doi:10.4149/gpb_2010_02_129

Analysis of rat papillary muscle transverse deformation by laser diffraction

Adolfo Virgen-Ortiz[1], Alejandro Apolinar-Iribe[2], Martín E. Ríos-Martínez[3], José L. Marín[4] and Jesús Muñiz[3]

[1] *Departamento de Ciencias Químico Biológicas y Agropecuarias, División de Ciencias e Ingeniería, Unidad Regional Sur, Universidad de Sonora. Blvd. Lázaro Cárdenas No. 100, Colonia Francisco Villa, CP 85880, Navojoa, Sonora, México*
[2] *Departamento de Física, Universidad de Sonora, Apdo. Postal 1626, CP 83000, Hermosillo, Sonora, México*
[3] *Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de Julio No. 965, Col. Villas San Sebastián, CP 28040, Colima, Colima, México*
[4] *Departamento de Investigación en Física, Universidad de Sonora, Apdo. Postal 5-088, CP 83190, Hermosillo, Sonora, México*

Abstract. We use laser diffraction in the analysis of the transversal deformation that the papillary muscle of the female and male Wistar rat may undergo when is subjected to different tension (tension range, 0–30 mN) in the longitudinal plane. Papillary muscles from the right ventricle were illuminated at normal incidence with a He-Ne laser lasing at 594 nm at room temperature. The far-field diffraction pattern projected to a screen was recorded with a digital camera for its analysis. The analysis of the stress-strain curves from the two experimental groups shows that the papillary muscles from male rats exhibit a higher stiffness in the transversal axis compared to the female rats.

Key words: Cardiac papillary muscle — Transverse deformation — Laser diffraction

Introduction

Papillary muscles play a vital role in the pumping efficiency of the heart. These cylindrically shaped muscles are attached at one end to the wall of the left or the right ventricle and at the other end to the mitral or tricuspid valve, respectively. During ventricular systole, the increasing pressure in the ventricles tends to push the valves upward into the atria, which would allow backflow of blood during ejection. The papillary muscles prevent this inversion by contracting and holding the valves closed. Due to their relatively simple geometry and muscle fibers aligned along their axes, papillary muscles have been popular in studies of the passive and active mechanical properties of heart muscle. For determining normal and shear properties, combined extension and torsion is a useful loading protocol (Taber 2004).

Correspondence to: Alejandro Apolinar-Iribe, Departamento de Física, Universidad de Sonora, Apdo. Postal 1626, CP 83000 Hermosillo, Sonora, México
E-mail: apolinar@ciencias.uson.mx

Both in human and animal models it has been observed that there are structural and functional differences in the female and male heart, and the understanding of these variations can be clinically important to elucidate the progress until a pathophysiological study is obtained. In the case of animals, it is known that the heart size is greater in male rats than in female rats, nevertheless the heart weight/body weight ratio is greater in females than in males (Capasso et al. 1983; Leblanc et al. 1998). Also, there have been reported differences in the contractility, for example, the papillary muscle of a male rat develops higher tension than females up to ages 6–14 months, and there are not significant differences observed in the cross sectional area of papillary muscle removed from the left ventricle (Leblanc et al. 1998). Recently, it has been reported that there are electrophysiological differences between male and female, especially in the electrocardiographical pattern of ventricular repolarisation. Females may have lengthening of the QT interval (time from electrocardiogram Q wave to the end of the T wave), when increased QT is observed, females are more susceptible to development polymorphic ventricular tachycardia (Abi-Gerges et al. 2004). Moreover, other aspect of relevance is the deformation of cardiac tissues. There are

