ORIGINAL ARTICLE

Distinct lethal arrhythmias susceptibility is associated with sex-related difference in myocardial connexin-43 expression

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Abstract

OBJECTIVES: To elucidate gender-related differences in occurrence of sudden cardiac death the myocardial connexin-43 (Cx43) and the susceptibility of male and female rat hearts to ventricular fibrillation (VF) were investigated.

METHODS AND RESULTS: Ventricular tissues taken from male and female normotensive Wistar and spontaneously hypertensive (SHR) rats were processed for immuno-fluorescence and immuno-blotting of Cx43. Susceptibility to ventricular fibrillation was examined in isolated heart preparation using either electrical stimulation or low K^+ perfusion. Results showed that VF susceptibility of male either normotensive or hypertensive rats was significantly increased comparing to female counterparts. In correlation, ventricular expression of Cx43 was markedly lower in males of both normotensive and hypertensive rats comparing to females. SHR in addition exhibited abnormal myocardial Cx43 distribution due to structural remodelling.

CONCLUSIONS: Findings indicate that higher level of myocardial Cx43 expression is linked with lower lethal arrhythmia susceptibility and vice versa. It appears that Cx43 can be involved in sex-related differences in incidence of life-threatening arrhythmias.

INTRODUCTION

Cardiovascular diseases as well as cardiac arrhythmias occur with different frequency and severity in man and woman [1,18]. These gender-specific differences are thought to be mediated by sex hormones (Pelzer 1996) since incidence of disease and/or arrhythmia rises after the menopausal transition [8] or ovariectomy [17]. Distinct gender hormones may explain, at least in part, some disparities in cardiac electrophysiology [15] that is crucial in the development of lethal arrhythmias such as ventricular fibrillation (VF). The general classification of cardiac arrhythmias assumed that all rhythm disturbances result from one of two primary abnormalities in electrical activity [14]. The first is an abnormality in impulse initiation and the second, an abnormality in impulse propagation, whereby both may co-exist. The former is associated particularly with triggered activity and/or abnormal automaticity, whereas the latter with block of conduction and re-entry, which facilitate occurrence of VF.

It is well established that intercellular coupling and direct communication mediated by gap junction connexin (Cx) channels ensure electrical impulse propagation and myocardial synchronisation [4]. We as well as others [14] have previously shown that disease- or noxious event-induced alterations in Cx expression and/or distribution affect cell-to-cell coupling and contribute to abnormal conduction facilitating the occurrence of re-entrant arrhythmias, as it is VF. In this respect, it is of interest to know whether there are some gender-related differences in myocardial Cx [5]. This study was, therefore, undertaken to compare Cx43 expression, a major cardiac gap junction channel protein, in the male and female rat heart in respect to its susceptibility to lethal arrhythmias.

MATERIAL AND METHODS

All animal experiments were performed in accordance with the rules issued by the State Veterinary Administration of the Slovak Republic, legislation No 289/2003 and with the regulations of the Animal Research and Care Committee of the Institute of experimental pharmacology. The experiments were conducted on aged (>1-year-old) Wistar and SHR male and female rats (n=10 per group). Hearts excised under anesthesia were perfused via cannulated aorta with an oxygenated Krebs-Henseleit (K-H) solution at the constant pressure (85 mmHg) and temperature. ECG, the left ventricular pressure and the coronary flow were continuously monitored. Upon 20-min stabilization, the occurrence of sustained VF was examined either by perfusion of the heart with K^+ deficient K-H solution (Wistar group) or by electrical stimulation (SHR group). Ventricular tissues excised from intact rat hearts were immediately frozen in liquid nitrogen and processed either for either western-blotting of Cx43 or immuno-fluorescence of Cx43 using mouse monoclonal anti-Cx43 antibody (Chemicon Inc., USA). Goat anti-mouse secondary antibody conjugated with fluorescein isotiocyanate (FITC) was used for *in situ* myocardial Cx43 labelling, while alkaline phosphatase was used for visualisation of western blots. Control of the amount of loading protein was performed via labelling of gel upon electrophoresis with Coomassie blue. Thereafter quantification of immuno-labelled Cx43 was performed as previously described [7,13]. Results are given as means ± SEM and significances were ascertained with Student's t-test when p values <0.05.

