Original Article

Angiotensin II Regulates Cardiac Hypertrophy *via* Oxidative Stress but Not Antioxidant Enzyme Activities in Experimental Renovascular Hypertension

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The aim of this study was to provide new insights into the role of angiotensin II and arterial pressure in the regulation of antioxidant enzyme activities in a renovascular model of cardiac hypertrophy. For this purpose, aortic coarcted rats were treated with losartan or minoxidil for 7 days. Angiotensin II induced cardiac hypertrophy and oxidative stress *via* Nox4, $p22^{phox}$ and $p47^{phox}$, which are components of the NAD(P)H oxidase. Antioxidant enzymes were regulated by arterial pressure and were not implicated in cardiac hypertrophy. Heme oxygenase-1, the rate-limiting enzyme in heme catabolism, behaved as a catalase and glutathione peroxidase, and is regulated by arterial pressure. In summary, the present report indicates that cardiac hypertrophy, induced by renovascular hypertension, depends on angiotensin II through reactive oxygen species and is not prevented by the action of antioxidant enzymes. (*Hypertens Res* 2008; 31: 325–334)

***Key Words*: angiotensin II, antioxidant enzymes, arterial pressure, cardiac hypertrophy, renovascular hypertension**

Introduction

Oxidative stress plays a key role in the development of cardiac hypertrophy and its progression to failure, but the sequence of events remains to be elucidated (*1*). It is well known that reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), nitric oxide, and peroxynitrite are implicated in the pathogenesis of hypertension and endothelial damage (*2*). ROS generation is enhanced by different mechanisms, including xanthine oxidase activation, NADH autooxidation, and superoxide dismutase (SOD) inactivation (*3*). Most vascular ROS are produced by NAD(P)H oxidase, a multisubunit enzyme that catalyzes $O_2^{\cdot -}$ production. The vascular NAD(P)H oxidase comprises at least four components: cell membrane-associated $p22^{phox}$ and $gp91^{phox}$ and cytosolic subunits $p47^{phox}$ and $p67^{phox}$ (*4*). The $O_2^{\cdot -}$ formed can dismutate to produce H_2O_2 and oxygen, either spontaneously or by the action of SOD. On the one hand, H_2O_2 can also be reduced to generate the highly reactive $HO^{\cdot}$, which induces local damage. On the other hand, it is scavenged by catalase (CAT) and

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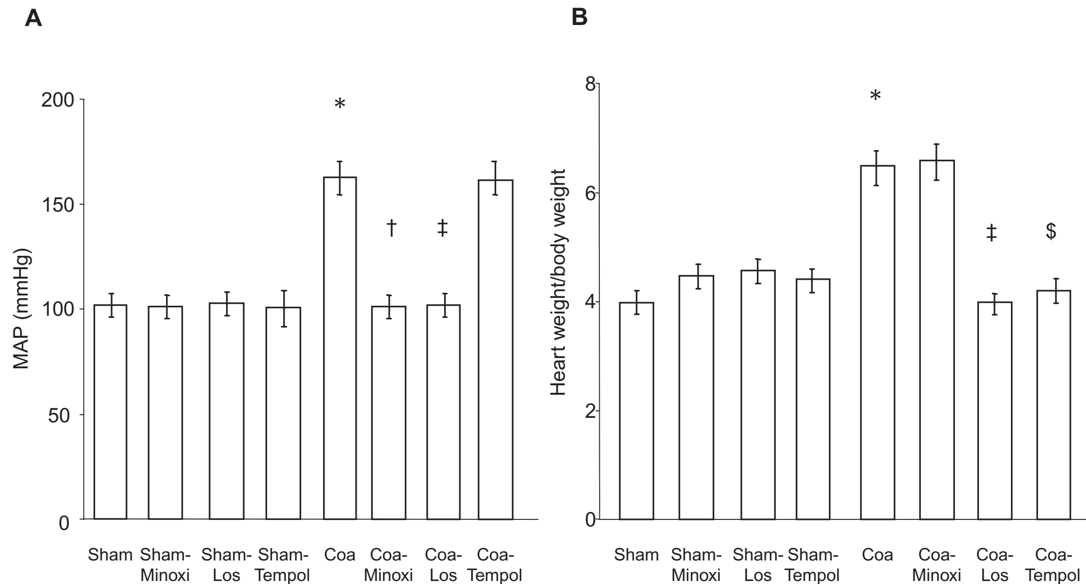

Fig. 1. *Mean arterial pressures (MAP) (A) and heart weight/body weight ratios (B) in the different animal groups. Rats were treated as described in Methods. Each value is the mean of 7 rats for each group, and bars indicate SD. *Significant differences ($p<0.001$) between untreated-Coa group and untreated-Sham group. †Significant differences ($p<0.001$) between minoxidil-treated Coa group and untreated-Coa group. ‡Significant differences ($p<0.001$) between losartan-treated Coa group and untreated-Coa group. $Significant differences ($p<0.001$) between tempol-treated Coa group and untreated-Coa group.*

glutathione peroxidase (Gpx) (*5*). Recently, heme oxygenase (HO), the rate-limiting enzyme in heme catabolism, was implicated in the antioxidant defense system (*6–9*).

