Biochemical and Biophysical Research Communications 406 (2011) 117–122

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Sex differences in expression and subcellular localization of heart rhythm determinant proteins

N.M. Thomas [a,*], J.F. Jasmin [b], M.P. Lisanti [b], D.A. Iacobas [a]

[a] Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Ave., Kennedy Center, New York, NY 10461, USA
[b] Thomas Jefferson University, Department of Stem Cell Biology and Regenerative Medicine, Bluemle Building, 233 South 10th St., Philadelphia, PA 19107, USA

ARTICLE INFO

Article history:
Received 18 January 2011
Available online 4 February 2011

Keywords:
Heart
Sex
Arrhythmia
Connexin
Caveolin

ABSTRACT

To evaluate sex differences in protein expression in the heart, we performed Western blot studies on a subset of Heart Rhythm Determinant (HRD) proteins. We examined key components of a variety of types of mechanical and electrical junctions including, connexin43, plakophilin-2, N-cadherin and plakoglobin, ankyrin-2 and actin. We describe novel findings in sex differences in cardiac protein expression and membrane localization. For most proteins examined, sex differences were significantly more pronounced in the membrane compartment than in overall expression. These studies extend our previous findings in microarray studies to demonstrate that sex differences in gene expression are likely to confer distinct functional properties on male and female myocardium.

1. Introduction

Significant differences exist between the sexes both in healthy cardiac function and in the prevalence and presentation of arrhythmia [1]. In physiological conditions, women display faster resting heart rates [2,3], longer rate-corrected QT intervals [4,5], and numerous other sex-associated electrophysiological differences [1]. Moreover, these properties may be altered during specific stages of the menstrual cycle [6]. In pathophysiological conditions, female sex appears cardioprotective. Malignant ventricular arrhythmias and sudden cardiac death are more common in men [7,8], as is atrial fibrillation, the most common sustained arrhythmia reported in clinical medicine [9,10]. Nonetheless, sick sinus syndrome, paroxysmal supraventricular tachycardia and arrhythmic events in the long-QT syndrome are more frequently observed in women [11]. For example, women are significantly more susceptible to drug-induced QT prolongation and Torsades de Pointes [12–14]. Apart from these observations, however, we have little mechanistic understanding of the molecular foundations that underlie sex differences in cardiac function and dysfunction.

To elucidate the molecular mechanisms that determine sex dichotomy, we and others have performed systematic microarray analyses of sexual dimorphism in gene expression in the healthy and failing heart [15–19]. As well as abundant differences in expression of genes located on the sex chromosomes, the expression levels of many autosomal genes were also found to differ substantially between the sexes. Genes encoding various adrenergic receptors, ion channels and transporters, and structural components of the intercalated discs form complex, intercoordinated sex-specific networks [19]. Despite these observations, little is known about sex differences in protein expression in the heart.

In our previous study, we profiled the ventricular transcriptomes of male and female adult mice with special emphasis on a defined cohort of Heart Rhythm Determinant (HRD) genes [19]. HRD genes have a demonstrated association with arrhythmia, either through altered function after mutation or in response to different expression patterns. In the present study, we performed quantitative Western blot studies of a small subset of HRD in order to evaluate sex differences in protein expression. The HRD cohort almost exclusively consists of membrane proteins and their binding partners in junctional complexes. Membrane pools of junctional proteins, including those that compose gap junctions, desmosomes, adherens junctions and tight junctions, can be biochemically distinguished from non-junctional, cytosolic pools using non-ionic detergents such as Triton X-100 [20,21]. In our present study, we also applied this method of subcellular fractionation to examine whether differences in HRD expression were reproduced in membrane compartments where they are more likely to have a functional role. Consonant with our findings in microarray experiments, we found that a number of HRD proteins display significant sex differences which are enhanced in functionally relevant subcellular compartments.

2. Materials and methods

2.1. Animals

Wildtype C57Bl/6J mice used in this study were purchased from Charles River Laboratories International, Inc. (Wilmington, MA,

* Corresponding author. Fax: +1 718 430 8594.
E-mail address: neil.thomas@einstein.yu.edu (N.M. Thomas).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.02.006

USA). C57Bl/6J mice heterozygous for caveolin-3 ($Cav3^{+/-}$) mice were kindly gifted by the laboratory of Dr. Michael P. Lisanti (Thomas Jefferson University, PA, USA). The experimental protocol was approved by the AECOM Institutional Animal Care and Use Committee (IACUC). We have followed the IACUC program for prevention of disease, daily observation and surveillance for assessment of animal health, and the methods of animal handling, restraint, anesthesia, and analgesia. Animal euthanasia respected the guidelines of the Panel on Euthanasia of the American Veterinary Medical Association. For microscopy studies, cell cultures were prepared from neonatal animals sacrificed on post-natal day 1. For Western blot studies of wildtype expression, male ($n = 8$) and female ($n = 8$ at diestrus and $n = 8$ at estrus) animals were sacrificed at 8 weeks of age. For studies of expression in transgenic animals, a separate set of male and female wildtype mice were selected ($n = 4$) for comparison with caveolin-3 knockdown animals ($n = 4$). To assess estrous cycle in females, vaginal lavage was performed daily and vaginal cytology examined microscopically [22].

