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Disease-associated changes in the expression of ion channels, ion receptors, ion exchangers and Ca^{2+}-handling proteins in heart hypertrophy

Carolin Zwadlo, Jürgen Borlak*

Fraunhofer Institute of Toxicology and Experimental Medicine, Center for Drug Research and Medical Biotechnology, Hannover, Germany

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Abstract

The molecular pathology of cardiac hypertrophy is multifactorial with transcript regulation of ion channels, ion exchangers and Ca^{2+}-handling proteins being speculative. We therefore investigated disease-associated changes in gene expression of various ion channels and their receptors as well as ion exchangers, cytoskeletal proteins and Ca^{2+}-handling proteins in normotensive and spontaneously hypertensive (SHR) rats. We also compared experimental findings with results from hypertrophic human hearts, previously published (Borlak, J., and Thum, T., 2003. Hallmarks of ion channel gene expression in end-stage heart failure. FASEB J. 17, 1592–1608). We observed significant ($P < 0.05$) induction in transcript level of ATP-driven ion exchangers (Atp1A1, NCX-1, SERCA2a), ion channels (L-type Ca^{2+}-channel, $K_{ir}3.4$, $Na_v1.5$) and RyR-2 in hypertrophic hearts, while gene expression was repressed in diseased human hearts. Further, the genes coding for calreticulin and calmodulin, PMCA 1 and 4 as well as α-skeletal actin were significantly ($P < 0.05$) changed in hypertrophic human heart, but were unchanged in hypertrophic left ventricles of the rat heart. Notably, transcript level of α- and β-MHC, calsequestrin, $K_{ir}6.1$ (in the right ventricle only), phospholamban as well as troponin T were repressed in both diseased human and rat hearts. Our study enabled an identification of disease-associated candidate genes. Their regulation is likely to be the result of an imbalance between pressure load/stretch force and vascular tonus and the observed changes may provide a rational for the rhythm disturbances observed in patients with cardiac hypertrophy.

Keywords: Ion channels; Ion exchangers; Ca^{2+}-handling proteins; Spontaneously hypertensive rats; Ventricular hypertrophy

Introduction

Heart hypertrophy is considered to be an adaptive response to undue stress and results in thickening of the ventricular wall. Despite intense research, the molecular pathology of this disease remains uncertain and appears to be driven by a multitude of factors resulting in enlargement of muscle fibers. Essentially, the prolonged unphysiological hemodynamic alterations are followed by edema of the lungs as well as pulmonary hypertension, which are considered to be progressive stages of disease. Further complications include stroke, myocardial necrosis and myocardial fibrosis as well as nephrosclerosis and various types of vascular lesions (Frohlich, 1982). To gain insight into the onset and progression of disease, various animal models were developed which mimic, at least in part, the clinical features observed with patients and the spontaneously hypertensive rat (SHR) is an accepted model for the study of essential hypertension and left ventricular hypertrophy (Ji et al., 2003; Minami et al., 2003; Rizzoni et al., 2003).

Recent evidence suggests ventricular hypertrophy to be associated with changes in transcript abundance of cardiac-specific genes. Specifically, hypobaric hypoxia resulted in a biphasic mRNA expression of SERCA2a and was associated with right ventricular hypertrophy (Sharma et al., 2004). In end-stage dilative cardiomyopathy (DCM), unilateral repression of a Ca^{2+}/calmodulin-dependent kinase, sarcoplasmic reticulum Ca^{2+} ATPase 2 (SERCA) (Barrans et al., 2002), ryanodine receptor (RyR-2) and phospholamban (Guo et al., 2003) was observed. Further, induction of ANP, cardiac troponin and tropomyosin (Barrans et al., 2002) as well as

* Corresponding author. Fraunhofer Institute of Toxicology and Experimental Medicine, Drug Research and Clinical Inhalation, Nicolai-Fuchs-Str. 1, D-30625 Hannover, Germany. Fax: +49 511 5350 155.
E-mail address: borlak@item.fraunhofer.de (J. Borlak).

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tumor necrosis factors (Dibbs et al., 2003; Diwan et al., 2004), calcineurin (Dong et al., 2003; Petrashevskaya et al., 2002) or calsequestrin (Ihara et al., 2002; Sato et al., 1998) was also associated with various stages of cardiomyopathy. Notably, these changes may simply be disease associated but not disease defining. Further, ion homeostasis is altered in cardiac hypertrophy, but the underlying pathologies for ion channel and ion transporter disease remain uncertain. Indeed, channels must inherently have the ability to open and close in response to the cells' need or appropriate stimuli. If the channel remains open, an ionic equilibrium would quickly be reached between the outside and inside of the cells and no ionic gradient or electrical potential across the membrane would be maintained. This would lead to cellular dysfunction and/or death. Further, the "gating" function of ion channels is intrinsic to the protein structure of the channel and can be regulated by several distinct signaling processes, including mechanical stimulation of the channel such as stretch (mechanically gated channel), voltage changes across the membrane (voltage-gated channels) or binding of hormones or drugs (ligand-gated channels). An improved understanding of ion channel disease in ventricular hypertrophy is in need and will aid the development of novel therapies and may provide a rational for the known ECG changes and rhythm disturbances in hypertrophic hearts. Here, we report gene expression of ion channels, ion exchangers and Ca^{2+}-handling proteins in spontaneously hypertensive and normotensive rats and compared findings with diseased human hearts (Borlak and Thum, 2003). Overall, we wished to further our understanding on disease-associated changes in the transcript regulation of cardiac-specific genes for its diagnostic value and for an improved knowledge on the molecular basis of disease.

