The Ultrastructure of the Heart in Systole and Diastole

CHANGES IN SARCOMERE LENGTH

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ABSTRACT

The relations between ultrastructure and function have been examined in the canine left ventricle under known hemodynamic conditions. Left ventricles of normal dogs were fixed acutely by rapid perfusions of the coronary arteries in either diastole or systole, and also following acute ventricular distension, or potentiation of contraction. Sarcomere lengths at the midwall of the left ventricle averaged 2.07 μ at end diastole and 1.81 μ at end systole, changes that are adequate to explain the degree of normal ventricular emptying. Following marked ventricular emptying induced by postextrasystolic potentiation, sarcomeres shortened to an average of 1.60 μ; with acute ventricular distension resulting from over-transfusion, average sarcomere length in diastole increased to 2.25 μ. In each instance, sarcomere lengths correlated with the changes in sarcomere length predicted from changes in the dimensions of a thick-walled sphere. The importance of dispersion of sarcomere length within a given layer of the ventricular wall was noted and its potential significance discussed.

ADDITIONAL KEY WORDS

myocardium
Starling's law of the heart
dog
heart muscle

The sarcomere comprises the basic ultrastructural unit of contraction in both skeletal and heart muscle (1, 2). Recent studies in the isolated cat papillary muscle preparation (3) and in the passively distended cat and dog left ventricle (4) have suggested that alterations of sarcomere length help to explain the length-tension relation of heart muscle and, in turn, form the basis of the Frank-Starling law of the heart (5). However, no information is available concerning the changes in sarcomere length which occur in the myocardium in vivo during contraction or following ventricular dilatation.

The recent development of a method for abruptly arresting the heart in various phases of the cardiac cycle has now permitted a direct approach to the problem of ultrastructure in relation to ventricular contraction (6). It is the purpose of this study to define the dimensions of the sarcomere in normal diastole and to delineate the changes in sarcomere length which occur during systole. Further, the changes which occur in sarcomere length during either marked potentiation of contraction or following acute ventricular dilatation have been explored.

Methods

Dog ventricles have been arrested and fixed in either diastole or during systolic contraction by the technique detailed in the preceding communication (6). In brief, glutaraldehyde fixative was introduced into the coronary circulation by a
power syringe at a predetermined time during diastole or systole (6). Only those ventricles that were clearly fixed either at end diastole or end systole were used in this analysis. In two instances, ventricles were fixed by chance during the potentiated beat following extrasystole.

Following fixation, the ventricle remained in glutaraldehyde for 4 hr and was then transferred to isotonic phosphate buffer. A segment of the anterior wall of the left ventricle was then removed for electron microscopic study. This sample was always taken from a site halfway between the apex and base of the heart, 1 cm to the left of the left anterior descending coronary artery (Fig. 1). Sections of 1 mm² were then selected from this tissue midway between the epicardium and endocardium; in this region circumferential fibers tend to be perpendicular to the long axis of the ventricle (8). The tissue was trimmed under a dissecting microscope to assure orientation of fibers within the plane of sectioning, and it was then fixed in 2% osmium with sucrose for 30 min. Subsequently, the tissue was dehydrated progressively in acetone and embedded in epon in a manner previously described (3). Shrinkage with similar techniques has been estimated to be less than 5% (7). Ultra-thin sections of the imbedded material were prepared using an LKB microtome and the sections were stained with lead citrate. Care was taken to section the tissue with the knife edge perpendicular to the long axes of the fibers to avoid artifacts resulting from tissue compression (3). The sections were then examined under a calibrated RCA EMU 3 electron microscope. Overall magnification was generally 20,000. A minimum of 25 electron micrographs was obtained for each heart. Sections of distinct tissue blocks were compared as a precaution against sampling errors. Sarcomere lengths (measured from the center of the Z line to the adjacent Z line) (Fig. 2) were determined on all photomicrographs and between 100 and 200 measurements were taken to obtain an average sarcomere length for the mid-ventricular wall of each heart.

Results

Of 46 dogs studied, the hearts from 22 were judged satisfactory for the present analysis, having been fixed at an appropriate time during contraction. The over-all results are summarized in Table 1.

Six hearts were fixed in diastole with an average filling pressure of 8 mm Hg (range 2 to 12 mm Hg) and an average intraventricular volume of 51.6 ml (range 40.0 to 66.5 ml). A typical electron micrograph from a heart fixed in diastole is shown in Figure 2. Sarcomere length is indicated by the distance between two Z lines; in this instance it was 2.07 μm. In hearts fixed in diastole, the sarcomere length from the left ventricular midwall averaged 2.07 ± 0.024 (SD) μm (range 2.03 to 2.10 μm) (Table 1 and Fig. 3).