2.2. Antibodies

Primary antibodies used for the studies described below included those raised against actin (AAN01-A; Cytoskeleton, Inc., CO, USA); connexin40, junction plakoglobin and N-cadherin (SC-20466; SC-7939; SC-7900; Santa Cruz Biotechnologies, CA, USA); caveolin-3 (610420; BD Transduction Laboratories, USA); connexin43 (C6219; Sigma, MO, USA); connexin45 (40-7000; Invitrogen, CA, USA); and, plakophilin-2 (K44262M, Meridian Life Science, Inc., ME, USA).

2.3. Specimen preparation for immunolabeling and microscopy studies

Cultures of mouse neonatal atrial cardiomyocytes were prepared on 12 mm glass coverslips as described previously [23]. In order to test *in situ* whether membrane complexes co-localizing specific HRD proteins were resistant to Triton X-100 solubilization, live cultures were treated according to the original protocol communicated by Musil and Goodenough [20] with minor modifications. Immunolabeling and epifluorescence microscopy were performed as described previously [24]. For confocal analysis, sections were examined using an Olympus Fluoview 500 Laser Confocal Microscope and Fluoview Software (Olympus America, Inc., PA, USA). Figures were prepared using ImageJ Software (National Institutes of Health, MD, USA).

2.4. Sample preparation for Western blot studies

In order to quantify separately proteins localized in membrane junctional structures from proteins localized elsewhere in cells, differential solubilization of snap-frozen, mouse ventricular tissue was performed using Triton X-100 [20,25,26]. Protein separation and subsequent Western blotting procedures were performed as described previously [27]. Bands obtained for immunolabeled proteins were normalized to Ponceau S stainings of total protein transferred to Western blot membranes to avoid artifacts due possible intrinsic sex differences in conventional housekeeping proteins.

2.5. Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc., CA, USA). Data are expressed as mean ± SD. Statistical significance was evaluated by two-way ANOVA. Statistical differences were judged significant at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

3. Results

Our initial studies investigated the extent to which the Triton X-100 differential solubilization protocol used by Musil and Goodenough [20] could distinguish between membrane and cytosol-localized connexin43. As illustrated in Fig. 1A, when cultured cardiac myocytes were immunolabeled for connexin43, abundant gap-junctional signal was visible at the interfaces between apposed cells (arrows) as-well-as profuse non-junctional signal apparently labeling intracellular connexin43 (asterisk). Orthogonal sections (Fig. 1C and D; planes indicated by arrow indents), confirmed the intracellular location of this connexin43 signal. Treatment of live cultures of cardiac myocytes with Triton X-100 prior to fixation dramatically changed the intracellular localization pattern of connexin43. As shown in Fig. 1B, a pattern of gap-junctional plaque labeling similar to that observed in untreated cells was detected between abutting cells incubated with Triton X-100 (arrows). In striking contrast, however, neither cytosolic nor perinuclear label for connexin43 was detected in *en face* or orthogonal sections, indicating complete removal of non-junctional connexin43 protein from Triton X-100 treated myocytes. Extension of these studies to other HRD membrane proteins such as connexin40, connexin45 and plakophilin-2 revealed similar results (see Supplementary Fig. 1). These results demonstrated that membrane-localized pools of certain junctional proteins were resistant to solubilization by Triton X-100, and offered a potential procedure whereby this subcellular compartment could be differentiated from intracellular pools.

The next step was to determine whether this technique could be applied to Western blot studies. To differentiate between subcellular pools, neonatal mouse heart tissue samples were lysed using Triton X-100, and detergent soluble and insoluble material was separated by centrifugation. The upper immunoblot in Fig. 1E shows a Western blot membrane illustrating connexin43 immunolabeling in each fraction. In Western blot studies, connexin43 typically presents as 1–3 bands which migrate within a range of 40–47 kDa according to the degree of phosphorylation of the protein [28]. A hallmark of membrane-localized, gap-junctional connexin43 is that it exclusively comprises the phosphorylated isotypes [20]. As highlighted by the magnified bands in the lower panels in Fig. 1E, connexin43 in total tissue lysates comprised three bands, representing phosphorylated (P1 and P2) and unphosphorylated (P0) protein. Bands obtained in Triton X-100 soluble fractions, taken to represent non-junctional, cytosolic connexin43 displayed a similar migration pattern. However, bands obtained in Triton X-100 insoluble fractions, presumed to represent gap-junctional connexin43, were the phosphorylated P1 and P2 isotypes alone. No unphosphorylated connexin43 was detectable. Thus, these results confirmed earlier findings by the Musil and Goodenough [20], and our immunoconfocal results, that membrane-localized, junctional connexin43 was resistant to Triton X-100 solubilization, and could be distinguished from cytosolic connexin43 due to this property.

The final stage to validate this technique was to assess the quantitative potential of Western blot data. As shown in Fig. 1E, insoluble and soluble fractions were immunoblotted alongside total lysate samples. In order to be truly quantitative, the sum signal of insoluble and soluble bands should match closely signal obtained for total bands. The bar chart in Fig. 1F proved this to be the case. Scanning densitometry of Western blot bands revealed that the difference between "total" and "insoluble + soluble" was approximately 10% and statistically insignificant. Taken together, these data confirm the validity of this technique for quantitative Western blot studies.

