Original Article

Is Cardiac Hypertrophy in Spontaneously Hypertensive Rats the Cause or the Consequence of Oxidative Stress?

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The aim of this work was to assess the possible correlation between oxidative damage and the development of cardiac hypertrophy in heart tissue from young (40-d-old) and older (4-, 11- and 19-month-old) spontaneously hypertensive rats (SHR) in comparison with age-matched Wistar (W) rats. To this end, levels of thiobarbituric acid reactive substances (TBARS), nitrotyrosine contents, NAD(P)H oxidase activity, superoxide production, and the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined. Compared to age-matched normotensive rats, SHR showed a significant increase in systolic blood pressure from 40 d of age and left ventricular hypertrophy (LVH) was significantly evident from 4 months of age. W rats (11- and 19-month-old) also showed an increase in LVH with aging. TBARS and nitrotyrosine levels were similar in young rats from both strains and were significantly increased with age in both strains, with the values in SHR being significantly higher than those in age-matched W rats. NAD(P)H activity was similar in young SHR and W rats, whereas it was higher in aged SHR compared with age-matched W rats. Compared to W rats, superoxide production was higher in aged SHR, and was abolished by NAD(P)H inhibition with apocynin. CAT activity was increased in the hearts of 4-month-old SHR compared to age-matched W rats and was decreased in the hearts of the oldest SHR compared to the oldest W rats. SOD and GPx activities decreased in both rat strains with aging. Moreover, an increase in collagen deposition with aging was evident in both rat strains. Taken together, these data showed that aged SHR exhibited higher cardiac hypertrophy and oxidative damage compared to W rats, indicating that the two undesirable effects are associated. That is, oxidative stress appears to be a cause and/or consequence of hypertrophy development in this animal model. (*Hypertens Res* 2008; 31: 1465–1476)

Key Words: antioxidant enzymes, hypertrophy, thiobarbituric acid reactive substances, nitrotyrosine, superoxide production

Introduction

The spontaneously hypertensive rat (SHR) is a laboratory model of naturally developing hypertension and heart failure that appears to be similar in many aspects to essential hypertension in humans (*1*). Systolic blood pressure (SBP) in SHR rapidly increases between 5 and 10 weeks of age and cardiac hypertrophy develops between 9 and 12 weeks of age (*2*). Increasing evidence from different experimental models supports the concept that oxidative stress contributes to the pathogenesis of myocardial hypertrophy and the process of
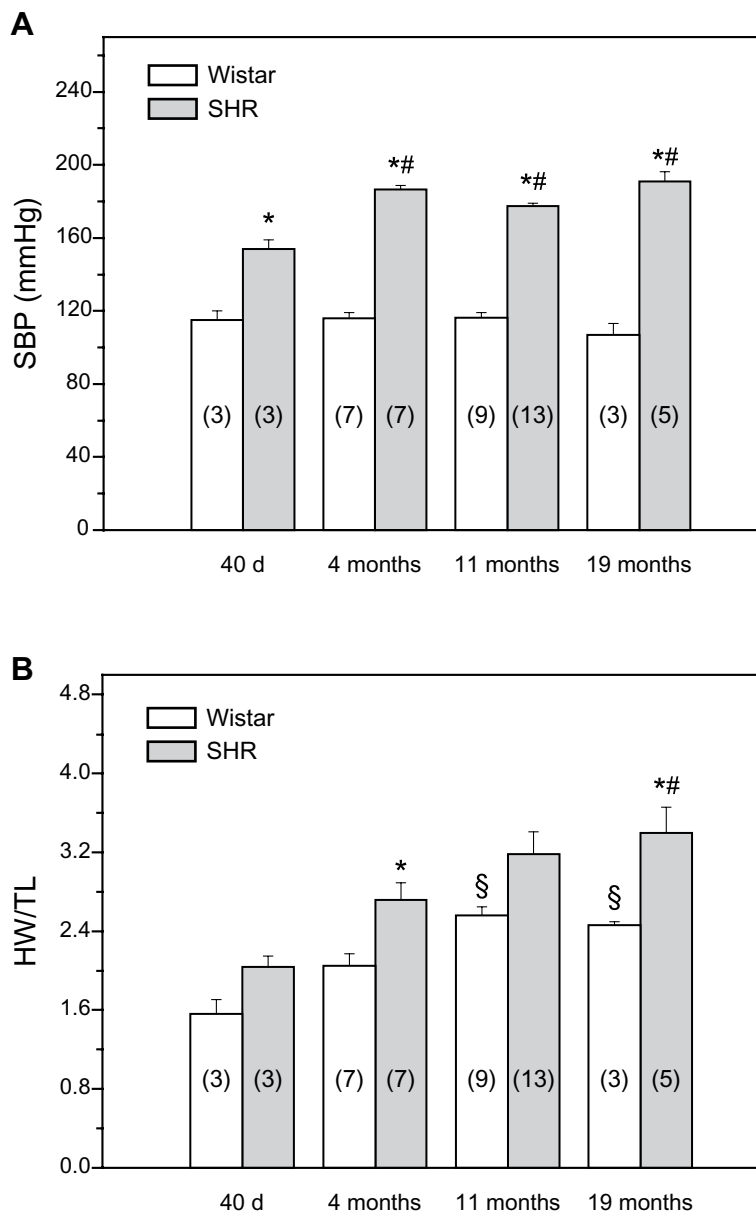
A