Coarctation of the abdominal aorta above the renal arteries is known to cause severe hypertension proximal to the level of constriction. This primary response occurs as a consequence of reduced renal perfusion and subsequent activation of the renin-angiotensin system during the first week of coarctation (*10*). Furthermore, we previously showed that cardiac and plasma levels of angiotensin II (Ang II) were increased in this renovascular model of hypertension (*11*), and several studies have demonstrated that aortic coarctation in rats caused cardiac hypertrophy at 2, 6, and 28 days after ligation of the abdominal aorta (*12*, *13*).

It is interesting that, in this model, cardiac hypertrophy occurs as a consequence of pressure overload (*14*) and/or Ang II overproduction (*15*). It has been shown that high pressure elicits ROS production that mediates vascular hypertrophy in vessels (*16*). Moreover, Ang II has direct and indirect actions on cardiac tissue. In vascular smooth muscle cells, Ang II induces cellular hypertrophy by acting through G protein–coupled Ang II type 1 (AT1) receptors (*17*).

This study was undertaken to investigate the roles of Ang II and arterial pressure on oxidative stress damage and cardiac hypertrophy in coarcted animals. To this end, the effects of the AT1 receptor blocker losartan were compared against those of the vasodilator minoxidil.

Methods

Chemicals

NADPH, reduced glutathione (GSH), 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid, glutathione reductase, tempol, and minoxidil were from Sigma-Aldrich (St. Louis, USA). All other chemicals were of analytical grade.

Animals and Tissue Preparation

Male Wistar rats (250 g) were anesthetized with ether and submitted to sham operation (Sham) or complete ligation of the abdominal aorta between the right and left renal arteries (Coa) according to the method described by Rojo-Ortega and Genest (*18*). The animals displayed lower limb paralysis following aortic ligature, but they had completely recovered their movements by 24 h post-operation. The rats were divided into six groups ($n=10$): 1) Sham group, 2) Coarted group (Coa), 3) Sham rats treated with minoxidil (120 mg/mL) in the drinking water (Sham-Minoxi), 4) Sham rats treated with losartan (10 mg/kg/day) in the drinking water (Sham-Los), 5) Coa treated with minoxidil (Coa-Minoxi), and 6) Coa treated with losartan (Coa-Los).

After 7 days of treatment, rats were decapitated. The hearts were excised, washed with ice-cold saline solution (0.9% w/v

Table 1. Lipid Peroxidation and H_2O_2 Levels in Heart Homogenates of Different Sham and Coa Groups

Groups	TBARS content (nmol/mg protein)	H_2O_2 (μmol/L)
Sham	0.080±0.003	0.40±0.03
Sham-Minoxi	0.082±0.004	0.42±0.04
Sham-Los	0.056±0.002*	0.32±0.01*
Coa	0.152±0.010[†]	0.81±0.08[†]
Coa-Minoxi	0.153±0.010	0.83±0.09
Coa-Los	0.070±0.007[‡]	0.41±0.02[‡]

Both oxidative stress parameters were assayed as described in Methods. Data are means±SD, $n=7$. *Significant differences ($p<0.05$) between losartan-treated Sham group *vs*. untreated-Sham group. [†]Significant differences ($p<0.001$) between Coa group *vs*. Sham animals. [‡]Significant differences ($p<0.01$) between losartan-treated Coa group *vs*. untreated-Coa group. TBARS, thiobarbituric acid–reactive substances; Sham-Minoxi, sham rats treated with minoxidil; Sham-Los, sham rats treated with losartan; Coa, coarted group; Coa-Minoxi, Coa treated with minoxidil; Coa-Los, Coa treated with losartan.

