Structural Basis for the Ascending Limb of Left Ventricular Function

By Chaim Yoran, James W. Covell, and John Ross, Jr.

ABSTRACT
Midwall sarcomere lengths near maximal have been reported at left ventricular end-diastolic pressures at the upper limits of normal, but an ascending limb of cardiac function can exist at much higher filling pressures. Accordingly, an analysis was made of sarcomere length distributions across the left ventricular wall over a range of end-diastolic pressures between 2 and 20 mm Hg. In 11 dogs, significant ascending limbs of ventricular function were documented at filling pressures between 2 and 12 mm Hg and between 12 and 20 mm Hg. Nine hearts were arrested and rapidly fixed in diastole with intracoronary glutaraldehyde perfusion, and tissues from five sites equally distributed across the left ventricular free wall were examined by electron microscopy. At filling pressures at the lower end of the normal range (2, 6, and 12 mm Hg), an uneven distribution of sarcomere lengths across the wall was noted: mean sarcomere lengths were shortest at the subendocardial layer, longest at a site between the subendocardial and the midwall layers, and then progressively shorter toward the epicardium. All differences were highly significant. At an end-diastolic pressure of 20 mm Hg, the difference in sarcomere lengths between layers was small; sarcomere lengths decreased only slightly from endocardium to epicardium. Despite maximal sarcomere lengths just inside the midwall in hearts fixed at 12 mm Hg (2.253 μm), sarcomere lengths in other layers—the subendocardial and the subepicardial regions—were increased substantially from 2.129 and 2.183 μm, respectively, at 12 mm Hg to 2.283 and 2.247 μm at 20 mm Hg (P < 0.001 and P < 0.002, respectively). Therefore, sarcomeres from all layers of the wall increase in length up to a filling pressure of 12 mm Hg; however, as filling pressure is increased further, only short sarcomeres from the subendocardial and the subepicardial layers are recruited. The latter mechanism forms the ultrastructural basis for the ascending limb of normal ventricular function at elevated left ventricular filling pressures.

KEY WORDS  dog  electron microscopy  sarcomere length  sarcomere distribution  cardiac performance  ultrastructure of the heart

According to Starling's law of the heart, ventricular performance is a function of initial muscle length: an increase in ventricular volume or filling pressure produces an increase in actively developed pressure or stroke volume (1, 2). In isolated cardiac muscle, over the physiological range of the length-tension curve (3, 4), sarcomere length tends to be a direct function of muscle length, providing an ultrastructural explanation for the Frank-Starling mechanism (5, 6). Previous studies in excised hearts demonstrated that, at the midwall of the left ventricle, mean sarcomere lengths were near 2.2 μm (a value at the apex of the sarcomere length-tension relation) when left ventricular end-diastolic pressure was at the upper limits of normal (12 mm Hg) (7). However, physiological studies in the intact normal animal and in the isolated dog heart have indicated that an ascending limb of left ventricular function can exist at much higher filling pressures, i.e., pressures in excess of 20 mm Hg (8–10).

Based on these considerations, it seems unlikely that a simple relation between midwall sarcomere length and left ventricular performance exists, particularly at elevated filling pressures. Accordingly, the experiments reported in this paper were designed to document the presence of an ascending limb of cardiac function at filling pressures above 12 mm Hg and then to examine sarcomere lengths and their distribution at sites across the left ventricular wall in hearts fixed rapidly in diastole.
over a range of filling pressures from 2 to 20 mm Hg.

Methods

VENTRICULAR FUNCTION STUDIES

In dogs (15.4-27 kg, average 20.6 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv), the chest was opened, ventilation was maintained, and the heart was supported in a pericardial cradle. Left ventricular pressure was measured through a short stiff tube inserted through the apex and connected directly to a Statham P23Db pressure transducer. Aortic pressure was also measured with a Statham transducer through a catheter inserted via the femoral artery and advanced to the aortic arch.

A series of near-isovolumic contractions of the left ventricle was obtained in each of 11 dogs by manually clamping the ascending aorta just above the aortic valve late in diastole. These contractions closely resembled those obtained when the aortic valve was occluded in diastole by an intra-aortic balloon (9), although slight ejection and some systolic coronary flow occurred with the present technique. With complete occlusions, the ensuing left ventricular pressure curve was rounded, its peak was higher compared with that of control contractions, and an uninterrupted fall in aortic blood pressure was demonstrable; ill-timed or incomplete occlusions were excluded. Filling pressure was raised by rapid volume transfusion (6% dextran) into the inferior vena cava, and in each experiment aortic occlusions were obtained initially at low filling pressures and subsequently as end-diastolic pressures were raised to 20 mm Hg.