B

Fig. 1. *A: Systolic arterial blood pressure (SBP) and B: cardiac hypertrophy measured as the ratio between heart weight (HW) and tibia length (TL) of SHR and W rats of 40 d, and 4, 11, and 19 months of age. *p < 0.05 in SHR vs. W rats, #p < 0.05 vs. 40 d-old SHR, §p < 0.05 vs. 40 d-old W rats. The number of animals is indicated in parentheses.*

myocardial remodeling leading to heart failure (*3*, *4*). The oxidative stress is the result of an increase of reactive oxygen species (ROS) production and/or inadequate antioxidant defense mechanisms. It has been shown that an increase in the activity and expression of myocardial NAD(P)H oxidase is the main source of ROS in cardiac hypertrophy (*5–7*). However, existing data about the antioxidant status in hypertension are inconsistent. Various studies have shown that the activities of one or more antioxidant enzymes are lower (*8*, *9*), higher (*10*) or unchanged (*11*, *12*) in hypertensives compared with normotensive controls. Although the underlying causes

of these discrepancies are unknown, they may be due to the use of different hypertension models, animals at different hypertensive stages and/or different experimental preparations.

On the other hand, lipid peroxidation and oxidative modification of proteins by reactive nitrogen species such as peroxynitrite—a product of the reaction between superoxide ($O_2^{-\cdot}$) and nitric oxide (NO)—have been implicated in the pathogenesis of cardiac hypertrophy and normal aging (*13*, *14*).

The aim of this study was to compare the oxidative stress in the hearts of young and old SHR and Wistar (W) rats and to
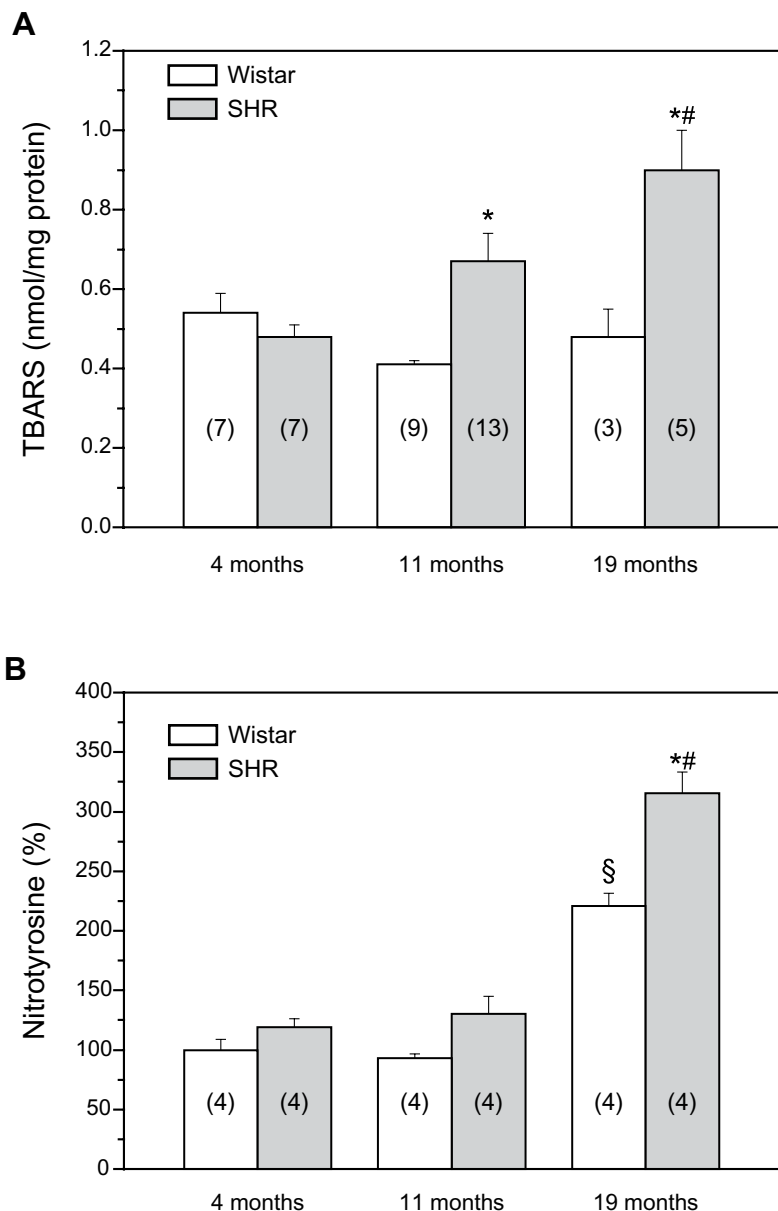
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Fig. 2. *A: TBARS concentration (nmol/mg protein) and B: nitrotyrosine content expressed as a percentage with respect to 4-month-old W rats in hearts from SHR and W rats at 4, 11, and 19 months of age. *p < 0.05 in SHR vs. W rats, #p < 0.05 vs. 4 months-old SHR, §p < 0.05 vs. 4-month-old W rats. The number of animals is indicated in parentheses.*

analyze the possible correlation between such stress and the development of cardiac hypertrophy.

