Myocardial Cell and Sarcomere Lengths in the Normal Dog Heart

By Michael M. Laks, M.D., Michael J. Nisenson, B.A., and H. J. C. Swan, M.B., Ph.D.

ABSTRACT

The purpose of this study was to measure myocardial cell and sarcomere lengths at the bases and apices of the right (RV) and left ventricle (LV) of 4 normal canine hearts. The RV and LV were simultaneously fixed with glutaraldehyde at zero transmural pressure. Sections of trabeculae carneae were taken from the 1 cm cut at the bases (free wall) of the RV and LV. They were further fixed with osmium tetroxide, embedded in Epon 812, sectioned at 1 &mu;m and stained with azure II and methylene blue. Measured from photomicrographs (X 1,000) taken with the phase microscope, mean cell length was 70.9 &mu;m ± 1.49 (SEM); no statistical difference existed between the cell lengths at the bases and apices of the RV and LV. From adjacent tissue, the measurements of sarcomere lengths made from photomicrographs (phase microscope, oil immersion lens, enlarged to X 4,000) were not statistically different from those made with the electron microscope (P > 0.9). The sarcomere lengths at the LV base were the shortest—2.16 &mu;m ± 0.002 (SEM), followed by the LV apex, 2.28 &mu;m ± 0.005 (SEM); RV base, 2.41 &mu;m ± 0.006 (SEM); and RV apex, 2.46 &mu;m ± 0.003 (SEM). These variations in sarcomere length show the importance of specifying the site of sampling of myocardial tissue. The sarcomere lengths were related inversely to the thickness of the ventricular wall. This observation may be considered the structural reflection of the lesser distensibility of the left base as compared with the right apex, and may be simply the manifestation of the fact that the left base contains more muscle fibers.

ADDITIONAL KEY WORDS
tissue fixation technique zero transmural pressure sarcomeres/cell left ventricular base right ventricular base right ventricular apex

In the last 20 years many investigators have described the fine structure of the myocardium with the aid of advanced techniques in staining and electron microscopy (1-7). Although there is general agreement on the basic structural characteristics of cardiac muscle, there is still a considerable amount of variation in the quantitative values reported for myocardial fiber size (8-10), cell length (1, 3, 11), and sarcomere length (3, 6, 12, 13). Previously reported determinations of myocardial fiber size have been based solely on cross-sectional estimations (8-11). The logical method of appraising myocardial fiber size would be to determine the volume of the myocardial cell. A necessary measurement, therefore, is cell length. However, the concept that the myocardium is not a syncytium but is composed of individually bounded cells is only about 15 years old. The few published values for myocardial cell length need revision partly because, until recently, no method has been available for the uniform stretching of the myocardial cell in both the right and left ventricles; and fixation and embedding materials that minimize shrinkage and distortion have not been available.
The purpose of this study is to report the myocardial cell and sarcomere lengths of the normal right and left ventricle of the fresh post mortem canine heart at zero transmural pressure.

Methods

Four mongrel dogs weighing between 15 and 23 kg were anesthetized with sodium pentobarbital (27 mg/kg iv). The hearts were prepared before fixation by the method for determination of ventricular volumes previously reported (14). The entire heart was submerged in a solution containing 8 g NaCl, 0.2 g KCl and 0.33 g CaCl₂ per liter of water. This solution at 4°C was simultaneously pumped into both ventricles to the point of overflow. The volumes remaining in the ventricles were designated the volumes at zero transmural pressure (P₀).

For histological studies, 6% glutaraldehyde in Palade's buffer at 4°C was exchanged for the same intraventricular volume of Ringer's solution. The procedure to this point required 30 to 45 min. To ensure good and rapid fixation, glutaraldehyde was also infused at 100 mm Hg pressure via polyethylene catheters (PE 160 o.d. = 1.57 mm, i.d. = 1.14 mm) inserted into the left anterior descending, left circumflex, and right coronary arteries. This fixation procedure was continued for at least 1½ hr. The atria were then removed at the atrioventricular groove. Approximately 1-cm sections were made from the base to the apex of the ventricles (Fig. 1). Tissues were then taken from the trabeculae carneae of the free lateral wall at the bases and apices of the right and left ventricles. These were fixed further in glutaraldehyde and subsequently by osmium tetroxide, then embedded in Epon 812, longitudinally sectioned at 5 μ, and stained with azure II and methylene blue. With phase microscopy, 4 × 5 inch photomicrographs were made with a Zeiss Universal microscope and a Brinkman camera. Using high contrast paper (Kodabromide F-4), the final prints of 1000 × and 4000 × magnification were made. Cell lengths were measured from intercalated disc to intercalated disc on the 1000 × magnification photographs (Fig. 2). Each measurement was repeated three times. All ambiguous measurements of intercalated discs were rechecked by direct visualization under oil with the light microscope (1200 ×). The sarcomeres were measured from Z line to Z line from the photographs with a final magnification of 4000 × (Fig. 3). The coefficient

![FIGURE 1](http://circres.ahajournals.org/)

Method of section and locations at base and apex of right and left ventricles at which tissue samples were taken for determination of sarcomere and cell lengths.

