Physiol. Res. 54: 705-708, 2005

RAPID COMMUNICATION

Sex Differences in Connexin-43 Expression in Left Ventricles of Aging Rats

N. TRIBULOVÁ[1], E. DUPONT[2], T. SOUKUP[3], L. OKRUHLICOVÁ[1], N.J. SEVERS[2]

[1]Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic, [2]Imperial College, Royal Brompton Hospital, London, UK and [3]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

Received August 19, 2005
Accepted September 5, 2005

Summary

Cardiac gap junctions have been implicated in maintaining intercellular electrical and metabolic couplings. The abnormalities in connexin-43 (Cx43) lead to conduction defects and contractile dysfunction. We have evaluated the expression and phoshorylation status of Cx43 in the left ventricular myocardium of male and female 16-month-old rats submitted to 14-week L-thyroxine (T_4) treatment. Western blot analysis revealed the presence of fully or intermediately phosphorylated and unphosphorylated forms of Cx43. We have found no significant differences in Cx43 expression and phosphorylation between T_4-treated and control untreated animals. However, expression of Cx43 was significantly higher in female compared to male rats. We conclude that T_4 administration has no effect on Cx43 expression, but there are sex-dependent differences in Cx43 expression in the left ventricles between aging male and female rats.

Key words

Connexin-43 • Rat cardiomyocytes • Steroid and thyroid hormones • Male and female specificity

Cardiac gap junctions form a low-resistance pathway for cell-to-cell electrical impulse propagation. They are composed of intercellular connexin channels that allow diffusion of ions and signaling molecules between cardiomyocytes. It has been shown that the expression and distribution of Cx43, the most abundant gap junction channel protein, was abnormal in diseased or failing hearts (Severs 1999, Dupont *et al.* 2001, Tribulová *et al.* 2002). Cx43 alterations have also been linked to cardiac arrhythmic and functional complications (Gutstein *et al.* 2001, Severs 2001, Tribulová *et al.* 2003). Since Cx43 is a phosphoprotein, the changes in phosphorylation status (Dhein 1998) or the inhibition of protein kinases (Duthe *et al.* 2000) reduce gap junctional intercellular communication. The function of gap junction channels is modulated by endogenous and exogenous substances (e.g. by angiotensin II, estrogens or thyroid hormones) under normal and pathophysiological conditions. It was shown that the addition of angiotensin II or T_3 enhanced Cx43 expression in cultured neonatal rat ventricular myocytes (Dodge *et al.* 1998, Tribulová *et al.* 2004b). Furthermore, we have found that T_4-treated aging 20-month-old females were less susceptible to malignant arrhythmias compared to 4-month-old rats (Tribulová *et al.* 2004a). In order to test possible involvement of Cx43 in such differences, we have

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

evaluated the expression and phosphorylation status of Cx43 in the left ventricle of aging 16-month-old male and female Wistar rats treated with L-thyroxine (T_4, 50 µg/100g/day) for 14 weeks compared to control untreated rats. Twenty-four rats (12 males and 12 females) obtained from the rat-breeding unit Dobrá Voda (Bratislava, SK) were used. Treatment of animals conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, Revised 1985) and the investigation was approved by the Expert Committee of the Institute for Heart Research, Slovak Academy of Sciences. Frozen ventricular tissue was homogenized and sonicated in 0.1 M Tris buffer (pH 6.8), containing 10 mM EDTA and 20 % SDS. Protein concentration was determinated using the DC protein assay kit (Bio-Rad). Five µg of total protein per lane was run on 10 % SDS polyacrylamide gels and electrophoretically transferred to a PVDF membrane. The resulting replica was reacted with an anti-Cx43 antibody (Chemicon, Inc. USA), incubated with an alkaline phosphatase-conjugated secondary antibody (goat antimouse IgG, Pierce) and the enzymatic activity was revealed using NBT and BICP substrate kits (Promega). Densitometric quantification of Cx43 band intensity normalized to myosin content was performed as previously described (Dupont *et al*. 2001).