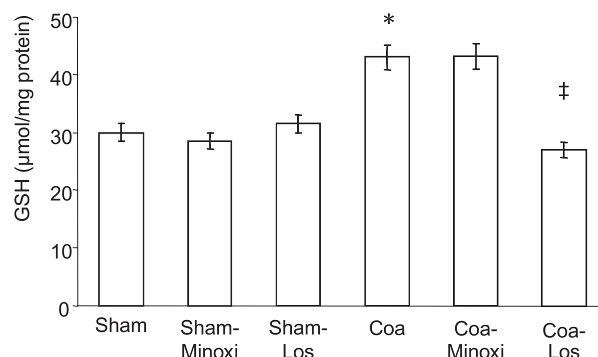

Fig. 2. *Reduced glutathione content in the different animal groups. Rats were treated as described in Methods. Each value is the mean of 7 rats for each group, and bars indicate SD. *Significant differences (p < 0.001) between untreated-Coa group and untreated-Sham group. [‡]Significant differences (p < 0.001) between losartan-treated Coa group and untreated-Coa group.*

NaCl), and weighed. Myocardial hypertrophy was evaluated using the heart weight/body weight ratio. Heart homogenates were then prepared in a Potter-Elvehjem homogenizer using a medium containing 140 mmol/L KCl and 25 mmol/L potassium phosphate buffer (pH 7.4), and they were centrifuged at 600 × *g* for 10 min. The supernatant, a suspension of preserved organelles, was used as heart homogenate. The animals were treated in accordance with the National Institute for Health (NIH) Guide for the Care and Use of Laboratory Animals.

To determine whether or not there is a relationship between oxidative stress and cardiac hypertrophy, two other groups, one Coa ($n=4$) and the other Sham ($n=4$), were treated with tempol (30 mg/kg/day) in the drinking water. After 7 days, mean arterial pressure and myocardial hypertrophy were evaluated as previously described.

Arterial Pressure Determination

A carotid artery was cannuled and connected to a Statham Gould P23ID pressure transducer coupled to a Grass 79D polygraph. Mean arterial pressure (MAP) was calculated according to the formula: diastolic pressure + (systolic pressure − diastolic pressure)/3.

Determination of Antioxidant Enzyme Activities

SOD, CAT, and Gpx activities were determined spectrophotometrically in tissue homogenates prepared in a medium containing 140 mmol/L KCl and 25 mmol/L potassium phosphate buffer (pH 7.4), and centrifuged at 600 × *g* for 10 min. The supernatant, a suspension of preserved organelles, was used as homogenate. CAT activity was determined by measuring the decrease in absorbance at 240 nm (*19*). Gpx activity was assayed by following NADPH oxidation at 340 nm; one unit of the enzyme represents a 1 mmol decrease in NADPH/min under assay conditions (*20*). SOD activity was determined by inhibition of the adrenochrome formation rate at 480 nm (*21*). One unit in the SOD assay is defined as the amount of enzymatic protein required to inhibit 50% epinephrine auto-oxidation.

Glutathione Content

Total glutathione (GSH plus GSSG) was determined in heart homogenates after precipitation with 2% w/v perchloric acid and using yeast-glutathione reductase, DTNB, and NADPH, at 340 nm. Oxidized glutathione (GSSG) was determined by the same method in the presence of 2-vinylpyridine, and reduced glutathione (GSH) was calculated as the difference between total glutathione and GSSG (*22*).

Lipid Peroxidation

Lipid peroxidation was determined by measuring the production rate of thiobarbituric acid–reactive substances (TBARS) (expressed as malondialdehyde equivalents). One volume of tissue homogenate was mixed with 0.5 volume of trichloroacetic acid (15% w/v) and centrifuged at 2,000 × *g* for 10 min. The supernatant (1 mL) was mixed with 0.5 mL thiobarbituric acid (0.7% w/v) and boiled for 10 min. After cooling, sample absorbance was determined spectrophotometrically at 535 nm. The malondialdehyde concentration was calculated using a ε value of 1.56×10^5 L/mol/cm (*23*).

Table 2. Activity of Classical Antioxidant Enzymes and HO-1 in Heart Homogenates of Different Sham and Coa Groups

Groups	SOD (U/mg protein)	CAT (pmol/protein)	Gpx (U/mg protein)	HO-1 (U/mg protein)[a]
Sham	4.78±0.30	0.118±0.011	0.041±0.001	0.50±0.01
Sham-Minoxi	4.16±0.40	0.109±0.011	0.044±0.004	0.52±0.03
Sham-Los	4.06±0.40	0.110±0.010	0.036±0.007	0.51±0.02
Coa	6.86±0.60*	0.085±0.010*	0.060±0.003*	0.80±0.02*
Coa-Minoxi	6.36±0.60	0.139±0.009†	0.040±0.005†	0.45±0.04†
Coa-Los	6.22±0.67	0.142±0.010†	0.045±0.001†	0.47±0.04†