In two experiments, ascending aortic flow was measured by a Biotronex electromagnetic flowmeter. The flowmeter was calibrated by determining the aortic output with the indicator-dilution method (9).

Ventricular ejection fraction was determined by induced isovolumic beats, and an uninterrupted fall in aortic blood pressure was demonstrable; ill-timed or incomplete occlusions were excluded. Filling pressure was raised by rapid volume transfusion (6% dextran) into the inferior vena cava, and in each experiment aortic occlusions were obtained initially at low filling pressures and subsequently as end-diastolic pressures were raised to 20 mm Hg.

MORPHOLOGIC STUDIES

In nine experiments, the hearts were rapidly arrested and fixed with glutaraldehyde (14), using a modified technique for the coronary arterial perfusion of the fixative. In brief, hearts were arrested in diastole with KCl; the great vessels were tied, and the left coronary artery was rapidly ligated and cannulated. The left ventricle was then filled with contrast material (75% Hypaque) to achieve the desired level of ventricular diastolic pressure, and 5% glutaraldehyde in sodium phosphate buffer (pH 7.4) was perfused directly into the coronary artery. Biplane cineangiograms were exposed, and left ventricular pressure was recorded to exclude changes in pressure or in the size and the shape of the left ventricle during fixation. Specimens were obtained from the nine hearts for electron microscopy. Two hearts were fixed at 2 mm Hg, two at 6 mm Hg, two at 12 mm Hg, and three at 20 mm Hg.

After coronary perfusion with glutaraldehyde, the hearts were left in 5% glutaraldehyde in phosphate buffer (4°C) for 12 hours (15, 16). Following two or three rinses with phosphate buffer, transmural specimens were obtained and cut under a dissecting microscope into blocks approximatively 1 x 0.5 mm so that the muscle fibers could be oriented longitudinally during embedding. Specimens were then postfixed in 1% osmium tetroxide in sodium phosphate buffer (pH 7.4) for 1 hour, rinsed several times with phosphate buffer, and dehydrated with ethanol. Specimens were embedded in a 3:2 mixture of Araldite 502 and dodecenyl succinic anhydride. Specimens were then postfixed in 1% osmium tetroxide in sodium phosphate buffer (pH 7.4) for 1 hour, rinsed several times with phosphate buffer, and dehydrated with ethanol. Specimens were embedded in a 3:2 mixture of Araldite 502 and dodecenyl succinic anhydride. Thin sections (500 Å) were cut on an ultramicrotome (Porter-Blum MT2B) with a diamond knife; cuts were made parallel to the fiber axis to avoid compression of the section in the longitudinal direction of the filaments. The sections were then mounted on 300-mesh copper grids and stained with uranyl acetate for 15 minutes and lead citrate for 2 minutes. Electron micrographs were taken with a Zeiss EM 9S electron microscope at a magnification of 4,000x and enlarged on prints to a final magnification of 16,500x. Sarcomere lengths were measured on the prints assuming a constant length of the A band. The A-band width measured at 14 sites in five hearts averaged 1.425 ± 0.006μ (range 1.386 to 1.473μ); a parallel-lined carbon grating replica was used for this calibration and was also photographed with each set of pictures to verify magnification. At each site, sarcomeres were counted in pictures taken from at least three grids obtained from each of two separate blocks. Approximately 600 sarcomeres were counted in each heart.
Results

PHYSIOLOGICAL STUDIES

The relation between peak isovolumic developed pressure and left ventricular end-diastolic pressure in 11 dogs is shown in Figure 1. Peak isovolumic developed pressure rose significantly from 177.3 ± 9.5 (SE) mm Hg at left ventricular end-diastolic pressures of 1-5 mm Hg to 240.0 ± 12.5 mm Hg at left ventricular end-diastolic pressures of 9-13 mm Hg (P< 0.001) and increased further to 269.9 ± 11.8 mm Hg at left ventricular end-diastolic pressures of 18-22 mm Hg (P<0.001). Stroke volume and stroke work also increased when the filling pressure was raised over this entire range, and in two other studies wall stress calculations showed that for each increment in peak isovolumic developed pressure the increase in developed wall stress was even greater (Fig. 2). Therefore, all measures of left ventricular performance indicated that an ascending limb of left ventricular function existed at filling pressures well in excess of 12 mm Hg.

