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Short communication

Sex differences in cardiac muscle responsiveness to Ca^{2+} and L-type Ca^{2+} channel modulation

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Abstract

This study investigated sex-related differences in rat papillary muscle force generation in response to altered extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$, 0.2 to 5.0 mM) and to L-type Ca^{2+} channel modulators (nifedipine and Bay K8644). At all $[Ca^{2+}]_o$ examined, contractile force was significantly greater in male than female papillary muscles. The $[Ca^{2+}]_o$ required for 50% maximum force was significantly lower in male [0.34±0.06 mM] than female [0.61±0.10 mM] papillary muscles. Nifedipine decreased contractile force in papillary muscles of both sexes in a concentration-dependent manner, but the extent of the contractile depression was more marked in male papillary muscles at all nifedipine concentrations examined. BayK 8644 produced a concentration-dependent increase in contractile force in male papillary muscles but notably, not in female papillary muscles. These findings show that sex differences in myocardial mechanical function are associated with sex-specific modulation of L-type Ca^{2+} channel responsiveness. Thus, the L-type Ca^{2+} channel could represent an important cellular locus from which sex-based differences in myocardial excitation–contraction coupling arise.

Keywords: Gender; Myocardial contractility; Papillary muscle; Calcium flux; Cardiovascular sex differences

1. Introduction

Sex differences in the incidence of cardiovascular disease are well documented, but poorly understood. There is mounting evidence of differences in myocardial function between male and female hearts under both physiological and pathophysiological circumstances (Czubryt et al., 2006; Du et al., 2006). Although limited information is currently available, some studies indicate that there are fundamental sex differences in cardiac myocyte Ca^{2+} handling. Curl et al. (2001) have shown that under comparable experimental conditions, both the peak and amplitude of the intracellular Ca^{2+} transient are higher in freshly isolated cardiac myocytes from male as compared to female rats. Sex-based differences in myocardial contractile reserve of feline cardiac muscle have also recently been reported with evidence presented that this was associated with sex differences in peak sarcoplasmic reticulum Ca^{2+} load and/or release during dynamic stress (Petre et al., 2007). In addition there are sex differences in age-related changes in excitation–contraction coupling in murine cardiac myocytes involving changes in both plasma membrane and sarcoplasmic reticulum Ca^{2+} fluxes (Grandy and Howlett, 2006).

One potential cellular site where sex-based differences in intracellular Ca^{2+} handling and contractility of cardiac muscle could arise is the L-type Ca^{2+} channel. There is evidence that both estrogen and testosterone can exert long-term influences on the cardiac L-type Ca^{2+} channel. Increased expression of the cardiac L-type Ca^{2+} channel has been demonstrated in estrogen receptor knockout mice (Johnson et al., 1997) and increased abundance of L-type Ca^{2+} channel protein is found in female rat heart after ovariectomy (Chu et al., 2006). Conversely, decreased L-type Ca^{2+} channel mRNA is observed in male rats following castration (Golden et al., 2003).

The goal of this study was to determine whether sex differences are evident in the contractile function of cardiac muscle under conditions where Ca^{2+} flux through the L-type

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Ca^{2+} channel is modulated. The results demonstrate, for the first time, that male and female myocardial tissues exhibit differential sensitivity to agents which pharmacologically target the L-type Ca^{2+} channel.

2. Methods

Male and female Wistar rats aged 3 months were housed separately and given free access to food and water. All experimental procedures complied with the guidelines of the National Health and Medical Research Council of Australia on the care and use of animals in research and had the approval of the Monash University Animal Ethics Committee.