RESULTS

As it is shown in Figure 1, *in situ* immunolabeling of Cx43 revealed end-to-end type (intercalated disc-related) Cx43-positive gap junctions in both normotensive and hypertensive rat heart ventricles. Besides, enhanced expression of side-to-side type (lateral) gap junctions was found in hypetrophied hearts of SHR. Western blotting of Cx43 demonstrated two forms of Cx43, i.e. phosphorylated and nonphosphorylated, in both Wistar and SHR (Figure 2.). The expression of Cx43 was significantly higher in females comparing to males regardles the rat strain, as evaluated by quantitative analysis of immunofluorescence images (Figure 3, upper panel) and immunoblots (Figure 4, upper panel).

Incidence of low potassium-induced sustained VF was elicited significantly after longer period in female comparing to male Wistar rat hearts (9.55±0.55 min vs. 6.78±0.28 min; Figure 3 bottom). Likewise the VF incidence in female SHR was significantly decreased compared to male counterparts, as indicated induction of sustained VF elicited by programmed stimulation, i.e. 20% vs 80% (Figure 4, bottom).

DISCUSSION

Differential responsiveness of male and female hearts to various pathophysiological stimuli has been established in previous studies by others [1,2,10,11] showing female advantages. In this study we demonstrated for the first time that the female heart of either healthy or hypertensive rats is less prone to lethal VF when compared to males. Likewise rabbit males with left ventricular hypertrophy were reported to be much susceptible to ventricular arrhythmias than females [3]. These findings fit well with Framingham population analysis revealing significantly higher incidence of sudden cardiac death (presumably due to VF) in man [18]. In addition, man were found to have higher risk for developing atrial fibrillation (the most common arrhythmia seen in clinical practice today) compared to woman. It should be noted, however, that in specific cases (e.g. long QT syndrom) woman arrhythmia prevalence can be observed [18].

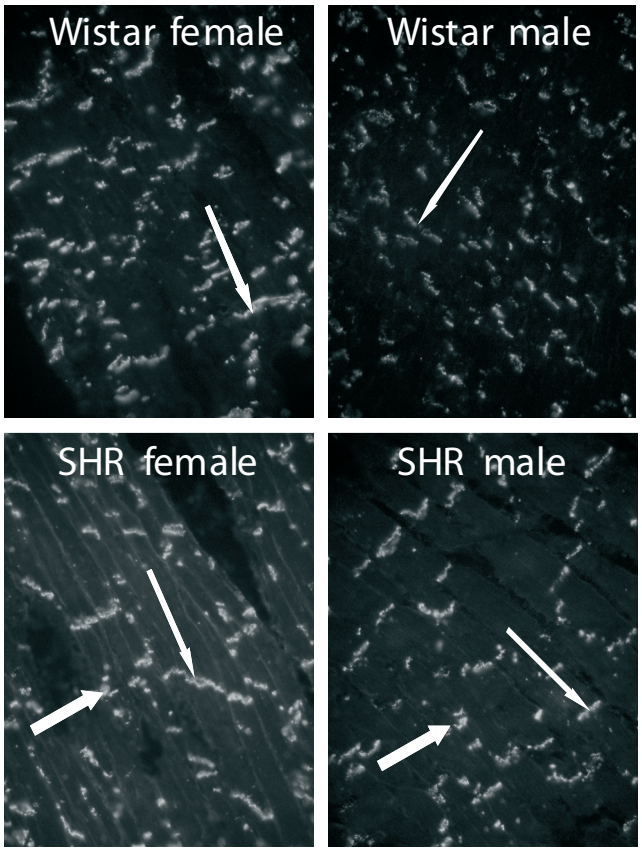

Figure 1. Immunofluorecence of Cx43 in female and male non-hypertensive (Wistar) and hypertensive (SHR) rat heart ventricles. Note Cx43 immunopositive intercalated disc-related gap junctions (thin arrows) in both strains and enhanced Cx43 immunopositivity in laterally located gap junctions (thick arrows) in SHR. Immunofluorescence signal is stronger in female compared to male counterparts. Objective 40×.