ELECTRON MICROSCOPY STUDIES

Sarcomere lengths and their distributions at each level of filling pressure are shown in Table 1 and Figure 3.

Sarcomere Lengths at Normal Filling Pressures.—Specimens were obtained at a filling pressure of 2, 6, and 12 mm Hg from five sites equally distributed across the ventricular wall (Fig. 3).
Table 1 shows the ranges and the differences between subendocardial, midwall, and subepicardial sites. At these normal filling pressures (2–12 mm Hg), the longest sarcomere lengths were found at site 2 (between the subendocardial and midwall layers). The mean values at this site at diastolic pressures of 2, 6, and 12 mm Hg were 2.074 ± 0.005, 2.142 ± 0.003, and 2.266 ± 0.002 /m, respectively. The mean sarcomere lengths decreased from site 2 towards the epicardium, and shorter sarcomeres from sites 1 and 3–5 increased in length at filling pressures between 2 and 12 mm Hg.

**Sarcomere Lengths at Elevated Filling Pressures**—In the ventricles fixed at 20 mm Hg, the sarcomere lengths at the endocardium and the midwall did not differ significantly within each heart, but the sarcomeres were slightly and significantly shorter between the midwall and the epicardium in two of the three hearts. The mean subendocardial sarcomere length in all three hearts was 2.283 ± 0.007 /m. This value is slightly longer than that at the midwall (2.274 ± 0.005 /m), but the difference is statistically insignificant (Table 1, Fig. 3). However, the mean subepicardial sarcomere length was slightly shorter than that at the midwall (P < 0.02), and the mean subendocardial sarcomere length was significantly longer than that at the epicardium (P < 0.002). Therefore, the highly significant differences in mean sarcomere lengths at all layers of the wall at a filling pressure of 12 mm Hg were largely abolished by increasing the filling pressure to 20 mm Hg.

Comparison of sarcomere lengths and their distributions across the left ventricular wall in ventricles fixed at 12 mm Hg with those in ventricles fixed at 20 mm Hg showed substantial differences. There was a large, significant difference at the subendocardial layer: mean values were 2.129 ± 0.005 and 2.283 ± 0.007 /m at filling pressures of 12 and 20 mm Hg, respectively (P < 0.001). A small difference existed at the midwall (2.233 ± 0.004 compared with 2.274 ± 0.005 /m, P < 0.02), and a sizable difference was apparent at the subepicardial layer (2.183 ± 0.005 compared with 2.247 ± 0.004 /m, P < 0.002). The mean sarcomere length at site 2 (midway between the subendocardial and the...
**TABLE 1**

<table>
<thead>
<tr>
<th>Filling pressure (mm Hg)</th>
<th>Subendocardial layer (µ)</th>
<th>Midwall layer (µ)</th>
<th>Subepicardial layer (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.781 - 1.979</td>
<td>1.950 - 2.137</td>
<td>1.916 - 2.071</td>
</tr>
<tr>
<td>Mean</td>
<td>1.869 ± 0.007</td>
<td>2.042 ± 0.004*</td>
<td>1.982 ± 0.005*</td>
</tr>
<tr>
<td>6</td>
<td>1.873 - 2.028</td>
<td>2.000 - 2.186</td>
<td>1.952 ± 2.111</td>
</tr>
<tr>
<td>Mean</td>
<td>1.946 ± 0.003</td>
<td>2.100 ± 0.007†</td>
<td>2.034 ± 0.004*</td>
</tr>
<tr>
<td>12</td>
<td>2.005 - 2.247</td>
<td>2.137 - 2.302</td>
<td>2.111 - 2.280</td>
</tr>
<tr>
<td>Mean</td>
<td>2.129 ± 0.005*</td>
<td>2.233 ± 0.004‡</td>
<td>2.183 ± 0.001</td>
</tr>
<tr>
<td>20</td>
<td>2.151 - 2.394</td>
<td>2.186 - 2.357</td>
<td>2.123 - 2.337</td>
</tr>
<tr>
<td>Mean</td>
<td>2.283 ± 0.001</td>
<td>2.274 ± 0.005‡</td>
<td>2.247 ± 0.004‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Statistical differences are between adjacent layers: midwall compared with endocardium and epicardium compared with midwall.

*P < 0.01. †P < 0.001.
‡No significant difference.
§P < 0.05.

midwall layers) in hearts fixed at 12 mm Hg was not significantly different from that at the midwall in hearts fixed at 20 mm Hg.