2.1. Papillary muscle dissection

Rats were killed by decapitation under deep chloroform anaesthesia. The heart was rapidly excised and washed in Krebs–Henseleit solution with the following composition (mM): 118.0 NaCl, 4.8 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 24.8 $NaHCO_3$, 1.6 Ca^{2+}, 10.0 glucose. Hearts were retrogradely perfused via an aortic cannula with 20 ml of Krebs–Henseleit solution containing 30 mM BDM (2,3-butanedione monoxime) to arrest mechanical activity. The left ventricle was opened to expose the papillary muscles that were then left to bathe in the BDM solution for 20 min. A suitable papillary muscle was identified, tied at either end with 4/0 non-capillary silk thread and placed under tension using a stainless steel spring clamp before being excised from the heart. Preparations of diameter <0.8 mm were chosen to ensure optimal superfusion conditions (Delbridge and Loiselle, 1981). The papillary muscles from the male (n=26) and female (n=25) animals used in the study had mean lengths of 5.6±0.2 mm and 4.7±0.2 mm and cross-sectional areas of 0.85±0.06 mm^2 and 0.86±0.09 mm^2 respectively. Muscle wet weights (5.2±0.3 vs 4.3±0.4 mg) were not significantly different. Muscle length was measured when the muscle was mounted on the force recording apparatus at optimal length. After removal from the apparatus, muscle blotted wet weight was determined. Cross-sectional area (CSA) was calculated from the length and mass, assuming a uniform cross-sectional area and a muscle density of 1.06 g/cm^3 according to the equation: CSA. (mm^2)=mass(mg)/(length (mm)×density)×(1.06 g/cm^3). All forces are reported normalised for cross-sectional area.

2.2. Force recordings

Isolated muscles were mounted on a force recording apparatus and immersed in a temperature regulated tissue bath maintained at 32 °C. Each muscle was placed under a preload of 10 mN and left to equilibrate in Krebs–Henseleit solution containing 1.6 mM Ca^{2+} at a stimulation frequency of 0.2 Hz. for 1 h. At the conclusion of this equilibration period muscle length was set to be optimal for force development and all subsequent recordings were of isometric force at this length. Data were acquired using a MacLab data and recording acquisition system with Chart software (ADInstruments, NSW, Australia). The sampling rate during recording for analysis purposes was 200 Hz.

2.3. Protocols

Following the equilibration period muscles were placed in Krebs–Henseleit solution containing 0.2 mM Ca^{2+} and left to contract in this solution until a steady-state level of developed force was attained. Further Ca^{2+} was then cumulatively added up to a final concentration of 5.0 mM. Muscles were allowed to contract at each concentration until developed force was stabilised at the new steady-state. At the conclusion of this procedure the solution was replaced with fresh Krebs–Henseleit solution containing 1.6 mM Ca^{2+}. After muscles were equilibrated to this concentration of $[Ca^{2+}]_o$, nifedipine was added cumulatively to final concentrations of: 10^{-7}, 3×10^{-7}, 10^{-6}, 3×10^{-6}, 10^{-5} and 3×10^{-5} M with the muscle allowed to contract until a new steady-state was attained at each nifedipine concentration.

The effects of Bay K8644 (1,4 Dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-3-pyridine carboxylic acid methyl ester(±enantiomer)) were investigated in separate preparations using muscles not previously exposed to nifedipine. Following equilibration in Krebs–Henseleit solution containing 0.8 mM Ca^{2+} Bay K8644 was added cumulatively to the tissue bath to give final concentrations of: 10^{-7}, 3×10^{-7}, 10^{-6} and 3×10^{-6} M with force being allowed to attain a new steady-state at each concentration of Bay K8644. The $[Ca^{2+}]_o$ concentration was reduced to 0.8 mM in these experiments to allow for greater potential expression of a positive inotropic response to Bay K8644. Concentrations of Bay K8644 above 3×10^{-6} M were not used as they produced a substantial decrease in force in both male and female papillary muscles (data not shown). This may be attributed to the antagonist action of the (±) enantiomer of Bay K8644, which can predominate at high concentrations (Bellemann and Franckowiak, 1985). Both nifedipine and Bay K8644 were initially dissolved in dimethyl sulfoxide (DMSO) and the final DMSO concentration in the experiments did not exceed 0.3%.

2.4. Statistics

All data are presented as mean±standard error of the mean (S.E.M) and were analysed using a one-way ANOVA with repeated measures. EC50 values were determined by linear interpolation between data points as depicted in Figs. 1 and 2. An EC50 value was determined for each muscle data set, and from these values the mean EC50 was calculated.

