ELSEVIER

European Journal of Pharmacology 361 (1998) 73–77

ejp

Short communication

Effects of gender on the sensitivity of rat cardiac muscle to extracellular Ca^{2+}

Shi-Nan Wang [a], Richard P. Wyeth [a,b], Richard H. Kennedy [a,b,*]

[a] *Department of Pharmacology and Toxicology, Mail slot 611, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, AR 72205, USA*

[b] *Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA*

Received 6 October 1998; accepted 9 October 1998

Abstract

Experiments were designed to determine if the inotropic response to increasing buffer calcium concentration differs in male and female cardiac muscle. Left atrial and papillary muscles were isolated from hearts of 3–4-month old male and female rats, bathed in Krebs–Henseleit solution (30°C), and stimulated at 1.5 Hz. Isometric developed tension was monitored continuously as extracellular Ca^{2+} was increased in a cumulative fashion. When compared to male atrial muscle, female atrial preparations were more sensitive to the resulting positive inotropic action; EC_{50} values were 2.89 ± 0.22 and 1.86 ± 0.21 mM in male and female atria, respectively. Two-way analysis of variance (ANOVA) also indicated that there was a significant gender-associated difference in the Ca^{2+} dose–response curves in atrial muscle. In contrast, papillary muscle did not show a significant gender-related difference in EC_{50} values (0.88 ± 0.07 and 0.74 ± 0.06 mM in males and females); however, the Ca^{2+} dose–response curves obtained from male and female preparations were found to be significantly different when compared by ANOVA.

Keywords: Atrial muscle; Papillary muscle; Female; Isometric developed tension; Male

1. Introduction

The literature is beginning to describe gender-related differences in cardiac muscle structure and function. For example, some investigators report that left ventricular mass is lower in women (Hinderliter et al., 1991), although others believe this difference is eliminated if ventricular mass is indexed to lean body mass (Daniels et al., 1995). Similarly, data regarding gender-related differences in resting cardiac contractile function in humans are inconsistent, with some investigators finding that men have a lower left ventricular ejection fraction at rest (Hanley et al., 1989; Wong et al., 1995; Merz et al., 1996) and others showing that gender has no influence (Adams et al., 1987). In spite of this disparity among reports on resting function, most studies show that men respond to stress with a greater increase in ejection fraction (Adams et al., 1987; Hanley et al., 1989; Merz et al., 1996). Thus, available data suggest that men have a greater cardiac reserve; however, documented gender-associated variations in neurohumoral control and hemodynamic responsiveness (Gotshall et al., 1991; Ryan et al., 1994; Du et al., 1995; Ettinger et al., 1996; Huikuri et al., 1996; Jones et al., 1996; Stein et al., 1997) make it difficult to determine the extent to which this difference is mediated by variations in the heart.

A limited number of studies have examined gender-related differences in contractile function in cardiac muscle isolated from animal models. Unfortunately, information from this in vitro experimentation is contradictory. Studies in isolated working rat heart suggest that basal cardiac function is somewhat greater in male as compared to female rats (Schaible et al., 1984), while experimentation in isolated rat papillary muscle indicates that the rate of tension development and the rate of relaxation are greater in female heart (Capasso et al., 1983). These contradictory reports may be a result of the different preparations utilized or variations in other experimental conditions. With relatively little known about possible gender-associated differences in cardiac biochemistry, physiology and elec-

* Corresponding author. Tel.: +1-501-686-8041; Fax: +1-501-686-5521; E-mail: kennedyrichardh@exchange.uams.edu

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00736-5

trophysiology it is difficult to understand observed differences in contractile function. The present study was designed to begin addressing this issue by determining if gender alters the response of cardiac muscle to increases in extracellular Ca^{2+}.

2. Materials and methods

2.1. Animals

Male and female, Sprague–Dawley rats (3–4 months of age) were purchased from Charles River Breeders (Wilmington, MA) and used to isolate tissues for all experiments. Female rats were used without regard to estrus cycle status. All protocols in this study were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences and were in accordance with the Guide for the Use of Laboratory Animals issued by the U.S. Department of Health and Human Services.