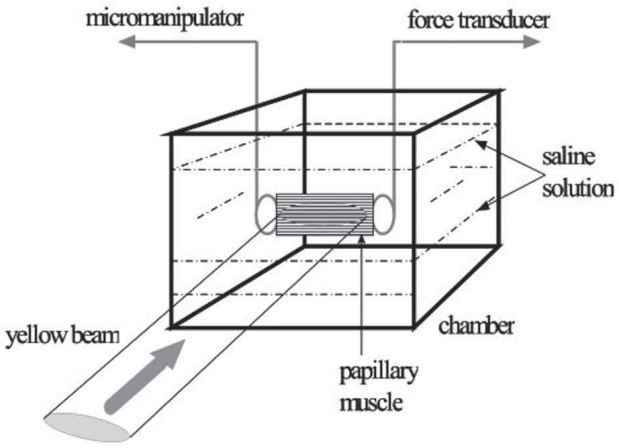

Figure 1. Scheme of the experimental setup. Muscle dimensions: length, 5.0 ± 0.5 mm; width, 1.1 ± 0.15 mm. Size of laser beam: length, 4 mm; width, 1 mm. Chamber dimensions: 2.5 × 2.5 × 4.0 cm. Dash-dotted lines: saline solution representative.

various studies performed in different orientation of cardiac tissue under passive stress. For example, the transverse stiffness was evaluated with the cell surface indented, using a small microsphere attached to carbon fiber. The shear stiffness was evaluated in transverse and longitudinal planes in single cardiac myocytes by applying shear stress. The results indicated that the transverse stiffness is greater than the shear stiffness in the longitudinal plane which is modulated by the cytoskeleton microtubules (Nishimura et al. 2006). Finally, although there are several studies that estimated the longitudinal stiffness in cardiac tissues (Litwin et al. 1991; Mirkovic et al. 2002), there is a lack of studies regarding the deformation in cardiac muscle related to gender differences. In the present work we evaluated the transverse deformation of papillary muscle, of both female and male rats, when it is subjected to longitudinal stress using the diffraction of laser.

Materials and Methods

Animal care and isolation of papillary muscle

We followed the procedures approved by the committee of ethics of the University of Colima in the care and handling on the surgery rats, fulfilling the recommendations of the Guide for the Care and Use of Laboratory Animals (US Department of Health, NIH). Female (F group, $n = 6$) and male (M group, $n = 6$) Wistar rats of the same age (two months of age, body weight 120–150 g,) were anesthetized with sodium pentobarbital (40 mg/kg b.w.). For each rat, the heart was quickly removed with a midline thoracotomy, and then the papillary muscle from the right ventricle was carefully dissected and immersed in Krebs buffer (in mmol/l: 115 NaCl, 4.8 KCl, 1.0 $MgSO_4 \cdot 7H_2O$, 2.0 $CaCl_2 \cdot 2H_2O$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 10 glucose; pH 7.4; oxygenated with 95% O_2 + 5% CO_2) on the experimental chamber without tension.

Muscle mounting and laser diffraction

Light diffraction by a grating with constant spacing obeys the grating equation: $d\sin\theta_m = m\lambda$, where θ_m are meridional angles of the diffracted beams, m is the diffraction order, λ is the wavelength of the irradiation and d is the grating spacing. The angle of the diffracted light by a grating depends on the incident wavelength, just as in a muscle fiber. For this purpose and to isolation, the whole papillary muscle was mounted in an experimental chamber with Krebs buffer. Then the one end of papillary muscle was attached to an isometric force transducer (Kent Scientific Corporation, USA) and the other end to a micromanipulator system for displacement (Narishigue, Japan). The geometry of the arrangement utilized in our experiments is depicted in Fig. 1. The papillary muscle was illuminated in its central region with a yellow beam of the He-Ne laser with ordinary polarization (λ = 594 nm and P = 10 mW, Melles-Griot, USA) at normal incidence. Distance from the laser to the papillary muscle was 2 meters. The setup included a cylindrical lens for diminishing the transversal dimension (4 mm to 1 mm) of the beam laser spot and the enlargement of the longitudinal dimension of the diffraction orders. The images of the diffraction orders generated by the papillary muscle were projected on a screen and taken with a digital camera for its analysis (muscle-screen distance was 6 meters). The screen had a graduated scale (mm), where the zero unit was the position of the 0^{th}-order diffraction. All measurements were made with respect to this reference and corroborated with a digital micrometer. After stretch, passive tension rises rapidly, and then declines over a long period. Forces were taken at the maximal point. The protocol of recording consisted in the acquisition of spectra of the diffraction orders using different tension of the papillary muscle (tension range: 0–30 mN; Δt = 3 min). Finally, all of the experiments were at room temperature (22°C) and the acquisition of the diffraction patterns was done in a dark room.

