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Regional Differences in Cardiac Myocyte Dimensions and Number in Sprague–Dawley Rats from Different Suppliers (42605)

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Abstract. Previous experiments conducted in this laboratory have indicated that adult Sprague–Dawley rats obtained from Holtzman Company (H) have hearts that are significantly larger than those obtained from Charles River (CR). To investigate potential differences in cell size and number, isolated myocytes were prepared by retrograde coronary perfusion with collagenase. Cell volume (V) was measured with a Coulter Channelyzer, cell length (L) was measured directly, and cross-sectional area (CSA) was calculated from V/L. Cell number was calculated using data from isolated cells and whole-sectioned tissue. Although V for left and right ventricular myocytes was similar in CR and H rats, myocytes from CR tended to be shorter in L ($P < 0.01$), but larger in CSA (N.S.) than myocytes obtained from H rats. In both H and CR, left ventricular V and CSA were greater ($P < 0.01$) in endomyocardium than epimyocardium and middle myocardium. Values for V and CSA of right ventricular myocytes were less ($P < 0.01$) than those from left ventricles in both H and CR. Hearts from H had 19% more myocytes than those from CR ($P < 0.05$). Values for cellular dimensions from CR generally showed less variability than those from H. We conclude that significant regional differences in myocyte size are present in rats from both H and CR. Hearts from H rats have significantly more myocytes than those from weight-matched CR rats. Therefore, investigators should be aware that differences in heart weight and myocyte number can be found in rats of the same strain, but obtained from different suppliers.

Many published reports based on experiments using the laboratory rat indicate that the old adage "a rat is a rat is a rat" is not true. For accurate comparisons to be made between studies of a similar nature using the laboratory rat, the genetic strain of the rat used must be taken into account. Strain differences in behavior, biochemistry, metabolism, body proportions, incidence of tumors, susceptibility to hypertension and/or vascular lesions, and heart weight, to name a few, have been reported (1–8). These reports underscore the importance of clearly stating the strain of rat used when experimental results are published.

Since it is necessary for similar strains of rats to be used to accurately compare experimental results, except if strain differences are being examined, does it then follow that all rats of a similar strain are identical? Numerous reports in the literature foster this belief. Indicative of this perception is the fact that many investigators do not identify the source of their experimental animals. Consequently, generalized statements are often made regarding a given strain of rat, irrespective of any potential supplier-related differences.

Over a period of time, investigations carried out in this laboratory using Sprague–Dawley rats obtained from two different suppliers have led to the observation that hearts from Sprague–Dawley rats obtained from one supplier seem smaller than those from rats of an identical strain and body weight, but obtained from a different source. Body mass (weight) is usually a reliable predictor of heart size under normal physiological conditions in the rat (9, 10). The observed disparity in heart size presents the possibility that Sprague–Dawley rats may not be as homogeneous a population of rats as might be expected. In an effort to quantitate possible intrastrain differences in hearts from the Sprague–Dawley rat, the present study was undertaken.

Materials and Methods. Female Sprague–Dawley rats weighing 275–300 g were obtained from Holtzman Company (Madison, WI) and Charles River Laboratories (Wilmington, MA). The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and hearts were quickly removed, blotted, and weighed. The aorta was cannulated for retrograde coronary perfusion

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with calcium-free Joklik media containing 0.1 m*M* ethyleneglycol-*bis*-(B-aminoethylether) *N,N'*-tetraacetic acid (EGTA), which was followed by Joklik media with collagenase. This procedure has been described previously (11).

Following collagenase treatment, hearts were divided into regions using sharp dissection. Hearts were initially divided into right ventricular free wall and left ventricle (plus septum). The left ventricle was then divided into outer (epimyocardium), middle (middle myocardium), and inner (endomyocardium) thirds. The tissue collected was minced in calcium-free, EGTA-containing Joklik media and poured through nylon mesh (250 μm). Freshly isolated cells were fixed immediately in 1.5% glutaraldehyde in 0.08 *M* phosphate buffer.