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FIGURE 2
Photomicrograph of a myocardial cell taken with the phase microscope at a magnification of 440 x. Note the readily identifiable nucleus, sarcolemma, and intercalated discs. The sarcolemma does not have the scalloped appearance of a tissue fixed in the unstretched state.

FIGURE 3
Photomicrograph of sarcomeres taken with the phase microscope (oil-immersion) at a magnification of 200 x. Note the clarity of the "Z" lines which permits accurate measurement of sarcomere length.
### Table 1

Statistical Data for Sarcomere Length, Cell Length, and Number of Sarcomeres per Cell in Four Normal Canine Hearts

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<tr>
<th>Region</th>
<th>Dog</th>
<th>N</th>
<th>Mean (a)</th>
<th>±SEM</th>
<th>N</th>
<th>Mean (a)</th>
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<td>79.9</td>
<td>6.54</td>
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</tbody>
</table>

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**Figure 4**

Frequency distribution of cell lengths measured at the bases and apices of the right and left ventricles (4 hearts, pooled data).

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of variation for the determination of sarcomere lengths was <1%. The thickness of the ventricular walls was measured from the sections made at the bases and apices of both ventricles.

Results

Cell Length

The cell lengths at the bases and apices of the left and right ventricles had a mean of 70.9±1.49 with a range of 22 to 147μ (Table 1). The measurements for cell length from the four regions within each heart were not statistically different (P > 0.4). There was also no statistical difference between mean values for the bases and apices of all four hearts (P > 0.5) (Fig. 4). The average coefficient of variation for all values was 35.2%. These distribution curves appear to be skewed to the left which may reflect a bimodal population.

Comparison of Sarcomere Length Measured with the Electron and Light Microscope

The mean of the sarcomere lengths measured in adjacent sections was 2.29±0.005 (N=65) with the electron microscope and 2.25±0.003 (N = 135) with the light microscope. The cell lengths measured with the light and electron microscope were not statistically different (P>0.9).

*All values are mean and standard error.

Sarcomere Length

The average sarcomere lengths at the left base were the shortest (2.16±0.002μ), followed in size by the left apex (2.28±0.005μ), the right base (2.41±0.006μ), and the right apex (2.48±0.003μ) was the longest (Table 1). These values are significantly different (P<0.001). Furthermore, there is an inverse relationship between sarcomere length and ventricular wall thickness (Fig. 5, Table 1).

Discussion

Cell Length

The few reports (1, 3, 11) of the measurements of myocardial cell lengths have lacked a presentation of specific methods used and details of the data obtained apart from a statement of the range of values. In this study the methods used ensured the following: (1) Straightening of the cells in the longitudinal axis so that sarcomeres could be clearly identified between two intercalated discs and (2) application of a constant and uniform transmural pressure to each region of the heart. The length of individual sarcomeres can vary between 1.5μ in the completely contracted state to 2.6μ in the noncontracted, stretched state (3). Since a 75% variation in length can occur in the measurement of a myocardial cell, we fixed the myocardial tissue...
under a standard condition of stretch—zero transmural pressure. In addition, our technique prevented the differential distortion of the ventricles by hydrostatic pressure effects.

In a previous study, we found that the diameter of the normal canine myocardial cell was the greatest at the right base, followed in magnitude by the left base, right apex, and the left apex. However, the myocardial cell volume, not the cell diameter, is the best theoretical measure of myocardial fiber size for the comparison of various hearts in the same or different species. An approximation of the cell volume can be obtained from the product of cell length and cell cross-sectional area. Although we are unable to demonstrate differences between the mean values for cell lengths at different locations in the normal canine heart, the greater cross-sectional diameter at the bases as opposed to the apices of the right and left ventricle would indicate that fiber volume differed in like manner. It is possible that the lengths of various cells in the hypertrophied myocardium are dissimilar.