Enzymatic activities were assayed as described in Methods. Data are means±SD, $n=7$. [a]One unit of the enzyme forms 1 nmol of bilirubin/30 min under assay conditions. *Significant differences ($p<0.001$) between Coa group *vs*. Sham animals. †Significant differences ($p<0.01$) between Coa-treated groups *vs*. untreated-Coa animals. HO-1, heme oxygenase-1; SOD, superoxide dismutase; CAT, catalase; Gpx, glutathione peroxidase; Sham-Minoxi, sham rats treated with minoxidil; Sham-Los, sham rats treated with losartan; Coa, coarted group; Coa-Minoxi, Coa treated with minoxidil; Coa-Los, Coa treated with losartan.

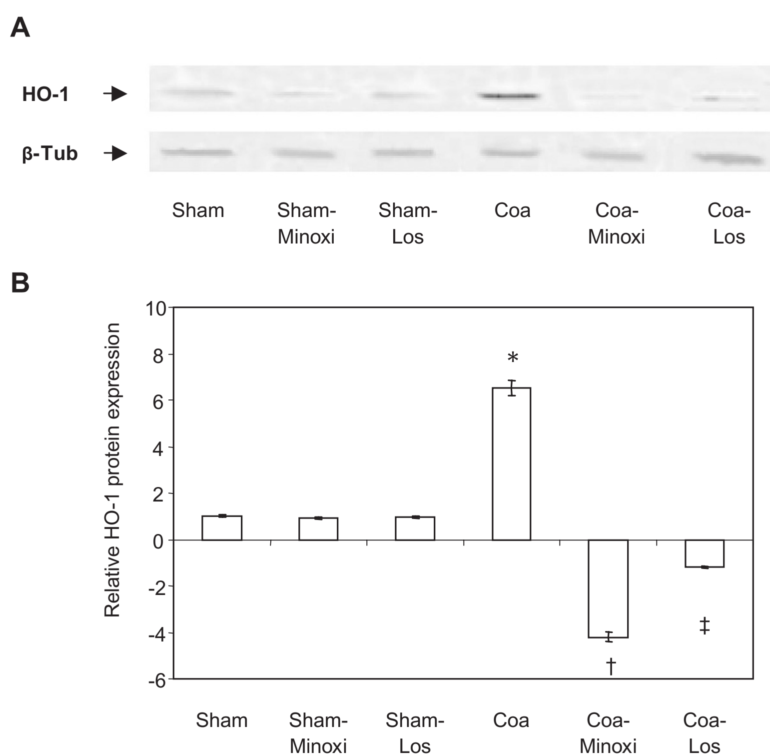

Fig. 3. *HO-1 protein abundance in heart homogenates. A: HO-1 protein abundance was analyzed by Western blotting as described in Methods. B: Relative HO-1 protein abundance taking control as 1 unit. Each value is the mean of 7 rats for each group, and bars indicate SD. *Significant differences ($p<0.001$) between untreated-Coa group and untreated-Sham group. †Significant differences ($p<0.05$) between minoxidil-treated Coa group and untreated-Coa group. ‡Significant differences ($p<0.001$) between losartan-treated Coa group and untreated-Coa group.*

Hydrogen Peroxide Production

H_2O_2 generation was determined in heart slices by the scopoletin–horseradish peroxidase (HRP) method (*24*), following the decrease in fluorescence intensity at 365–450 nm (exc–em) at 37°C. A calibration curve was run using H_2O_2 (0.05–0.35 μmol/L) as a standard.

Western Blot Analysis for Heme Oxygenase-1

Samples of homogenate were analyzed for heme oxygenase-1 (HO-1) using the Western immunoblot technique as previously described (*25*). Immunoblot with anti-β-tubulin (Sigma, St. Louis, USA) was used as an internal control of protein loading.