2.2. Cardiac tissue preparations

Rats were anesthetized with a volatile anesthetic, and their hearts were excised and immediately perfused via the aorta with a Krebs–Henseleit (KH) solution (30°C) of the following composition (in mM): 118.0 NaCl, 25.0 $NaHCO_3$, 3.7 KCl, 1.0 KH_2PO_4, 1.4 $CaCl_2$, 1.2 $MgCl_2$, and 11.0 Dextrose. The solution was buffered to pH 7.4 by saturation with 95% O_2/5% CO_2 gas. After the heart was free of residual blood, left atrial and papillary muscles (< 0.7 mm diameter) were dissected and hung vertically in muscle baths containing the oxygenated solution described above. Nadolol (1 μM; a β-adrenoceptor antagonist) was included in the buffer to prevent possible interference from endogenous catecholamines. Preparations were paced at 1.5 Hz via platinum contact electrodes using 2.0 ms square wave pulses set at 50% above threshold voltage. Isometric tension was monitored by force-displacement transducers (Type FT03, Grass Instrument, Quincy, MA) and recorded continuously on a polygraph (Grass Model 7 D). A 'length–tension' relationship was determined for each preparation, and resting tension was subsequently maintained at that level which elicited 90% of maximum observed contractile force (L_{max}). After establishing resting tension, tissues were equilibrated for 60 min in a low Ca^{2+} (0.4 mM) KH buffer with the bathing solution being changed every 15 min. Concentration–response curves for Ca^{2+} were then obtained by cumulative addition; each concentration was added to the medium only after the tissue reached a steady-state response at the previous level. After completing the concentration–response curve, tissues were dried to constant weight at 100°C. Developed tension was expressed per unit tissue dry weight. EC_{50} values were obtained by graphical evaluation of individual log concentration–response curves using regression analysis of the linear phase.

2.3. Statistical evaluation

Data were analyzed by analysis of variance (ANOVA) and by student's *t*-test where appropriate. All data are presented as means ± S.E.M. The criterion for significance was a P value less than 0.05.

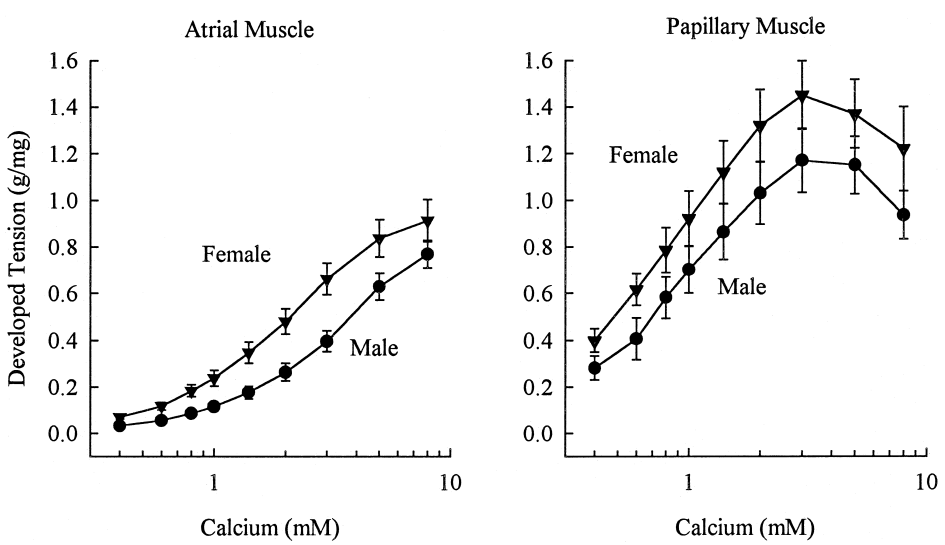

Fig. 1. Inotropic effects of extracellular calcium (Ca^{2+}) in left atrial (left panel) and papillary (right panel) muscles isolated from male (circles) and female (inverted triangles) rats. Preparations were bathed in a Krebs–Henseleit buffer (30°C) and stimulated at 1.5 Hz. After equilibration in buffer containing 0.4 mM Ca^{2+}, $CaCl_2$ was added cumulatively to attain each concentration. Each data point represents the mean of 10 preparations; vertical bars represent S.E.M. Developed tension is expressed relative to tissue dry weight.

