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Differential metal content and gene expression in rat left ventricular hypertrophy due to hypertension and hyperactivity

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ABSTRACT

The spontaneously hypertensive rat (SHR) has been studied extensively as a model of left ventricular hypertrophy (LVH) and associated cardiac dysfunction due to hypertension (HT). The SHR also possesses a hyperactive trait (HA). Crossbreeding SHR with Wistar–Kyoto (WKY) control rats, which are nonHT and nonHA, followed by selected inbreeding produced two additional homozygous strains: WKHT and WKHA, in which the traits of HT and HA, respectively, are expressed separately. WKHT, WKHA and SHR all display LVH, but only the SHR exhibits cardiac dysfunction. We hypothesized that cardiac dysfunction in the SHR is uniquely characterized by calcium overload. We measured total cardiac Ca, Cu, Fe, K, Mg and Zn in the four strains. We found elevated Ca and depressed Cu, Mg and Zn with HT, but not unique to SHR. We surmise that HT promotes aberrant regulation of cardiac Ca^{2+}, Cu^{2+}, Mg^{2+} and Zn^{2+}, which does not necessarily result in cardiac dysfunction. Interestingly, Cu was elevated in HA strains compared to nonHA counterparts. We then analyzed gene expression as mRNA of Cu-containing proteins, most notably mitochondrial-Cox, Dbh, Lox, Loxl1, Loxl2, Sod1 and Tyr. The gene expression profiles of Lox, Loxl1, Loxl2 and Sod1 were found especially high in the WKHA, which if reflective of protein content could account for the high Cu content in the WKHA. The mRNA of other genes, notably Mb, Fxyd1, Maoa and Maob were also examined. We found that Maoa gene expression and monoamine oxidase-A (MAO-A) protein content were low in the SHR compared to the other strains. The finding that MAO-A protein is low in the SHR and normal in the WKHT and WKHA strains is most consistent with the idea that MAO-A protects against the development of cardiac dysfunction in LVH but not against LVH in these rats.

Introduction

The spontaneously hypertensive rat (SHR) is characterized by hypertension (HT) and hyper-reactivity to stress associated with hyperactivity (HA). While the SHR has been used extensively as a model of left ventricular hypertrophy (LVH) and associated dysfunction due to severe HT, the cause of LVH in this strain is not solely due to HT [1–4]. The HT and HA traits of the SHR were segregated into two new homozygous strains: the WKHT, which is HT and not hyperactive (nonHA), and the WKHA, which is HA and not hypertensive (nonHT) [5,6]. By examining four genetically related strains it was possible to look for correlations of biological findings with the HT trait, if present in SHR and WKHT but not in WKY and WKHA, or with the HA trait, if present in SHR and WKHA but not in WKY and WKHT.

Both the WKHT and WKHA strains develop LVH by mechanisms independent of HA and HT, respectively [5,7]. Yet, neither the WKHT nor WKHA strain develops cardiac dysfunction at a cellular level like that observed in the SHR [8]. In the current study we used these four strains to test hypotheses regarding the role of metal regulation, particularly calcium overload, in the development of LVH and cardiac dysfunction in the SHR.

It has been suggested that LVH and associated dysfunction like that in the SHR are attributable to suppressed function of calcium regulatory proteins that remove Ca^{2+} from cardiomyocytes [8,9]. Excess calcium load may also initiate gene expression leading to LVH [10,11]. We have recently reported that Zn^{2+} can displace intracellular Ca^{2+} in isolated cardiomyocytes [12]. We therefore

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hypothesized that cellular calcium overload and/or zinc deficiency, measurable as total cardiac content of Ca and Zn, are present in the SHR. We found elevated Ca and depressed Cu, Mg and Zn with HT, but not unique to SHR. We also found elevated Cu with HA. Further examination of gene expression, especially for copper–proteins, revealed an elevated gene expression of the family of lysyl oxidase enzymes and superoxide dismutase in the WKHA. The gene expression and protein content of monoamine oxidase-A was found especially reduced only in the SHR, which suggests protection against development of cardiac dysfunction with LVH in the other strains.

Methods

Rat models

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of The University of Vermont College of Medicine and complied with the *Guide for the Use and Care of Laboratory Animals* published by the National Institutes of Health. Male rats of four inbred (fully homozygous) strains were used in this study. SHR and WKY were obtained commercially from Harlan Sprague–Dawley (Indianapolis, IN). Two other inbred strains were derived previously from crossing the SHR and WKY: WKHA, hyperactive, and WKHT, hypertensive rats [6]. WKHA (F45–F46 generations) and WKHT (F42–F45 generations) were attained from the colony maintained at the University of Vermont since 1980 and presently available from the Rat Resource and Research Center, Columbia, MO. At 10 weeks of age, four male rats from each strain were anesthetized with isoflurane, and hearts were removed, flash frozen in liquid nitrogen and stored at −80 °C.