**Discussion**

Fixation by direct coronary perfusion immediately after cardiac arrest probably provides the best available method for fixation (17). Although the preparation for electron microscopy induces some shrinkage, glutaraldehyde appears to cause the least change (5, 17, 18).

Our data for midwall sarcomere lengths at normal filling pressures are similar to those reported previously (7, 20), although Laks et al. (19) found that the mean sarcomere length was 2.16 ± 0.002 (2.09-2.20) in dog left ventricles fixed at 0 mm Hg. However, these specimens were taken from trabeculae carneae of the free lateral wall at the base of the left ventricle. The extent of sarcomere length change at the midwall in the minor ventricular circumference at normal filling pressures appears to account for the ventricular wall shortening necessary to explain the left ventricular ejection fraction in the cat, dog, and man (20-22). Thus, over the range of normal filling pressures the changes in midwall sarcomere length during the cardiac cycle can provide an ultrastructural basis for Starling's law of the heart. However, the fact that sarcomere lengths in different layers change to a varying degree over the range of normal filling pressures indicates that function curves of the whole heart do not necessarily bear a simple relation to the midwall sarcomeres. Moreover, a full understanding of such function curves will require knowledge of sarcomere lengths at different sites as well.

In this study, at elevated filling pressures (12-20 mm Hg) left ventricular performance increased, confirming previous findings in anesthetized dogs, conscious animals, and man (8-10, 23, 24). However, this enhanced ventricular function at high filling pressures cannot be explained on the basis of previous data, since midwall sarcomere lengths are reportedly maximal at a filling pressure of 12 mm Hg. We observed a small increase in the mean midwall sarcomere length between filling pressures of 12 and 20 mm Hg; however, the sarcomere lengths at the midwall at 20 mm Hg were essentially the same as those just inside the midwall (site 2) at 12 mm Hg. Therefore, recruitment of short sarcomeres appears to constitute the functional reserve of Starling's curve at elevated filling pressures. Thus, although the mean sarcomere length was already maximal just inside the midwall at filling pressures of 12 mm Hg, much shorter sarcomeres from both the subendocardial and the subepicardial layers were present, and, with an increase in filling pressure to 20 mm Hg, sarcomeres in these layers were considerably elongated together with a slight additional lengthening at the midwall layer. Obviously, only consideration of the entire sarcomere population across the ventricular wall, rather than the mean midwall sarcomere length alone, can allow an explanation of the ultrastructural basis for the ascending limb of
normal left ventricular function at elevated filling pressures.

Our data concerning sarcomere distribution across the ventricular wall between the subendocardial layer and site 2 at normal filling pressures differ from those reported by Spotnitz et al. (7) and by Anversa et al. (25), although our statistical analysis of the data published by the latter group reveals no significant differences in mean sarcomere lengths obtained from different wall layers. In the study of Spotnitz and co-workers (7), the data were of borderline statistical significance between mid- and outer-wall samples but not significant between mid- and inner-wall samples; the determinations were made with the paired t-test, using all data from hearts fixed over the range of filling pressures from 0 to 50 mm Hg. No statistically significant differences were apparent when a group t-test was applied, although the data suggest that the longest sarcomeres were subendocardial. Possibly our subendocardial sampling site was closer to the endocardium than in the reports mentioned above or the subendocardial site in earlier studies corresponded more closely to our site 2 in hearts fixed at normal filling pressures; however, precise location of the sampling sites in previous studies is not reported.

Our finding that the mean sarcomere length was longest just inside the midwall layer at normal filling pressures correlates with findings by Streeter et al. (28). In that study, myocardial fiber angles and distributions were considered in calculating the curve for circumferential wall stress vs. wall thickness at end-diastolic pressures. The curve was convex with the maximal length just inside the midwall (28, 27).

Despite significant differences between mean sarcomere lengths at individual sites from the different layers of the left ventricular wall at normal filling pressures, considerable overlap was present between sites (Table 1). Probably, sarcomere distributions form a continuum across the ventricular wall similar to that observed with myocardial fiber angles (27).

Acknowledgment

We are grateful for the technical assistance of Lana F. Nimmo in electron microscopy and the help of Donald F. Rippon.

References


Structural Basis for the Ascending Limb of Left Ventricular Function

CHAIM YORAN, JAMES W. COVELL and JOHN ROSS, Jr.

Circ Res. 1973;32:297-303
doi: 10.1161/01.RES.32.2.297

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1973 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/32/2/297

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/