The Mechanical Characteristics of Hypertrophied Rabbit Cardiac Muscle in the Absence of Congestive Heart Failure

The Contractile and Series Elastic Elements

BURT B. HAMRELL AND NORMAN R. ALPERT

SUMMARY Right ventricular papillary muscles from normal rabbits and rabbits with sustained pulmonary artery constriction (67% decrease in external diameter) were studied at several resting muscle lengths and at an early instant in the isometric twitch. Instantaneous force-velocity data were obtained at 30-38% of time to peak tension (TPT) and at 96%, 98%, and 100% of the resting muscle length at which active twitch tension was maximal. Unloaded shortening velocity ($V_{\text{max}}$) was estimated with a linearized form of the Hill hyperbolic formula, and was depressed in hypertrophy to 36% less than normal. We found that $V_{\text{max}}$ did not change with muscle length in the normal or hypertrophied muscles; therefore there was a length- and time-independent depression of contractile element shortening capacity that was consistent with previous work from this laboratory which demonstrated a depression of myosin and actomyosin ATPase activity in hypertrophy.

IN PREVIOUS studies of cardiac hypertrophy the force-velocity relationship has been measured for afterloaded isotonic twitches in which the total load lifted by the muscle and the moment during the rising phase of the twitch when shortening commences were directly related; each force-velocity point occurred at a unique time during the twitch and, therefore, at a particular level of internal activity. An instantaneous measure of force and velocity early in the twitch would allow an estimate of $V_{\text{max}}$ at a time when inactivation related to muscle shortening would be minimal. Series elasticity has been evaluated in normal and hypertrophied myocardium but there are no reports of quantification of contractile element function simultaneously with series elasticity at a relatively early instant in the twitch for normal and hypertrophied heart muscle. Therefore, in the experiments reported here, an isotonic "quick release" technique was used to obtain force-velocity and stress-strain data early in the rising phase of the isometric twitch of normal and hypertrophied isolated myocardium, at a time preset relative to time to peak tension (TPT).

In normal heart muscle the extrapolated value of shortening velocity at zero load ($V_{\text{max}}$) was independent of initial external length over a range from 96% to 100% of the length at which maximal peak active isometric tension occurs ($L_{\text{max}}$). In the experiments reported here quick releases were used to determine the force-velocity relationship at 96-100% $L_{\text{max}}$. If the length-independence of $V_{\text{max}}$ were confirmed for normal and observed for hypertrophied heart muscle, then a difference in $V_{\text{max}}$ levels between normal and hypertrophied myocardium would suggest an intrinsic change in contractile element $V_{\text{max}}$. The sarcomere ultrastructure in hypertrophy, in particular the average sarcomere length at $L_{\text{max}}$, is unchanged from normal. We hypothesized that $V_{\text{max}}$ would be independent of initial muscle length in the normal and hypertrophied papillary muscles and would be depressed in the latter.

Methods

PREPARATION OF RABBITS

Male Canadian albino rabbits, housed under constant environmental conditions for several weeks, underwent thoracotomy when body weight reached 2,000 g. Intravenous anesthetization with promethazine HCI, 25 mg, and pentobarbital sodium, 25 mg/kg, was followed rapidly by endotracheal intubation and methoxyflurane inhalational anesthesia delivered via positive pressure ventilation. Small intravenous doses (2 mg) of succinylcholine were administered when necessary for relaxation during surgical anesthesia. A left lateral thoracotomy in the 3rd interspace exposed the proximal main pulmonary artery. A Monel metal spiral with an inner diameter of 3.0 mm and 2.5 turns was "twirled" around the proximal main pulmonary artery, the average external diameter of which was 9.0 mm; this resulted in a 67% reduction in vessel diameter. Normal male rabbits matched for body weight were maintained in the same environment with the thoracotomized rabbits.