Isolated myocytes were centrifuged (International Equipment Company, Model HN-SII with rotor No. 221) through 4% Ficoll (Sigma Chemical Co., St. Louis, MO) in 0.15 *M* phosphate buffer at a force of 10× gravity. This effectively removed most endothelial cells, blood cells, and other unwanted debris without altering the undamaged myocyte (rod cell) population. Rod cells with normal sarcomere structure and no visible membrane damage were classified as undamaged. Freshly isolated cells meeting these criteria typically excluded trypan blue. Each region of all preparations characteristically contained greater than 70% undamaged cells.

Cell volume of isolated myocytes was determined using a Coulter Channelyzer (12, 13). The Coulter system determines cell volume by measuring the change in electrical resistance across an aperture resulting from the displacement of electrolyte as cells move through the aperture. Gerdes *et al.* (13) have recently reported that the Coulter Channelyzer method is an expeditious, reproducible, and consistent means for measuring cell volume. Based on the work of Hurley (14), a shape factor of 1.05, representing a cell length/width ratio of 7, was used. Since volume distribution curves were often slightly skewed to the right, median cell volume values were used.

Cell length, defined as the longest length parallel to the longitudinal axis of the myocyte, was measured directly using a phase contrast microscope. A minimum of 35 myocytes from each region of each animal was measured. Myocyte cross-sectional area was calculated from cell volume/cell length. Additionally, the percentage of binucleated and mononucleated cells was determined for all hearts from 200 myocytes isolated from both the left (100 myocytes each from epimyocardium and endomyocardium) and the right ventricles using hematoxylin and eosin-stained cells.

Total myocyte number was calculated using a previously described method (13, 15). The mean number of myocytes for left and right ventricles for animals from each supplier was calculated by dividing the product of myocyte volume fraction (MVF) and ventricular tissue volume (VTV) by the average myocyte volume (MV):

$$\frac{\text{MVF} \times \text{VTV}}{\text{MV}}.$$

VTV was calculated by dividing ventricular weight by tissue specific gravity (1.06). The left ventricle accounts for approximately 70% and the right ventricle approximately 20% of total heart weight in Sprague–Dawley rats obtained from Holtzman (13). Similar percentages of total heart weight for left and right ventricles have been previously determined in this laboratory for Sprague–Dawley rats obtained from Charles River. Since accurate regional weights cannot be obtained from hearts perfused with collagenase, ventricular weights were determined by multiplying the above percentages by total heart weight. A MVF value of 74% for left and right ventricles, previously determined morphometrically from whole-sectioned, perfusion-fixed hearts from Sprague–Dawley rats obtained from Holtzman (13) and Charles River (unpublished results), was used in cell number calculations. Mean MV for left ventricles in each animal was determined by averaging cell volumes for the three separate regions.

Student's *t* test for unmatched pairs was used to compare heart weight, body weight, heart weight/body weight ratio, percentages of binucleation and mononucleation, and total cell number data. In order to examine the differences in cell length, cell volume, and cross-sectional area as a function of supplier and region, data were subjected to a mixed-design two-way analysis of variance with one replicate. If significant regional differences were indicated with the two-way analysis of vari-

ance, multiple comparisons were made using a Scheffe a posteriori analysis.

Results. Comparisons of heart weight and heart weight-to-body weight ratios (HW/BW) in weight-matched rats obtained from Holtzman and Charles River indicate that hearts from Charles River rats are significantly smaller ($P < 0.001$) than those from Holtzman rats (Table I). Hearts from Holtzman rats weighed 15.6% more than those from Charles River rats and HW/BW differed by 16.9%.

Differences in cell volume, cell length, and cross-sectional area were analyzed as a function of supplier and region (Table II). No significant differences existed in regional ventricular cell volumes between Holtzman and Charles River rats. However, myocytes from Charles River rats had significantly shorter ($P < 0.01$) cell lengths (right ventricle, 9.6%; left ventricle, 7.2%), but larger cross-sectional areas (not significant) than myocytes obtained from Holtzman rats. Thus, cardiac myocytes from rats obtained from the two different suppliers differ in shape. Myocytes from Charles River rats have a smaller length–width ratio than the more elongated myocytes from Holtzman rats.