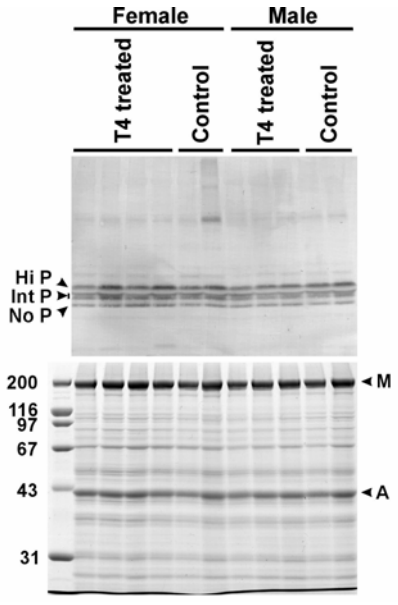

Fig. 1. A representative immunoblot showing Cx43 expression in female and male T_4-treated and control (T_4 untreated) rat left heart ventricles (upper panel). Fully (HiP) and intermediately (IntP) phosporylated, as well as unphosphorylated (NoP) forms of Cx43 can be recognized after staining with an anti Cx43 antibody. Corresponding gel stained with Coomassie Blue indicating myosin band (M) and actin band (A) comigrating with Cx43 isoforms is shown in the bottom panel.

Western blot analysis (Fig. 1) revealed three forms of Cx43 corresponding to fully or intermediately phosphorylated forms and to an unphosphorylated form of this protein. We have found no significant differences between T_4-treated and control rats either in total Cx43 expression or in the content of phosphorylated or unphosphorylated forms (Fig. 2, upper panel). In contrast, the total expression of Cx43 as well as the expression of individual isoforms (HiP, IntP and NoP) was significantly higher in female compared to male hearts (Fig. 2, lower panel). However, the percentage of NoP form (calculated from the total content of either male or female rats) was relatively higher in female (14.5 %) than in male (9.0 %) ventricles.

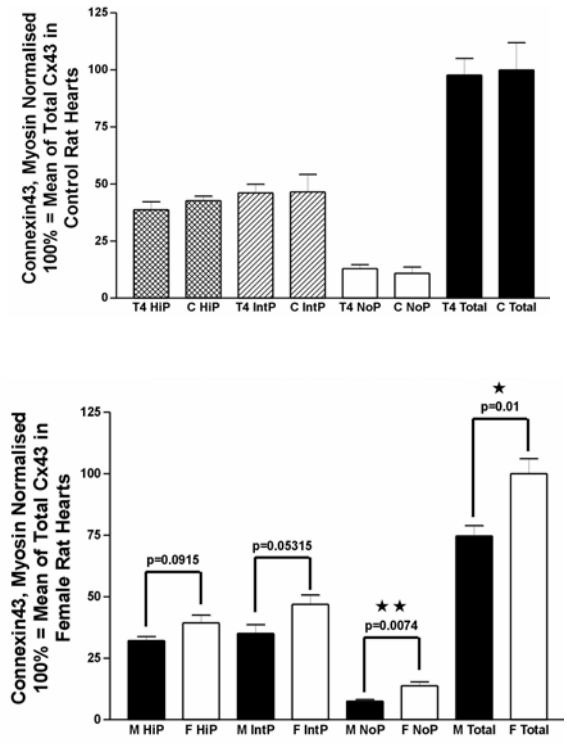

Fig. 2. Densitometric quantification of Cx43 immunoblot band intensities in T_4-treated (T_4) vs. control untreated (C) rat left heart ventricles (upper panel) and in male (M) versus female (F) control left heart ventricles (lower panel). Note that there is no significant difference between T_4 treated and control animals, but significant differences are present between male and female groups in NoP Cx43 isoform and total Cx43 content. Data are means ± S.E.M.

Data in the literature suggest direct tissue-specific regulation of the Cx43 gene by thyroid hormones. It was demonstrated that thyroid hormone administration increased gap junctional communication by elevating Cx43 mRNA and protein levels in rat liver epithelial cells, but not in adult rat heart cells (Stock and

Sies 2000). We have also found that thyroid hormones did not induce significant alterations of Cx43 expression in aging male and female rat hearts. The different effect of thyroid hormones on immature (Tribulová *et al.* 2004b) and adult hearts can reflect changes of hormone concentration, as the T_4 and T_3 levels are low in 5-day-old rats, they reach a peak at the age of about 2-4 weeks and then slightly decline towards adult values (Dubois and Dussault 1977). Alterations of thyroid hormone levels lead to MyHC changes both in the heart and skeletal muscles (Morkin 1993, Danzi *et al.* 2003, Soukup *et al.* 2003, Vadászová *et al.* 2004a,b, for review see Baldwin and Haddad 2001). Thyroid hormones are associated with striking alterations in cardiac contractile function and energy metabolism, as they increase myocardial contractility, systolic contraction speed and diastolic relaxation, cardiac output and heart rate. There are several T_3-regulated cardiac-specific genes including α- and β-MyHC, phospholamban or SR Ca^{2+}-ATPase (SERCA 2). Cardiac MyHC genes are thus controlled by T_3 which stimulates transcription of α-MyHC and inhibits β-MyHC mRNA expression both *in vivo* and in cultured heart cells (Morkin 1993, Klein and Ojamaa 2001).

