Transition from compensated hypertrophy to systolic heart failure in the spontaneously hypertensive rat: Structure, function, and transcript analysis

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ABSTRACT

Gene expression, determined by micro-array analysis, and left ventricular (LV) remodeling associated with the transition to systolic heart failure (HF) were examined in the spontaneously hypertensive rat (SHR). By combining transcript and gene set enrichment analysis (GSEA) of the LV with assessment of function and structure in age-matched SHR with and without HF, we aimed to better understand the molecular events underlying the onset of hypertensive HF. Failing hearts demonstrated depressed LV ejection fraction, systolic blood pressure, and LV papillary muscle force while LV end-diastolic and systolic volume and ventricular mass increased. 1431 transcripts were differentially expressed between failing and non-failing animals. GSEA identified multiple enriched gene sets, including those involving inflammation, oxidative stress, cell degradation and cell death, as well as TGF-β and insulin signaling pathways. Our findings support the concept that these pathways and mechanisms may contribute to deterioration of cardiac function and remodeling associated with hypertensive HF.

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Background

The spontaneously hypertensive rat (SHR) is a useful laboratory model of genetic hypertension and naturally evolving hypertensive heart disease [1] that has enabled investigators to study changes in structure and function during the transition to heart failure (HF) [2-4].

More recent studies have shown alterations in cardiac gene expression in association with phenotypic changes related to ventricular remodeling associated with HF. Clinical studies of gene expression have been carried out in failing human hearts with coronary artery disease [5] and dilated cardiomyopathy [6]. Experimental studies have examined a number of models of HF , including transgenic rats with overexpression of the human renin (Ren) and angiotensinogen (Agt) genes [7], Dahl salt-sensitive rats [8,9], TNFα (Tnf) overexpressing mice [10], transgenic mice with compensated hypertrophy and HF [11], and dogs with chronic rapid ventricular pacing [12,13]. Differences with respect to identified transcripts have been described in different models of HF. When all transcript analysis of HF are taken together, however, a common HF profile emerges that appears to indicate that genes encoding transcription factors, remodeling/repair, the immune system, cell communication and cell death, including apoptosis, and stress response genes, were up-regulated [6–13] whereas potassium current transcripts [14,15], mitochondrial transcription factors, lipid and glucose metabolism transcripts [7,12,16–19] were down-regulated in failing compared to non-failing controls. Despite the many transcript changes found, the mechanism(s) underlying transition from adaptive cardiac hypertrophy to HF remains poorly understood.

Despite the importance of the SHR model, to our knowledge, only one study has been carried out using microarray gene chip analysis of the aging SHR to study HF [20]. This study compared differences between 12- and 16-month-old SHR with compensated LV hypertrophy and 20-month-old animals with diastolic dysfunction and HF; these SHR exhibited changes in diastolic properties of the heart, while systolic function was unimpaired. Changes were attributed to upregulation of the extracellular matrix (ECM) while the broad array of transcript changes found in other studies of HF [6–13,18,19] were not found. The apparent divergence between these findings and those in other models of HF may be related to differences between diastolic and systolic HF, species differences, or possibly differences in statistical analysis.

In an effort to improve the discovery of differentially expressed transcripts in the SHR with systolic HF, as demonstrated by LV and isolated papillary studies, individual LV samples from six SHR and six age-matched SHR with compensated hypertrophy were studied by micro-array gene expression analysis. In addition, Gene Set Enrichment Analysis (GSEA) was carried out in order to elucidate pathways involved [21].

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Results

Development of HF

Sixteen 12-month-old SHR were initially entered into the study; four died prior to study. Of the surviving twelve SHR, six developed evidence of HF (SHR-F), and were compared to six additional SHR without HF (SHR-NF). Animals were considered to have HF when based on documentation of left ventricular dysfunction echocardiography findings, specifically LV enlargement with LV ejection fraction <55% (normal >80%). Pathological findings associated with heart failure included pleural and/or pericardial effusions, left atrial or LV thrombi, and right ventricular hypertrophy, as described previously [3,4,22–24]. Peak systolic blood pressure was 145 ± 8 mm Hg in SHR-F, as compared to 185 ± 5 mm Hg in SHR-NF. The mean age at the time of study (19 ± 1 months) was not significantly different between groups.

Echocardiography

Serial echocardiographic measurements of LV ejection fraction (LVEF) and LV volumes were carried out beginning at 12 months of age and approximately every 8 weeks thereafter until the development of HF. LVEF in 12-month-old animals (without HF) is approximately 90%, gradually declined with age to approximately 83 to 84% in non-failing SHR at 17 months of age, and to $54.2 \pm 4\%$ with HF. Echocardiographic findings of SHR hearts without HF (SHR-NF) and SHR at the time of HF (SHR-F) are presented in Table 1. LV ejection fraction and fractional shortening, used as measures of systolic function, decreased significantly, while LV end-diastolic and end-systolic chamber dimensions increased with HF (Fig. 1).

Pathological parameters

Body weight, cardiac chamber weights, and chamber weights normalized for body weight are presented in Table 2. Body weight decreased, and the LV and, to a greater extent, RV weight increased in SHR with HF relative to non-failing SHR ($p<0.01$). Body weight was significantly reduced ($p<0.05$), and LV/BW and RV/BW ratio significantly increased, in SHR-F relative to SHR-NF. Myocardial and liver water content data is also presented in Table 2. Left ventricular and liver water content (expressed as g water/g dry wt) was increased in SHR with HF compared to SHR with compensated hypertrophy ($p<0.05$).