3. Results

Increasing Ca^{2+} elicited a concentration-dependent positive inotropic effect in all preparations. The magnitude of the response was not affected by gender in either atrial or papillary muscle (Fig. 1). Maximum observed developed tension in the presence of high Ca^{2+} was 0.77 ± 0.06 and 0.92 ± 0.09 g/mg dry weight. in male and female atrial muscles, respectively. Corresponding values in papillary muscle were 1.20 ± 0.13 and 1.47 ± 0.19 g/mg dry weight. However, female atrial muscle was more sensitive to the positive inotropic effect of Ca^{2+}. EC_{50} values were 2.89 ± 0.22 and 1.86 ± 0.21 mM in male and female atria, respectively, and two-way ANOVA indicated that there was a significant gender-related difference in the dose–response curves. In contrast, the EC_{50} values in papillary muscle were not significantly different when compared between genders (0.88 ± 0.07 and 0.74 ± 0.06 mM in males and females); however, as with atrial muscle, two-way ANOVA indicated that there was a significant gender-associated difference in the dose–response curves in papillary preparations.

4. Discussion

Results of current experiments comparing male and female rat atrial muscle indicate that female preparations are more sensitive to extracellular Ca^{2+}; EC_{50} values for the Ca^{2+} dose–response curves were lower in female atria with no difference observed in the maximum response. Two-way ANOVA also indicated that the Ca^{2+} dose–response curves were different in male and female preparations. The observed difference in sensitivity to extracellular Ca^{2+} suggests that female atrial muscle is closer to its maximum contractile state when monitored at physiological extracellular Ca^{2+} concentrations and in the absence of neurohumoral control.

In contrast to atrial muscle, gender was not associated with a significant difference in the EC_{50} values in rat papillary preparations. However, two-way ANOVA did indicate that there was a statistically significant gender difference in the Ca^{2+} dose–response curves in papillary muscle. This difference results primarily from the fact that female preparations tended to develop greater tension per unit tissue weight at every Ca^{2+} concentration examined (P values ranged between 0.08 and 0.24 when the two groups were compared by t-test at individual Ca^{2+} concentrations). The maximum observed developed tension in papillary muscle was not significantly different in the two genders ($P = 0.24$).

It is not surprising that gender would have differing effects on contractile function in atrial and papillary muscle. Excitation–contraction (E–C) coupling is known to vary in these two tissues with reports from studies in various species suggesting that the differing function may be mediated by variations in sarcoplasmic reticular content and t-tubule density (Berlin, 1995), myosin isoform expression (Morano et al., 1991), and/or voltage-dependent Ca^{2+} influx (Beuckelmann, 1997). These differences between atrial and ventricular muscles apparently result in atrial myocardium being more dependent on the sarcoplasmic reticulum as a source for activator Ca^{2+} (see Asgrimsson et al., 1995). Similarly, these differences between atrial and ventricular muscles may play a role in the fact that gender-related differences in protein expression affect contractile function and responsiveness to extracellular Ca^{2+} differently in the two tissues. In addition, tissue differences in E–C coupling are probably involved in the fact that the EC_{50} values for extracellular Ca^{2+} were found in the present study to be greater in atrial than papillary muscle (e.g., mean values were 2.89 and 0.88 in male atrial and papillary preparations). Although observed routinely in our laboratory in rat myocardium monitored under the conditions used in this study, this tissue difference in the Ca^{2+} EC_{50} value has not been observed in cardiac tissue of all species (Kafiluddi et al., 1989; Tanaka et al., 1995) and is not observed in rat cardiac preparations monitored under different experimental conditions (Ruch et al., 1992).

Current results provide little insight into the cause of the disparity in previous studies comparing cardiac contractility in male and female rats. As discussed above, studies in isolated working rat heart suggested that basal cardiac function is somewhat greater in male as compared to female rats when examined at high afterload (Schaible et al., 1984). In contrast, experimentation by Capasso et al. (1983) with isolated rat papillary muscle suggested that basal contractility is greater in female heart. As indicated by results of the present study, these investigators found no significant difference in peak isometric developed tension; however, they reported that both $+dT/dt_{max}$ and $-dT/dt_{max}$ were lower in male than female myocardium. The studies by Schaible et al. (1984) and Capasso et al. (1983) both utilized tissues isolated from Wistar rats of approximately the same age (15–25 weeks). However, the isolated working heart was monitored at 37°C using a physiological buffer containing 1.5 mM Ca^{2+} and a stimulation frequency of 340 beats/min, while the papillary muscle was examined in a 2.4 mM Ca^{2+}-containing physiological solution at 30°C using a stimulation frequency of 30 beats/min. Current experiments utilized atrial and papillary preparations from 3–4 month old Sprague–Dawley rats that were bathed in a KH solution at 30°C and stimulated at 90 beats/min. It is possible that the disparities among these studies were the result of differences in the tissue studied, stimulation frequency or temperature. Additional studies are required to examine these possibilities.

