Cell size of mammalian myocardia is not related to physiological demand

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Received 24 August 1990; accepted 4 February 1991

Abstract. Morphological characteristics of myocardial ventricular myocytes have been evaluated from 5 mammalian orders with resting heart rates ranging from 51 to 475 bpm. The purpose was to determine if morphological characteristics of the myocardia are related to the functional demand imposed on the cell as represented by the resting heart rate. Cell size is a constant among mammals of different sizes which have different physiological demands. In contrast, there is more mitochondrial area and less myofibrillar area per cell in animals with rapidly beating hearts than in animals with slower heart rates. Additionally, the mean cross sectional area of individual myofibrils is 30% larger in the cow as compared to the mouse. These findings combined with our previous studies indicate that the different functional requirements of myocardia from different mammalian orders are satisfied by intracellular adaptations of both a structural and biochemical nature.
Key words. Cell size; cross sectional area; myofibril; mitochondria; morphology.

The cardiac myocyte is a fatigue-resistant muscle cell capable of accomodating a wide range of physiological demands. The myocardium is able to relax and contract so as to provide adequate filling and ejection to satisfy blood flow requirements of the animal both at rest and during times of stress. The cardiac minute work rate (CMWR) is primarily determined by the heart rate[1]. Within an animal, the CMWR can increase dramatically and the heart still functions without threatening the animal's survival. Among different mammalian species, the complexity of this task is even greater since resting heart rate correlates inversely with animal mass and ranges, in this study for example, from 51 bpm in the cow to 475 bpm in the mouse. Thus, we postulated that to accomplish adequate contraction and relaxation over this wide range of heart rates, the cardiac myocyte from different mammals must differ in structure and/or function. Previously[1,2] we have established that the glycolytic capacity of myocardia from different mammals does not scale with size. On the other hand, aerobic capacity is highly correlated with heart rate. Contractile capacity (myosin isoforms and ATPase activity) and calcium uptake (sarcoplasmic reticulum ATPase activity) are also correlated with heart rate[1,3]. These observations indicate that myocytes from different mammals function optimally in part by making subcellular adaptations to suit the physiological load.

The purpose of the present study was to morphologically examine ventricular myocytes from several different sized mammals in order to determine if there is also a structural component associated with cardiac function in the face of very different physiological requirements. We have evaluated cell size, and the relative contributions of mitochondria and myofibrils to the total cell area in 5 different mammalian orders with heart rates ranging from 51 to 475 bpm. We have found that cell size is constant among mammals. However, there is a tendency for mitochondria to contribute relatively more and myofibrils relatively less to the total cell longitudinal area in animals with high heart rates. Additionally, the average myofibril is 30% larger in cross section in the cow as compared to the mouse.

Methods

Animals. Morphological criteria were evaluated from ventricular myocardium from young adult males of 5 different mammalian orders: C.D.-1 mouse, Sprague Dawley rat, New Zealand white rabbit, mongrel dog and Hereford cattle. Mean resting heart rates of these animals have been established previously[4,5] and range from 475 to 51 beats per min from the mouse to the cow. Mean heart weights have been published previously[1,2] and ranged from 0.1 to 2583.3 g. At the time of tissue sampling, animals were anesthetized with sodium pentobarbitol except for cows which were killed by exsanguination. Not all of the mammals were assayed for all of the components of the study.

Evaluation of cell size. Fresh transmural tissue samples from the left ventricular free wall of mouse, rat, rabbit and cow were excised and immediately placed in minimum essential medium (90% Earle's salts, 10% horse serum, 4 mM L-glutamine, 20 mM HEPES, 16 mM $NaHCO_3$ at pH 7.3) and finely minced. Cells were then dissociated by collagenase digestion in successive 15-min. incubations in a 95% O_2, 5% CO_2 atmosphere[6,7]. The initial incubation was discarded as it contained many fibroblasts and red blood cells. Subsequent incubations were collected in culture media and ultimately resuspended at cell concentrations of 10^6 – 10^7 cells/ml. Viability of cells determined by Trypan Blue dye exclusion[7] was approximately 80%.

Samples of the cell suspension were placed on a counting chamber and examined under a light microscope equipped with a video recorder. Multiple images were recorded on video, projected, traced and digitized along with a light microscope micrometer image for calculation of magnification. Samples from 2–6 of each mammal

were examined and 143–489 cells per mammal were sized. Because the suspended cells are nearly symmetrical, orientation of cells was assumed to be random.