Material and methods

Animals. All animal procedures described in this report were approved by the local authorities and the investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

A total of $n = 6$ male Sprague–Dawley (SD) rats weighing 277 g ± 16 g and male spontaneously hypertensive rats (SHR) weighing 245 g ± 3 g were obtained from Charles River (Sulzfeld, Germany). Food and water were given ad libitum.

Heart explantation. Rats were anesthetized with 0.1 ml of Ketamin (Medistar, Hannover, Germany) per 100 g body weight and 50 μl of Rompun (Medistar, Hannover, Germany) per 100 g body weight. In addition, 2000 international units of Heparin (Roche, Mannheim, Germany) were given i.p. prior to surgery. The hearts were prepared anatomically and immediately frozen to await further analysis.

Table 1
Used oligonucleotide primers in the RT-PCR

Gene	Forward primer	Reverse primer	bp	Cycler program
Calsequestrin	tca aag acc cac cct acg tc	cca gtc ttc cag ctc ctc ag	352	57 °C, 60 s/30 cyc.
Calreticulin	atgaccccacagattccaag	gcataggcctcatcattggt	339	55 °C, 60 s/33 cyc.
Calmodulin	gaagcaggccagtcaaagac	cgaatttggaagccaacact	348	55 °C, 60 s/33 cyc.
SERCA 2a	tcc atc tgc ctg tcc at	gcggttactccagtattg	196	55 °C, 60 s/35 cyc.
SERCA 2b	tcc atc tgc ctg tcc at	aga cca gaa cat atc act	324	55 °C, 60 s/35 cyc.
PMCA 1	tgc ctt gtt ggg att tct ct	cac tct ggt tct ggc tct cc	351	55 °C, 60 s/35 cyc.
PMCA 2	att gat gga gct ggg atc ag	act tca ccg tgg aca cct tc	350	57 °C, 60 s/35 cyc.
PMCA 4	agc agt tgc gtc agt cag aa	gct ttg tag agg gct gtt gg	351	57 °C, 60 s/38 cyc.
Na^+–K^+-ATPase	tgt gat tct ggc tga gaa cg	agg aca gga aag cag caa ga	352	57 °C, 60 s/35 cyc.
NCX-1	tgt ctg cga ttg ctt gtc tc	tca ctc atc tcc acc aga cg	364	55 °C, 60 s/35 cyc.
K_{ir} 3.4	gga tgg cag aca gga aag ag	acc acc tgt tgg agc tgt tc	351	56 °C, 60 s/35 cyc.
K_{ir} 6.1	tga gtc tag gac gcg ttg tg	cac cag cca cca cat gat ag	351	55 °C, 60 s/35 cyc.
L-type Ca^{2+}-Channel	tgt cac ggt tgg gta gtg aa	ttg agg tgg aag gga ctt tg	346	55 °C, 60 s/35 cyc.
Na_v 1.5	cag ctg aca tga cca aca cc	ttg agc agc atc tcc aac ac	349	55 °C, 60 s/35 cyc.
Ryanodine receptor 2	cca aca tgc cag acc cta ct	ttt ctc cat cct ctc cct ca	351	57 °C, 60 s/35 cyc.
Ryanodine receptor 3	cgt ctc tgg tgt cat ggc ta	cac act tca tat ccg gct ca	347	57 °C, 60 s/38 cyc.
Phospholamban	gct gag ctc cca gac ttc ac	gcg aca gct tgt cac aga ag	339	57 °C, 60 s/30 cyc.
Troponin T	gggtacatccagaaggctca	gtgcctggcaagacctagag	374	55 °C, 60 s/33 cyc.
Troponin I	atctccgcctccagaaaact	cagtagtgcctgcatcatgg	352	55 °C, 60 s/33 cyc.
α-cardiac Actin	actcctatgtaggtgacgaggc	gacgttatgagtcacaccgtcg	337	57 °C, 60 s/33 cyc.
α-skeletal Actin	atctcacgttcagctgtggtca	accaccggcatcgtgttggat	182	55 °C, 60 s/33 cyc.
α-MHC	ggaagagcgagcggcgcatcaagg	ctgctggacaggttattcctca	304	65 °C, 60 s/35 cyc.
β-MHC	gccaacaccaacctgtccaagttc	ttcaaaggctccaggtctcagggc	202	55 °C, 60 s/35 cyc.
ANP	gccggtagaagatgaggtca	gggctccaatcctgtcaatc	269	60 °C, 60 s/35 cyc.
BNP	tctgctcctgcttttcctta	gaactatgtgccatcttgga	258	60 °C, 60 s/35 cyc.
GATA 4	agcaaggactaggcacctctagc	atagccaggctttggtacatcgc	410	55 °C, 60 s/35 cyc.
MEF 2C	ccg atg cag acg att cag tag	gtg tca cac cag gag aca tac	260	58 °C, 60 s/35 cyc.
GAPDH	ggccaaggtcatccatga	tcagtgagcccaggatg	353	55 °C, 60 s/32 cyc