The mechanism underlying the observed gender-related differences in cardiac contractile function is not known. Experiments in rabbits have shown that estradiol treatment

elicits a down-regulation of the delayed rectifier potassium current (I_K) (Drici et al., 1996). The resulting increase in action potential duration could enhance voltage-dependent Ca^{2+} influx and thus the sensitivity to Ca^{2+}. However, the role of I_K in rat myocardial repolarization may be minimal. Studies in guinea pigs have demonstrated that estradiol treatment increases Ca^{2+}-dependent nitric oxide synthase levels in the heart (Weiner et al., 1994). Since NO has been reported by some investigators to elicit negative inotropic actions and inhibit voltage-dependent L-type Ca^{2+} current (I_{Ca}) (see Wyeth et al., 1996), an estradiol-mediated up-regulation of nitric oxide synthase should act to diminish, not enhance, contractile function in the female. Data have also suggested that estrogen acts to control the expression of L-type Ca^{2+} channels; however, available results are somewhat contradictory. Johnson et al. (1997) reported that cardiac myocytes from male mice with a disrupted estrogen receptor gene show increases in both dihydropyridine binding site density and I_{Ca}. In contrast, binding studies by Ishii et al. (1988) suggested that estrogen increases the expression of dihydropyridine binding sites in rat heart. An estrogen-mediated increase in I_{Ca} would enhance the response to Ca^{2+}. Finally, steady state mRNA levels for α- and β-myosin heavy chain and sarcomeric actin are greater in female than male rat heart (Rosenkranz-Weiss et al., 1994). Differences in myofibrillar protein content could affect contractility; however, further work is required to demonstrate if gender affects myofibrillar expression in a manner that affects the sensitivity to Ca^{2+}.

Acknowledgements

This work was supported in part by a grant from the American Heart Association, Arkansas Affiliate.

References

Adams, K.F., Vincent, L.M., McAllister, S.M., El-Ashmawy, H., Sheps, D.S., 1987. The influence of age and gender on left ventricular response to supine exercise in asymptomatic normal subjects. Am. Heart J. 113, 732–742.

Asgrimsson, H., Johannsson, M., Arnardottir, S.A., 1995. Excitation and contraction in atrial and ventricular myocardium of the guinea pig. Acta Physiol. Scand. 153, 133–141.

Berlin, J.R., 1995. Spatiotemporal changes of Ca^{2+} during electrically evoked contractions in atrial and ventricular cells. Am. J. Physiol. 269, H1165–H1170.

Beuckelmann, D.J., 1997. Contributions of Ca^{2+}-influx via the L-type Ca^{2+}-current and Ca^{2+}-release from the sarcoplasmic reticulum to $[Ca^{2+}]_i$-transients in human myocytes. Basic Res. Cardiol. 92 (1), 105–110, Suppl.

Capasso, J.M., Remily, R.N., Smith, R.H., Sonnenblick, E.H., 1983. Sex difference in myocardial contractility in the rat. Basic Res. Cardiol. 78, 156–171.

Daniels, S.R., Kimball, T.R., Morrison, J.A., Khoury, P., Meyer, R.A., 1995. Indexing left ventricular mass to account for differences in body size in children and adolescents without cardiovascular disease. Am. J. Cardiol. 76, 699–701.

Drici, M.D., Burklow, T.R., Haridasse, V., Glazer, R.I., Woosley, R.L., 1996. Sex hormones prolong the QT interval and down-regulate potassium channel expression in the rabbit heart. Circulation 94, 1471–1474.

Du, X.-J., Riemersma, R.A., Dart, A.M., 1995. Cardiovascular protection by oestrogen is partly mediated through modulation of autonomic nervous function. Cardiovasc. Res. 30, 161–165.

Ettinger, S.M., Silber, D.H., Collins, B.G., Gray, K.S., Sutliff, G., Whisler, S.K., McClain, J.M., Smith, M.B., Yang, Q.X., Sinoway, L.I., 1996. Influences of gender on sympathetic nerve responses to static exercise. J. Appl. Physiol. 80, 245–251.

Gotshall, R.W., Tsai, P.F., Frey, M.A., 1991. Gender-based differences in the cardiovascular response to standing. Aviat. Space Environ. Med. 62, 855–859.