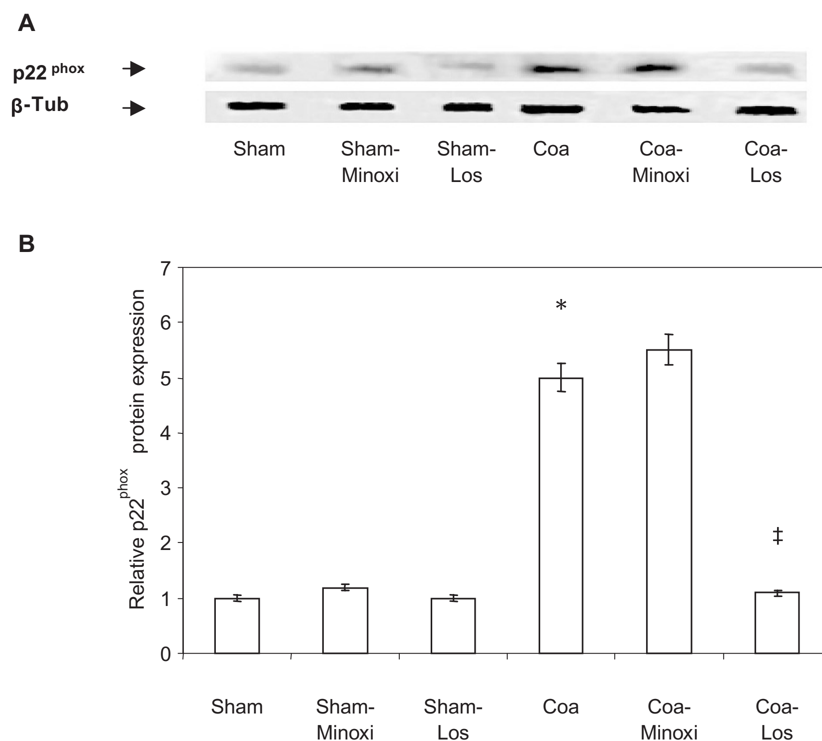

Fig. 4. *p22 subunit of NAD(P)H oxidase protein abundance in heart homogenates. A: p22 protein abundance was analyzed by Western blotting as described in Methods. B: Relative p22 protein abundance taking control as 1 unit. Each value is the mean of 7 rats for each group, and bars indicate SD. *Significant differences ($p < 0.05$) between untreated-Coa group and untreated-Sham group. ‡Significant differences ($p < 0.05$) between losartan-treated Coa group and untreated-Coa group.*

HO-1 Assay

For the heme oxygenase assay, the homogenate was prepared using 4 V of ice-cold 0.25 mol/L sucrose solution containing 1 mmol/L phenylmethyl sulfonyl fluoride, 0.2 mmol/L EDTA, and 50 mmol/L potassium phosphate buffer (pH 7.4). Homogenates were centrifuged at $20{,}000 \times g$ for 20 min and supernatant fractions were centrifuged at $150{,}000 \times g$ for 90 min. The microsomal pellet obtained was washed and resuspended in 20 mmol/L potassium phosphate buffer (pH 7.4) containing 135 mmol/L KCl, 1 mmol/L phenylmethylsulfonyl fluoride, and 0.2 mmol/L EDTA. Heme oxygenase activity was determined as described elsewhere (*26*, *27*).

Measurements of NAD(P)H Oxidase Subunits

Total tissue protein was electrophoresed in 4–20% Tris–glycine sodium dodecyl sulfate (SDS) polyacrylamide gels (Mini Protean II System, BioRad Laboratories, Hercules, USA), then transferred onto nitrocellulose membranes blocked in 5% w/v dry milk in T-TBS (0.02 mol/L Tris/0.15 mol/L NaCl, pH 7.5 containing 0.1% Tween 20) at room temperature for 3 h. The membrane was washed three times with T-TBS and incubated with the primary antibodies to Nox2, Nox4, $p47^{phox}$, and $p22^{phox}$ for 3 h at room temperature. The polyclonal antibodies against Nox2, Nox4, $p47^{phox}$, and $p22^{phox}$ were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). After washing five times with T-TBS, the blots were incubated with HRP-conjugated secondary antibodies (antigoat for NAD(P)H oxidase subunits) at room temperature for 2 h. Immunoblot with anti-β-tubulin (Sigma) was used as an internal control of protein loading. Thereafter, the membrane was washed five times with T-TBS, developed using enhanced chemiluminescent (ECL) reagent (Amersham Life Science, Arlington Heights, USA), and subjected to autoluminography for 1 to 5 min. Band intensity was analyzed with Gel-Pro® analyzer 3.1 version (Media Cybernetics, Bethesda, USA). In all instances, the membranes were stained with Ponceau S stain to verify the uniformity of protein load and transfer efficiency across the test samples.

Protein Determination

Protein concentration was evaluated by the method of Lowry *et al*. (*28*) using bovine serum albumin as a standard.