Cardiac elemental analysis

Polypropylene bottles were soaked in 0.4 mM EGTA overnight, then rinsed four times with ddH_2O and allowed to drip dry according to published recommendations [13]. Between 40–80 mg of each LV sample was placed in its own tube and lyophilized by 90 min of vacuum freeze drying. Samples were digested in 10 μL nitric acid (15.8 normal) per mg tissue wet weight. ddH_2O was added to bring total volume to 1000× dilution of original tissue wet weight including final concentrations of 0.8 N nitric acid. Standards were prepared for elements Ca, Cu, Fe, K, Mg and Zn including equivalent concentration of nitric acid. Detection of elemental content was measured in duplicate using inductively coupled plasma atomic emission spectroscopy (ICP-AES) performed by the ULTIMA2C operated in mono-mode (Horiba Scientific, Edison, NJ).

mRNA analysis and quantitative PCR

Total RNA was extracted from the rat hearts using Qiagen RNeasy kit and cDNA was synthesized using Invitrogen Superscript III first-strand synthesis system according to manufacturer instructions. Sequences for the Taqman specific primers were obtained from Applied Biosystems (http://www.appliedbiosystems.com.). mRNA expression levels were measured in duplicate using a ABI Prism 7900HT sequence detection system based by polymerase chain reactions (PCR). ΔCt was calculated relative to average Ct value for HPRT expression. The average ΔCt for all WKY samples was used as the normalization value to calculate ΔΔCt. ΔΔCt was then calculated for relative fold difference in transcription levels, and relative quantification (RQ) values calculated as $2^{-\Delta\Delta Ct}$.

Single nucleotide polymorphisms (SNP) rs8148546 and rs13458141 for Maoa and rs64072569 for Loxl2 were tested in cardiac cDNA of all rat strains by PCR. The PCR conditions included denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 72 °C for 30 s and extension at 72 °C for 30 s with the final extension of 72 °C for 10 min. Restriction pattern was determined on a 2% agarose gel using restriction digestion enzymes specific for the polymorphism. These SNPs were not detected and therefore did not influence the gene expression levels reported here.

Analysis

All analyses were performed using SPSS v. 19 (SPSS Inc.). Values reported in tables and figures as mean ± sem. Rats were identified for hypertensive trait as HT or nonHT and activity trait as HA or nonHA. We applied a 2(HT, nonHT) × 2(HA, nonHA) repeated-measures ANOVA to all variables to differentiate effects on elemental content and gene expression. Post hoc analyses were applied among groups using Student–Newman–Keul's post hoc test. Statistical significance is reported at $P < 0.05$ level and also at $P < 0.10$ to show strong trends which are in agreement with statistically significant results and thus to minimize the possibility of committing a Type II (false-negative) error [14]. Specifically, we note a $P < 0.10$ statistic if it is in support of other conventionally significant findings at the $P < 0.05$ level, such as when ANOVA results are conventionally significant, and the post hoc analyses show trends in support of the ANOVA results.

Results

Rat characteristics

Characteristics of the four homozygous rat strains measured over a ten year period including activity, blood pressure, heart rate and body mass are presented in Table 1 as previously reported [8]. The WKY strain is considered the normal control and thus displays normal blood pressure and activity. Activity is detected by movements recorded in an activity cage. WKHT rats are severely hypertensive and display blood pressures similar to those of the SHR. While the WKHT rats are somewhat more active than the WKY, the WKHT rats are clearly not hyperactive on the order of the WKHA and SHR and do not display elevated catecholamine release with stress as observed in the WKHA and SHR [15]. The WKHA rats are hyperactive on the order of the SHR, but are clearly not hypertensive on the order of the WKHT and SHR.

Other characteristics of the four strains of particular relevance to cardiac function include the following: (1) WKHT, WKHA and SHR all display LVH compared to WKY controls (Fig. 1A); (2) hypertension in the SHR and WKHT is associated with hypertrophy of stellate ganglion cells that innervate the heart [16]; and (3) HA in the WKHA and SHR rats is associated with a hyper-reactivity to stress and elevated catecholamine release during stress [15].

Cardiac elemental analysis

We had expected to find elevated Ca content in the SHR compared to the other strains. We found Ca content elevated by ∼25% with HT as determined by ANOVA, but not unique to the SHR strain (Table 2 and Fig. 1B). In other words, the significant HT main effect refers to Ca in the WKHT and SHR being statistically higher than that in the normotensive WKY and WKHA control strains, respectively, and there were no unique post hoc findings to highlight a significant difference between any two groups. We also found a significant reduction in cardiac content of Cu by ∼25%, Mg by ∼10% and Zn by ∼13% in HT strains compared to their nonHT controls. There was also a tendency ($P = 0.127$) for HT associated reduction in K by ∼10%, but no statistical significance. There were no significant effects of HT or HA on cardiac content of Fe.

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