Methods

Experiments were conducted using 40-d-old and 4-, 11- and 19-month-old male SHR and age-matched W rats. All animals were identically housed under controlled lighting and temperature conditions with free access to standard rat chow and tap water. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Ani-

mals published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996). SBP was recorded by the tail-cuff method (*15*). Left ventricular hypertrophy (LVH) was evaluated by the ratio between heart weight (HW) and tibia length (TL) as previously described (*16*). The W rats were used as normotensive controls. The animals were decapitated and their hearts quickly removed and perfused with ice-cold saline solution (0.9% NaCl) to remove the blood. Left ventricle (LV) samples (LVS) were taken to assay NAD(P)H oxidase activity, superoxide production, protein nitration and collagen deposition. The rest of the heart was homogenized in
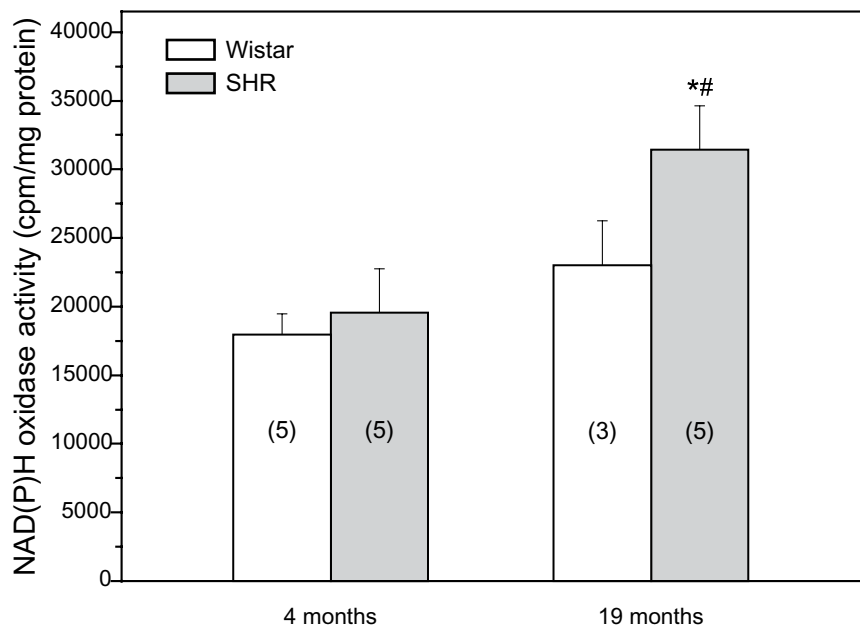
Fig. 3. *NAD(P)H oxidase activity, expressed as cpm/mg protein, in hearts from SHR and W rats at 4 and 19 months of age. *p < 0.05 in SHR vs. W rats, #p < 0.05 in 19- vs. 4-month-old SHR. The number of animals is indicated in parentheses.*

5 volumes of 25 mmol/L KH_2PO_4–140 mmol/L KCl (pH=7.4) containing a protease inhibitors cocktail (Complete Mini; Roche Diagnostics, Mannheim, Germany) with a Polytron homogenizer. An aliquot of the heart homogenate was used to assess lipid peroxidation. The remaining homogenate was centrifuged at $12,000 \times g$ for 5 min at 4°C and the supernatant stored at −70°C until the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were assayed. The protein concentration was evaluated by the Bradford method (*17*) using bovine serum albumin as a standard.

Lipid Peroxidation

Lipid peroxidation was determined by measuring the rate of production of thiobarbituric acid reactive substances (TBARS), expressed as nmol/mg protein. Heart homogenates were centrifuged at $2,000 \times g$ for 10 min. Supernatants (0.5 mL) were mixed with 1.5 mL trichloroacetic acid (30% w/v), 1 mL thiobarbituric acid (0.7% w/v) and 0.5 mL water, followed by boiling for 15 min. After cooling, absorbance was determined spectrophotometrically at 535 nm, using a ε value of 1.56×10^5 $(mol/L)^{-1}$ cm^{-1} (*18*).

Protein Nitration

The interaction of peroxynitrite leads to nitrotyrosine formation that is considered to be an indirect marker of oxidative stress (*19*). Thus, we assessed nitrotyrosine levels by Western blot analysis. A sample of the left ventricle was homogenized in lysis buffer (300 mmol/L sacarose; 1 mmol/L DTT; 4 mmol/L EGTA; protease inhibitors cocktail: 1 tablet/15 mL of buffer; 20 mmol/L Tris-HCl, pH 7.4). After a brief centrifugation the supernatants were denatured and equal amounts of protein were subjected to PAGE and electrotransferred to PVDF membranes. The membranes were incubated with an anti-nitrotyrosine polyclonal antibody (Cayman Chemicals, Ann Arbor, USA). A peroxidase-conjugated, anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, USA) was used as secondary antibody, and finally bands were visualized with an ECL-Plus chemiluminescence detection system (Amersham, Uppsala, Sweden). Autoradiograms were analyzed by densitometric analysis using Scion Image software (Scion, Frederick, USA) (*14*).