3. Results

3.1. Effects of varying extracellular [Ca^{2+}] on contractile force

Fig. 1 illustrates original force recordings from a male (1A) and female (1B) papillary muscle exposed to different extracellular [Ca^{2+}]. As expected, contractile force increased with rising $[Ca^{2+}]_o$ in both male and female papillary muscles (P<0.01), the increase being steeper at the lower Ca^{2+} concentrations and attaining a plateau as higher $[Ca^{2+}]_o$ levels were applied (Fig. 1C). No differences in the time course of

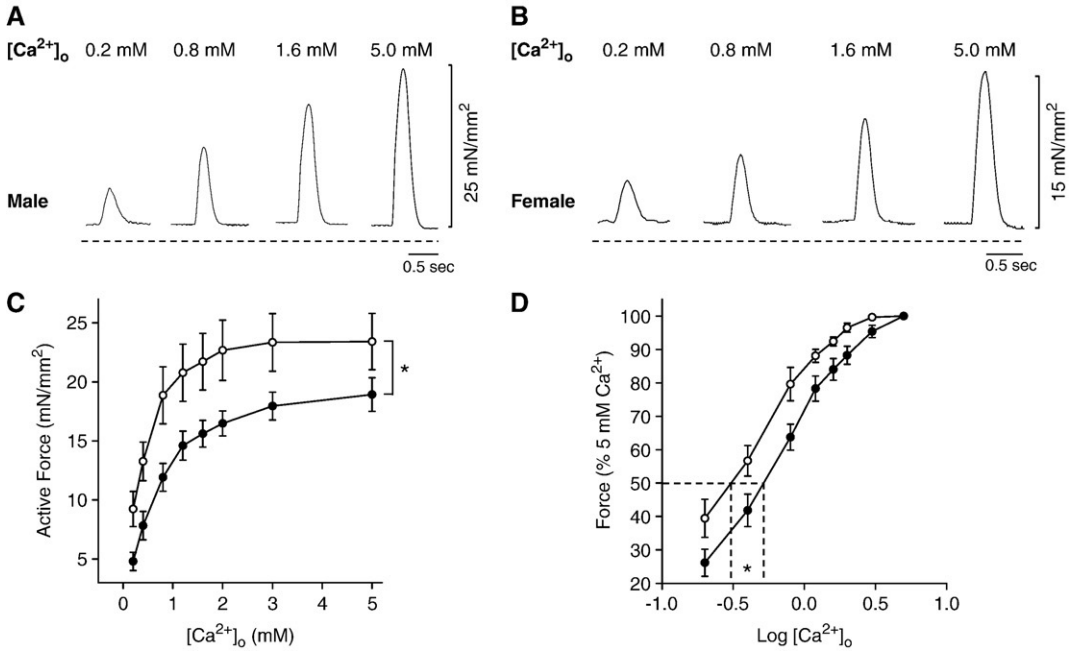

Fig. 1. Representative force records from male (A) and female (B) papillary muscles stimulated to contract at 0.2 Hz steady-state and exposed to different extracellular $[Ca^{2+}]$. Average developed force in male (○, $n=8$) and female (●, $n=9$) rat papillary muscles stimulated to contract at 0.2 Hz steady-state in varying extracellular $[Ca^{2+}]$. Panel C shows absolute force while in panel D force for each muscle has been expressed relative to that developed in the presence of 5.0 mM $[Ca^{2+}]_o$ by that muscle. Values are mean±S.E.M.

contraction were apparent between the sexes. Pilot experiments indicated that force did not significantly increase further in either male or female papillary muscles when $[Ca^{2+}]_o$ was raised above 5 mM. Over the range of $[Ca^{2+}]_o$ levels tested, mean force was significantly higher ($P<0.04$) in the male papillary muscles than the female papillary muscles. In Fig. 1D the relative force responses for each muscle are expressed as a percentage of maximum force developed by the muscle in the presence of 5 mM $[Ca^{2+}]_o$. At $[Ca^{2+}]_o$ below 5 mM the relative force developed by the female papillary muscles was significantly ($P<0.05$) less than that developed by the male muscles. The $[Ca^{2+}]_o$ required for 50% maximum force in female papillary muscles was almost double that required in male muscles (0.61±0.10 vs 0.34±0.06 mM; $P<0.05$, unpaired *t*-test), highlighting the greater apparent sensitivity to extracellular Ca^{2+} of the male muscles.

3.2. Effects of nifedipine and Bay K8644 on contractile force

Nifedipine significantly ($P<0.001$) decreased developed force in both male and female papillary muscles in a concentration-dependent manner (Fig. 2A). There was a significant sex difference ($P<0.02$) in the concentration-dependence of this decrease, with force proportionately decreasing to a greater extent at each nifedipine concentration in the female papillary muscles. The nifedipine concentration required to reduce force to 50% of control in female papillary muscles was less than half that required in male muscles (7.3±1.7 vs 18.4±2.7 μM; $P<0.05$). As shown in Fig. 2B, Bay K8644 produced a modest but significant ($P<0.001$) concentration-dependent increase in active force in male papillary muscles, but not in female papillary muscles ($P=0.690$). As a consequence the concentration-dependence of the effect of Bay K8644 on force development was also significantly different between male and female papillary muscles ($P<0.003$).

