained from patients with congenital heart disease. The essence of the present study is that the defect in ATPase activity in failure is the metabolic counterpart of the defect in contractility in failure. We
have used clinical and pathologic criteria for failure and, when these criteria were met, we have assumed that the ventricles of such patients had demonstrated a defect in contractility, i.e., had performed less work at a given fiber length.

The difficulty of clinical assessment of failure in infants and children is well known. The interesting studies of Miller and Swan (personal communication) have shown clearly that children judged clinically to be in congestive failure may have normal myocardial contractility as evaluated by more precise angiocardioangiographic technics. Their studies suggest further that the hearts of children with congenital heart disease handle volume and pressure loads better in terms of work performed at a given end diastolic volume than do hearts of older patients with acquired heart disease, even though clinical failure may be present in both groups. While there is no direct correlation between age and ATPase activity in any of the populations reported herein, it is interesting that the mean ATPase activity of specimens obtained from children is 25% higher than that obtained from adults in right ventricular failure.

The depression of ATPase activity during failure seems clearly a function of failure and not of hypertrophy per se. The ATPase activity of hypertrophic nonfailing ventricles obtained at postmortem was in the normal range. The ATPase activity of hypertrophic nonfailing pulmonary infundibula obtained at operation was statistically the same as that of the normal ventricles obtained at necropsy. This finding supports the work of Brown and associates, who also found no difference between myofibrillar ATPase activity of normal and that of hypertrophic human hearts. They also found no correlation between age and ATPase activity. Meerson and co-workers found decreased actomyosin ATPase activity in chronic (2.5 years) hypertrophic hearts of dogs in the absence of clinical failure as well as in the late stages of hypertrophy in rabbit hearts. They suggest that this change may "herald the onset of the stage of gradual exhaustion of the myocardium." A depression of ATPase activity seems, therefore, to appear in late hypertrophy or early failure. A clear distinction between the two is most difficult.

The observation that infundibular ATPase activity did not change up to 4 hours after the tissue was obtained at operation, coupled with previous observations of the stability of myofibrillar ATPase activity from 2.5 to 13 hours after death, supports the validity of comparing surgical to postmortem specimens. Finally, the ATPase activity of the limited number of failing papillary muscles studied is clearly in the range of failing postmortem ventricular ATPase activity. That the low activity is a function of failure and not of the site from which the specimen was obtained is supported by the distinctly higher values found in normal papillary muscles at necropsy.

It would seem reasonable that digitalis, or digitalis and calcium, might act to correct a defect in ATPase activity in failing hearts. This view was supported by Kako and Bing, who found that the defect in contractility at necropsy in failing human hearts was corrected by both calcium and digitalis together but not by either alone. In our study, however, ouabain did not affect ATPase activity in any group at any calcium chloride concentration. The pattern of calcium chloride activation of myofibrillar ATPase activity in the presence of magnesium found in our studies is similar to that previously described by Perry and Grey. There is no difference between normal hearts and failing hearts with regard to their response to calcium chloride.

The negative character of our findings in the face of a significant decrease in myofibrillar ATPase activity in the failing group, supports the conclusion that digitalis does not act directly on the contractile proteins to enhance the contractility of the myofibril. Whether the ultimate site of action of digitalis is on the membrane or sarcotubular relaxing factor, and whether it is mediated by an action on the ATPases present at these sites or through shifts of calcium between bound and unbound sites, remains for solution by future investigations.
Summary

Ouabain had no effect on myofibrillar adenosine triphosphatase (ATPase) activity in normal, hypertrophic, and failing hearts with concentrations of calcium chloride varying from 0 to 5.0 mM. The quantitative effects of CaCl₂ on myofibrillar ATPase were the same in all groups studied.

At necropsy it was found that myofibrillar ATPase activity of heart tissue from patients with clinical and pathologic diagnoses of failure from left ventricular volume and pressure overload was significantly lower than that of normal controls. There was no difference in myofibrillar ATPase activity between normal left and normal right and between failing left and failing right ventricles.

The difference demonstrated at necropsy between normal and failing hearts was not a function of age or of hypertrophy per se. Myofibrillar ATPase activity of hypertrophic nonfailing surgical pulmonary infundibular tissue was the same as that of normal postmortem ventricles. The ATPase activity of failing surgical papillary muscle in a small number of experiments was in the range of that found in failing postmortem ventricles.

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