NAD(P)H Oxidase Activity

Left ventricular slices (1×5 mm) were incubated for 5 min at 37°C in Krebs-HEPES buffer (in mmol/L: 99 NaCl, 4.69 KCl, 1.87 $CaCl_2$, 1.2 $MgSO_4$, 1.03 K_2PO_4, 25 $NaHCO_3$, 20 HEPES, 11.1 glucose) bubbled with 95% O_2–5% CO_2 to maintain pH 7.4 and then transferred to glass scintillation vials containing the same buffer with 5 μmol/L lucigenin. Chemiluminescence was assessed at 37°C over 15 min in a scintillation counter (Packard 1900 TR; Packard Instruments, Meriden, USA) at 1-min intervals. Vials containing all components without tissue were previously counted, and these values were subtracted from the chemiluminescence signals obtained in the presence of LVS. NAD(P)H oxidase activity was measured in the presence of 100 mmol/L NAD(P)H and
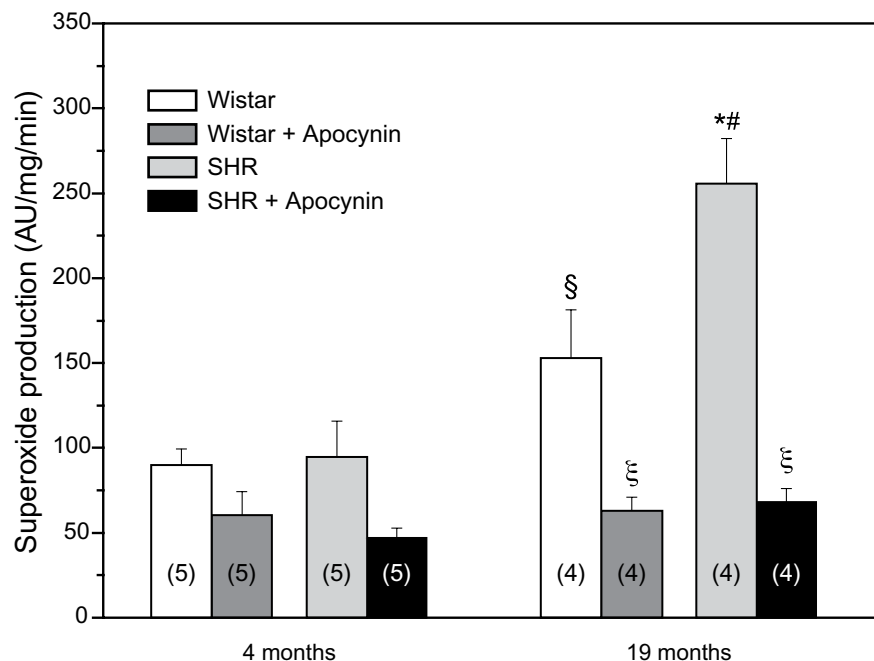
Fig. 4. *Superoxide production, expressed as AU/mg/min, in hearts from SHR and W rats at 4 and 19 months of age in the absence and presence of apocynin. *p<0.05 in SHR vs. W rats, #p<0.05 in 19- vs. 4-month-old SHR, §p<0.05 in 19- vs. 4-month-old W rats, ξp<0.05 in 19-month-old SHR and W rats in the presence vs. absence of apocynin. The number of animals is indicated in parentheses.*

expressed as cpm/mg dry weight of LVS (*20*).

Measurements of $O_2^{-\cdot}$ production

Superoxide production was measured in tissue slices from LV with lucigenin-enhanced chemiluminescence in Krebs-HEPES buffer with 5 μmol/L lucigenin (*21*). The chemiluminescence was recorded in arbitrary units (AU) with a luminometer (Chameleon; Hidex, Turku, Finland) for 30 s each with 4.5 min interval during 30 minutes. $O_2^{-\cdot}$ production was expressed as AU per mg dry weight per min. To determine the involvement of NAD(P)H oxidase in $O_2^{-\cdot}$ production, the slices were pretreated for 30 min with 300 μmol/L apocynin.

SOD, CAT and GPx Activities Assays

SOD activity was determined by inhibition of formazan production (produced by nitroblue tetrazolium [NBT] reduction by superoxide anion) at pH 10.2 and 25°C. The reaction mixture consisted of 100 μmol/L xanthine, 100 μmol/L EDTA, 25 μmol/L NBT, and 50 mmol/L Na_2CO_3 (pH 10.2). The reaction was started by the addition of xanthine oxidase, and then the absorbance at 560 nm was read every 30 s for 5 min (*22*). One unit of SOD assay was defined as the amount of enzymatic protein required to inhibit 50% of NBT reduction.

CAT activity was determined by the procedure of Aebi (*23*). The decrease in absorbance at 240 nm by the addition of

30 mmol/L H_2O_2 was monitored each 15 s for 30 s. One unit of CAT assay was defined as the amount of enzyme that decomposes 1 μmol of H_2O_2.

The GPx activity was measured according to the method of Lawrence and Burk (*24*). The assay reaction comprised 50 mmol/L K_2HPO_4 buffer, 1 mmol/L EDTA, 1 mmol/L NaN_3, 1 mmol/L reduced glutathione, 0.2 mmol/L NADPH, 0.25 mmol/L H_2O_2 and 1 U/mL glutathione reductase. Gpx activity was assayed by monitoring NADPH oxidation at 340 nm, measuring the absorbance every 15 s for 5 min. The activity was calculated using a molar extinction coefficient for NADPH of 6.22×10^3 $(mol/L)^{-1}$ cm^{-1} at 340 nm. One unit of the enzyme represented a decrease of 1 μmol of NADPH/min under the assay conditions used.

Myocardial Collagen Content

Samples of LV were fixed in 10% buffered formaldehyde for 24 h and embedded in paraffin. To estimate the amount of collagen present, serial sections of 4 μm were stained for 1 h in 0.2% picrosirius red in aqueous saturated picric acid (Direct Red 80; Aldrich, Milwaukee, USA). The images were analyzed under a microscope at magnification ×200. The area of the myocardial collagen network was determined using the image-processing program Image-Pro Plus v. 4.5 and expressed as the percentage of picrosirius red–positive pixels among the total pixels in each image stack.

Statistics

Data are presented as the mean±SEM. Differences between young or old SHR and age-matched W rats, were analyzed using two-way analysis of variance (ANOVA) with the Newman-Keul's post test used for multiple comparisons among groups. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Compared to age-matched W rats, the SBP of SHR was higher at all ages examined (Fig. 1A). The analysis of the time course of SBP showed that as early as 40 d of age the SHR exhibited higher SBP values compared to age-matched W rats. At 4 months of age the SBP increased further in comparison to the youngest rats and it remained elevated throughout the last stage studied. Figure 1B shows that LVH increased significantly in SHR at 4, 11, and 19 month of age compared to age-matched W rats. Higher values were obtained in 11- and 19-month-old SHR than in younger SHR. Although lower than that in SHR, an increase in LVH was also observed in W rats with aging (at 11 and 19 months of age *vs.* 4 months of age).