Data analysis

The ImageJ software (NIH, USA), was used to obtain densitometer profiles from the diffraction patterns (Fig. 2) and to determine the distance between each diffraction order (Δx). Then the thickness of papillary muscle was calculated using the following approximated equation:

$$d = (D\lambda) / (\Delta x) \quad (1)$$

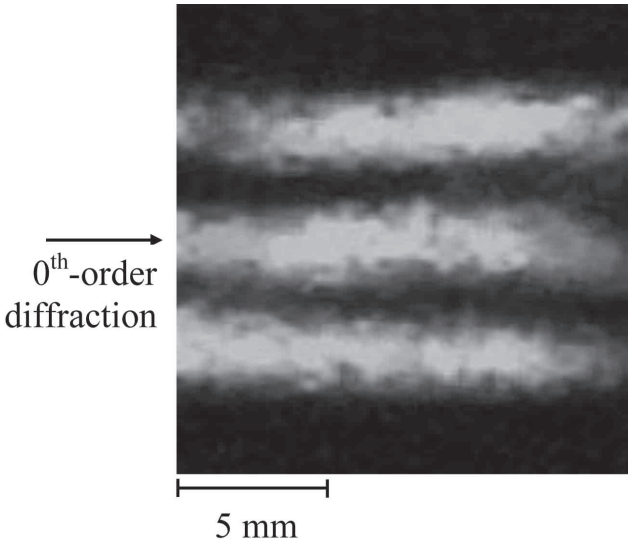

Figure 2. Experimental diffraction spectra obtained from a papillary muscle of rat right ventricle. The central order is the 0^{th}-order diffraction. The measurements of the distance of the first-order diffraction were made with respect to the center of the 0^{th}-order diffraction in a graduated scale on the screen (not seen in photo).

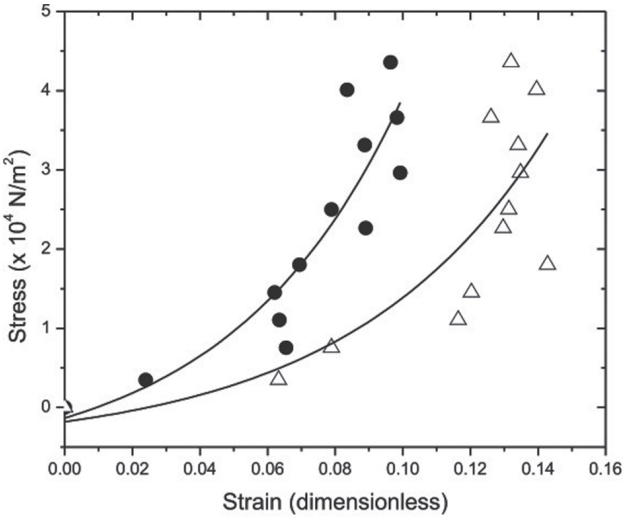

Figure 3. Comparative analysis of stress-strain curves of papillary muscle from the rat right ventricle. The circles represent the group of male rats and the triangles correspond to female rats. All data are expressed as means. The points represent the mean of six different experiments with same tension applied.

where d corresponds to thickness of diffracted object (papillary muscle), D is the distance from the diffracting object to the screen, λ is the laser wavelength used, and Δx is the distance between zero and first diffraction orders.

Assuming that the papillary muscle has symmetry similar to a cylinder lens, the cross-sectional area was evaluated by the equation:

$$A = \pi\theta^2 /4 \quad (2)$$

where A corresponds to the cross-sectional area, and θ is the diameter of the papillary muscle.

In addition, the tension data were analyzed by means of stress-strain curves with Magid and Law equation

$$\varepsilon = (Ee/\alpha)[\exp(\alpha\delta) - 1] \quad (3)$$

where ε is stress, δ is strain (δ = Δd/d, deformation *per* length unit of the material), Ee is elastic modulus or Young modulus, and α corresponds to deformation rate. The stress was calculated from F/A ratio, where force F corresponds to applied tension to the muscle for the isometric force transducer and A is cross-sectional area.