We found a great and equal variability in cell length in all locations ranging from 22 to 147 μ. Since this range of cell lengths exceeds that reported previously, we reexamined the photomicrographs with particular attention to those cell lengths that were at the extremes of our range. We were unable to identify a nucleus in cells less than 54 μ in length. However, 80% of the cells in the range of 54 to 147 μ also lacked a nucleus. Since the nucleus lies in the center of a cell, a given plane of section may not transect it. The intercalated discs which formed the transverse borders of the cells were often arranged in a staircase sequence (Fig. 2). There was no distinct pattern of ascent or descent as the intercalated discs randomly coursed in and out as they divided the cells. This irregular configuration of the intercalated discs imposes a variation as to the precise length. The values tabulated are the distances between two points—one at each end of the cell, each representing the average of all steps of the staircase. The variability between steps was small. Furthermore, because of the staircase distribution of the intercalated disc, longitudinal sections of the cell may have different apparent lengths. The variability between different cells that is not due to technical problems in measurement is far greater and probably due to a true biological difference; it apparently is not unimodal in distribution.

What is the functional significance of this great variation in cell length? Do the shortest cell lengths characterize older or younger cells? What is the upper limit to the normal cell length? Answers to these and other related questions are not available at present.

**Sarcomere Length**

As in the case of measurements of cell length, the preparation of tissue for the determination of sarcomere lengths must be done under constant conditions of transmural pressure. Furthermore, for the determination of sarcomere lengths, the specific location of tissue sampling is of extreme importance (Fig. 1). Spotnitz et al. (6) have demonstrated that the sarcomere length of the inner wall of the myocardium exceeded the sarcomere length of the middle and outer layers. In our study samples were taken from only the trabeculae carneae of the free lateral wall. The lengths of sarcomeres obtained from such tissue at the left ventricular base (2.09 to 2.20 μ) fell within the higher portion of the range reported by Sonnenblick et al. (6, 15) but showed less variability. The great variation in sarcomere length reported by Sonnenblick et al. (approximately 1.85 to 2.25 μ with clusters at the high and low extremes) may be due to sampling of tissues from different portions of the left ventricle. Our results demonstrate a significant difference between sarcomere lengths at the base and apex of the left ventricle. Furthermore, if their thesis were correct that the innermost wall of the myocardium has the longest sarcomere, we would have anticipated our sarcomere lengths would have been in the higher range. This follows since we specifically chose the innermost tissue, the trabeculae carneae, while they chose the inner one-third of the
left ventricular wall. In addition, no comparable measurements have been reported of sarcomere lengths from the right ventricle.

The fact that we observed the smallest sarcomere length at the left base may be considered the structural reflection of its least distensibility. This can be compared with the longest sarcomere length at the right apex, the region of greatest distensibility. The left base showed the least distensibility; this may be because it contains the largest number of muscle fibers. This is demonstrated by the inverse relationship between sarcomere lengths and ventricular wall thickness at the bases and apices of the ventricles.

What is the functional basis for the differences in sarcomere lengths observed in the four areas of myocardial sampling? Two possibilities appear worthy of consideration. First, more contractile units (fibers) are present in the thick than in the thin portions of the ventricular walls. Our data demonstrate that the shorter sarcomeres are found in the thicker portions of the ventricular walls. Since shorter sarcomere lengths result in a lesser work output on the ascending limb of the Frank-Starling curve, the sarcomeres in this region may be required to perform less mechanical work. At normal filling pressures, and during contraction (P > 0), the wall tension at the bases exceeds that at the apices as a consequence of the Laplace relationship. It is, therefore, possible that for filling pressures in excess of P0 these sarcomere lengths would approach the same value. Further, the shorter sarcomere lengths at the bases under conditions of P0 may be entirely related to the greater amount of parallel elastic elements—the major determinant of sarcomere length in the resting state (15). Between bases and apices the relative or absolute distribution of the anatomic elements thought to comprise the parallel elastic elements, sarcolemma, mitochondria, and connective tissue, have not been defined: A second possibility is that early changes associated with rigor mortis may result in such changes in sarcomere length. In our previous studies (13), no gross change in overall ventricular compliance could be demonstrated in studies carried out within 90 min of death. The tissue under study herein was fixed between 30 and 45 min after death. If this remote possibility pertains, its effect would appear insufficient to account for the changes observed.

References
intravenous and intra-arterial epinephrine on O2 consumption and CO2 production of human forearm skeletal muscle were studied in relation to the vasodilator effect of the hormone.

**Methods**

Experiments were performed on 20 young, healthy, male volunteers. All subjects were studied in the postabsorptive state while lying recumbent on a table in an air-conditioned laboratory (room temperature 23 to 24°C). Experiments on the same subject were done at least 3 weeks apart and none of the subjects was used for the same type of experiment more than once. All subjects gave their informed consent to the studies.