TBARS

Figure 2A shows TBARS production in hearts from 4-, 11- and 19-month-old SHR and W rats. In hearts from SHR there was a significant increase in TBARS at 11- and 19-months of age compared to the level in age-matched W rats, with the highest value occurring at 19 months of age. No changes in TBARS with age were observed in W rats.

Protein Nitration

The nitrotyrosine levels in hearts of 4, 11 and 19-month-old W rats and SHR are depicted in Fig. 2B. Immunoblotting assays showed a statistically significant increase of nitrotyrosine levels in 19-month-old SHR compared to age-matched W rats. The oldest SHR and W rats exhibited an increase of nitrotyrosine levels compared to their respective younger group.

The highest degree of oxidative damage, as evaluated by TBARS and nitrotyrosine levels, was seen in the oldest SHR and W rats. Therefore, the following analyses were conducted using hearts from 19 and 4-month-old rats from both strains.

NAD(P)H Oxidase Activity and $O_2^{-\cdot}$ Production

Although there were no significant differences in NAD(P)H oxidase activity between SHR and W rat hearts from young animals, aged SHR showed an increase in NAD(P)H oxidase activity compared to age-matched W rats (Fig. 3). Similar $O_2^{-\cdot}$ production was obtained in hearts from W rats and SHR
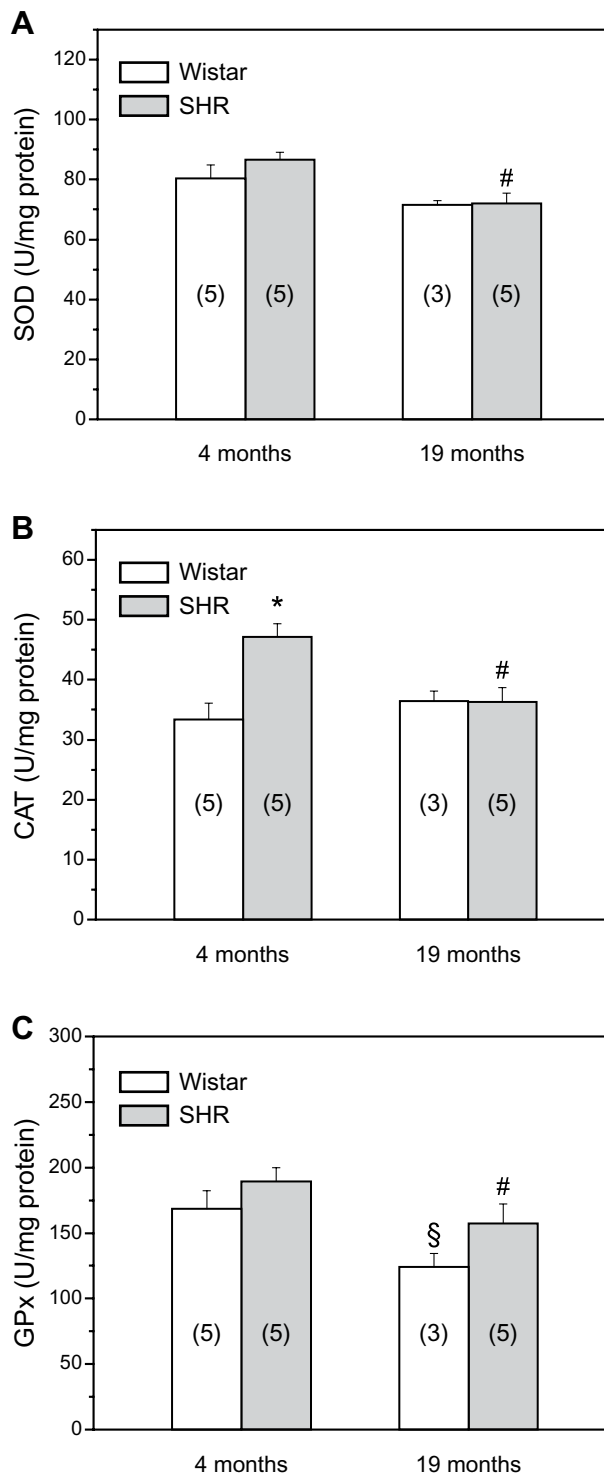

Fig. 5. *A: Superoxide dismutase (SOD), B: catalase (CAT), and C: glutathione peroxidase (GPx) activities, expressed as U/mg protein, in hearts of 4- and 19-month-old SHR and W rats. *p<0.05 in SHR vs. W rats, #p<0.05 in 19- vs. 4-month-old SHR, §p<0.05 in 19- vs. 4-month-old W rats. The number of animals is indicated in parentheses.*

Table 1. NAD(P)H oxidase/SOD, SOD/CAT and SOD/GPx Ratios in 4-month-old and 19-month-old SHR and W Rats

	4-month-old		19-month-old	
	W rats	SHR	W rats	SHR
NAD(P)H oxidase/SOD	223±27	235±51	323±60	458±82[#]
SOD/CAT	2.33±0.07	1.84±0.08*	1.97±0.06[§]	2.04±0.16
SOD/GPx	0.46±0.05	0.47±0.02	0.59±0.05	0.51±0.04

SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; SHR, spontaneously hypertensive rat; W, Wistar. *$p<0.05$ SHR *vs*. W rats, [#]$p<0.05$ *vs*. 4-month-old SHR, [§]$p<0.05$ *vs*. 4-month-old W rats.

at 4 months of age, whereas in older animals SHR showed a significantly higher $O_2^{-\cdot}$ production in comparison with age-matched W rats (Fig. 4). The addition of the selective NAD(P)H oxidase inhibitor apocynin decreased $O_2^{-\cdot}$ production in hearts of aged SHR and W rats. In 4-month-old SHR and W rats $O_2^{-\cdot}$ production was lower in the presence of apocynin, but the reduction was not statistically significant. This may have been because the lucigenin method was unable to detect very slight differences in $O_2^{-\cdot}$ levels that were only slightly above the background levels (*25*).

