A method to collect isolated myocytes and whole tissue from the same heart

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Chen Y-F, Said S, Campbell SE, Gerdes AM. A method to collect isolated myocytes and whole tissue from the same heart. Am J Physiol Heart Circ Physiol 293: H2004–H2006, 2007. First published May 18, 2007; doi:10.1152/ajpheart.00479.2007.—A technique for isolation of cardiac myocytes and collection of whole heart tissue from individual hearts of adult rats is described in this study. After excision of the apical half of the left ventricle (LV) and cauterization of the cut edge, aortas were cannulated and high-quality isolated cardiac myocytes were collected after collagenase perfusion of the basal portion. Myocyte dimensions from the basal portion of cauterized and noncauterized hearts from matching rats were identical. Additionally, myocyte dimensions from the basal and apical halves of the LV were compared with the use of whole heart-isolated myocyte preps. No regional differences between basal and apical LV myocyte size were found. Therefore, this cauterization method can be used to collect isolated myocytes from the basal half and whole heart tissue from the apical half, with each half being representative of the other with respect to myocyte dimensions.

MATERIALS AND METHODS

Experimental design. Adult male Sprague-Dawley rats weighing between 400 and 500 g were used for this study. After ketamine-xylazine anesthesia, animals were heparinized and hearts were then quickly removed, trimmed, blotted, and weighed. The apical one-half to one-third of the heart was excised. The cut edge of the remaining basal portion was cauterized with an electric soldering iron obtained from a local hardware store (model L25; Wall Lenk, Kinston, NC), and the aorta was cannulated for retrograde coronary perfusion (Fig. 1). Cardiac myocytes were then isolated from the basal part used in the method described previously (5). Isolated myocyte preps from cauterized hearts were compared with isolated cells collected by the standard whole heart-isolated myocyte method. After collagenase perfusion of whole hearts, the digested heart was divided into basal and apical portions for comparison. All experiments and protocols were approved by the University of South Dakota Animal Care and Use Committee and followed institutional guidelines for animal care.

Myocyte morphometry. Freshly isolated cardiac myocytes were quickly prepared and fixed in 2.0% glutaraldehyde, and dimensions were determined as described previously (5). Briefly, cell volume (V) was measured with a Coulter Channelizer system (model Z2; Coulter Electronics; Miami, FL), myocyte length (L) was determined via microscopy, and cross-sectional area (CSA) was calculated as V/L. Based on the formula for an elliptical cylinder, which most closely approximates myocyte shape, myocyte major and minor diameters were determined in the following manner: major diameter (A) was determined by tracing isolated myocytes (A = profile area/L). Minor diameter (B) was subsequently determined from the formula for V relative to an elliptical cylinder (V = πA × B/L) and by substituting the Coulter values for V (4, 11). All data from a given animal were excluded from analysis if isolated cell preparations were of poor quality (e.g., <60% rod cells).

Statistical analysis. All data are expressed as means ± SD and were compared with Student’s t-test. A value of P < 0.05 was considered statistically significant.

RESULTS

Table 1 shows that there are no significant differences in cell volume, cell length, and CSA in myocytes isolated from the LV basal regions of cauterized and noncauterized hearts. Table 2 indicates that myocytes isolated from the LV basal portion have the same cell volume, cell length, CSA, major dimension A, minor dimension B, and A-to-B ratio as those isolated from the LV apical portion.

DISCUSSION

This study shows that excision of the apex of the heart followed by cauterization and cardiac myocyte isolation of the basal portion yields myocytes of similar quality (e.g., 80–90% rod-shaped cells) and shape compared with isolated myocytes.
collected from noncauterized hearts. Additionally, there appears to be uniformity of cardiac myocyte dimensions in the LV basal and apical portions of the heart.

Myocyte isolation techniques have been used for research work for many years. In general, it has proven difficult to obtain high-quality isolated myocyte preps from mammals without prior perfusion with collagenase before mincing tissues to release cells. The collagenase digestion procedure used in this study has proven to be efficient and to produce a high yield of isolated rod-shaped adult mammalian cardiomyocytes. However, the requirement of collagenase perfusion of the whole heart with subsequent breakdown of extracellular matrix precludes the use of such hearts for whole tissue analyses. In this study, we cauterized the cutting edge of the basal portion to help maintain perfusion pressure and prevent leaking through larger, low-resistance vessels. This method allows analysis of isolated myocytes as well as myocardial tissue from the same heart. The excised apical portion can be fixed or frozen for histological, biochemical, or molecular studies.