Similar regional differences in myocyte dimensions were noted in animals obtained from either supplier. Right ventricular cell length was significantly smaller ($P < 0.05$) than all left ventricular regions. There were no significant differences in cell length of the epimyocardium, middle myocardium, and endomyocardium of the left ventricle. Regional differences in cell volume and cross-sectional area for both Holtzman and Charles River rats were similar. Right ventricular cell volume and cross-sectional area were significantly smaller ($P < 0.01$) than all left ventricular regions. Although cell volume and cross-sectional area values became progressively larger across the left ventricular wall from epimyocardium to endomyocardium, epimyocardial values were not significantly different from middle myocardial values. However, endomyocardial cell volume and cross-sectional area values were significantly greater ($P < 0.01$) than all other regions.

TABLE I. ANIMAL DATA[a]

	Body wt (g)	Heart wt (mg)	Heart wt/body wt (mg/g)
H ($n = 10$)	290 ± 18	1002 ± 89	3.46 ± 0.27
CR ($n = 9$)	293 ± 16	867 ± 38[b]	2.96 ± 0.18[b]
% Difference	1.0	15.6	16.9

Note. H, Holtzman; CR, Charles River.
[a] All values, except % difference, are means ± SD.
[b] Significantly different, $P < 0.001$.

The nucleation pattern of cardiac myocytes isolated from different regions of hearts from Holtzman and Charles River rats was analyzed. No significant differences existed in the percentage of binucleated and mononucleated cells isolated from the epimyocardium and endomyocardium of the left ventricle. Therefore, left ventricular values are an average of percentages calculated from cells in these two regions. The percentage of binucleation was not significantly different between Holtzman and Charles River rats in either the left (96% vs 95%, respectively) or the right (91% vs 93%, respectively) ventricles. In Holtzman rats, the left ventricle had a significantly higher ($P < 0.001$) percentage of binucleated cells than the right ventricle (96% vs 91%). However, no significant difference was seen between left and right ventricles in Charles River rats (95% vs 93%).

Calculation of total myocyte number for left and right ventricles (Table III) indicated a significantly higher number of cells in the hearts from Holtzman rats compared to Charles River rats. Hearts from Holtzman rats contained approximately 17% more ($P < 0.05$) cells in the left ventricle and 25% more ($P < 0.001$) cells in the right ventricle than those from Charles River rats.

Discussion. When Evan Carl Holtzman left Sprague–Dawley, Inc., in the 1940s to establish his own business, he took with him some Sprague–Dawley rats to use as a seed stock (16). Charles River Laboratories originally obtained their Sprague–Dawley rat seed stock from Sprague–Dawley, Inc., in 1950 (17). Therefore, Sprague–Dawley rats available through both companies are derived from the same stock of animals. Furthermore, both suppliers adhere to the guidelines for housing found in the *Guide for the Care and Use of Laboratory Animals* published by the U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Both diet and litter size are similar for

TABLE II. ISOLATED CELL DATA[a]

	RV	EPI	MM	ENDO
CV				
H	18,275 ± 1418[b]	23,878 ± 3918	25,082 ± 3979	27,903 ± 4974[b]
CR	20,023 ± 2077[b]	24,241 ± 1541	25,687 ± 2848	26,788 ± 2449[b]
%DIF	9.6	1.5	2.4	4.2
CL				
H	121.6 ± 5.1[c]	125.1 ± 5.8	124.1 ± 5.8	123.7 ± 6.2
CR	110.9 ± 10.6[c,d]	115.9 ± 8.7[d]	116.0 ± 6.2[d]	115.8 ± 7.6[d]
%DIF	9.6	7.9	7.0	6.8
CSA				
H	150 ± 9[b]	191 ± 28	202 ± 28	226 ± 40[b]
CR	181 ± 17[b]	209 ± 10	222 ± 30	232 ± 19[b]
%DIF	20.7	9.4	9.9	2.7

Note. H, Holtzman; CR, Charles River; CV, cell volume in μm^3; CL, cell length in μm; CSA, cross-sectional area in μm^2; RV, right ventricle; EPI, epimyocardium; MM, middle myocardium; ENDO, endomyocardium; EPI, MM, and ENDO were collected from the left ventricle; %DIF, % difference between Holtzman and Charles River values.