SOD, CAT and GPx Activities

The activities of antioxidant enzymes are shown in Fig. 5. SOD activity significantly decreased in older hearts from SHR (approximately 17%) (Fig. 5A), whereas a small but not significant decrease was detected in W rats with aging.

Hearts from 4-month-old SHR exhibited a higher CAT activity in comparison to hearts from age-matched W rats, and this activity decreased in 19-month-old SHR. In W rats CAT activity did not change with aging (Fig. 5B).

Compared to younger animals a significant decrease of GPx activity was detected in hearts from 19-month-old SHR and W rats. No differences in GPx activity were detected between SHR and age-matched W rats (Fig. 5C).

Table 1 shows NAD(P)H oxidase/SOD, SOD/CAT and SOD/GPx ratios. The NAD(P)H oxidase/SOD ratio in 19-month-old SHR was significantly higher than those obtained in the other groups. Lower values of the SOD/CAT ratio were detected in 4-month-old SHR and 19-month-old W rats compared to 4-month-old W rats. The SOD/GPx ratio was similar in all groups examined.

Myocardial Collagen Content

Representative microphotographs of LV specimens obtained from young and aged SHR and W rats are shown in Fig. 6 (upper panel). The images illustrate the moderate increase of fibrosis in hearts from 4-month-old SHR in comparison to the thin collagen filaments detected in cardiac tissue from age-matched W rats. A marked fibroblast proliferation was also evident in hearts from aged SHR. As shown in the lower panel of Fig. 6, both SHR and W rats showed an increase in collagen content with aging. However, the collagen content in

young and old SHR was higher than that in age-matched W rats, and this difference was more pronounced in the oldest animals.

Discussion

The present study showed an increase of oxidative stress associated with higher levels of hypertrophy in hearts from aged SHR in comparison to age-matched W rats. Oxidative stress develops when the well-regulated balance between pro-oxidants and antioxidants becomes uncontrolled and tips in favor of pro-oxidants. Oxidative stress is a major contributor to the aging process (*26*) and appears to be a common feature of hypertensive disorders from diverse origins (*9, 27–29*). ROS have also been shown to be involved in cardiac hypertrophy induced by several stimuli, such as mechanical stretch, endothelin and angiotensin II (*30*). The damage caused by oxidative stress during aging becomes more evident when analyzing the effect of ROS on organic macromolecules, such as proteins and lipids. Lipid peroxidation is a major contributor to the age-related loss of membrane fluidity, especially with respect to the two aldehydic lipid peroxidation products, malonyldialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). Therefore, it is not surprising that lipid peroxidation is increased in the aged heart as demonstrated by the higher levels of MDA (*31*) or HNE (*32*). However, in the present study, in accordance with previously reported data (*33–35*), we did not find an increase of TBARS in hearts from normotensive rats with aging. These results can be attributed to the fact that normal hearts have a reduced amount of substrate for the lipid peroxidation (*35*) and/or the fact that the end products of lipid peroxidation are readily metabolized (*33*). Although 4-month-old SHR showed a higher rate of cardiac hypertrophy compared to age-matched W rats, the TBARS content was similar between the two. However, we detected an increase in TBARS content with aging (*i.e.*, in 11 and 19-month-old animals) in the hearts from SHR compared to age-matched W rats. Moreover, 19-month-old SHR exhibited the highest hypertrophy index and level of lipid peroxidation, suggesting that an increase of oxidative damage may be the consequence of the persistent elevated SBP and/or increased cardiac hypertrophy in addition to aging.

