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Age-related difference in cardiac adaptation to chronic hypertension in rats, with and without nifedipine treatment

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Abstract

Three myosin isozymes, V1 (αα MHC = Myosin Heavy Chain gene), V2 (αβ MHC) and V3 (ββ MHC) that are identified in the cardiac ventricles of most mammals have been shown to shift to a V3 predominance pattern during cardiac growth and in response to left ventricular pressure overload, and to V1 predominance following anti hypertensive treatment. This study examined whether long-term hypertension impairs the ability of the adult heart to restructure myosin isozyme proportions. Using pyrophosphate gel electrophoresis, we studied proportions of cardiac myosin isozymes (V1 and V3) in young (16 weeks) and adult (36 weeks) spontaneously hypertensive rats (SHR), and following 12 weeks of nifedipine (N) treatment in age-matched SHR rats (SHR-N). The values of V1 and V3 myosin isozymes were derived by adding half of the value of V2 to each isozyme proportion. The V3 proportion in the young SHR control (SHR-C) group (49%) was 34% higher ($p < 0.05$) than in the young Wistar Kyoto control (WKY-C) group (37%). However, the proportion was similarly high, though not statistically significant, in both the adult SHRC (73%) and WKY-C (71%) groups. The proportion in the young SHR-N group (29%) was 41% lower ($p < 0.05$) than in the young SHR-C group (49%), and the proportion in the adult SHR-N group (47%) was 34% lower ($p < 0.05$) than in the adult SHR-C group (73%). The ratio of left ventricular weight to body weight (LVW/BW), which determines left ventricular hypertrophy (LVH), was higher in both young and adult SHR-C (26%, $p < 0.05$, and 42%, $p < 0.05$, respectively) than in WKY-C groups. The mean LVW/BW was 27% ($p < 0.05$) greater in adult than in young SHR-C rats. The LVW/BW in both age groups of treated SHR-N was similar to that in age matched WKY-C rats. Conclusion: Our study showed that a rise in the V3 level occurs in young hypertensive rats, but no rise occurs in the V3 level in adult hypertensive rats. High blood pressure seems to contribute to the high V3 level in young hypertensive rats, but in adult hypertensive rats, high blood pressure does not accentuate the V3 rise already acquired due to the aging process. Nifedipine treatment in both young and adult hypertensive rats prevented the V3 rise due to hypertension and to the aging process. This effect of nifedipine seems to be through its antihypertensive action. (Mol Cell Biochem **198**: 109–112, 1999)

Key words: LVH, hypertension, aging, myosin isozyme, nifedipine

Introduction

During the three stages of growth, development, and aging, the rat myocardium undergoes structural, molecular genetic, and protein synthetic changes which could cause its ability to adapt to a variety of stimuli to be different in younger and older rats [1]. Throughout the aging process, the myocardial structural alterations include the development of left ventricular hypertrophy (LVH) associated with cell loss, and an increase in collagen deposition and fibrosis. At the

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biochemical and molecular genetic levels, of the three myosin isozymes (V1, V2, V3), the V1, with higher ATPase and higher V_{max}, predominates throughout both the young and adult life of the rats [2, 3]. With aging, however, the V1 level decreases, while the V3 level, with low ATPase and low V_{max} increases, resulting in V3 predominance in old rats [3, 4]. The shift in myosin isozyme distribution toward a higher level of V3 has been observed in response to mechanical pressure overload due to aortic banding and renal hypertension, regardless of age [1, 3]. In a recent study in old rats with chronic genetic hypertension, we did not observe an increase in V3 level [4]. In that study, the rise in V3 level in both old normotensive and old hypertensive rats was only minimally reduced with nifedipine; however, in another study we observed the V3 rise to be completely reversed in normotensive younger adult rats [5]. From these findings, it appears that the strong adaptive capacity with respect to myosin isozyme shift observed in young rats becomes diminished in old rats. The hypothesis based on this observation in rats is that the myocardium is well able to adapt to chronic hypertension and to reverse consequent cardiac abnormalities in young rats, but its ability diminishes with advancing age.

The aim of the present study was to characterize the myosin isozyme adaptations in rats in response to (1) chronic hypertension and (2) antihypertensive treatment as young rats mature to adulthood. We used spontaneously hypertensive rats (SHR) for this study because they are hypertensive from an early age and throughout their life time, and are, therefore, conducive to a study of evident myosin isozyme changes due to on-going hypertension during growth and aging.