Having confirmed the efficacy of differential Triton X-100 solubilization of different subcellular pools of HRD proteins, we used

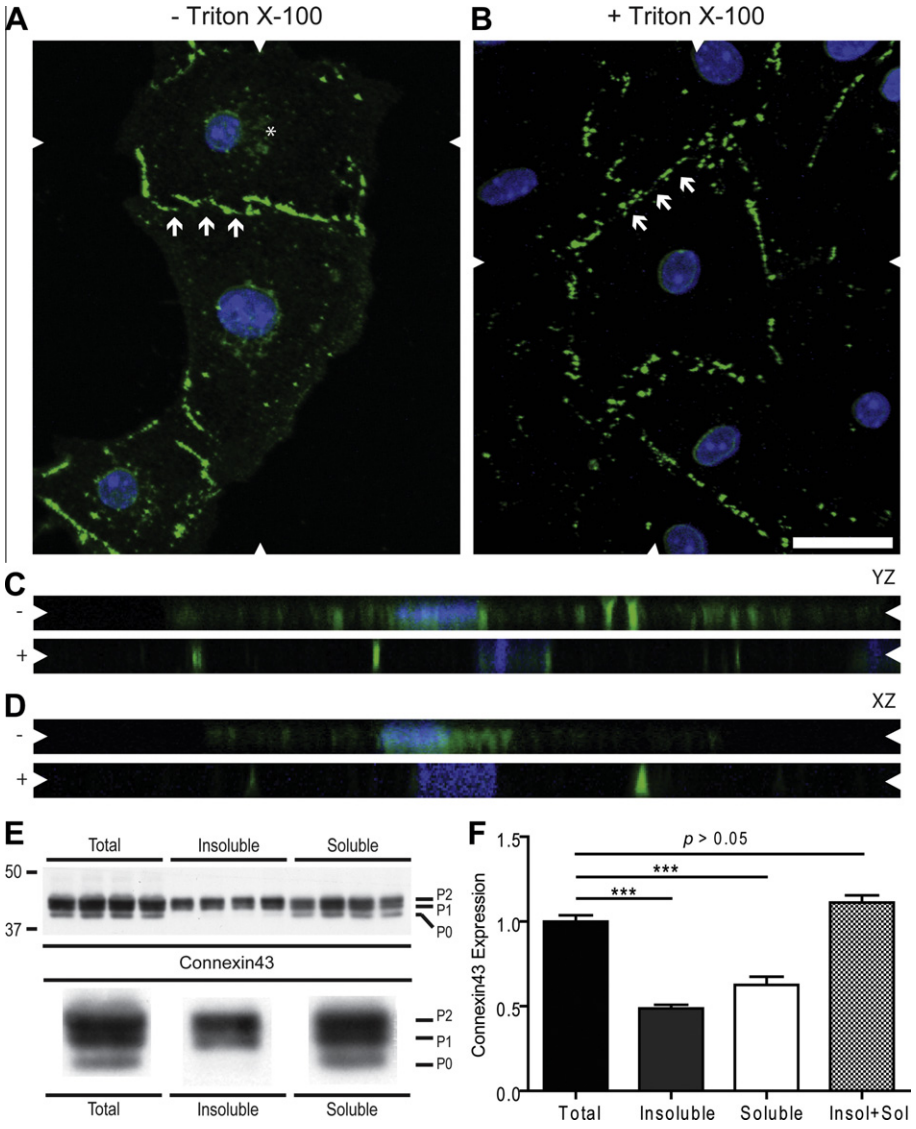

Fig. 1. Differential solubilization of junctional and non-junctional pools of connexin43 using Triton X-100. Panels (A) and (B) depict confocal micrographs of immunofluorescent label for connexin43 (green) in isolated mouse neonatal atrial cardiomyocytes. Gap-junctional plaques (arrow heads) and intracellular connexin43 (asterisk) are indicated. +/− TX-100 denotes incubation with or without Triton X-100 prior to fixing. XZ and YZ orthogonal sections are shown adjacent to each micrograph. Planes in which slices were made are indicated by arrow indents. Bar: 25 μm. Note, complete absence of intracellular connexin43 after Triton X-100 treatment. The immunoblot shows Western blot analysis of connexin43 in mouse neonatal cardiac tissue. Note, different phosphorylation profiles in insoluble and soluble fractions. Quantification of bands shows that similar quantities of connexin43 protein are localized in both the insoluble and soluble fractions. The sum of band intensities for the junctional and non-junctional samples closely matches the signal obtained for the total samples demonstrating the high accuracy of quantification attainable using this method and represents a powerful internal control ($n = 4$; $p < 0.001^{***}$, $p > 0.05$ demonstrates no significant difference). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

this technique to examine sex differences in HRD expression. Female animals were subdivided according to their estrous cycle stage in order to examine whether this parameter altered expression and localization levels. Fig. 2 shows sex and estrous cycle stage differences in expression of the HRD proteins connexin43 (A), plakophilin-2 (B), N-cadherin (C) and plakoglobin (D) in adult mouse ventricle. While little difference in overall expression of connexin43 and plakophilin-2 was detected between the sexes, protein localized in junctional fractions displayed a significant predominance in both female sets of samples. Compared to males, connexin43 localization was approximately 70–80% higher in diestrus ($p < 0.01$) and estrus ($p < 0.001$) females. Plakophilin-2 localization was approximately 25% higher in diestrus ($p < 0.01$) and estrus ($p < 0.05$) females.