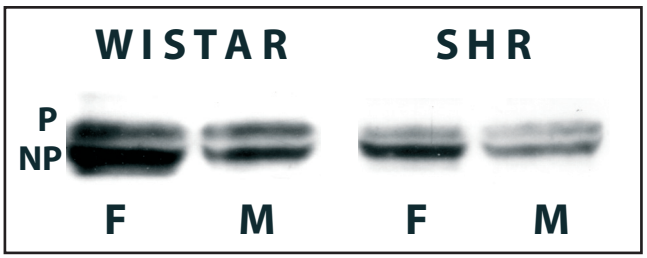

Figure 2. Representative immunoblots of Cx43 in female and male Wistar and SHR heart ventricles showing phosphorylated (P) and non-phosphorylated forms of Cx43. Note increased expression of Cx43 in female normotensive as well as hypertensive rat hearts, despite Cx43 is down-regulated in the latter.

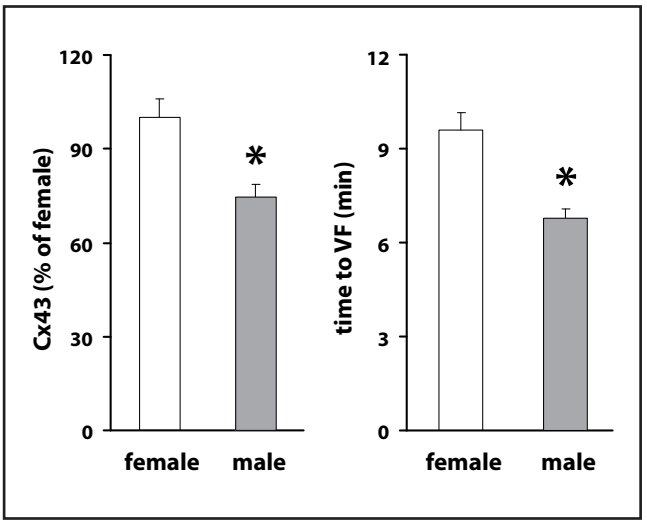

Figure 3. Myocardial Cx43 expression (upper panel) and susceptibility of the heart to ventricular fibrillation (bottom) in female and male normotensive Wistar rat hearts. Note sex-related differences in Cx43 expression, whereby its decrease in male correlates with shorter time to induce VF compared to female. *p<0.05.

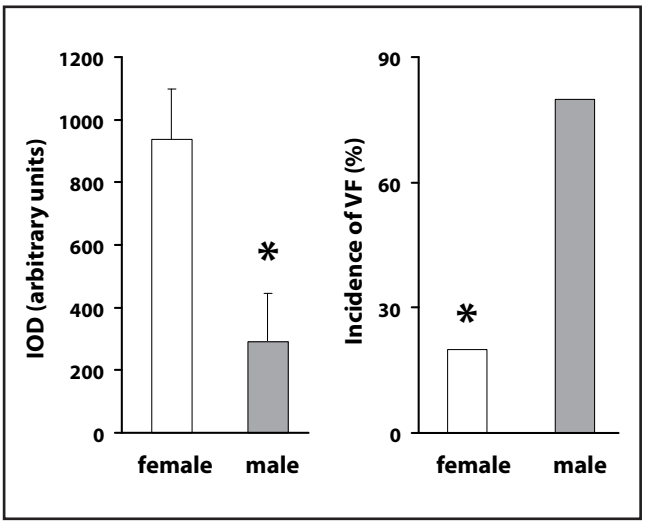

Figure 4. Myocardial Cx43 expression (upper panel) and susceptibility of the heart to ventricular fibrillation (bottom) in female and male hypertensive rat hearts. Note sex-related differences in Cx43 expression, whereby its decrease correlates with higher incidence of VF. *p<0.05.

Basic electrophysiology points out sex-related differences in the expression and/or function of ion channels or ion transporters [3,16] likely due to differences in gender hormones [8]. The heart is a target organ for oestrogen action [8,9] and Cx43 gene was responsive to oestrogen [19]. It can results in modulation of cardiac phenotype [10]. Indeed, we have found the sex-related differences in the expression of intercellular channel protein, Cx43 in favour of females. Interestingly, the expression of total Cx43 was higher not only in healthy but also hypertensive female rat hearts. Whereby, the down-regulation of Cx43 in male rat hearts was linked with increased susceptibility to electrically (Figure 4) or low K^+-induced VF [12]. The phosphorylating status of Cx43 was also enhanced in female normotensive [13] as well as hypertensive rats compared to males (Figure 2). It indicates sex-related differences in posttranslational processes, while the higher expression of mRNA for

Cx43 in females points out the differences in gene transcription [10]. Altogether the data strongly indicate modulation of Cx43 gene expression by oestrogen.