Materials and methods

Cardiac myosin isozyme proportions were studied in four groups of SHR: (1) two age groups of controls (SHR-C), one group aged 16 weeks (young) and one aged 36 weeks (adult), and (2) two groups of SHR receiving nifedipine (SHR-N) for 12 weeks, one group from age 4–16 weeks (young), and one from age 24–36 weeks (adult). The findings from the four SHR groups were compared with the findings from two groups of normotensive Wistar Kyoto control (WKY-C) rats of the same age groups, 16 and 36 weeks from a previous study [5]. The treatment regimen consisted of 26 ± 0.2 mg/g body weight/day of nifedipine mixed with rat chow. Nifedipine dosage was the same as that used to lower blood pressure in spontaneously hypertensive rats in our laboratory [4, 5]. Systolic arterial pressure (SAP) mmHg by tail-cuff was measured before the initiation of the drug and at the end of the treatment period in the treatment group, and in all other groups.

At the end of the 12-week treatment period, at ages 16 and 36 weeks, all rats were sacrificed under anesthesia (nembutal 50 mg/kg intraperitoneal). The heart was then removed and the left ventricle (LV) including the interventricular septum was dissected free from the atria, great vessels, and right ventricle. The LV was dropped in a cryotube immersed in liquid nitrogen and was used for myosin isozyme studies. The body weight (BM) and LV weight (LVW) were measured, and LV weight-to-body weight (LVW/BM ratio was calculated. The protocol was approved by the University of New Mexico Laboratory Animal Care and Use Committee.

Myosin isozyme study

Isolation of myosin and separation of myosin isozymes by gel electrophoresis were accomplished as described in previous studies [6, 7]. The gels were stained and destained, and their densitometric scans were recorded on an E-C apparatus attached to a Hewlett-Packard integrator (Model 3390A). The relative estimate of each isozyme was calculated from the areas under the peak height. For the calculation of V1 ($\alpha\alpha$ Myosin Heavy Chain (MHC) gene) and V3 ($\beta\beta$ MHC) content, the V2 (heterodimer, $\alpha\beta$ MHC) isozyme was assumed to be equally distributed between V1 and V3 isozymes. The values of V1 and V3 myosin isozymes were derived by adding half of the value of V2 to each isozyme proportion.

Statistical analysis

All results are expressed as the mean ± S.E.M. One-way analysis of variance followed by the Newman-Keul's pairwise comparisons test was used to compare mean V3 levels among the groups [8]. The same method was used to compare systolic arterial pressure, LVW, BW, and the LVW/BW ratio among the groups. Comparisons between time periods within the same animal species were made using *t*-tests. Results are reported as statistically significant if $p < 0.05$, or as NS if they were not significant. Spearman correlation coefficients were calculated to evaluate associations between V3 myosin isozyme, systolic arterial pressure (SAP) both before and after treatment, LVW, BW, and LV/BW in all subgroups of young and adult WKY-C, SHR-C, WKY-N and SHR-N rats. The analyses were carried out using the SAS statistical package for personal computers.

Results

The SAPmmHg in the SHR-C groups at both ages (16 and 36 weeks) was higher (170 ± 7 and 167 ± 5, respectively, $p < 0.05$ for both groups) than in the age-matched WKY-C (112 ± 4, 130 ± 3, respectively). For SHR-C, no significant difference in the SAP was observed between the two age groups. The post-treatment SAP in both young and adult age groups of the SHR-N rats was lower (152 ± 5 and 150 ± 1,

respectively, $p < 0.05$ for both groups) than in the age-matched SHR-C. The SAP level in both age groups of SHR-N was lower relative to the age matched SHR-C groups (18 at 16 weeks and 17 at 36 weeks, $p < 0.05$ for both values). However, the level in the SHR-N groups was significantly higher ($p < 0.05$) than in the age-matched WKY-C.