Statistics

Values in the figures and tables are expressed as mean±SD. Differences between groups were analyzed using two-way

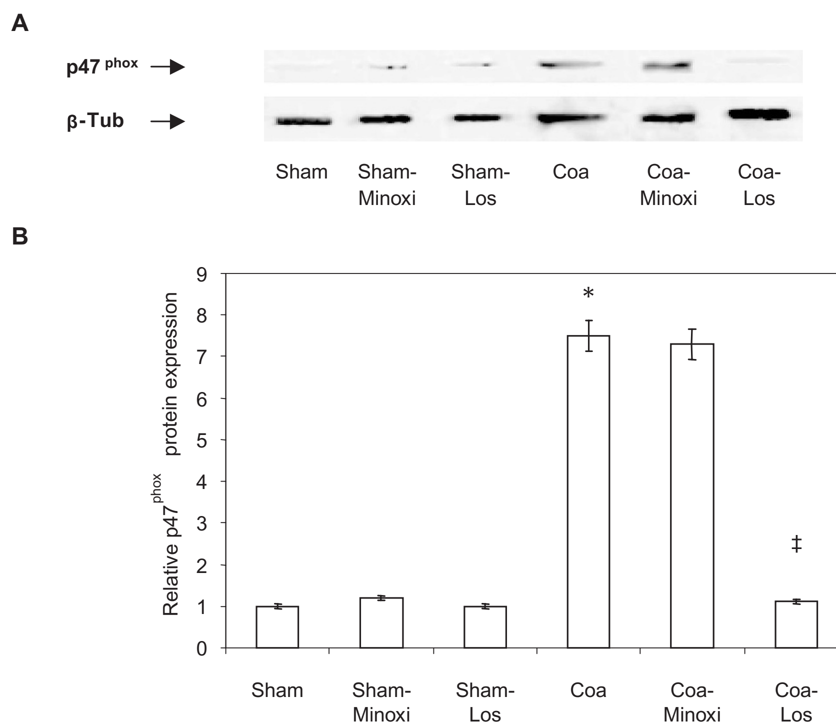

Fig. 5. *p47 subunit of NAD(P)H oxidase protein abundance in heart homogenates. A: p47 protein abundance was analyzed by Western blotting as described in Methods. B: Relative p47 protein abundance taking control as 1 unit. Each value is the mean of 7 rats for each group, and bars indicate SD. *Significant differences ($p < 0.05$) between untreated-Coa group and untreated-Sham group. ‡Significant differences ($p < 0.05$) between losartan-treated Coa group and untreated-Coa group.*

ANOVA, with $p<0.05$ considered statistically significant.

Results

MAP and Myocardial Hypertrophy

Mean arterial pressure in the Sham group was 102.0±3.9 mmHg. Neither the Sham-Minoxi nor the Sham-Los group showed any difference with respect to Sham animals (Fig. 1A). However, an increase was evidenced in Coa rats (162.5±5.3 mmHg). This enhancement in arterial pressure was totally avoided by administration of losartan or minoxidil. Therefore, the Coa-Los and Coa-Minoxi groups showed similar arterial pressure values as the Sham rats (Fig. 1A). Treatment with tempol did not modify MAP values in Sham or Coa animals (Fig. 1A).

Myocardial hypertrophy was observed in Coa animals. In this group, the heart weight/body weight ratio increased 50% more than it did in controls (Fig. 1B). Our data also indicated that losartan and tempol avoided changes in the heart weight/body weight ratio of Coa rats produced by coarctation (Fig. 1B). A different response was obtained in animals treated with minoxidil. In this case, there were no changes in the heart weight/body weight ratio in Coa rats (Fig. 1B).

On the other hand, neither minoxidil, losartan, nor tempol administration in the Sham groups had any effect on this parameter in this period of treatment.

Oxidative Stress Generation

TBARS formation is a reliable indicator of free radical generation in tissues. As shown in Table 1, a significant increase in TBARS content (90%) occurred in the Coa group with respect to controls. Treatment with losartan decreased this parameter in the Coa-Los group (54%) and Sham-Los group (30%) with respect to the Coa and Sham groups, respectively. On the other hand, minoxidil did not modify TBARS content in the Coa-Minoxi or Sham-Minoxi group compared with the respective untreated groups.

H_2O_2 was markedly increased (100%) in the Coa groups compared to controls, while treatment with losartan significantly diminished this oxidant compound in both the Sham group (20%) and the Coa animals (50%) (Table 1). Moreover, minoxidil treatment did not affect H_2O_2 amount in either the Sham or the Coa groups compared with the untreated animals.