[a] All values, except % difference, are means ± SD.

[b] Significantly different from all other regions within the supplier, $P < 0.01$.

[c] Significantly different from all other regions within the supplier, $P < 0.05$.

[d] Significantly different from same region of H, $P < 0.01$.

animals supplied by both companies. As is the case with any outbred population, a certain amount of inherent variability is to be expected in these rats. However, the significant difference in heart size reported in this study goes beyond what is attributable to inherent variability alone. Although the source of this variability is unknown, it may be due to selective breeding practices.

Differences in heart size within a strain of rat is not without precedent. The spontaneously hypertensive rat (SHR), developed from Wistar Kyoto (WKY) rats by Okamoto and Aoki (3), develops cardiomegaly. Blocking the development of hypertension in the SHR with immunosympathectomy (18) or treatment of neonates with reserpine, hydralazine, and chlorothiazide (19) does not prevent the development of larger hearts. Thus, factors other than increased blood pressure may contribute to the development of cardiomegaly in the SHR (20).

TABLE III. CELL NUMBER DATA[a]

	Left ventricle	Right ventricle
H	20.35 ± 3.19	7.11 ± 0.61
CR	17.43 ± 2.01[b]	5.67 ± 0.78[c]
% Difference	16.8	25.4

Note. H, Holtzman; CR, Charles River.

[a] All values, except % difference, are means ± SD in millions (10^6).

[b] Significantly different from Holtzman left ventricle, $P < 0.05$.

[c] Significantly different from Holtzman right ventricle, $P < 0.001$.

Normotensive WKY rats used as controls for SHR have also been reported to exhibit variation in heart size. Pfeffer *et al.* (21) have identified a subset of the WKY rat, originally obtained from NIH, demonstrating an inheritable transmission of biventricular cardiac hypertrophy (BVH). They have also observed BVH in WKY rats commercially obtained from Charles River Laboratories. Ventricular weight/body weight ratios in weight-matched, normotensive, BVH animals was 74% larger in males and 97% larger in females compared to WKY controls. The cardiac hypertrophy appears to be correlated to an increased cardiac output in these animals. Therefore, increased heart size in different groups of rats of the same strain, as evidenced by studies in inbred SHR and WKY rats, has been reported. What is unique about the data presented in the present study using Sprague–Dawley rats is that the intrastrain difference is found in an outbred population.

Dependable measurement of cardiac myocyte volume is necessary to calculate myocyte

number and hence, to accurately evaluate hyperplastic, hypertrophic, or atrophic changes in the myocyte population due to physiological or pathological adaptations. The feasibility of using Coulter analysis to determine cell volume has been reported (12, 14, 22). The reliability of the Coulter Channelyzer method used to determine myocyte dimensions in the present study has been recently documented (13). Briefly, data obtained from Coulter Channelyzer analysis of isolated myocytes were compared with data obtained morphometrically from isolated myocytes and whole perfusion-fixed tissue. Virtually identical results were obtained with all three independent methods. The Coulter Channelyzer technique was the method of choice because results comparable to the anatomical methods can be obtained quickly and with greater objectivity.

A shape factor of 1.05, based on a length–width ratio of approximately 7, was used for cell volume determinations with the Coulter Channelyzer in this investigation. Previous reports have indicated that the length–width ratio for the rat is slightly above 5 (11, 23–25). However, because we are reporting maximum cell length, a shape factor corresponding to a length–width ratio of approximately 7 was determined to be more appropriate.

Most calculations of cross-sectional area, especially those based on isolated myocytes, assume a cylindrical shape of the cardiac myocyte. Although this is not realistic, it is the best approximation of cardiac myocyte geometry available. We have used this assumption of cylindrical shape for our determination of cross-sectional area. However, because we were able to directly measure the two factors necessary for doing the calculation, i.e., cell volume and length, the resulting cross-sectional area values were considered to be just as, if not more, accurate than values previously reported in the literature.