Many results point to the existence of sex-specific differences in expression of mRNAs for functional and structural proteins in rat ventricular myocardium (e.g. Rosenkranz-Weiss *et al*. 1994). Our results revealed such differences also in myocardial Cx43 expression and phosphorylation. Furthermore, chronic hyperthyroidism led to an absolute and relative rat heart weight increase (Soukup *et al*. 2001) that was higher in males than in females (unpublished observation). It also caused relatively larger body weight loss and higher mortality rate in male than female rats, probably in relation to higher cardiac arrhythmias in males (Yu *et al.* 1998). Interestingly, the data of the Framingham Heart Study suggested that the left ventricular mass is significantly greater in men compared to women even after adjusting for body surface area (Levy *et al.* 1990). Since thyroid and sex hormone receptors belong to the same nuclear receptor protein superfamily, direct effects of estrogen on cardiomyocytes containing functional estrogen receptors can be expected. The increase in Cx43 expression observed after 17 β-estradiol administration (Grohe *et al*. 1997) suggests that estrogens might play a part in sex differences in Cx43 level. We conclude that the T_4 application has no significant effect on Cx43 expression in the left ventricles of aging male and female rats, but that there are sex-dependent differences in Cx43 level, which may, in general, favor females by decreasing their propensity to cardiac diseases.

Acknowledgements

This study was supported by VEGA, MYORES No. 511978 and GACR 304/05/0327 grants and by the Research Project AV0Z 50110509.

References

BALDWIN KM, HADDAD F: Plasticity in skeletal, cardiac, and smooth muscle. Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle. *J Appl Physiol* **90**: 345-357, 2001.

DANZI S, OJAMAA K, KLEIN I: Triiodothyronine-mediated myosin heavy chain gene transcription in the heart. *Am J Physiol* **284**: H2255-H2262, 2003.

DHEIN S: Gap junction channels in cardiovascular system: pharmacological and physiological modulation. *Trends Pharmacol Sci* **19**: 229-241, 1998.

DODGE SM, BEARDSLEE MA, DARROW BJ, GREEN KG, BEYER EC, SAFFITZ JE: Effect of angiotensin II on expression of the gap junction channel protein connexin43 in neonatal rat ventricular myocytes. *J Am Coll Cardiol* **32**: 800-807, 1998.

DUBOIS JD, DUSSAULT JH: Ontogenesis of thyroidal function in the neonatal rats. Thyroxine (T_4) and triiodothyronine (T_3) production rates. *Endocrinology* **101**: 435-441, 1977.

DUPONT E, MATSUSHITA T, KABA R, VOZZI C, COPPEN SR, KHAN N, KAPRELIAN R, YACOUB MH, SEVERS NJ: Altered connexin expression in human congestive heart failure. *J Mol Cell Cardiol* **33**: 359-371, 2001.

DUTHE F, DUPONT E, VERRECCHIA F, PLAISANCE I, SEVERS NJ, SARROULHE D, HERVÉ JC: Dephosphorylation agents depress gap junctional communication between rat cardiac cells without modifying the connexin43 phosphorylation degree. *Gen Physiol Biophys* **19**: 441-449, 2000.

GROHE C, KAHLERT S, LOBBERT K, STIMPEL M., KARAS RH, VETTER H, NEYSES L: Cardiac myocytes and fibroblasts contain functional estrogen receptors. *FEBS Lett* **416**: 107-112, 1997.

GUTSTEIN DE, MORLEY GE, VAIDYA D, LIU F, CHEN FL, STUHLMANN H, FISCHMAN GI: Heterogenous expression of gap junction channels in the heart leads to conduction defects and ventricular dysfunction. *Circulation* **104**: 1194-1199, 2001.

KLEIN I, OJAMAA K: Thyroid hormone and the cardiovascular system. *N Engl J Med* **344**: 501-509, 2001.