Soluble Antioxidant Defenses

Reduced glutathione, the major low molecular weight antioxidant hydrosoluble thiol inside cells, increased 45% in the

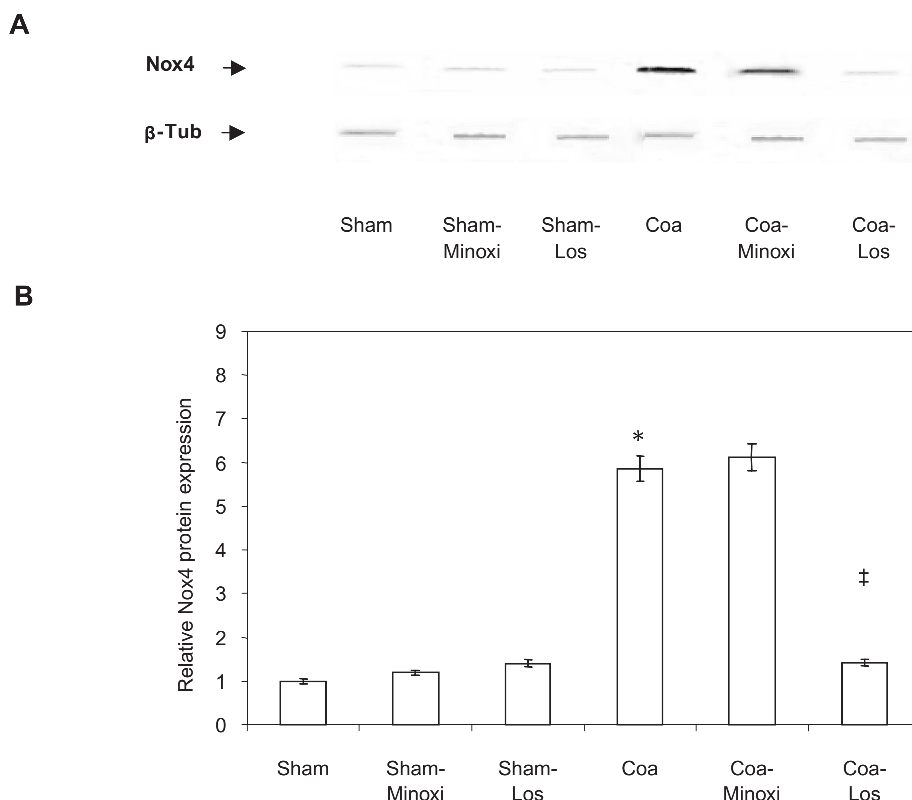

Fig. 6. *Nox4 subunit of NAD(P)H oxidase protein abundance in heart homogenates. A: Nox4 protein abundance was analyzed by Western blotting as described in Methods. B: Relative Nox4 protein abundance taking control as 1 unit. Each value is the mean of 4 rats for each group, and bars indicate SD. *Significant differences ($p < 0.05$) between untreated-Coa group and untreated-Sham group. ‡Significant differences ($p < 0.05$) between losartan-treated Coa group and untreated-Coa group.*

Coa groups compared to the control Sham rats. Interestingly, losartan avoided this enhancement. Furthermore, losartan administration to Sham rats did not modify GSH levels (Fig. 2). On the other hand, minoxidil treatment did not have any effect on GSH content in the normotensive or hypertensive groups (Fig. 2).

Antioxidant Enzymatic Activities

Superoxide dismutase activity increased 43% in the Coa group compared to control Sham rats. Treatment with losartan or minoxidil did not modify SOD activity in normotensive or hypertensive groups (Table 2). CAT activity decreased 28% in the Coa group compared to controls; however, the administration of losartan or minoxidil in the Coa group increased CAT activity up to control Sham values (Table 2). Gpx and HO-1 activities increased (45% and 60%, respectively) in Coa animals compared to the corresponding values in controls (Sham group), whereas both enzyme activities decreased in the Coa-Los and in Coa-Minoxi groups, reaching control Sham values (Table 2).

Western Blot Analysis of HO-1

HO-1 abundance increased 65% in the Coa group with respect to Sham rats. Losartan and minoxidil treatment each avoided this enhancement (Fig. 3). Minoxidil administration to Coa rats brought about a 98% decrease in the protein abundance with respect to untreated-Coa rats (Fig. 3).

Western Blot Analysis of Nox2, Nox4, p22 and p47 Subunits of NAD(P)H Oxidase

A significant upregulation was found in the protein expressions of Nox4, $p22^{phox}$ and $p47^{phox}$ subunits of NAD(P)H oxidase in the Coa group with respect to Sham animals (Figs. 4–6). Treatment with losartan significantly decreased this expression to control values (Sham group). However, minoxidil did not modify this upregulation in the Coa group (Figs. 4 and 5). Furthermore, the $gp91^{phox}$ (Nox2) protein expression remained unaltered in the Coa group as well as in Sham animals (Fig. 7). On the other hand, the expression of these subunits was not modified by losartan or minoxidil in the normotensive animals.