Physical characteristics and V3 proportion for all groups are given in Table 1, with values of these parameters in age-matched WKY rats from our previous published study [5]. The question(s) posed in the present work concerns whether or not the change in the V3 level and LVH with hypertension and anti hypertensive treatment is different in the young vs. the adult SHRs. In the young SHR-C, mean LVW was 26% higher ($p < 0.05$) than in the young WKY-C, and the mean BW was similar to that in the young WKY-C. In the adult SHR-C, mean LVW was 31% higher and mean BW was 8% lower than in the adult WKY-C. Mean LVW/BW ratio was higher in both the young and adult SHR-C (26 and 42% higher, respectively, $p < 0.05$ for both values) than in the age-matched WKY-C. The mean LVW/BW ratio in adult SHR-C was 27% ($p < 0.05$) greater than in the young SHR-C. No statistically significant difference in the mean LVW/BW ratio was found between the adult and young SHR-N. Mean LVW/BW ratio was 18% ($p < 0.05$) higher in young SHR-N than in the young WKY-C; but not statistically different between the adult SHR-N and the adult WKY-C.

The V3 proportion was substantially increased in the young SHR-C vs. the young WKY-C (34% higher, $p < 0.05$), but was not significantly increased in the adult SHR-C vs. the adult WKY-C (3% higher; NS). The proportion was lower in both young and adult SHR-N vs. age-matched SHR-C (41 and 34% lower, respectively, $p < 0.05$ for both values). Furthermore, in both young and adult SHR-N, the V3 proportion was lower than that in the age-matched WKY-C (21%, NS, and 35%, $p < 0.05$, respectively).

Table 1. Physical characteristics and V3 myosin isozyme level in untreated control (SHR-C) and nifedipine treated (SHR-N) spontaneously hypertensive rats, and age-matched Wistar Kyoto control *(WKY) rats

	Age/wks	WKY-C	SHR-C	SHR-N
LVW	16	0.70 ± 0.02[a,d]	0.88 ± 0.06[d]	0.86 ± 0.02[d]
	36	1.03 ± 0.05	1.35 ± 0.03[b]	0.98 ± 0.03
BW	16	362 ± 8[d]	362 ± 8[d]	379 ± 8[d]
	36	477 ± 4[a]	437 ± 7	445 ± 5
LVW/BW	16	1.92 ± 0.05[a,d]	2.42 ± 0.18[d]	2.27 ± 0.05
	36	2.17 ± 0.10	3.08 ± 0.06[b]	2.20 ± 0.05
V3	16	36.60 ± 2.79[d]	49.17 ± 5.33[b,d]	29.00 ± 2.94[d]
	36	70.60 ± 1.12	72.63 ± 1.71	46.88 ± 2.72[c]

Results from a previous study [5]. Data, mean ± S.E.M.; [a]$p < 0.05$ vs. SHR-C and SHR-N within the same age group; [b]$p < 0.05$ vs. WKY-C and SHR-N within the same age group; [c]$p < 0.05$ vs. SHR-C and WKY-C within the same age group; [d]$p < 0.05$ 16 vs. 36 weeks within the specific animal group; LVW – left ventricular weight; BW – body weight.

Because of the observed difference in the V3 level between the young SHR-C vs. the adult SHR-C, correlations were calculated to determine whether the V3 level in the SHR-C and SHR-N groups was associated with SAP and LVH, with and without treatment. The V3 level in young SHR-C was significantly related only to pretreatment SAP ($r = 0.97$, $p < 0.001$), and not to LVW ($r = –0.21$, $p = 0.74$), BW ($r = 0.48$, $p = 0.33$), or to LVW/BW ratio ($r = 0.10$, $p = 0.87$). The V3 level in the adult SHR-C was related neither to pretreatment SAP nor to any parameter of LVH: pretreatment SAP ($r = –0.40$, $p = 0.33$), LVW ($R = –0.04$, $p = 0.93$), BW ($r = 0.42$, $p = 0.30$), or to LVW/BW ($r = –0.50$, $p = 0.20$). The V3 level in the young SHR-N was significantly related to the post-treatment SAP ($r = 0.84$, $p = 0.04$), but not to pretreatment SAP ($r = –0.34$, $p = 0.51$), LVW ($R = 0.14$, $p = 0.78$), BW ($r = 0.64$, $p = 0.17$), or to LVW/BW ($r = –0.26$, $p = 0.62$). Similarly, the V3 level in the adult SHR-N was significantly related to the post-treatment SAP ($r = 0.80$, $p = 0.02$), but not to pretreatment SAP ($r = –0.06$, $p = 0.88$), LVW ($r = 0.14$, $p = 0.74$), BW ($r = 0.46$, $p = 0.26$), or to LVW/BW ($r = –0.24$, $p = 0.57$).