In contrast to connexin43 and plakophilin-2, sex dichotomy of expression and localization of N-cadherin appeared heavily dependent on the estrous cycle stage of the female animals. Inspection of data for total samples indicated that males and diestrus females express similar quantities of N-cadherin. However, expression of N-cadherin was significantly lower in estrus females (~50%; $p < 0.001$). More dynamic changes were observed in N-cadherin localization. Lower total expression of N-cadherin was replicated precisely in junctional fractions of estrus females (~50%; $p < 0.001$). But in counterpoint, far greater quantities of N-cadherin were localized in junctional fractions in diestrus females (~40%; $p < 0.001$) than total expression suggested. By comparison, no discernable patterns of sexual dimorphism were observed in plakoglobin, ankyrin-2 (not shown) and β-actin (not shown) expression or subcellular localization.

A key observation in these experiments was that quantities of proteins localized in junctional fractions were not well predicted by total cellular expression of those proteins. These data suggested

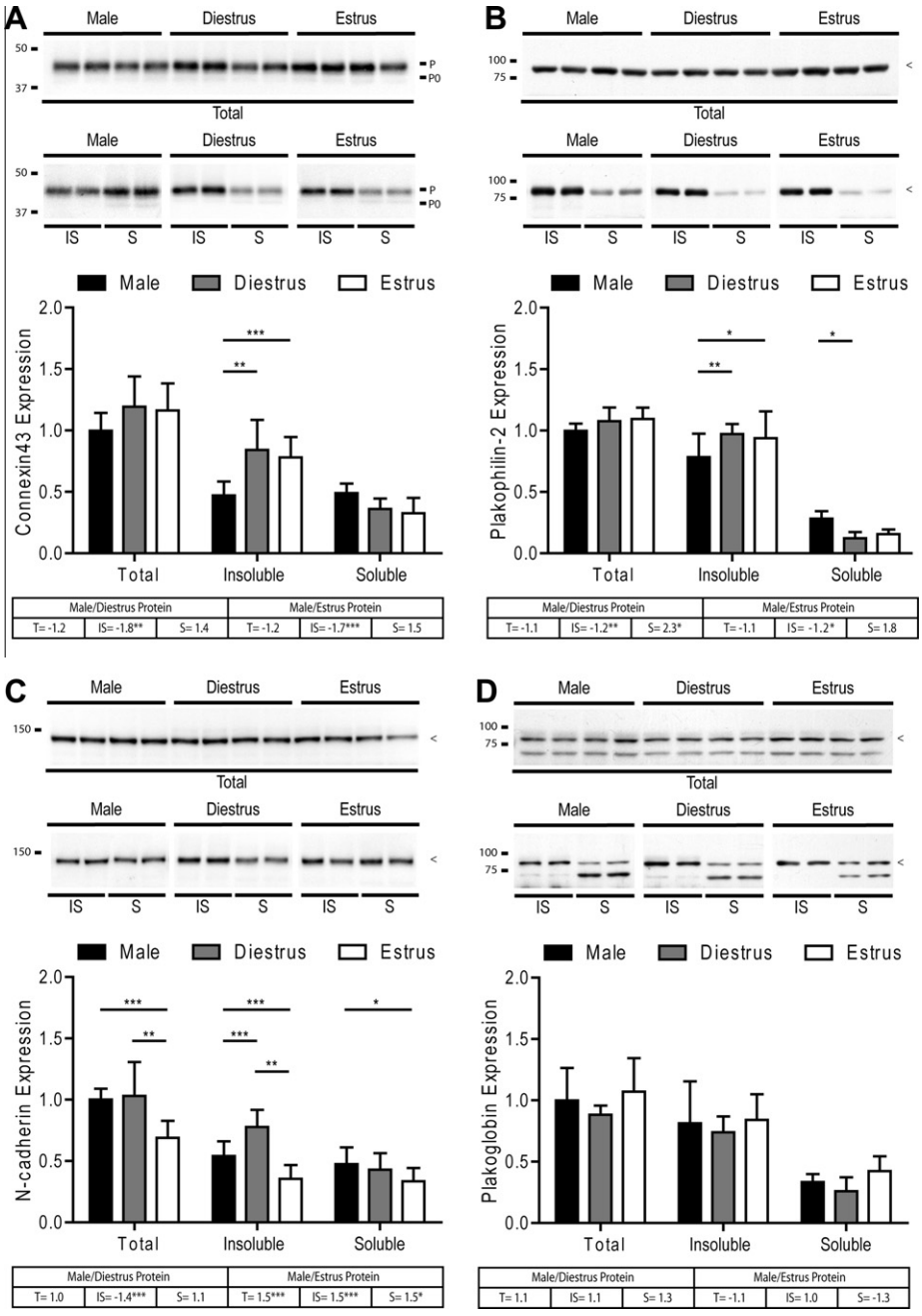

Fig. 2. Heart rhythm determinants display prominent sex differences in expression and subcellular localization. Panels (A)–(D) show Western blot experiments and quantitative analysis of connexin43, plakophilin-2, N-cadherin and plakoglobin in adult mouse heart (8 weeks). Example immunoblots illustrate differences in total expression and in insoluble:soluble ratios between the sexes (arrows indicate relevant bands; lower plakoglobin band was assumed non-specific). Bar charts demonstrate significant sex differences in total expression and junctional localization for connexin43, plakophilin-2 and N-cadherin between males (black bars), diestrus females (gray bars) and estrus females (white bars). No significant sex differences were detected for plakoglobin. Relative expression ratios are tabulated below. Positive fold values are given in instances of higher male quantities, or their negative reciprocals in instances of higher female quantities, e.g. for connexin43—2.1 denotes 2.1-fold higher expression in females ($n = 8$; $p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$).