The effect of epinephrine on the metabolism and blood flow of forearm skeletal muscle was studied following complete suppression of the circulation to forearm skin by epinephrine iontophoresis. The technique of epinephrine iontophoresis was similar to that used by Cooper et al. (14) with the important exceptions that the current intensity was higher (20 ma) and the duration of iontophoresis was longer (20 to 25 min). As described elsewhere (15), these modifications result in more complete suppression of the cutaneous circulation.

Epinephrine iontophoresis was carried out in 22 experiments. In 4 of these, epinephrine infusion was not given because iontophoresis resulted in incomplete suppression of skin blood flow or because there was absorption of epinephrine into the systemic circulation. Epinephrine iontophoresis was considered complete when the skin was uniformly blanched and remained so after release of 3 min of arterial occlusion.

Since the major components of total forearm blood flow are represented by cutaneous and skeletal muscle flows (14), the plethysmographically measured forearm blood flow following epinephrine iontophoresis was considered to represent muscle blood flow entirely. Forearm blood flow was measured by venous occlusion plethysmography using a plethysmograph filled with water whose temperature was maintained thermostatically at 33 to 34°C. The circulation to the hand was arrested by inflating a sphygmomanometer cuff around the wrist to a pressure whose temperature was maintained thermostatically at 33 to 34°C. The circulation to the hand was arrested by inflating a sphygmomanometer cuff around the wrist to a pressure whose temperature was maintained thermostatically at 33 to 34°C. The circulation to the hand was arrested by inflating a sphygmomanometer cuff around the wrist to a pressure whose temperature was maintained thermostatically at 33 to 34°C. The circulation to the hand was arrested by inflating a sphygmomanometer cuff around the wrist to a pressure whose temperature was maintained thermostatically at 33 to 34°C.

Arterial blood was obtained from a 18-gauge Cournand needle placed into the brachial artery of the opposite arm. Arterial blood pressure was measured with a Statham P23-Db strain gauge connected to the Cournand needle via polyethylene tubing. Mean arterial blood pressure was obtained by electronic damping. Forearm vascular resistance was calculated as the ratio of mean arterial blood pressure divided by forearm blood flow. Venous blood was obtained from a polyethylene catheter placed into a deep forearm vein in a retrograde direction so that its tip lay within the portion of the forearm contained in the plethysmograph. The oxygen and carbon dioxide contents of blood samples were determined by the method of Van Slyke and Neill (16).

Expired air CO2 concentration was monitored continuously with a Liston-Becker infrared CO2 analyzer.

Epinephrine was administered by a Harvard constant infusion pump either intravenously at a rate of 10 μg/min or into the brachial artery at a rate of 0.1 μg/min. These dose levels were selected because in the intact forearm they give consistent and approximately equal increases in blood flow (17). Intra-arterial infusions were given into the brachial artery of the experimental arm through a Riley needle placed into the vessel at the upper part of the bicipital groove. For intravenous infusion, epinephrine was dissolved in 0.9% NaCl solution to give a concentration of 1 μg/ml; for intra-arterial infusions a concentration of 0.05 μg/ml was used.

The experimental design was as follows: After control measurements of forearm blood flow and two or three sets of arterial and venous blood samples were obtained, the infusion of epinephrine was begun while blood flow measurements were continued. After the blood flow and blood pressure became stable two or three more sets of arterial and venous blood samples interspersed with blood flow determination were obtained. The O2 consumption and CO2 production of forearm skeletal muscle were calculated from the blood flow and the corresponding arteriovenous differences by application of the Fick principle. In the experiments reported, the requirements from the application of the Fick principle, namely constant arterial and venous concentrations and constant blood flow, were met.

In view of the observation by Lundholm and Svedmyr (11) that epinephrine infusion caused an increase in arterial and deep forearm venous blood Pco2 and their suggestion that this might be causally related to the associated vasodilation, the response to intravenous infusions of epinephrine was compared before and during hypocapnia induced by voluntary hyperventilation. We thought it unlikely that epinephrine iontophoresis would give complete suppression of skin blood flow for a sufficiently long period to perform these studies. For this reason we carried out these experiments in the intact forearm without epinephrine iontophoresis. Epinephrine was infused at a rate of 10 μg/min iv for 10 min during normal breathing and the infusion was repeated during
left ventricular wall. In addition, no comparable measurements have been reported of sarcomere lengths from the right ventricle.

The fact that we observed the smallest sarcomere length at the left base may be considered the structural reflection of its least distensibility. This can be compared with the longest sarcomere length at the right apex, the region of greatest distensibility. The left base showed the least distensibility; this may be because it contains the largest number of muscle fibers. This is demonstrated by the inverse relationship between sarcomere lengths and ventricular wall thickness at the bases and apices of the ventricles.

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