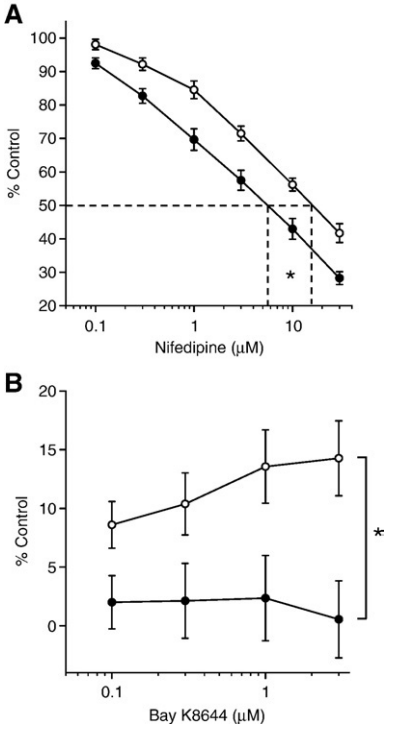

Fig. 2. Effects of increasing concentrations of (A) nifedipine (male ○, $n=8$; female ●, $n=10$) and (B) Bay K8644 (male ○, $n=9$; female ●, $n=11$) on rat papillary muscle developed force. For each muscle force has been expressed as a percentage of that developed in the absence of drug (control). Values are mean±S.E.M.

4. Discussion

It is becoming increasingly evident that several aspects of the cardiac phenotype show important pathophysiological differences between the sexes. In this context roles for both estrogen and testosterone in regulating myocardial Ca^{2+} handling, and consequently cardiac muscle contraction, have been suggested (Czubryt et al., 2006). The present study provides support for this proposition by demonstrating sex differences in the contractility of rat papillary muscles under conditions where Ca^{2+} flux through the L-type Ca^{2+} channel is modulated.

Under comparable experimental conditions, male papillary muscles in the present study consistently developed higher contractile force than female papillary muscles. No differences in the time course of contraction were apparent between the sexes. This coincides with some earlier reports of studies involving rat (Leblanc et al., 1998) and cat (Petre et al., 2007) tissues. In contrast, other investigators have found no significant difference in isometric force development in female compared to male papillary muscles (Wang et al., 1998; Chu et al., 2005). We have previously reported that in freshly isolated rat cardiac myocytes, the amplitude of the Ca^{2+} transient is significantly greater in myocytes of males compared with myocytes of females at the same $[Ca^{2+}]_o$ (Curl et al., 2001). Thus our current finding of higher force development in male cardiac muscle, relative to female, provides direct evidence of a functional mechanical outcome which reflects sex differences in underlying myocyte Ca^{2+} handling processes.

The sex differences in contractile force reported here, and those in the Ca^{2+} transient amplitude reported earlier, could both result from differences in Ca^{2+} entry through the L-type Ca^{2+} channel. The first direct evidence for long-term genomic modulation of the cardiac L-type Ca^{2+} channel by estrogen was provided by Johnson et al. (1997) who demonstrated increased Ca^{2+} channel ligand binding and Ca^{2+} channel current in estrogen receptor knockout mice. Patterson et al. (1998) subsequently showed that 17β-estradiol treatment decreased the number of nitrendipine binding sites in left ventricular membrane preparations from ovariectomised rabbits. More recently Kam et al. (2005) and Chu et al. (2006) reported increased L-type Ca^{2+} channel activity and protein levels respectively in ovariectomised rats when compared with sham and estrogen-replaced animals. We have also shown that the amplitude of the Ca^{2+} transient is greater in cardiac myocytes isolated from ovariectomised female rats compared to myocytes from sham or estrogen-replaced rats (Curl et al., 2003). Cell shortening was also augmented in myocytes from the ovariectomised animals.

There is also some evidence which indicates regulation of cardiac L-type Ca^{2+} channel expression by testosterone. In male rats, castration was reported to lead to decreased expression of the cardiac L-type Ca^{2+} channel and sodium–calcium exchanger which was in turn reflected in depressed contraction and relaxation in isolated myocytes (Golden et al., 2003). Increases in L-type Ca^{2+} channel mRNA and contractility have also been reported in cultured rat cardiac myocytes exposed to testosterone for 8–24 h (Golden et al., 2004, 2005).