that additional post-translational mechanisms regulated HRD abundance at cell membranes. Recently, caveolin-3 was discovered to regulate gap junction remodeling through interactions with estrogen receptors [44]. Therefore, to investigate whether caveolae play a role in sex differences in membrane localization, the expression levels and localization caveolin-3 were evaluated in male and female animals. As shown in Fig. 3A, caveolin-3 displayed similar expression and detergent solubility profiles in both sexes. To probe deeper, we examined how alterations to caveolin-3 expression affected patterns of HRD expression and detergent solubility. Fig. 3B and C shows results obtained for caveolin-3 and connexin43 in mice heterozygous for the caveolin-3 gene and a new group of wildtype mice. Heterozygous mice were chosen in order to avoid potential unrelated, pleiotropic effects which may come about due to complete knock-out of caveolin-3. Wildtype and transgenic female mice were all in the diestrus phase of the estrous cycle. Together, these data demonstrate that ~70% reduction in caveolin-3 expression has little effect on connexin43. Identical to results shown in Fig. 2A, ratios of Triton X-100 insoluble:soluble connexin43 indicated higher quantities of gap-junctional connexin43 in

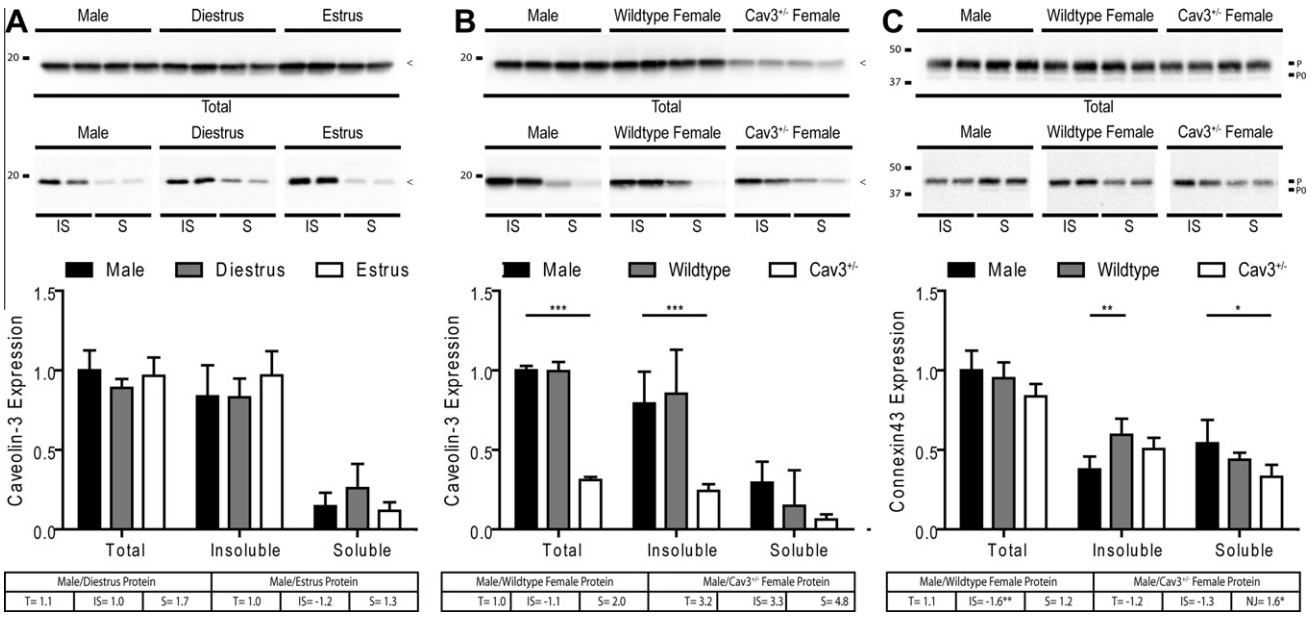

Fig. 3. Caveolin-3 displays little involvement in HRD sex differences. (A) Quantitative Western blot analyses of caveolin-3 in adult wildtype mouse ventricle show no apparent sex differences in expression or detergent solubilization. In caveolin-3 heterozygotes, substantial reduction in caveolin-3 (B) protein quantities has little impact on connexin43 (C) expression and subcellular localization ($n = 4$; n.d.—not done; $p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$).

female ventricle. This distinct pattern was retained in caveolin-$3^{+/-}$ tissue, potentially excluding the possibility that this phenomenon is regulated by caveolin-3 expression. Although, it is indeed possible that there is a specific threshold that lies below ~70% wherein caveolin-3 expression become paramount sex dichotomy of HRD expression.