Relative surface areas of myofibrils and mitochondria. Samples were obtained from mouse, rat, dog and cow and immediately fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, 300 mOsm (using sucrose) and postfixed as described by Lee et al.[9]. Fixed cells were oriented longitudinally and embedded in epoxy-resin[10], mounted on single slot formvar coated copper grids, sectioned on a Porter-Blum MT2B ultramicrotome and stained with lead citrate[11]. An electron microscope (Philips 201 or 200) image of each cell was photographed, printed at a final magnification of 9000 and digitized using a Neumonics electronic graphics calculator No. 1224. Total cell area was determined excluding nuclei. The total mitochondrial and myofibrillar areas were then determined and calculated as a percentage of total cell area (fractional volume). Two cells were evaluated from each mammal type and this included tracing 200–300 mitochondria and 200–300 myofibrils from each cell.

Cross sectional area of myofibrils. Tissue samples from the mouse and the cow were fixed and prepared for electron microscopy as described above except that cells were oriented for cross sectioning and images were printed at a final magnification of 14 300. A carbon grating replica standard was used to calculate magnification. Two of each mammal were evaluated and this involved sizing 200–400 individual myofibrils.

Protein and DNA determinations. Tissues and cell suspensions from mouse, rabbit and cow were stored frozen at −70 °C until analyzed. Protein was determined by the method of Lowry et al.[12] using the Folin phenol reagent. DNA was determined fluorometrically as described previously[13].

Statistical analysis. Dual means were compared using a *t*-test at the $p < 0.05$ level of significance. Multiple means were compared using a one-way analysis of variance at the $p < 0.05$ level with a Duncan's post hoc test. Data are expressed as mean ± SEM.

Results

Figure 1 illustrates the cell size frequency distributions from four mammals. Mean cell size was statistically similar in all animals (1429 ± 59, 1371 ± 36, 1333 ± 23 and 1426 ± 30 μm^2 in mouse, rat, rabbit and cow, respectively) and the distributions were all essentially normal and superimposable indicating that across a wide range of heart size and heart rate, individual cell size is a constant. Figure 2 depicts the relative contributions of myofibrils and mitochondria to the entire cell area excluding nuclei. Mitochondria comprised 33% of the cell area in the mouse, but only 19% in the cow ($p < 0.05$). Myofibrils tended to comprise a greater relative area (56.5% in the cow as compared to the mouse (47.5%). Also the average

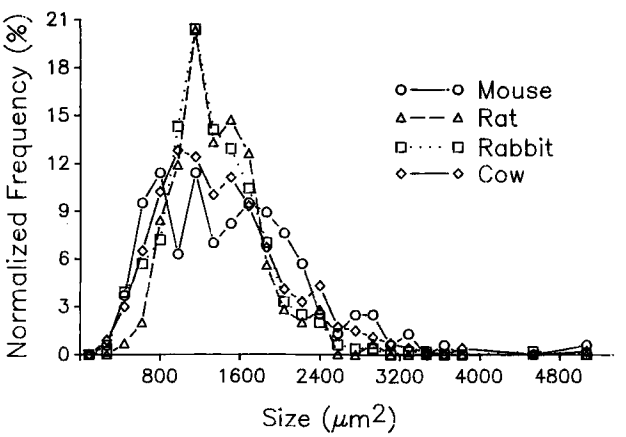

Figure 1. Normalized cell size frequency distribution of cells from four different mammalian orders. Mouse, n = 6, number of cells sized = 165. Rat, n = 2, number of cells sized = 143. Rabbit, n = 4, number of cell sized = 489. Cow, n = 4, number of cells sized = 460.

myofibril was 30% larger in cross section in the cow (55.9 ± 2.9 nm^2) than in the mouse (42.6 ± 2.3 nm^2) ($p < 0.05$). (These data must be interpreted with caution since the number of cross sections examined is small. However, the uniform composition of heart muscle reduces the chance of error to a great extent.)

DNA concentration was evaluated in both tissue samples and isolated cell suspensions in three of the mammals studied (table). The tissue concentration of DNA, expressed in μg DNA per mg of tissue protein, did not differ among mammals. Isolated rabbit cells contained significantly more DNA per cell than did cow cells.

Discussion

This comparative biological approach has been useful in gaining insight into the possible role of physiological demand in regulating the structure and function of the myocyte.