Isolated muscle data

Isometric papillary muscle data from SHR-NF and SHR-F are summarized in Table 3; peak active isometric stress and quick-release
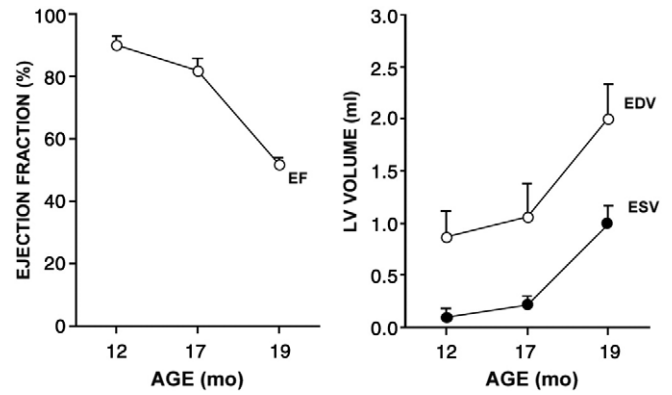

Fig. 1. Serial echocardiographic measurements in SHR. LV ejection fraction (left panel) and end-systolic and end-diastolic LV volume (right panel) of male SHR during the transition to heart failure (12, 17, and 19 months of age). EF, indicates LV ejection fraction (%); ESV, end-systolic volume (ml; black circle); EDV, end-diastolic volume (ml; open circle). LV function remains compensated until approximately 17 months, and then rapidly declines; mean age at onset of HF 19 ± 1 months.

force–velocity relationships are presented in Fig. 2. Papillary muscle cross-sectional area was not significantly different between SHR groups. In SHR-F, active isometric stress (σ_{active}, active developed tension normalized for muscle cross-sectional area) and maximum rate of stress development ($+d\sigma/dt$) were depressed, and relaxation time significantly abbreviated, relative to SHR-NF. Myocardial stiffness was significantly increased in SHR-F compared to SHR-NF (Table 3). Velocity of shortening was less at all loads examined in papillary muscles from SHR-F than SHR-NF ($p<0.01$).

Response to β-adrenergic stimulation

Peak σ, $+d\sigma/dt$, and time to peak σ were determined at muscle bath isoproterenol (ISO) concentrations of 10^{-8} and 10^{-7} mmol/l. In SHR-F, 10^{-7} ISO decreased peak σ by $16 \pm 2\%$ of pre-ISO control, and $+d\sigma/dt$ was unchanged. In SHR-NF, peak σ was unchanged and $+d\sigma/dt$ increased by $14 \pm 4\%$ at 10^{-7} ISO. Time to peak σ and relaxation time index decreased by approximately 15% and 25%, respectively, with ISO in both groups (NS).

Histology

Fig. 3 is a cross-section of the LV from SHR-NF and SHR-F stained with Masson's trichrome. Representative sections of LV-free wall and LV papillary muscles from SHR-NF and SHR-F were assessed by quantitative histological analysis. Table 4 presents quantitative connective tissue and myocyte area data. Myocardial fibrosis was increased in LV and papillary muscle samples from SHR-F relative to SHR-NF ($p<0.05$) and to a greater extent relative to age-matched normotensive WKY (LV average $8.3 \pm 4.0\%$; WKY data not shown).

Differentially expressed LV transcripts between SHR-F and SHR-NF

A total of 1,431 transcripts were found to be differentially expressed in LV samples from SHR-F relative to SHR-NF ($p<0.05$) by microarray analysis. Of these, 484 transcripts (294 upregulated and 190 downregulated) were positively identified with known biological function. Fig. 4 is an example of four highly expressed transcripts that were down- and five up-regulated with HF, obtained from six individual LV samples from SHR-F (left panel) and SHR-NF (right panel). In general, the extent of expression changes appeared to be related to the extent of myocardial impairment. The top 25 identified transcripts that were most substantially up- and down-regulated are presented in Tables 5 and 6, respectively. (See supplemental tables for a complete list of all identified transcripts differentially expressed with HF).

Table 1
Echocardiographic measurements.

	SHR-NF		SHR-F	
	Initial	Time of study	Initial	Time of study
LVEF%	89.2 ± 6.0	83.7 ± 5.2	90.9 ± 4.4	54.2 ± 4.5*
LVFS%	55.5 ± 5.5	46.8 ± 7.8	56.7 ± 5.3	23.1 ± 2.6*
LVEDD$_{mm}$	7.32 ± 0.66	7.54 ± 0.78	7.23 ± 0.89	9.75 ± 0.72*
LVESD$_{mm}$	3.40 ± 0.39	4.00 ± 0.66	3.25 ± 0.41	7.45 ± 0.69*
EDVtz$_{ml}$	0.89 ± 0.22	0.98 ± 0.27	0.88 ± 0.32	1.93 ± 0.40*
ESVtz$_{ml}$	0.14 ± 0.06	0.17 ± 0.07	0.10 ± 0.05	0.93 ± 0.24*

SHR-NF indicates spontaneously hypertensive rats without HF, initial echocardiographic data obtained at 12 months of age, and at time of study; SHR-F, spontaneously hypertensive rats with HF, initial echocardiographic data obtained at 12 months of age, and at time of study. LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; EDVtz, end-diastolic volume (Teichholz); ESVtz, end-systolic volume (Teichholz).
Values are mean of six rats per group $\pm$ SD. *$p<0.05$ SHR-F vs. SHR-NF at the time of study.