Over the past century, the literature has been filled with many reports of changes in cardiac myocyte size. In these studies, whole tissue sections were typically used to measure cellular dimensions (e.g., cell length, cell diameter, CSA). However, there are many sources of error associated with this approach. For instance, myocyte dimensional measurements collected from tissue sections may be affected by tissue shrinkage artifacts from processing, variation in sectioning angle, tissue compression from sectioning, and changes in contractile phase (e.g., myocyte CSA increases ~40% during contraction) (5). Very few studies have corrected for such sources of error. With tissue sectioning methods, it is common to observe published values for myocyte CSA in normal mammals ranging from 100 to as high as 1,500 \( \mu m^2 \). Actually, values for LV myocyte CSA are generally in the range of ~180–280 \( \mu m^2 \) in adults, with males having larger values than females. Additionally, reported increases in myocyte CSA after mechanical overloading (e.g., aortic constriction) may differ by many fold from increases in ventricular mass. Obviously, such data do not make sense. We have intentionally avoided referencing specific examples. There are simply too many, and it would be unfair to single out a specific lab when this flawed technology is so widely accepted and used.

Because of the stair step nature of intercalated disks and the complex shape of cardiac myocytes, estimation of myocyte length using tissue sections provides values that underestimate true myocyte length. Remarkably, many current medical histology texts report cardiac myocyte lengths ranging from ~80 to 100 \( \mu m \), when isolated myocyte data have clearly shown that true length ranges from ~100 to 150 \( \mu m \) in adult mammals. In summary, collecting comprehensive and reliable data for myocyte length, CSA, and volume with tissue sectioning technology presents a formidable, if not impossible, challenge.

Table 1. Myocyte dimensions from LV basal regions

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Cell Volume, ( \mu m^3 )</th>
<th>Cell Length, ( \mu m )</th>
<th>CSA, ( \mu m^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cauterized samples</td>
<td>8</td>
<td>37,647±3,216</td>
<td>140.2±10.6</td>
<td>269±25</td>
</tr>
<tr>
<td>Noncauterized samples</td>
<td>17</td>
<td>38,487±3,598</td>
<td>139.0±8.6</td>
<td>277±28</td>
</tr>
</tbody>
</table>

Values are means ± SD for n rats. LV, left ventricle; CSA, cross-sectional area.
These potential problems have been avoided by our group by using isolated myocytes and measuring myocyte dimensions with a Coulter Channelyzer and measuring microscope (5). The reliability of this approach has been confirmed with three independent measures with corrections of all known sources of error with the morphometric methods (5). Using this method, we have demonstrated that right ventricular myocytes are generally smaller than LV myocytes in rats and hamsters (2, 3, 5). There are also some transmural differences in LV from endomyocardium to epimyocardium in rats, with myocytes from endomyocardium being greater than cells from epimyocardium as indicated by larger volume and CSA (2, 3, 5). However, regional differences in myocyte size have varied somewhat between species (3). We have also found that myocyte size can be affected by age, growth, sex, and different pathological conditions (1, 6–8, 10, 12). Pressure overload leads to an increase in CSA without a change in length during the compensatory phase of hypertrophy. Volume overloading tends to increase both CSA and length proportionally. A key feature of progression to dilated heart failure is an increase in LV myocyte length only, without a change in CSA.

To our knowledge, this is the first report examining potential differences in the basal and apical halves of the LV with a well-characterized technique of proven reliability. We believe the technique documented here may be of considerable utility. It allows the subsequent use of isolated myocytes for Western blots, cell mechanics, cellular electrophysiology, and confocal imaging. With other applications, however, it would be advisable for users to first confirm that heat injury from cauterization of the cut edge does not trigger associated signaling events that may alter data. Should this occur, it is likely that such changes may be minimized by avoiding cells adjacent to the cauterized edge. Again, this will need confirmation.

REFERENCES