These studies are suggestive of increased L-type Ca^{2+} channel expression in male cardiac muscle, relative to female. Our present finding of the greater mechanical sensitivity of male papillary muscles to $[Ca^{2+}]_o$ reported here is entirely consistent with this proposition. By comparing the effects of nifedipine and Bay K8644 on contractile force in male and female papillary muscles we have confirmed that the sex difference in the responsiveness to variations in $[Ca^{2+}]_o$ are likely modulated through the L-type Ca^{2+} channel. Papillary muscles from female rats were more sensitive to nifedipine than those from male rats. At equivalent concentrations of nifedipine force was reduced proportionately more in the female muscles and a significantly higher concentration of nifedipine was required to reduce force to 50% of the control level in male as compared to female papillary muscles. This would be expected if there was greater expression of the L-type Ca^{2+} channels in the male heart. A sex difference was also found in the response to Bay K8644, an agent known to increase influx of Ca^{2+} into the cardiomyocyte by prolonging the open time and promoting bursting activity of the L-type Ca^{2+} channel (Rampe et al., 1993). Whilst a significant positive inotropic action of Bay K8644 was observed in the male papillary muscles, it is particularly notable that no such response was detected in the female muscles. An increased number of targets (i.e., L-type Ca^{2+} channels) for Bay K8644 action in male cardiac muscle may facilitate an increased responsiveness to this Ca^{2+} channel agonist. This interpretation is supported by the finding that modulation of the sensitivity to Bay K8644 by estrogen has been reported in the rabbit where ovariectomy decreased the ED_{50} for the Bay K8644-induced positive inotropic response in papillary muscles (Patterson et al., 1998). This effect was reversed by estrogen replacement and was attributed to an estrogen-induced alteration in L-type Ca^{2+} channel expression.

The lower developed force observed in female papillary muscles compared with male could reflect decreased sarcoplasmic reticulum Ca^{2+} content and/or reduced Ca^{2+} release coincident with, or contingent on, a reduced Ca^{2+} entry through the L-type channel. A significant reduction in the rate of decay of the Ca^{2+} transient in female cardiac myocytes compared with male has been reported, indicative of a reduction in sarcoplasmic reticulum Ca^{2+}-ATPase activity (Curl et al., 2001; Leblanc et al., 1998). This is consistent with the finding that the isolated sarcoplasmic reticulum Ca^{2+}-ATPase activity tends to be lower in membrane preparations from female rats. (Penpargkul et al., 1981). In contrast, the myocardial SERCA2a mRNA levels have been reported to be similar for male and female rats (Tappia et al., 2007). Notwithstanding a possible difference in sarcoplasmic reticulum Ca^{2+} uptake in male and female myocytes, no sex difference in the sarcoplasmic reticulum Ca^{2+} content as assessed by caffeine contracture can be detected (Curl et al., 2001; Grandy and Howlett 2006). Paradoxically, an increased ryanodine receptor mRNA level in females has been reported (Tappia et al., 2007) — a finding which is not consistent with the diminished force production measured in female myocardium in the present study. Thus, while this study clearly establishes that the L-type Ca^{2+} channel is important in contributing to the sex differences in myocardial contractility, further work is required to resolve how sex influences the complex interactions between the various Ca^{2+} transport processes involved in regulating excitation–contraction coupling.

In summary, the present study provides the first direct experimental evidence that sex differences in myocardial mechanical function are associated with sex-specific modulation of L-type Ca^{2+} channel responsiveness. In the context of other evidence indicating that the expression of the cardiac L-type Ca^{2+} channel is regulated by endogenous sex hormone levels, our findings support the conclusion that the L-type Ca^{2+} channel could represent an important cellular locus from which sex-based differences in myocardial excitation–contraction coupling arise. These findings may have potential implications in the usage of L-type Ca^{2+} channel blockers in cardiac disease conditions in male and females.

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References

Bellemann, P., Franckowiak, G., 1985. Different receptor affinities of the enantiomers of BAY K 8644, a dihydropyridine Ca channel activator. Eur. J. Pharmacol. 118, 187–188.