Discussion

Two major cardiac adaptations to pressure overload are LVH and a myosin isozyme shift toward V3 predominance [1, 3, 7]. The rat heart at all ages, including very young and very old, responds in this manner to acute pressure overload [1, 3, 7]. However, our data show that with chronic pressure overload as in systemic hypertension, both these responses do not occur at all ages. Our study showed that in chronically hypertensive rats, the young rats developed both LVH and a rise in V3, but the adult rats developed LVH with no concomitant rise in V3.

The specific triggers leading to up-regulation of the β-MHC gene and an increase in V3 level in response to left ventricular pressure overload are not known. However, the stimulus of high LV wall stress resulting from an acute increase in LV pressure has been linked with the α- to β-MHC switch and greater synthesis of V3 isozyme, and with LVH development [9]. In support of this view, studies [9, 10] have demonstrated an accumulation of β-MHC mRNAs after aortic banding first in the endocardium and in the areas surrounding coronary arteries and then in the rest of the myocardium [9], an increase in myocardial protein synthesis following acute increases in aortic pressure in isolated heart preparations, and an increase in the mRNA synthesis in isolated cardiac nuclei subjected to hydrostatic pressure [10]. Based on these findings, we believe that high LV wall stress is a major stimulus for the observed rise in V3 in young SHRs with recent onset hypertension. It is likely that in this age group, the moderate LVH is not sufficient to normalize the wall stress. With long-term hypertension as in the adult SHR

group in the present study, further increase in left ventricular wall thickness by hypertrophy could restore the wall stress to within normal limits, and thus reduce the stimulus responsible for a V3 rise. Consistent with this view, our data demonstrated an association between V3 level and SAP in young SHRs ($r = 0.97$, $p < 0.001$), but not in adult SHRs ($r = 0.40$, $p = 0.33$).

The absence of a rise in V3 proportion in adult SHRs raises the question of whether adult chronically hypertensive rats are unable to alter myosin isozymes. This question is based on several earlier studies [11, 12, 13] according to which both aging and hypertension impair the ability of the heart to alter gene expression and to synthesize proteins. In the present study, the adult SHRs were able to achieve a V3 level as high as their age-matched normotensive counterparts. In addition, the level was substantially lower in adult SHRs treated with nifedipine. These findings and the 43% increase in LVH in adult SHRs vs. adult WKYs argue against an age- and hypertension-associated impairment in molecular and biochemical adaptations in the adult rat heart.

Nifedipine treatment resulted in lower V3 proportion, and SAP and LVH reduction in both young and adult SHRs. The V3 proportion in both young and adult SHRs was related to post-treatment SAP, but not to LVH. Based on these data, the lower V3 level in nifedipine-treated SHRs could be attributed to the drug's anti hypertensive action. Because the decrease in the V3 level with nifedipine occurred despite moderate residual hypertension, the V3 to V1 shift could also be due to a direct action of the drug on the heart which was in addition to its anti hypertensive effect. This view is supported by findings in our previous study [5], in which nifedipine prevented the age-related rise in V3 levels in WKYS. Nifedipine has been shown to decrease a number of functional calcium channels secondary to myocardial hypertrophy [14], thereby decreasing calcium entry into the myocytes and ameliorating the calcium overload, and thus inhibiting the factor(s) that contribute to greater β-MHC synthesis. Another possible mechanism is that nifedipine enhances synthesis of the α-myosin gene by a direct effect on gene transcription [15].

The present study suggests that the ability to restructure myosin isozyme composition and to modify LVH in response to chronic hypertension and anti hypertensive treatment is not impaired with hypertension and the aging process. However, the pattern of these adaptive responses in chronic hypertension is not similar in the young and the adult rats. High blood pressure contributes to high V3 level in young SHRs but not in adult SHRs. The high V3 level in adult SHRs is principally due to the aging process, without any additional contribution from the high blood pressure or LVH. Treatment with nifedipine prevents a rise in V3 in both young and adult SHRS. This effect of nifedipine seems to be through its anti hypertensive action.

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