In the 8 ventricles fixed in systole, the intraventricular volume averaged 21.4 ml (range 17.0 to 23.9). The end-diastolic pressure prior to fixation averaged 6 mm Hg and was not significantly different from that of the ventricles fixed in diastole, which averaged 8 mm Hg. In the ventricles fixed in systole, left ventricular midwall sarcomere length averaged 1.81 ± 0.096 μm (range 1.68 to 1.91 μm). A typical electron micrograph taken from a left ventricle fixed in systole is shown in Figure 4. Of note, in ventricles fixed in systole, sarcomeres less than 1.8 μm in length were commonly observed and in these sarcomeres an additional band at the center of the A band termed the A contraction band could usually be perceived (Fig. 4). In 2 hearts fixed dur-
TABLE I

Correlations between the Intact Left Ventricle and Sarcomere Dimensions

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>LV/D (mm Hg)</th>
<th>Fixation pressure (mm Hg)</th>
<th>LV wt (g)</th>
<th>LV vol (ml)</th>
<th>Sarcomere length (μ)</th>
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Abbreviations: LV = left ventricular; S/D = systolic/end diastolic pressure.

Figure 3. Using the average sarcomere length at normal diastolic volume as the midpoint for calculation, the 50% decrease in ventricular volume which was observed in systole should have been accompanied by a 17% decrease in sarcomere length. Experimentally, a 13% average decrease in sarcomere length was observed. In Figure 3, the close approximation between predicted sarcomere lengths (dashed line) and those sarcomere lengths observed experimentally between systole and diastole...
Electron micrograph from a left ventricle fixed in diastole (Expt. 41). The A and I bands of the sarcomere are noted on the figure. Sarcomere length is represented by the distance between two Z lines. M = mitochondrion. A 1-μ marker is shown on the figure.

is apparent. Those hearts fixed during post-extrasystolic potentiation had the smallest intraventricular volumes (average 7.2 ml). Nevertheless, changes in sarcomere length followed the line of prediction for sarcomere length relative to intraventricular volume that was previously calculated (Fig. 3).

Following acute dilatation of the left ventricle induced by over-transfusion of the dog,
left ventricular volume increased to an average of 72.1 ml (range 54.8 to 83.0 ml) while the diastolic filling pressure increased to an average of 32 mm Hg (range 24 to 40 mm Hg). Sarcomere length in the mid-ventricular wall increased to an average of $2.25 \pm 0.081 \mu$ (range 2.13 to 2.32 $\mu$) (Fig. 5). This 8.7% increase in average sarcomere length correlates well with the 8.6% change in sarcomere length which would be predicted from the change in midwall circumference (Fig. 3). In the dilated left ventricle, H zones were commonly observed at the center of the A bands of the sarcomeres as shown in Figure 5, but only in sarcomeres whose length was in excess of 2.20 $\mu$. The H zones appeared as lighter areas in the central portion of the A band; they were irregular and difficult to define precisely, even in longer sarcomeres ($>2.25 \mu$).

It was consistently found that within the midwall of the left ventricle there was considerable variation in sarcomere lengths. In Figure 6 the dispersion of sarcomere lengths is shown for a left ventricle fixed in diastole (Fig. 6A), systole (Fig. 6B) and in diastole following acute dilatation (Fig. 6C). The dispersion of sarcomere lengths about the mean values (vertical arrows in Fig. 6) was similar in normal systole and diastole. However, with acute left ventricular dilatation, the dispersion of sarcomere lengths was somewhat greater, as is apparent from Figure 6C.

**Discussion**

In the present study, hearts were fixed in
situ and the changes in sarcomere length that occur in the normal heart during the cardiac cycle were defined. Further, the changes in sarcomere length that occur with either marked potentiation of contraction or with acute left ventricular dilatation have been delineated. In normal diastole, with an average left ventricular filling pressure of 6 mm Hg, sarcomere length averaged 2.07 μ with a range from 2.03 to 2.10 μ. Previous studies in vitro have shown that maximum active tension is developed by the isolated cat papillary muscle preparation with a sarcomere length of 2.20 μ, and that actively developed tension decreases along with sarcomere length as initial muscle length is shortened (3). Further, in the arrested and passively distended dog and cat left ventricle, it has been demonstrated that sarcomere length is a function of filling pressure and that a sarcomere length of 2.20 μ corresponds to a ventricular filling pressure of 12 to 15 mm Hg. Thus, the present study indicates that in the normal contracting ventricle, with sarcomeres of 2.03 to 2.10 μ, contraction ensues with sarcomeres on the ascending limb of their length-active tension curve, somewhat below the apex of this curve. The increase in sarcomere length between the observed 2.07 μ sarcomere length and the 2.20 μ at the apex of the sarcomere length-tension curve permits increased muscle shortening for a given load and represents reserve provided by the Frank-Starling mechanism (9), although it should be pointed out that more reserve may be available in the intact animal than is indicated in these experiments performed in open-chest animals.