These curves were fitted with Magid and Law equation (Eq. (3)) to estimate the papillary muscle elastic modulus (Magid and Law 1985). After taking logarithms, Eq. (3) becomes (when exp (αδ) >> 1)

$$1n(\varepsilon) = \alpha\delta + 1n(Ee/\alpha) \quad (4)$$

Statistical analysis

The difference between the stress-strain curves were analyzed by One-way analysis with a Tuckey's *post hoc* test. Moreover, the differences of area between experimental groups were compared using paired Student's *t*- test. The differences were considered significance to $p \leq 0.05$ for both tests. All data are expressed as mean ± SE. The analyses were performed in Minitab software for windows.

Results

The optical technique of laser diffraction allowed estimating the cross-sectional area of papillary muscle. The area data obtained from female muscles was greater than that obtained from male muscles (F group: 0.67 ± 0.08 mm^2; M group: 0.41 ± 0.01 mm^2; tension to 0 N). Subsequently, tension was applied in the longitudinal axis from the papillary muscle increasing progressively until it reached 30 mN. During this protocol the strain was evaluated in the transversal axis of the papillary muscle by monitoring the diffraction patterns (Fig. 3). The adjustment of the experimental data showed an exponential behavior. The analysis of the stress-strain curves from the two experimental groups (Fig. 3) clearly shows that the papillary muscles from male rats have higher stiffness in the transversal axis compared to the female rats. Besides, the Young modulus in this orientation was also smaller in the F group (Young modulus: 18.3 ± 3.0 kPa) compared to the

M group (Young modulus: 51.3 ± 7.0 kPa). This parameter permits an estimation of rigidity grade of a tissue; in this case the obtained values support the conclusion that the papillary muscle from F group is less rigid.

Discussion

It is becoming evident that there is considerable sexual dimorphism in the pathogenesis of cardiovascular disease. Thus findings from past studies, obtained mostly from experiments with male rats, could not be automatically applied to female.

A new method was developed using laser diffraction in the analysis of the transversal deformation that the papillary muscle may undergo when subjected to different tension in the longitudinal plane. This new method of analysis has not been reported in previous studies; therefore we consider that the method of analysis presented here using optical techniques offers other ways to perform studies concerning the mechanical behavior in cardiac tissues. The papillary muscles in female and male rats do not have significant mechanical differences when are subjected to small deformations, but exhibit very different mechanical properties at higher deformations, as shown in Fig. 3. Moreover, an important aspect of this investigation is the difference found between the female and male rats, which clearly show that the papillary muscle from female rats is more elastic compared to male rats.

The clinical data reported in different investigation about human heart failure showed differences in the percentage of prevalence between men and women (Sussman 2003). Additionally it has been documented that both hearts respond differently to physiological and pathological stress. The incidences of coronary artery diseases are lower in pre-menopausal women than men of similar age, but in the post-menopause the women require hormone therapy to reduce the risk of developing coronary artery disease (Tobin et al. 1987; Grady et al. 1992). These reports showed that sexual hormone have an essential function in the physiology normal heart in both genders. Thus the differences between female and male, reported in this work, could be regulated under a hormonal control, and the differences in stiffness may represent – in the case of female rat cardiac tissues – a functional advantage for various physiological conditions such as pregnancy, and heavy and intense effort that induce an overload of blood volume during the ventricular filling.

Our present data about the stiffness in the heart papillary muscle in female and male rats and previous data of tension reported by Capasso et al. (1983) and Leblanc et al. (1998) can help to understand the mechanical behavior of heart papillary muscle in both genders. Also differences in the sarcoplasmic reticulum calcium handling reported in female and male may explain the changes in the sex-based mechanical properties showed in this work and in others (Leblanc et al. 1998; Chen et al. 2003). Moreover, from pathophysiological point of view, our data support the hypothesis that there are sex-based differences in heart function and these differences will impact the risk for the development of heart failure. For example, females are more resistant to ischemia-reperfusion injury (Imahashi et al. 2004) and are less prone to heart failure during chronic pressure overload (Douglas et al. 1998).