Chu, S.H., Sutherland, K., Beck, J., Kowalski, J., Goldspink, P., Schwertz, D.W., 2005. Sex differences in expression of calcium-handling proteins and b-adrenergic receptors in rat heart ventricle. Life Sci. 76, 2735–2749.

Chu, S.H., Goldspink, P., Kowalski, J., Beck, J., Schwertz, D.W., 2006. Effect of estrogen on calcium-handling proteins, b-adrenergic receptors, and function in rat heart. Life Sci. 79, 1257–1267.

Curl, C.L., Wendt, I.R., Kotsanas, G., 2001. Effects of gender on intracellular [Ca^{2+}] in rat cardiac myocytes. Pflugers Arch. 441, 709–716.

Curl, C.L., Wendt, I.R., Canny, B.J., Kotsanas, G., 2003. Effects of ovariectomy and 17b-estradiol replacement on [Ca2+]i in female rat cardiac myocytes. Clin. Exp. Pharmacol. Physiol. 30 (7), 489–494.

Czubryt, M.P., Espira, L., Lamoureux, L., Abrenica, B., 2006. The role of sex in cardiac function and disease. Can. J. Physiol. Pharmacol. 84 (1), 93–109.

Delbridge, L.M., Loiselle, D.S., 1981. An ultrastructural investigation into the size dependency of contractility of isolated cardiac muscle. Cardiovasc. Res. 15, 21–27.

Du, X.-J., Fang, L., Kiriazis, H., 2006. Sex dimorphism in cardiac pathophysiology: experimental findings, hormonal mechanisms, and molecular mechanisms. Pharmacol. Ther. 111, 434–475.

Golden, K.L., Marsh, J.D., Jiang, Y., Brown, T., Moulden, J., 2003. Gonadectomy of adult male rats reduces contractility of isolated cardiac myocytes. Am. J. Physiol. 285 (3), E449–E453.

Golden, K.L., Marsh, J.D., Jiang, Y., 2004. Testosterone regulates mRNA levels of calcium regulatory proteins in cardiac myocytes. Horm. Metab. Res. 36 (4), 197–202.

Golden, K.L., Marsh, J.D., Jiang, Y., Moulden, J., 2005. Acute actions of testosterone on contractile function of isolated rat ventricular myocytes. Eur. J. Endocrinol. 152 (3), 479–483.

Grandy, S.A., Howlett, S.E., 2006. Cardiac excitation–contraction coupling is altered in myocytes from aged male mice but not in cells from aged female mice. Am. J. Physiol. 291, H2362–H2370.

Johnson, B.D., Zheng, W., Korach, K.S., Scheur, 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.

Kam, K.W.L., Kravtsov, G.M., Liu, J., Wong, T.M., 2005. Increased PKA activity and its influence on isoprenaline-stimulated L-type Ca^{2+} channels in the heart from ovariectomized rats. Br. J. Pharmacol. 144, 972–981.

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.

Patterson, E., Ma, L., Szabo, B., Robinson, C.P., Thadani, U., 1998. Ovariectomy and estrogen-induced alterations in myocardial contractility in female rabbits: role of the L-type calcium channel. J. Pharmacol. Exp. Ther. 284, 586–591.

Penpargkul, S., Fein, F., Sonnenblick, E.H., Scheuer, J., 1981. Depressed cardiac sarcoplasmic reticular function from diabetic rats. J. Mol. Cell. Cardiol. 13, 303–309.

Petre, R.E., Quaile, M.P., Rossman, E.I., Ratcliffe, S.J., Bailey, B.A., Houser, S.R., Margulies, K.B., 2007. Sex-based differences in myocardial contractile reserve. Am. J. Physiol. 292, R810–R818.

Rampe, D., Anderson, B., Rapien-Pryor, V., Li, T., Dage, R.C., 1993. Comparison of the in vitro and in vivo cardiovascular effects of two structurally distinct Ca^{2+} channel activators, Bay K8644 and FPL 64176. J. Pharmacol. Exp. Ther. 265, 1125–1130.

Tappia, P.S., Dent, M.R., Aroutiounova, N., Babick, A.P., Weiler, H., 2007. Gender differences in the modulation of cardiac gene expression by dietary conjugated linoleic acid isomers. Can. J. Physiol. Pharmacol. 85, 465–475.

Wang, S.N., Wyeth, R.P., Kennedy, R.H., 1998. Effects of gender on the sensitivity of rat cardiac muscle to extracellular Ca^{2+}. Eur. J. Pharmacol. 361, 73–77.