LEVY D, GARRISON RJ, SAVAGE DD, KANNEL WB, CASTELLI WP: Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* **322**: 1561-1566, 1990.

MORKIN E: Regulation of myosin heavy chain genes in the heart. *Circulation* **87**: 1451-1460, 1993.

ROSENKRANZ-WEISS P, TOMEK RJ, MATHEW J, EGHBAL M: Gender-specific differences in expression of mRNAs for functional and structural proteins in rat ventricular myocardium. *J Mol Cell Cardiol* **26**: 261-270, 1994.

SEVERS NJ: Cardiovascular disease. In: *Gap Junction-Mediated Intercellular Signaling in Health and Disease.* G CARDEW (ed), Kluwer Academic Publishers, Boston, 1999, pp 188-206.

SEVERS NJ: Gap junction remodeling and cardiac arrhythmogenesis: cause or coincidence? *J Cell Mol Med* **5**: 355-366, 2001.

SOUKUP T, ZACHAŘOVÁ G, SMERDU V, JIRMANOVÁ I: Body, heart, thyroid gland and skeletal muscle weight changes in rats with altered thyroid status. *Physiol Res* **50**: 619-626, 2001.

SOUKUP T, ZACHAŘOVÁ G, VADÁSZOVÁ A, JIRMANOVÁ I, SMERDU V, ASMUSSEN G, KRIŽANOVÁ O, NOVOTOVÁ M: Contribution of innervation and thyroid hormones to the differentiation of muscle fibre phenotype. *Physiol Res* **52**: 19P, 2003.

STOCK A, SIES H: Thyroid hormone receptors bind to an element in the connexin43 promoter. *Biol Chem* **381**: 973-979, 2000.

TRIBULOVÁ N, OKRUHLICOVÁ L, VARON D, MANOACH M, PECHÁŇOVÁ O, BERNÁTOVÁ I, WEISMANN P, BARANČÍK M, STYK J, SLEZÁK J: Structural substrates involved in the development of severe arrhythmias in hypertensive rat and aged guinea pig hearts. In: *Cardiac Remodeling and Failure.* NS DHALLA, P SINGAL (eds), Kluwer Academic Publishers, Boston, 2002, pp 377-398.

TRIBULOVÁ N, OKRUHLICOVÁ L, IMANAGA I, HIROSAWA N, OGAWA K, WEISMANN P: Factors involved in the susceptibility of spontaneously hypertensive rats to low K^+-induced arrhythmias. *Gen Physiol Biophys* **22**: 369-382, 2003.

TRIBULOVÁ N, KNÉZL V, OKRUHLICOVÁ L, DŘÍMAL J, LAMOŠOVÁ D, SLEZÁK J, STYK J: L-thyroxine increases susceptibility of adult rats to low K^+-induced ventricular fibrillation and sinus rhythm restoration in old rats. *Exp Physiol* **89**: 629-636, 2004a.

TRIBULOVÁ N, SHNEYVAYS V, MAMEDOVÁ LK, MOSHEL S, ZINMAN T, SHAINBERG A, MANOACH M, WEISMANN P, KOSTIN S: Enhanced connexin-43 and alpha-sarcomeric actin expression in cultured heart myocytes exposed to triiodo-L-thyronine. *J Mol Histol* **35**: 463-470, 2004b.

VADÁSZOVÁ A, ZACHAŘOVÁ G, MACHÁČOVÁ K, JIRMANOVÁ I, SOUKUP T: Influence of thyroid status on the differentiation of slow and fast rat muscle phenotypes. *Physiol Res* **53** (Suppl 1): S57-S61, 2004a.

VADÁSZOVÁ A, ZACHAŘOVÁ G, ASMUSSEN G, HUDECOVÁ S, KRIŽANOVÁ O, SOUKUP T: Thyroid hormones regulate differently muscle MHC isoforms and their mRNA levels. *Physiol Res* **53**: 42P, 2004b.

YU F, DEGENS H, LI X, LARSSON L: Gender- and age-related differences in the regulatory influence of thyroid hormone on the contractility and composition of single rat soleus muscle fibres. *Pflügers Arch* **437**: 21-30, 1998.

Reprint requests

N. Tribulová, Institute for Heart Research, Slovak Academy of Sciences, Dúbravská cesta 9, 840 05 Bratislava, P.O. Box 104, Slovakia. E-mail: narcisa.tribulova@savba.sk