HEMODYNAMIC STATUS AND EXTENT OF HYPERTROPHY

At 37–80 days after surgery, the hemodynamic status was evaluated in a series of rabbits with pulmonary artery constriction and in normal rabbits, including some of the rabbits used in the present experiments.
The displacement transducer was a photoelectric system in through a mercury seal to a stable parallel plate capacitor, which a segment of the long arm of the lever modulated on the basis of the values for equivalent mass and resulting acceleration, was 52 mg. Compliance was evaluated by substituting a rigid link for the muscle and recording the displacement that resulted with a series of weights suspended from the short arm of the lever; the compliance of the lever, measured by suddenly applying a constant weight.

### MECHANICAL STUDIES

Immediately following the pressure measurements the heart was removed and a suitable right ventricular papillary muscle was excised, as described below. The right ventricular free wall was weighed separately from the left ventricular wall and septum; the blotted weights of both portions were obtained, then samples were dried to constant weight.

#### Table 1 Isometric Twitch Data

<table>
<thead>
<tr>
<th>Length (%)</th>
<th>Force (g/mm²)</th>
<th>dP/dtmax (g/mm²/sec)</th>
<th>TPT (msec)</th>
<th>TV2R* (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td><strong>H</strong></td>
<td><strong>N</strong></td>
<td><strong>H</strong></td>
<td><strong>N</strong></td>
</tr>
<tr>
<td>100%</td>
<td>4.71±0.47</td>
<td>4.63±0.30</td>
<td>0.87±0.20</td>
<td>1.10±0.12</td>
</tr>
<tr>
<td>98%</td>
<td>4.33±0.45</td>
<td>3.73±0.23</td>
<td>0.43±0.03</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>96%</td>
<td>3.87±0.40</td>
<td>3.15±0.26</td>
<td>0.23±0.04</td>
<td>0.18±0.05</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* Time from peak active isometric tension to 1/2 that value (time to 1/2 relaxation).

** N = normal (n = 5); H = hypertrophy (n = 9).”
After each experiment the papillary muscle was blotted and weighed. Average cross-sectional area was estimated on the basis of muscle weight, length, and an assumed specific gravity of 1.05. All force levels were expressed in grams, i.e., gram weight, and were normalized to cross-sectional area. Cross-sectional areas did not differ significantly between muscles from normal hearts (0.72 ± 0.06 mm²) and hypertrophied hearts (0.80 ± 0.17 mm²) \( (P > 0.05) \); as noted above, papillary muscles from normal and hypertrophied ventricles were selected so as to maximize length and minimize diameter.

All shortening velocity values (millimeters per second) were divided by initial muscle length to convert them to lengths per second to permit comparisons of velocity data from different length muscles and in the same muscle at different lengths. No correction for system compliance was made in the calculations of muscle elasticity. Analysis of variance and linear regression analysis were used in the data analysis.

**Results**

**HEMODYNAMIC STATUS**

There were no signs of congestive heart failure at the time the rabbits were killed. Right ventricular intraluminal end-diastolic pressures were significantly elevated in the banded rabbits. The end-diastolic pressure levels were similar to those reported for cats with compensated right ventricular hypertrophy caused by pulmonary artery banding and were less than the levels reported for cats with right ventricular failure.

**CARDIAC TISSUE STUDIES**

The right ventricular free wall weight, expressed as the percentage of total heart weight, was significantly increased in the rabbits with pulmonary artery constriction (34.0 ± 0.5%) as compared to the percentage in normal rabbit hearts (21.0 ± 0.8%) \( (P < 0.001) \). The water content of hypertrophied myocardium (81.4 ± 0.9%), calculated as [(wet weight - dry weight)/wet weight] \times 100, did not significantly differ from that of normal myocardium (80.2 ± 1.2%) \( (P > 0.05) \), therefore there was no cardiac muscle edema. Right ventricular free wall mass was 1.86 ± 0.07 g in the hypertrophied hearts and 1.08 ± 0.06 g in the normals.