NO plays pivotal roles in the maintenance of blood pressure and vascular tone (*36*). Superoxide avidly reacts with NO and
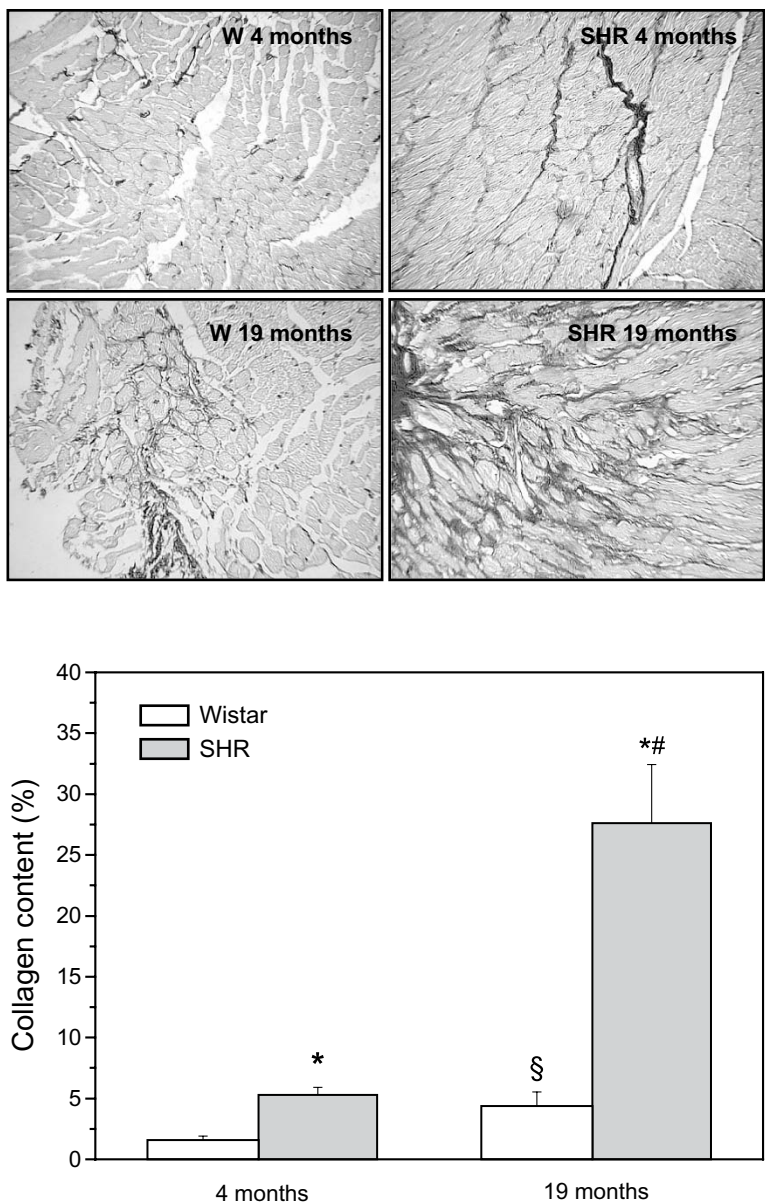
Fig. 6. *Microphotographs of picrosirius red–stained histological LV sections and collagen content of young and aged SHR and W rats. A few isolated collagen fibers are seen in the LV of young W rats, whereas the highest collagen deposition (the darkest areas) is observed in aged SHR. *p < 0.05 in SHR vs. W rats, #p < 0.05 in 19- vs. 4-month-old SHR, §p < 0.05 in 19- vs. 4-month-old W rats.*

in the process produces highly reactive and cytotoxic products, such as peroxynitrite (ONOO⁻) and peroxynitrous acid (ONOOH). Peroxynitrite in turn reacts with and modifies various molecules, namely lipids, DNA, and proteins. For instance, peroxynitrite reacts with the tyrosine and cysteine residues in protein molecules to produce nitrotyrosine and nitrocystein, leading to inactivation of important antioxidant enzymes, such as SOD (*37, 38*). In addition to these and other harmful biochemical reactions, the oxidation of NO by ROS inevitably results in functional NO deficiency, which can

contribute to pathogenesis and maintenance of hypertension and its long-term consequences. In agreement with previous findings in the vasculature of hypertensive animals (*39, 40*), we detected a higher $O_2^{-\cdot}$ production in cardiac tissue of aged SHR compared to age-matched normotensive W rats. The fact that hypertension improves with antioxidant therapy implies that oxidative stress is involved in that pathology (*41*). Recent investigations using hypertensive models other than SHR have shown that a decrease in oxidative stress is one of the mechanisms responsible for the efficacy of anti-

hypertensive treatments such as calcium antagonists (*42*, *43*), angiotensin II type 1 receptor antagonists, or angiotensin-converting enzyme inhibitors (*44*, *45*).

In our study, hearts from 4-month-old SHR and W rats showed a similar nitrotyrosine content. In addition to lipid peroxidation data, this result is another demonstration that the higher LVH observed in young SHR relative to age-matched W rats was not accompanied by higher nitrosative damage. Aged W rats exhibited an increase in nitrotyrosilation compared with young animals. This increase was lower than that detected in aged SHR, indicating that the addition of hypertrophy to the aging process leads to a high degree of nitration due to an increased misbalance in myocardial production of either NO or $O_2^{-\cdot}$. Although we did not measure the expression or activity of NO synthase (NOS), it has been reported that aged hearts exhibit increased myocardial NOS-cGMP signaling associated with an up-regulation of NOS (*46*, *47*). Therefore, higher levels of nitrotyrosine in aged SHR hearts would be attributed to an increase of peroxynitrite derived from an excessive production of both reactive species, NO and $O_2^{-\cdot}$. Another possibility for explaining the higher oxidative and nitrosative stress of aged SHR compared to W rats is a decrease in NO availability due to an increase in $O_2^{-\cdot}$ production.

Mitochondria occupy a central position in the metabolism of ROS, supporting the so-called "free radical theory of aging" (*48*–*50*). Other cardiovascular sources of ROS include the enzymes xanthine oxidoreductase (*51*) and NAD(P)H oxidase (multisubunit membrane complexes) (*6*) and NOS uncoupling (*52*, *53*). Abnormal activation and expression of myocardial NAD(P)H oxidase have been suggested to be the main sources of ROS in the hypertrophic and failing myocardium (*5*, *54*). A recent paper of Miyagawa *et al*. (*55*) showed that the production of $O_2^{-\cdot}$ by NAD(P)H oxidase in femoral arteries of SHR is enhanced in comparison with that in WKY, resulting in the inactivation of NO and impairment of endothelial modulations of vascular contractions. In our study, whereas young SHR showed a similar NAD(P)H oxidase activity as age-matched W rats, an increase in the activity of this enzyme was detected in aged SHR. Apocynin is a well-characterized inhibitor of NAD(P)H oxidase (*56*). It acts by impeding the assembly of the p47-phox and p67-phox subunits within the membrane NAD(P)H oxidase complex (*56*, *57*). Some of the effects of apocynin treatment are protection of the endothelium from the initiating events of atherosclerosis (*57*), a reduction of p22-phox mRNA expression and cardiac hypertrophy in aldosterone-infused rats (*58*), and a prevention of hyperglycemia-induced intracellular ROS elevation and myocyte dysfunction (*59*). Aponycin has also been shown to reduce oxidative stress in stroke-prone spontaneously hypertensive rats, leading to the suppression of cardiac hypertrophy, inflammation and fibrosis (*60*). Under our experimental conditions, apocynin blunted the $O_2^{-\cdot}$ production in hearts from aged SHR and W rats. Although a significant increase in NAD(P)H oxidase activity was only evident

in aged SHR hearts, we suggest that ion NAD(P)H oxidase–dependent ROS production would mediate both the hypertrophic response and aging.