4. Discussion

Few studies have investigated sex differences in gene expression in the heart [15–19,29,30]. Fewer still have examined sex differences in cardiac protein expression [30,31]. In the present study, we report novel data for connexin43, plakophilin-2, N-cadherin, plakoglobin and caveolin-3, demonstrating significant sex dichotomy in the protein expression in the heart. These proteins represent key components of a variety of mechanical and electrical junction types, each of which uphold pivotal roles in healthy cardiac function.

In the mouse heart, we found that localization of connexin43 in the junctional compartment was substantially higher in females. No differences were detected in comparisons between diestrus and estrus females. Our findings are supported by previous studies connexin43 expression in female rat ventricle which reported similar elevations [31]. Superior expression of connexin43 is associated with a reduced susceptibility to ventricular fibrillation in female rats [32]. This raises the possibility that elevated connexin43 expression is cardioprotective. In the heart, a high conduction reserve means that much of the electrical coupling available to the myocardium is redundant [33]. Thus, the benefits of higher quantities of gap junction channels composed of connexin43 may only become apparent under pathophysiological conditions where connexin43 expression and localization is altered. An observation that supports this notion is that conduction velocity of impulse propagation, as measured by optical mapping, is comparable in male and female rat ventricles under healthy conditions [34]. However, conduction of the cardiac impulse under diabetic conditions is more sensitive to perturbation in male rats than in females [34]. These findings were associated with significantly higher quantities of connexin43 localized to the intercalated discs of female cardiomyocytes, supporting the hypothesis that elevated connexin43 expression contributes to cardioprotection under disease conditions.

Besides gap junctions, the plasma membranes of cardiomyocytes are populated by a variety of mechanical junctions including adherens junctions and desmosomes. We examined the expression and subcellular localization of three protein components of the desmosomes and adherens junctions: plakophilin-2, N-cadherin and plakoglobin, ankyrin-2 and actin. Adherens junctions and desmosomes are responsible to withstand mechanical stresses within the myocardium as it undergoes contraction. Thus, the differences in expression of these junctional proteins potentially represent the different mechanical demands of male and female ventricles. Indeed, most studies into the pathophysiology of ARVC have focused on the disruptions to intercellular adhesion by mechanical stress [35–37]. Targeted disruption of plakophilin-2, N-cadherin, plakoglobin and ankyrin-2 expression in transgenic mouse models is associated with dramatic reorganization of gap junction size, number and distribution [38,39]. Therefore, an additional hypothesis is that defects in cell–cell adhesion may in turn destabilize gap junctions and potentially alter electrical coupling [40,41].

It is noteworthy that in most cases described in this study, the quantities of heart rhythm determinant proteins that were localized to cardiomyocyte membranes were largely different from those predicted by overall expression amounts. On most occasions, it was only upon inspection of membrane pools that significant sex differences arose. These findings also suggested that sex differences in protein localization may be mediated by mechanisms other than expression alone. To probe this hypothesis further, we examined whether interactions with caveolae could bring about the sexual dimorphism we detected in membrane-localization patterns. Caveolin-3 is a binding partner of connexin43 [42,43]. Recently, it was shown that interaction of estrogen receptor α and caveolin-3 regulates connexin43 phosphorylation and membrane localization in metabolic inhibition-treated rat cardiomyocytes [42]. Disappointingly, we found scant evidence to implicate caveolin-3 involvement in connexin43 sex differences, indicating that any role for caveolin-3 in sex dichotomy of HRD localization is likely independent of its expression levels.

In conclusion, we have shown that significant sex differences exist in the localization of key proteins at the intercalated discs between cardiac myocytes. Moreover, we have also shown that patterns of protein expression and localization may also be affected by the estrous cycle stage in females. Together, these studies extend our previous findings in microarray studies to demonstrate that sex differences in gene expression are likely to confer distinct functional properties on male and female myocardium.

Acknowledgments

The authors gratefully thank Dr. David C. Spray for his invaluable input and Sanda Iacobas for her technical support. The research was supported by the Award No. R01HL092001 from the National Heart, Lung, and Blood Institute (NHLBI). The content is solely the responsibility of the authors and does not necessarily represent the NHLBI official views. The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.02.006.