**MECHANICAL STUDIES**

**Isometric Results**

At 96%, 98%, and 100% \( L_{max} \) peak active isometric tension attained by papillary muscles from hypertrophied ventricles (\( n = 9 \)) was not significantly different from the levels observed in normal muscle (\( n = 5 \)) (Table 1). The maximum rate of isometric tension development (\( \text{dP/dt}_{max} \)) in the hypertrophied muscles was significantly reduced below normal levels \( (P < 0.01) \) and TPT was significantly greater than normal at all the lengths studied \( (P < 0.01) \) (Table 1). TPT was measured from the onset of the isometric twitch and excludes the latent period. There was no significant difference between the normal and hypertrophied muscles in the time from the peak of the isometric twitch to 1/2 that value on the descending limb (time to 1/2 relaxation; \( T_{1/2} \)).

Passive isometric tension levels in the hypertrophied muscles were not different from normal at all the lengths studied. However, the tangent modulus of the parallel elastic component, determined with quick stretches of the resting muscles, was increased in the hypertrophied preparations.

**Isotonic Results**

The relationship of muscle shortening velocity to the load moved can be described by the Hill rectangular hyperbola:

\[
(P + a)(V + b) = b_0(P_o + a)
\]

where \( a \) and \( b \) have the dimensions of force and velocity, respectively, and are the asymptotes. The force and velocity data in these experiments were analyzed with linearized form of this hyperbola as follows:

\[
\frac{L(1 - [P/P_{max}])}{V} = \frac{L}{b} \left( \frac{P}{P_{max}} \right) + \frac{a}{P_{max}} \frac{b}{L}
\]

The external length of the muscle (\( L \)), in millimeters, appears because all velocity values, including the constant \( b \), were normalized to the initial muscle length. Brady reported that quick stretches during the onset of a cardiac muscle isometric twitch elicited force levels no greater than peak active tension (\( P_{max} \)). Therefore, \( P_{max} \) was a close estimate of the maximal force (\( P_o \)) developed by a papillary muscle. Relative force was expressed as the ratio of the total load lifted by the muscle (\( P \)) to \( P_{max} \) at that initial length. The linearized form of the hyperbola was used to estimate \( V_{max} \); the reciprocal of the slope of the linear regression of \( L(1 - [P/P_{max}])/V \) on \( P/P_{max} \) was \( b/L \), and \( V_{max} \) was the reciprocal of the intercept with the ordinate, that is, \( (b/L)/(a/P_{max}) \). Data from a representative normal and hypertrophied muscle selected for similarity of cross-sectional area illustrate the extent of depression of the force-velocity relationship in hypertrophy as compared with normal myocardium at 96%, 98%, and 100% \( L_{max} \) (Figs. 1 and 2). All the force-velocity data for a muscle were included in the linear regression analyses. The data from the three initial muscle lengths were superimposable within the normal and hypertrophied groups. There was minimal deviation from a hyperbolic force-velocity relationship (Fig. 1) and there was no overlap of the data for normal and hypertrophied muscles. The intercept at the ordinate in Figure 1 is the reciprocal of \( V_{max} \); \( V_{max} \) of the hypertrophied preparation was 0.78 L/sec and the normal was 1.31 L/sec. Coordinates predicted by the regression formulas were used to plot the curves in Figure 2.

When all force-velocity data from all muscles were analyzed as above, \( V_{max} \) of the hypertrophied muscles was 0.83 ± 0.04 L/sec and was significantly less than the normal level of 1.19 ± 0.07 L/sec \( (P < 0.001) \).

**The Series Elastic Element**

The elastic properties of the activated muscles were assessed by analyzing the initial undamped shortening phase of the quick release perturbations at \( \text{dP/dt}_{max} \). Figure 3A illustrates all stress and
MECHANICS OF HYPERTROPHIED MYOCARDIUM/Hamrell and Alpert

4.0 -  
L(1 - P/Pmax)

V

P/Pmax

FIGURE 1 Linearized force-velocity data at 96-100% Lmax. All force-velocity data at 96% (stars), 98% (triangles), and 100% (circles) Lmax are presented in a normal and a hypertrophied muscle selected for similarity of cross-sectional area: normal = 0.61 mm²; hypertrophy = 0.51 mm². Pmax = peak active isometric tension at each initial length. Pmax at Lmax in the normal is 3.87 g/mm² and in the hypertrophy is 4.62 g/mm². See text for further discussion of the linearization analysis.