During normal systolic contraction, which
Examples of the nomograms of sarcomere frequency in a diastolic (A), a systolic (B) and a dilated diastolic (C) left ventricle. The vertical heavy arrows indicate the average sarcomere length.

was initiated from left ventricular end-dias-
tolic pressures approximately the same as
those of ventricles studied in diastole, aver-
age sarcomere length decreased to 1.81 μ. This 13% decrease in sarcomere length is ap-
propriate to explain the observed decreases in intraventricular volume that occurred
during systole. As seen in Figure 3, the close

approximation of changes in midwall circum-
ference of the left ventricle and in the sarco-
mere length with changes in ventricular volume supports the view that changes in sarcomere
length can explain observed stroke volume/ end-diastolic volume relations in the intact heart (5).

The length of sarcomeres in the ventricle
yields added information when related to the
disposition of myofilaments within the sarco-
mere. It is now well recognized in both
skeletal (1) and heart (2) muscle that the
sarcomere is composed of two sets of partially
overlapping filaments. The thin actin filaments
which extend from the Z lines, through the I
band and into the A band are 1.0 μ in length,
while the thicker filaments of myosin, which
are 1.5 μ in length (3), extend the length
of the A band. The length of these filaments
is constant despite changes in sarcomere
length (1, 2, 7), a fact that supports the sliding
filament hypothesis for muscle contraction.

In diastole, with a sarcomere length of 2.07
μ, thin actin filaments lie near the center of
the A band. During systole, these thin fila-
ments move into the center of the sarcomere
and begin to bypass one another as the sarco-
mere length becomes less than 2.0 μ. In the
presence of a potentiated contraction, the
thin filaments may penetrate the opposite
half of the A band far enough to form a
prominent additional dark band flanking the
center of the A band, termed an A contraction
band. As noted in Figure 4, the distance
from the Z line to the edge of the A contrac-
tion band in the opposite half of the sarcomere
is 1.0 μ, which is equal to the length of the
actin filament. Thus, although such extensive
shortening of the sarcomere has been known
to exist in heart muscle in vitro (2, 3), the
documentation of this phenomenon in the
intact heart demonstrates the range of sarco-
mere shortening that may occur physiologi-
ically, i.e., from 2.20 μ to about 1.60 μ and
shows that a double overlapping of thin fila-
ments at the center of the sarcomere may
occur during potentiated contractions. Fur-
ther, such a 30% reduction in sarcomere length
theoretically could permit stroke volume to
end diastolic volume ratios of 75% or more during markedly potentiated contractions.

Following ventricular dilatation, thin filaments may be withdrawn from the center of the A band creating an H zone. The production of H zones, with a decrease in the overlap of thick and thin filaments, could in itself result in a decrease in force development, since force depends on the degree of interdigitation of the two sets of myofilaments (1, 2, 10). However, such H zones were only observed with marked acute ventricular distension, at ventricular filling pressures beyond those usually compatible with life; it would, therefore, appear unlikely that the production of H zones plays a primary role in the etiology of myocardial decompensation.

The demonstration of dispersion of sarcomere length presents certain interesting problems in the relation of ultrastructure to ventricular performance. It is evident that effective sarcomere length is a derived value in the intact heart and that the force generated at any given time will reflect the average sarcomere length. Further, this finding suggests that tension may not be uniformly distributed across all sarcomeres in the ventricle in a similar manner, even within the same layer of the wall, or indeed, even within a given fiber. The possibility exists that some of the dispersion of sarcomere lengths during systole may reflect some delay in fixation, which could permit some sarcomeres to begin to relax and lengthen. Although this possibility cannot be excluded, it would not explain the dispersion of sarcomere length in diastole, nor the finding of sarcomere length dispersion in the totally excised ventricle (4, 11).

In the dilated ventricle the interpretation of sarcomere dispersion is even more complex. Following acute overdistension of the left ventricle, average sarcomere length commonly exceeded 2.20 μ, a value at the apex of the sarcomere length-active tension curve. However, a considerable portion of the sarcomeres were shorter than 2.20 μ even in the presence of ventricular dilatation. Thus, with contraction it would be anticipated that the sarcomeres at the apex of the curve (being strongest) would tend to extend further the already overstretched, and therefore weakened, sarcomeres. This might tend to increase the sarcomere dispersion further.

Following acute ventricular dilatation, there was no disparity of theoretical and observed sarcomere lengths, a phenomenon previously observed in the passively overdistended left ventricle and ascribed to possible fiber slippage or shear within the wall (4). In the present study the changes in sarcomere length follow the line of prediction for changes in midwall circumference and are entirely adequate to explain observed changes in ventricular volume (Fig. 3). Differences in methodology may help to explain this discrepancy, since in the studies on the passive ventricle, multiple pressure-volume curves were obtained prior to fixation (4). This procedure could have induced progressive stress-relaxation and fiber slippage, and the lack of active contraction in these previous studies could have prohibited compensation for excessive distension.

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References


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