References

[1] A.F. James, S.C. Choisy, J.C. Hancox, Recent advances in understanding sex differences in cardiac repolarization, Prog. Biophys. Mol. Biol. 94 (2007) 265–319.
[2] W. Adams, The normal duration of the electrocardiographic ventricular complex, J. Clin. Invest. 15 (1936) 335–342.
[3] H. Bazett, An analysis of the time-relations of electrocardiograms, Heart 7 (1920) 353–370.
[4] M. Merri, J. Benhorin, M. Alberti, E. Locati, A.J. Moss, Electrocardiographic quantitation of ventricular repolarization, Circulation 80 (1989) 1301–1308.
[5] P.M. Rautaharju, S.H. Zhou, S. Wong, H.P. Calhoun, G.S. Berenson, R. Prineas, A. Davignon, Sex differences in the evolution of the electrocardiographic QT interval with age, Can. J. Cardiol. 8 (1992) 690–695.
[6] J.H. Burke, F.A. Ehlert, J.T. Kruse, M.A. Parker, J.J. Goldberger, A.H. Kadish, Gender-specific differences in the QT interval and the effect of autonomic tone and menstrual cycle in healthy adults, Am. J. Cardiol. 79 (1997) 178–181.
[7] C.M. Albert, B.A. McGovern, J.B. Newell, J.N. Ruskin, Sex differences in cardiac arrest survivors, Circulation 93 (1996) 1170–1176.
[8] W.B. Kannel, P.W. Wilson, R.B. D'Agostino, J. Cobb, Sudden coronary death in women, Am. Heart J. 136 (1998) 205–212.
[9] E.J. Benjamin, P.A. Wolf, R.B. D'Agostino, H. Silbershatz, W.B. Kannel, D. Levy, Impact of atrial fibrillation on the risk of death: the Framingham Heart Study, Circulation 98 (1998) 946–952.
[10] R.W. Peters, M.R. Gold, The influence of gender on arrhythmias, Cardiol. Rev. 12 (2004) 97–105.
[11] M.K. Jonsson, M.A. Vos, G. Duker, S. Demolombe, T.A. van Veen, Gender disparity in cardiac electrophysiology: implications for cardiac safety pharmacology, Pharmacol. Ther. 127 (2010) 9–18.
[12] J. Reinoehl, D. Frankovich, C. Machado, R. Kawasaki, J.J. Baga, L.A. Pires, R.T. Steinman, B.S. Fromm, M.H. Lehmann, Probucol-associated tachyarrhythmic events and QT prolongation: importance of gender, Am. Heart J. 131 (1996) 1184–1191.
[13] M.D. Drici, B.C. Knollmann, W.X. Wang, R.L. Woosley, Cardiac actions of erythromycin: influence of female sex, JAMA 280 (1998) 1774–1776.
[14] M.H. Lehmann, S. Hardy, D. Archibald, D.J. MacNeil, JTc prolongation with D,L-sotalol in women versus men, Am. J. Cardiol. 83 (1999) 354–359.
[15] J.S. Gilbert, M.J. Nijland, Sex differences in the developmental origins of hypertension and cardiorenal disease, Am. J. Physiol. Regul. Integr. Comp. Physiol. 295 (2008) R1941–R1952.
[16] K. Silander, M. Alanne, K. Kristiansson, O. Saarela, S. Ripatti, K. Auro, J. Karvanen, S. Kulathinal, M. Niemela, P. Ellonen, E. Vartiainen, P. Jousilahti, J. Saarela, K. Kuulasmaa, A. Evans, M. Perola, V. Salomaa, L. Peltonen, Gender differences in genetic risk profiles for cardiovascular disease, PLoS ONE 3 (2008) e3615.
[17] H. Witt, C. Schubert, J. Jaekel, D. Fliegner, A. Penkalla, K. Tiemann, J. Stypmann, S. Roepcke, S. Brokat, S. Mahmoodzadeh, E. Brozova, M.M. Davidson, P. Ruiz Noppinger, C. Grohe, V. Regitz-Zagrosek, Sex-specific pathways in early cardiac response to pressure overload in mice, J. Mol. Med. 86 (2008) 1013–1024.
[18] J. Isensee, H. Witt, R. Pregla, R. Hetzer, V. Regitz-Zagrosek, P.R. Noppinger, Sexually dimorphic gene expression in the heart of mice and men, J. Mol. Med. 86 (2008) 61–74.
[19] D.A. Iacobas, S. Iacobas, N. Thomas, D.C. Spray, Sex-dependent gene regulatory networks of the heart rhythm, Funct. Integr. Genomics 10 (2010) 73–86.
[20] L.S. Musil, D.A. Goodenough, Biochemical analysis of connexin43 intracellular transport, phosphorylation, and assembly into gap junctional plaques, J. Cell Biol. 115 (1991) 1357–1374.
[21] K.S. Grossmann, C. Grund, J. Huelsken, M. Behrend, B. Erdmann, W.W. Franke, W. Birchmeier, Requirement of plakophilin 2 for heart morphogenesis and cardiac junction formation, J. Cell Biol. 167 (2004) 149–160.
[22] J.F. Nelson, L.S. Felicio, P.K. Randall, C. Sims, C.E. Finch, A longitudinal study of estrous cyclicity in aging C57BL/6J mice: I. Cycle frequency, length and vaginal cytology, Biol. Reprod. 27 (1982) 327–339.
[23] S.O. Suadicani, M.J. Vink, D.C. Spray, Slow intercellular Ca(2+) signaling in wild-type and Cx43-null neonatal mouse cardiac myocytes, Am. J. Physiol. Heart Circ. Physiol. 279 (2000) H3076–H3088.