FIGURE 2 The force-velocity relationship at 96% (stars), 98% (triangles), and 100% (circles) Lmax in the same muscles as in Figure 1. Pmax and P are defined as in Figure 1. The normalization of velocity to initial muscle length is discussed in the text. The curves are constructed using coordinates predicted by the linear regression analyses in Figure 1.

strain data obtained from all the muscles. The nonlinear stress-strain relationship was studied further by analysis of the linear regression of the ratio of stress to strain on stress (Fig. 3B). There was no significant difference between the linear regressions of the data for normal and hypertrophied muscles. Consequently, in agreement with previous observations, there was no significant change in the elastic properties of the series elastic component as a result of hypertrophic growth.

Discussion

Within minutes after the onset of systolic ventricular overload there is an increase in myocardial protein synthesis. Eventually ventricular wall cell enlargement results from the addition of sarcomeres in series and parallel. The sarcoplasmatic reticulum and transverse tubular system are increased in proportion to the cellular enlargement. The organization of the contractile apparatus in hypertrophied muscle is not changed from normal; left ventricular wall sarcomere length assessed at low end-diastolic pressures is unchanged in hypertrophy when compared with normal. There is muscle fiber enlargement in papillary muscles from right ventricles in cats with sustained pulmonary artery banding.

There was no difference in the cross-sectional areas of the normal and hypertrophied papillary muscles in the

FIGURE 3 A: series elastic stress-strain relationship. These are all the data from all muscles studied at Lmax. Stress is the total load across the muscle. L0, an estimate of the unstressed length of the series elasticity, is the muscle length after the early rapid shortening phase following the quick release to the smallest total load. The curves are constructed with coordinates predicted from the linear regression analyses discussed below. B: linearized series elastic stress-strain relationship. These are the same data as in panel A, but plotted as the inverse slope of the stress-strain relationship on the ordinate vs. stress on the abscissa. The lines are constructed on the basis of linear regression analyses of the data. Hypertrophy (closed circles): Ŷ = 7.0 + 18.8X. Normal (open circles): Ŷ = 4.0 + 14.4X.
present study because we selected the longest, thinnest muscles in order to avoid the problems of hypoxia of the central fibers and less active force development in thicker muscles. There were no reported data regarding the uniformity of cellular enlargement in papillary muscles within a ventricle and we chose muscles entirely on the basis of suitability for isolated mechanical studies.

Contractile element shortening was characterized by the force-velocity relationship at the same early instant in the twitch relative to TPT for hypertrophied and normal muscles and at a time when the ability to shorten had reached a maximum. The significant depression of the instantaneous force-velocity relationship in hypertrophied as compared with normal myocardium at L_max agreed with most mechanical observations utilizing an afterloaded isotonic technique.\textsuperscript{1,4,6} \( V_{\text{max}} \) was depressed in hypertrophied muscles to 36% below normal levels (calculated as \( [\text{normal } V_{\text{max}} - \text{hypertrophy } V_{\text{max}}]/\text{normal } V_{\text{max}} \times 100 \)), but in normal and hypertrophied myocardium \( V_{\text{max}} \) was constant at initial external lengths from 96% to 100% \( L_{\text{max}} \) (Figs. 1 and 2).

At low loads muscle function was not altered by the isotonic quick release perturbations\textsuperscript{17} nor at higher loads by the extent of internal shortening as has been described for isotonic afterloaded twitches.\textsuperscript{8} The velocities reported here for normal rabbit right ventricular papillary muscles were comparable to velocity levels obtained for rabbit myocardium by others with lever-damping techniques (9, 28) and for cat right ventricular papillary muscles at 20°C. Bodem and Sonnenblick\textsuperscript{30} reported that deactivation of the contractile system due to quick releases was minimized at 24°C, the temperature used in the experiments reported here. During the first third of TPT the velocity-length trajectory following a quick release became coincident with an isotonic afterloaded twitch, i.e., an early quick release such as was used in the experiments reported here did not alter the isotonic activity in the remainder of the twitch in cat papillary muscles.\textsuperscript{21} Releases were not done later than the first third of TPT.\textsuperscript{28,30}