Furthermore, this study will allow us to perform future studies in both female and male rats to explore the role of cytoskeleton in the regulation mechanisms associated to the transverse deformation of papillary muscle.

References

Abi-Gerges N., Small B. G., Lawrence C. L., Hammond T. G., Valentin J. P., Pollard C. E. (2004): Evidence for gender differences in electrophysiological properties of canine Purkinje fibres. Br. J. Pharmacol. **142,** 1255–1264; doi:10.1038/sj.bjp.0705880

Capasso J. M., Remily R. M., Sonnenblick E. H. (1983): Age-related differences in excitation-contraction coupling in rat papillary muscle. Basic Res. Cardiol. **78,** 492–504; doi:10.1007/BF01906460

Chen J., Petranka J., Yamamura K., London R. E., Steenbergen C., Murphy E. (2003): Gender differences in sarcoplasmic reticulum calcium loading after isoproterenol. Am. J. Physiol. Heart Circ. Physiol. **285,** H2657–2662

Douglas P. S., Katz S. E., Weinberg E. O., Chen M. H., Bishop S. P., Lorell B. H. (1998): Hypertrophic remodeling: gender differences in the early response to left ventricular pressure overload. J. Am. Coll. Cardiol. **32,** 1118–1125; doi:10.1016/S0735-1097(98)00347-7

Grady D., Rubin D. B., Fox C. S., Black D., Ettinger B., Ernster V. L., Cummings S. R. (1992): Hormone therapy to prevent disease and prolong liege in postmenopausal women. Ann. Intern. Med. **117,** 1016–1037

Imahashi K., London R. E., Steenbergen C., Murphy E. (2004): Male/female differences in intracellular Na+ regulation during ischemia/reperfusion in mouse heart. J. Mol. Cell. Cardiol. **37,** 747–753; doi:10.1016/j.yjmcc.2004.06.010

Leblanc N., Chartier D., Gosselin H., Rouleau J. L. (1998): Age and gender differences in excitation-contraction coupling of the rat ventricle. J. Physiol. **511,** 533–548; doi:10.1111/j.1469-7793.1998.533bh.x

Litwin S. E., Raya T. E., Warner A., Litwin C. M., Goldman S. (1991): Effects of captopril on contractility after myocardial infarction: experimental observations. Am. J. Cardiol. **68,** D26–34; doi:10.1016/0002-9149(91)90258-M

Magid A., Law D. J. (1985): Myofibrils bear most of the resting tension in frog skeletal muscle. Science **230,** 1280–1282; doi:10.1126/science.4071053

Mirkovic S., Seymour A. M., Fenning A., Strachan A., Margolin S. B., Taylor, S. M., Brown, L. (2002): Attenuation of cardiac fibrosis by pirfenidone and amiloride in DOCA-salt hypertensive rats. Br. J. Pharmacol. **135,** 961–968; doi:10.1038/sj.bjp.0704539

Nishimura S., Nagai S., Katoh M., Yamashita H., Saeki Y., Okada J., Hisada T., Nagai R., Sugiura S. (2006): Microtubules modulate the stiffness of cardiomyocytes against shear stress. Circ. Res. **98,** 81–87; doi:10.1161/01.RES.0000197785.51819.e8

Sussman M. A. (2003): Gender differences in heart failure. In: Cardiac Drug Development Guide. (Ed. Pugsley M. K.), pp. 389–405, Edited Humana Press; doi:10.1385/1-59259-404-2:389

Taber L. A. (2004): Nonlinear theory of elasticity. Applications in Biomechanics. pp. 266, World Scientific Publishing Co.; doi:10.1142/9789812794222

Tobin J. N., Wassertheil-Smoller S., Wexler J. P., Steingart R. M., Bunder N., Lense L., Wachspress J. (1987): Sex bias in considering coronary bypass surgery. Ann. Intern. Med. **107,** 19–25

Received: August 13, 2009
Final version accepted: December 21, 2009