Other factors to be considered are antioxidant enzymes, a primary defense that helps prevent oxidative damage to biological molecules. SOD rapidly converts $O_2^{-\cdot}$ to H_2O_2, which is further degraded by CAT and GPx. The levels of the antioxidant enzymes are sensitive to the oxidative stress, and increased or decreased levels have been reported in different pathologies in which an enhancement of ROS is a cause or consequence of the disease (*34*, *61*).

Our data show that SOD activity in hearts from young SHR was slightly but not significantly higher than that in W rats. The lack of significant difference in the SOD activity between the hearts of these two rat strains agrees with previous findings (*11*, *62*, *63*). GPx activity was slightly but not significantly higher in the hearts from young SHR compared to those of age-matched W rats, whereas CAT activity showed a significant increase in the former. An opposite result was recently demonstrated in the thoracic aortas of SHR, in which CAT activity decreased along with a concomitant increase of H_2O_2 (*61*). Although we do not have experimental evidence, the increase in CAT activity detected in our young SHR suggests that H_2O_2 production was decreased in our preparations. Analyzing the NAD(P)H oxidase/SOD, SOD/CAT and SOD/GPx ratios in 4-month-old rats showed that only the SOD/CAT ratio was significantly lower in young SHR compared to W rats. Since the SOD/GPx ratios were the same for all the groups, the decrease in the SOD/CAT ratio in young SHR indicates that CAT became the compensatory mechanism removing H_2O_2. Moreover, CAT activity was shown to be increased in young SHR. Thus, the development of a compensatory mechanism apart from the generation of reactive oxygen species could explain our finding that the TBARS and nitrotyrosine contents in the hearts of young rats were similar between the two rat strains. In hearts from aged rats, a decrease of SOD, CAT and GPx activities was evident in accordance with the data obtained by Ito *et al*. (*8*) and in contradiction to the recent observations of Csonka *et al*. (*10*). Aged W rats exhibited a small but not significant decrease in SOD and GPx activities. However, a significant diminution of antioxidant enzymes was evident in aged SHR compared to younger SHR. In addition, both rat strains showed similar antioxidant enzyme activities at 19 months of age. Therefore, this fact could not explain the differences of oxidative damage detected between aged SHR and W rats. These differences could be attributed to a significantly higher NAD(P)H oxidase/SOD ratio in aged than young SHR in accordance with the increased $O_2^{-\cdot}$ production with aging, indicating that the compensatory mechanism detected in young rats will be abnormal in cardiac tissue from aged SHR. In this regard, it is worth noting a previous report that an increase of SOD pharmacology potency by lecithinization is able to protect endothelial cells against alterations induced by ROS (*64*). Another explanation for the observed differences would be related to

angiotensin II content, which appears to be involved in the genesis of oxidative stress in tissues other than cardiac tissue in SHR (*65*). This hypothesis is supported by recent experiments performed using the vascular tissue of stroke-prone SHR (*44, 45*), in which the inhibition of angiotensin receptors or the angiotensin-converting enzyme system reduced ROS production. Our results are also consistent with investigations showing that cardioprotective treatments are mediated by a restoration or up-regulation of antioxidant enzymes (*42, 45*). It is recognized that an increase in collagen concentration is an integral part of the extracellular matrix remodeling that takes place in the LVs during the natural aging process (*66*) and as well as in response to a variety of pathologies resulting in hypertrophy of this chamber (*67, 68*). In accordance with these studies, we showed evidence of an increase of collagen deposition with aging and its exacerbation in aged SHR. This histological feature of the oldest SHR would be related to the higher oxidative stress detected in these rats. This hypothesis is supported by recent investigations in other hypertrophy models (*69, 70*), in which a correlation between ROS production and cardiac collagen accumulation was found.

Accumulating evidence has suggested that ROS are capable of directly activating intracellular cascades involved in the regulation of hypertrophic growth (*71*). It has been reported that Rho family proteins, especially Rac1, play critical roles in mechanical stress-induced hypertrophy responses and are involved in ROS-mediated activation of MAP kinases (such as p38, ERK1/2) and activation of nuclear factor-κB. Moreover, Rac 1 is essential for the assembly of plasma membrane NAD(P)H oxidase (*6*). Thus, under our experimental conditions, the sustained hemodynamic load in SHR would modulate the action of extracellular stimuli (such as angiotensin II, norepinephrine, tumor necrosis factor-α, and epidermal growth factor) on Rac1 activation, leading to NAD(P)H oxidase activation. The increase in $O_2^{-\cdot}$ production by NAD(P)H oxidase would, in the presence of a deficient endogenous antioxidant system, activate redox-sensitive kinase cascades and transcription factors. These actions would induce immediate early genes, reexpression of fetal genes, increased mRNA content and protein synthesis, thereby leading to an increase in myocyte cross-sectional area and fibrosis in the hearts of aged SH.

In conclusion, the present study demonstrated that an elevated level of $O_2^{-\cdot}$ production and higher oxidative damage were associated with an increase in histologically assessed cardiac hypertrophy in the hearts of aged SHR compared to age-matched W rats. Thus, oxidative stress appears to be a cause and/or consequence of hypertrophy development in the SHR model.

Acknowledgements

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