Hanley, P.C., Zinsmeister, A.R., Clements, I.P., Bove, A.A., Brown, M.L., Gibbons, R.J., 1989. Gender-related differences in cardiac response to supine exercise assessed by radionuclide angiography. J. Am. Coll. Cardiol. 13, 624–629.

Hinderliter, A.L., Light, K.C., Willis, P.W., 1991. Left ventricular mass index and diastolic filling. Relation to BP and demographic variables in a healthy biracial sample. Am. J. Hypertens. 4, 579–585.

Huikuri, H.V., Pikkujämsä, S.M., Airaksinen, K.E.J., Ikäheimo, M.J., Rantala, A.O., Kauma, H., Lilja, M., Kesäniemi, Y.A., 1996. Sex-related differences in autonomic modulation of heart rate in middle-aged subjects. Circulation 94, 122–125.

Ishii, K., Kano, T., Ando, J., 1988. Sex differences in [^{3}H]nitrendipine binding and effects of sex steroid hormones in rat cardiac and cerebral membranes. Jpn. J. Pharmacol. 46, 117–125.

Johnson, B.D., Zheng, W., Korach, K.S., Scheuer, T., Catterall, W.A., Rubanyi, G.M., 1997. Increased expression of the cardiac L-type calcium channel in estrogen receptor-deficient mice. J. Gen. Physiol. 110, 135–140.

Jones, P.P., Spraul, M., Matt, K.S., Seals, D.R., Skinner, J.S., Ravussin, E., 1996. Gender does not influence sympathetic neural reactivity to stress in healthy humans. Am. J. Physiol. 270, H350–H357.

Kafiluddi, R., Kennedy, R.H., Seifen, E., 1989. Effects of buffer magnesium on positive inotropic agents in guinea pig cardiac muscle. Eur. J. Pharmacol. 165, 181–189.

Merz, C.N.B., Moriel, M., Rozanski, A., Klein, J., Berman, D.S., 1996. Gender-related differences in exercise ventricular function among healthy subjects and patients. Am. Heart J. 131, 704–709.

Morano, I., Bletz, C., Wojciechowski, R., Ruegg, J.C., 1991. Modulation of crossbridge kinetics by myosin isoenzymes in skinned human heart fibers. Circ. Res. 68, 614–618.

Rosenkranz-Weiss, P., Tomek, R.J., Mathew, J., Eghbali, M., 1994. Gender-specific differences in expression of mRNAs for functional and structural proteins in rat ventricular myocardium. J. Mol. Cell. Cardiol. 26, 261–270.

Ruch, S., Kennedy, R.H., Seifen, E., 1992. Aging: inotropic effects of Bay K-8644 and nifedipine on rat cardiac muscle. Eur. J. Pharmacol. 216, 393–400.

Ryan, S.M., Goldberger, A.L., Pincus, S.M., Mietus, J., Lipsitz, L.A., 1994. Gender- and age-related differences in heart rate dynamics: are women more complex than men?. J. Am. Coll. Cardiol. 24, 1700–1707.

Schaible, T.F., Malhotra, A., Ciambrone, G., Scheuer, J., 1984. The effects of gonadectomy on left ventricular function and cardiac contractile proteins in male and female rats. Circ. Res. 54, 38–49.

Stein, P.K., Kleiger, R.E., Rottman, J.N., 1997. Differing effects of age on heart rate variability in men and women. Am. J. Cardiol. 80, 302–305.

Tanaka, H., Takagi, N., Shigenobu, K., 1995. Difference in excitation–contraction mechanisms between atrial and ventricular myocardia of hatched chicks. Gen. Pharmacol. 26, 45–49.

Weiner, C.P., Lizasoain, I., Baylis, S.A., Knowles, R.G., Charles, I.G., Moncada, S., 1994. Induction of calcium-dependent nitric oxide syntheses by sex hormones. Proc. Natl. Acad. Sci. U.S.A. 91, 5212–5216.

Wong, N.D., Gardin, J.M., Kurosaki, T., Anton-Culver, H., Sidney, S., Roseman, J., Gidding, S., 1995. Echocardiographic left ventricular systolic function and volumes in young adults: distribution and factors influencing variability. Am. Heart J. 129, 571–577.

Wyeth, R.P., Temma, K., Seifen, E., Kennedy, R.H., 1996. Negative inotropic actions of nitric oxide require high doses in rat cardiac muscle. Pflug. Arch. 432, 678–684.