On the basis of the above arguments and the lack of significant fall-off of velocity at low loads (Figs. 2 and 3), the muscle shortening velocities at low total loads (low preloads at 96% and 98% \( L_{\text{max}} \)) in normal and hypertrophied papillary muscles were a close approximation of unloaded contractile element velocity and minimized the extent of extrapolation to obtain \( V_{\text{max}} \) (Figs. 1 and 2). The estimated velocity of muscle shortening at “zero” contractile element load should represent the maximal rate of cross-bridge turnover, unimpeded by tension maintenance, and be related to the ability of the contractile proteins to hydrolyze ATP. \( V_{\text{max}} \) has been directly correlated with the ability of skeletal muscle myosin to hydrolyze ATP and release the energy for shortening.\textsuperscript{32} Hypertrophied cat left ventricular myocardium\textsuperscript{23} and right ventricular muscle from rabbits used in these experiments, as well as myocardium from other rabbits with similar pulmonary artery bands,\textsuperscript{34} manifested depressed actomyosin ATPase activity, and the extent of depression was similar to that of \( V_{\text{max}} \) reported here.

The resting tension at the shorter muscle lengths was low in hypertrophied and normal muscles (Table 1). Furthermore, in three-element mechanical analogs of cardiac muscle the tension across parallel elastic elements was discharged during an isotonic quick release to a total load substantially less than the preload.\textsuperscript{18} At a low tension (stress) across the parallel elastic elements the tangent moduli of normal and hypertrophied muscles were similar.\textsuperscript{16} Consequently the possible effects of parallel elasticity on muscle shortening velocity\textsuperscript{25} were minimized in the experiments reported here.

In the studies reported here the changes in the isometric twitch that occur with hypertrophic cardiac muscle growth\textsuperscript{15,16} have been confirmed (Table 1). In addition, the shortening capacity of hypertrophied, as compared to normal, muscle has been studied at a constant early time in the twitch and was characterized as follows: (1) \( V_{\text{max}} \) in hypertrophied muscles was significantly depressed when measured at a constant relative time in the twitch, and (2) \( V_{\text{max}} \) was independent of initial muscle length from 96% to 100% \( L_{\text{max}} \) in hypertrophied and normal muscles, and therefore was a reflection of maximal speed of contractile element motion in the region of optimal myofilament overlap. The intrinsic defect in the ability of the contractile element to shorten may be related to alterations in the myosin molecule.\textsuperscript{25,26} The changes from normal in the isotemic twitch in hypertrophy may in part be related to depressed contractile element function or altered internal Ca\textsuperscript{2+} movement, or both,\textsuperscript{25} but were not the result of changes in series elasticity (Fig. 3).

Congestive heart failure was not present in the rabbits studied in our experiments, but there were substantial changes in myocardial mechanical properties. The depression of shortening velocity with hypertrophy was substantial but when time constraints were not severe, as in an isotemic twitch with a prolonged time to peak tension, hypertrophied myocardium generated close to normal active isometric force levels. In the intact mammal with cardiac hypertrophy, without failure, extrinsic factors, particularly the sympathetic nervous system, compensated for the intrinsic functional deficit, at least when the rabbit was at rest. Under conditions of stress, such as endurance exercise when the heart must beat faster, maintain or increase stroke volume, and pump at higher systolic pressures, the patient with a hypertrophied “non-failed” heart performed less well than the normal subject.\textsuperscript{28}

References

The mechanical characteristics of hypertrophied rabbit cardiac muscle in the absence of congestive heart failure: the contractile and series elastic elements.

B B Hamrell and N R Alpert

doi: 10.1161/01.RES.40.1.20

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1977 American Heart Association, Inc. All rights reserved.
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:
http://circres.ahajournals.org/content/40/1/20

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/