[24] M.M. Thi, T. Kojima, S.C. Cowin, S. Weinbaum, D.C. Spray, Fluid shear stress remodels expression and function of junctional proteins in cultured bone cells, Am. J. Physiol. Cell Physiol. 284 (2003) C389–C403.
[25] A.F. Bruce, S. Rothery, E. Dupont, N.J. Severs, Gap junction remodelling in human heart failure is associated with increased interaction of connexin43 with ZO-1, Cardiovasc. Res. 77 (2008) 757–765.
[26] K. Grikscheit, N. Thomas, A.F. Bruce, S. Rothery, J. Chan, N.J. Severs, E. Dupont, Coexpression of connexin 45 with connexin 43 decreases gap junction size, Cell Commun. Adhes. 15 (2008) 185–193.
[27] D.A. Iacobas, S.O. Suadicani, S. Iacobas, C. Chrisman, M.A. Cohen, D.C. Spray, E. Scemes, Gap junction and purinergic P2 receptor proteins as a functional unit: insights from transcriptomics, J. Membr. Biol. 217 (2007) 83–91.
[28] J.L. Solan, P.D. Lampe, Connexin43 phosphorylation: structural changes and biological effects, Biochem. J. 419 (2009) 261–272.
[29] P. Rosenkranz-Weiss, R.J. Tomek, J. Mathew, M. Eghbali, Gender-specific differences in expression of mRNAs for functional and structural proteins in rat ventricular myocardium, J. Mol. Cell. Cardiol. 26 (1994) 261–270.
[30] S.H. Chu, K. Sutherland, J. Beck, J. Kowalski, P. Goldspink, D. Schwertz, Sex differences in expression of calcium-handling proteins and beta-adrenergic receptors in rat heart ventricle, Life Sci. 76 (2005) 2735–2749.
[31] N. Tribulova, E. Dupont, T. Soukup, L. Okruhlicova, N.J. Severs, Sex differences in connexin-43 expression in left ventricles of aging rats, Physiol. Res. 54 (2005) 705–708.
[32] V. Knezl, B. Bacova, L. Kolenova, M. Mitasikova, P. Weismann, J. Drimal, N. Tribulova, Distinct lethal arrhythmias susceptibility is associated with sex-related difference in myocardial connexin-43 expression, Neuro Endocrinol. Lett. 29 (2008) 798–801.
[33] R.M. Shaw, Y. Rudy, Ionic mechanisms of propagation in cardiac tissue. Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling, Circ. Res. 81 (1997) 727–741.
[34] Y. Shimoni, T. Emmett, R. Schmidt, A. Nygren, G. Kargacin, Sex-dependent impairment of cardiac action potential conduction in type 1 diabetic rats, Am. J. Physiol. Heart Circ. Physiol. 296 (2009) H1442–H1450.
[35] P. Kirchhof, L. Fabritz, M. Zwiener, H. Witt, M. Schafers, S. Zellerhoff, M. Paul, T. Athai, K.H. Hiller, H.A. Baba, G. Breithardt, P. Ruiz, T. Wichter, B. Levkau, Age- and training-dependent development of arrhythmogenic right ventricular cardiomyopathy in heterozygous plakoglobin-deficient mice, Circulation 114 (2006) 1799–1806.
[36] E. Garcia-Gras, R. Lombardi, M.J. Giocondo, J.T. Willerson, M.D. Schneider, D.S. Khoury, A.J. Marian, Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy, J. Clin. Invest. 116 (2006) 2012–2021.
[37] K.R. Nilsson Jr., V. Bennett, Ankyrin-based patterning of membrane microdomains: new insights into a novel class of cardiovascular diseases, J. Cardiovasc. Pharmacol. 54 (2009) 106–115.
[38] S.R. Kaplan, J.J. Gard, N. Protonotarios, A. Tsatsopoulou, C. Spiliopoulou, A. Anastasakis, C.P. Squarcioni, W.J. McKenna, G. Thiene, C. Basso, N. Brousse, G. Fontaine, J.E. Saffitz, Remodeling of myocyte gap junctions in arrhythmogenic right ventricular cardiomyopathy due to a deletion in plakoglobin (Naxos disease), Heart Rhythm 1 (2004) 3–11.
[39] S. Rohr, Molecular crosstalk between mechanical and electrical junctions at the intercalated disc, Circ. Res. 101 (2007) 637–639.
[40] M. Delmar, The intercalated disk as a single functional unit, Heart Rhythm 1 (2004) 12–13.
[41] J.E. Saffitz, Dependence of electrical coupling on mechanical coupling in cardiac myocytes: insights gained from cardiomyopathies caused by defects in cell–cell connections, Ann. N. Y. Acad. Sci. 1047 (2005) 336–344.
[42] T.H. Chung, S.M. Wang, J.Y. Liang, S.H. Yang, J.C. Wu, The interaction of estrogen receptor alpha and caveolin-3 regulates connexin43 phosphorylation in metabolic inhibition-treated rat cardiomyocytes, Int. J. Biochem. Cell Biol. 41 (2009) 2323–2333.
[43] L. Liu, Y. Li, J. Lin, Q. Liang, X. Sheng, J. Wu, R. Huang, S. Liu, Connexin43 interacts with caveolin-3 in the heart, Mol. Biol. Rep. 37 (2010) 1685–1691.
[44] C.K. Liao, S.M. Wang, Y.L. Chen, H.S. Wang, J.C. Wu, Lipopolysaccharide-induced inhibition of connexin43 gap junction communication in astrocytes is mediated by downregulation of caveolin-3, Int. J. Biochem. Cell Biol. 42 (2010) 762–770.