In an effort to account for the supplier-related disparity in heart size between Holtzman and Charles River Sprague–Dawley rats, regional myocyte size was compared in this study. When comparing similar regions between the two suppliers, cell volume and cross-sectional area values were not significantly different. However, cell length in Charles River rats was significantly less than that in Holtzman rats. Cell volume and cross-sectional area values calculated in the present study are in good agreement with values previously reported for Sprague–Dawley rats obtained from Holtzman Company (13).

Differences in myocyte size between right and left ventricles in adult rat hearts within a specific strain have been reported. Gerdes *et al.* (13) determined that right ventricular cell length, cell volume, and cross-sectional area values were significantly smaller than corresponding values in left ventricular epimyocardium and endomyocardium in the Sprague–Dawley rat. Bishop *et al.* (25) also reported that right ventricular myocytes were significantly smaller than those from the left ventricle in Fischer 344, WKY, and SHR rats. Comparison of right and left ventricular cell length, cell volume, and cross-sectional area values for Sprague–Dawley rats in this study agree with the above-mentioned findings.

In addition to right and left ventricular myocyte differences, regional differences within the left ventricle have been reported. Using whole-sectioned tissue from adult WKY rats, Loud *et al.* (26) found that cross-sectional area was 26% larger in endomyocardium than in epimyocardium. It has been reported, using morphometric analysis of whole-sectioned tissue (27) and isolated myocytes (13), that cross-sectional area and cell volume are significantly larger in endomyocardium compared with the epimyocardium in Sprague–Dawley rats. Tomanek (19) and Bishop *et al.* (25) have also reported myocyte cross-sectional area to be larger in endomyocardium compared to epimyocardium in adult male WKY rats, but these differences were not statistically significant. Regional differences in left ventricular myocyte size are not an exclusive feature of the laboratory rat. Significantly greater cross-sectional areas have been reported for endomyocardium compared to epimyocardium of left ventricles in dogs (28) and humans (29). This regional variation in left ventricular myocyte size, however, may not be a general mammalian feature since regional differences in the left ventricle were not found in hearts from ferrets (22) or guinea pigs (30).

In the present study, values for cross-sectional area and cell volume of endomyocardium were found to be significantly greater

than those determined for the epimyocardium and middle myocardium. Investigators have previously reported regional myocyte data on the epimyocardium and endomyocardium of the left ventricle. The inclusion of myocyte size data for the middle myocardium in the present study indicates that a transmural gradient in myocyte size is present. Cell volume and cross-sectional area tend to increase from epimyocardium to endomyocardium in normal Sprague–Dawley rats. In addition, the calculated values for cross-sectional area and cell volume in Charles River rats tended to be less variable (standard deviations were lower) than values determined for Holtzman rats. Therefore, although Charles River and Holtzman Sprague–Dawley rats are an outbred strain, the Charles River rat colony may be a more homogeneous population than that from Holtzman.

No significant differences in binucleation were seen between Holtzman and Charles River rats. The incidence of binucleation reported in the present study, ranging from 91 to 96%, is similar, but slightly higher, than values previously reported for adult rats (24, 25, 31). The estimation of total cell number in hearts of Holtzman rats agrees with values previously reported for Sprague–Dawley rats (13). However, the estimated cell number for Charles River rats is significantly less than that of Holtzman rats.

This study was undertaken because we had noticed differences in heart mass between rats of the same strain and body weight, but obtained from two different suppliers. Our results indicate that hearts from Charles River rats are significantly smaller and contain significantly fewer myocytes than Holtzman rats. Accordingly, caution must be exercised when using allometric equations to predict heart weight and cardiac myocyte size based on body weight in the Sprague–Dawley rat (24). Allometric equations may be generally applicable for a specific strain of rat, but significant variability can occur. This variability in heart size within a specific strain of laboratory rat makes it necessary for the investigator doing cardiac research not only to define the strain of rat being used, but also to identify the supplier.

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