The cell-to-cell communication, i.e. electrical and metabolic signal propagation, ensured by gap junction connexin channels is crucial for the myocardial tissue homeostasis and synchronisation. Our findings suggest that higher level and/or up-regulation of Cx43, major cardiac gap junction protein, can support electrical and functional stability of the heart and consequently to decrease its vulnerability to lethal arrhythmias.

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REFERENCES

1 Barrett-Connor E. (1997). Sex differences in coronary heart disease. Circulation **95:** 252–264.
2 Besik J, Szaroszoi O, Kunes J, Netuka I, Maly J, Kolar F, Pirk J, Ostadal B. (2007). Tolerance to acute ischemia in adult male and female spontaneously hypertensive rats. Physiol. Res **56:**267–274.
3 Biagetti MO, Quinteiro RA. (2006).Gender differences in elctrical remodeling and susceptibility to ventricular arrhythmias in rabbits with left ventricular hypertrophy. Heart Rhythm **3:** 840–1.
4 Dhein S. (1998). Gap junction channels in cardiovascular system: pharmacological and physiological modulation. TiPS **19:** 229–241.
5 Knezl V, Drimal J, Fialova M, Dlugosova K, Okruhlicova L, Tribulova N. (2007). Connexin-43 expression is increased in female compared to male rat hearts and correlate with lethal arrhythmias susceptibility. Acta Physiol **191:** Suppl 658**,** 88 Abstract.
6 Lee T, Lin M, Chou T, Tsai CH, Chang N. (2004). Adjunctive 17 beta-estradiol administartion reduces infarct size by altered expression of canine myocardial connexin-43 protein. Cardiovasc Res **63:** 109–117.
7 Mitašíková M, Smidová S, Macsaliová A, Knezl V, Dlugošová K, Okruhlicová L, Weismann P, Tribulová N. (2008). Aged male and female spontaneously hypertensive rats benefit from n-3 polyunsaturated fatty acids supplementation. Physiol Res. [Epub ahead of print].
8 Mendelsohn ME, Karas RH. (2005). Molecular and cellular basis of cardiovasular gender differences. Science **308:** 1583–1587.
9 Pelzer T, Shamim A, Neyses L. (1996). Estrogen effects in the heart. Mol Cell Biochem **160–161:** 307–313.
10 Rosenkranz-Weiss P., Tomek R.J., Mathew J., Eghbal 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.
11 Schwanke ML, Dutta K, Podolin DA, Davidoff AJ. (2006). Cardiomyocyte dysfunction in insulin-resistant rats: a female advantage. Diabetologia **49:** 1097–1105.
12 Tribulova **N,** Okruhlicova L, Novakova S, Pancza D, Bernatova I, Pechanova O, Weismann P, Manoach M, Seki S, Mochizuki S. (2002). Hypertension-related intermyocyte junction remodeling is associated with a higher incidence of low-K(+)-induced lethal arrhythmias in isolated rat heart. Exp Physiol **87:** 195–205.
13 Tribulova N, Dupont E, Soukup T, Okruhlicova L, Severs NJ. (2005). Sex differences in connexin-43 expression in left ventricles of aging rats. Physiol. Research **54**: 705–708.
14 Tribulova N, Knezl V, Okruhlicova L, Slezák J. (2008). Myocardial gap junctions: targets for novel approaches in the prevention of life-threatening cardiac arrhythmias. Physiol Res [Epub ahead of print].
15 Verkerk AO, Wilders R, Veldkamp MW, deGringel W, Kirkels JH, Tan HL. (2005). Gender disparities in cardiac cellular electrophysiology and arrhythmia susceptibility in human failing ventricular myocytes. Int Heart J **46:** 1105–1117.
16 Vlkovicova J, Javorkova V, Pechanova O, Vrbjar N. (2005). Gender difference in functional properties of Na,K-ATPase in the heart of spontaneously hypertensive rats. Life Sci **76:** 971–982.
17 Wattanapermpool J, Reiser P. (1999). Differential effects of ovariectomy on calcium activation of cardiac and soleus myofilamnets. Am J Physiol **277:** H467–H473.
18 Yarnoz MJ, Curtis AB. (2008). More reasons why man and woman are not the same (Gender differences in electrophysiology and arrhythmias). A J Cardiol **101:** 1291–1296.
19 Yu W, Dahl G, Werner R. (1994). The connexin43 gene is responsive to oestrogen. Proc Biol Sci **255:** 125–132.