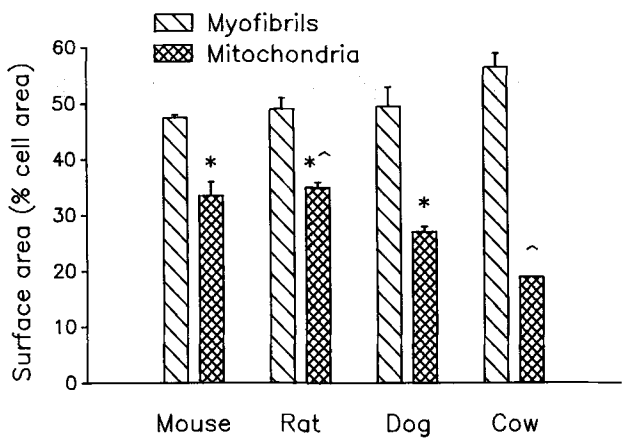

Figure 2. Surface areas of myofibril and mitochondrium profiles as a percentage of the total cell surface area (excluding nuclei) of a longitudinal section in cells from four different mammalian orders. * Significantly different from cow, $p < 0.05$. ^ Significantly different from dog, $p < 0.05$. n = 2, number of cells evaluated = 2 for each mammal. Approximately 200–300 mitochondria and 200–300 myofibrils from each cell were digitized.

DNA content of tissues and isolated cells from three mammalian orders

Animal	Tissue (μg DNA/mg protein)	Isolated cells (μg × 10^{-6} DNA/cell)
Cow	9.27 ± 1.29	3.34 ± 0.28
Rabbit	6.83 ± 1.18	8.64 ± 0.93*
Mouse	6.97 ± 1.00	6.28 ± 1.66

*Rabbit is significantly different from cow, $p < 0.05$. $n = 3-6$ for each preparation. Data are expressed as mean ± SEM.

Maximum rates of mass-specific oxygen consumption scale with body size. The mouse has nearly 10 times the rate of oxygen consumption as does the cow[14]. In order to maintain their higher metabolic rates, the hearts of small mammals must pump relatively more blood per unit of time and thus perform more work than large mammals. As a number of hemodynamic parameters remain constant among different species the primary determinant of CMWR is heart rate[1]. There was a 10-fold difference in heart rate among the mammals studied in the present investigation and thus one would expect to observe significant differences among these mammals if indeed physiological demand dictates structure and function. Others[15] have shown that the volume density of mitochondria in heart muscle scales with resting CMWR. We speculate that in addition to biochemical adaptations of the energy supplying and utilizing systems[1-3], the cardiac myocyte has adapted to its role in mammals with high heart rates by developing its internal components to facilitate chemical flux from the intermyofibrillar space into the myofibril. Enhanced movement of high energy phosphates and calcium would help to ensure both adequate contraction and relaxation in a rapidly beating heart.

Cell sizes were estimated by determining the area of two-dimensional images of suspended isolated cells. This unit of size is not equivalent to a cross sectional area since it is obtained from a more spherical cell than would be observed in fixed tissue. However, since preparations from different animals were similar in cell concentration and viability, it seems to be a valid conclusion that there is no observable difference in cell size among the mammals studied. From this it may be interpreted that cell size does not create a diffusion problem even in small mammals with high metabolic demands. This interpretation is consistent with observations[16] that during stimulated cardiac growth, cell size can increase without apparently compromising the function of the cell.

A biochemical indicator of cell size which is often used is the DNA concentration of the cell or the protein to DNA ratio in the tissue. The underlying assumption of these measurements is that the amount of DNA per nuclei is constant[17] and that there is a set volume of mass of cytoplasm over which one nucleus has jurisdiction[18]. Thus since the different cell populations studied had similar proportions of mono- and binucleated cells (unpublished observations), the similarities in our calculated DNA content per cell further support the morphological data obtained from our light microscope evaluation from which we conclude that cell size is similar among species. As has been observed previously[15], mitochondria comprised a greater proportion of the total cell area in small mammals with high resting heart rates than in larger animals. Conversely, myofibrils tended to contribute slightly less to the total cell area in small animals as compared to large and the individual myofibrils of the mouse were smaller in cross sectional area than those of the cow. These data suggest that an adaptive strategy of the cardiac myocyte in a rapidly beating heart may have been to increase mitochondrial density to facilitate energy production while decreasing the size of the myofibril to enhance the movement of both energy and calcium to their respective sites. In conjunction with biochemical adaptations observed previously[1-3] (increased sarcoplasmic reticulum ATPase and myosin and myofibrillar ATPase activities) a rapidly beating heart can thus perform a more rapid contraction and relaxation cycle while allowing adequate coronary blood flow and ventricular filling time.

Thus it appears that adaptation of cell size is not required to fulfill the different functional demands of hearts of different sized mammals. Over a 10-fold difference in metabolic rate, myocardial demands seem to be accomodated instead by extracellular (blood flow) and intracellular (structural and biochemical) adaptations.

Acknowledgments. The authors wish to thank Kate Matthews for technical assistance. The study was supported by grants from NSERC and the Ontario Heart and Stroke Foundation.

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0014-4754/91/101070-03$1.50 + 0.20/0