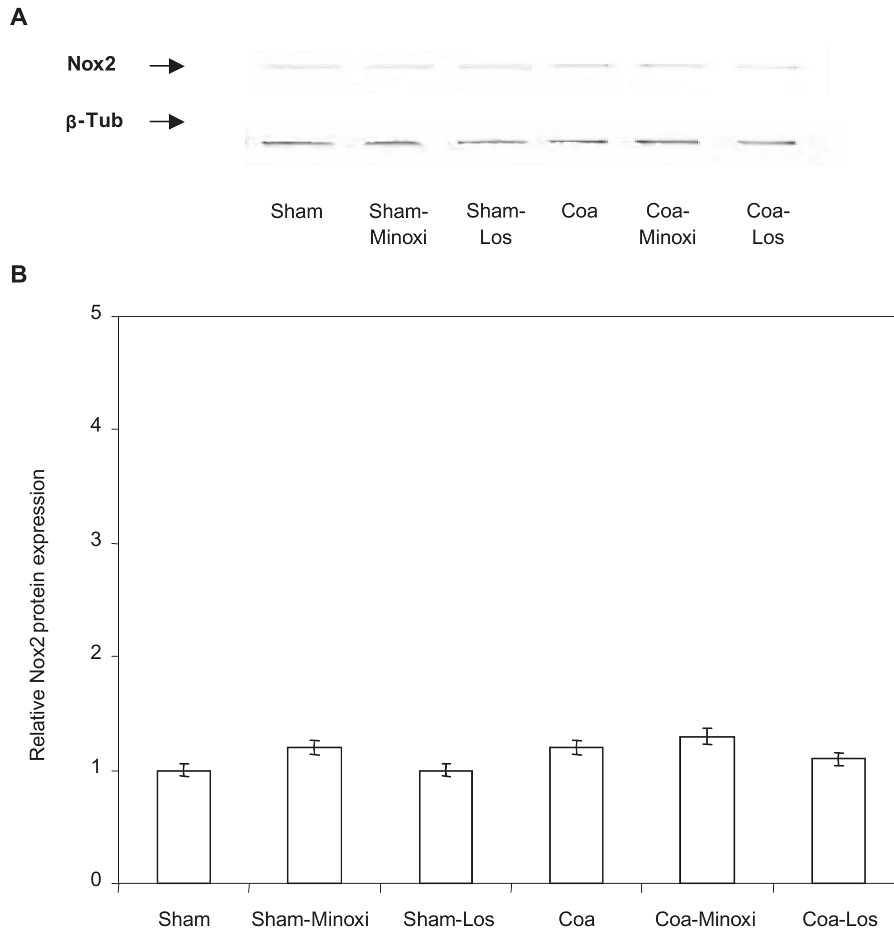

Fig. 7. *Nox2 subunit of NAD(P)H oxidase protein abundance in heart homogenates. A: Nox2 protein abundance was analyzed by Western blotting as described in Methods. B: Relative Nox2 protein abundance taking control as 1 unit. Each value is the mean of 7 rats for each group, and bars indicate SD.*

Discussion

It was previously demonstrated in rats that aortic coarctation brings about cardiac hypertrophy, oxidative damage and changes in antioxidant enzyme activities (*13*). The present results clearly suggest that, in coarcted rats, the action of antioxidant enzymes does not prevent cardiac hypertrophy caused by ROS *via* NAD(P)H oxidase.

The NAD(P)H oxidases are a predominant source of ROS, and activation of these enzymes leads to a variety of intracellular signaling events that ultimately cause dysfunction of the endothelium, proliferation of vascular smooth muscle cells, and reconstruction of the extracellular matrix (*29*, *30*). A major stimulus for activation of NAD(P)H oxidases is Ang II (*31*). On the other hand, these enzymes are also activated by mechanical forces, hormones, and cytokines. Several studies have demonstrated the role of Ang II in hypertrophy (*32–34*). Hypertrophy was attenuated by employing DPI (a nonspecific inhibitor of NAD(P)H oxidase) or by diminishing transfection of the antisense $p22^{phox}$. Moreover, CAT overexpression abolished the effects of Ang II on growth (*32–34*). In the present study, we have demonstrated that, as a consequence of coartation, Nox4, $p22^{phox}$, and $p47^{phox}$ subunits were increased. Because minoxidil treatment in Coa animals did not decrease these expressions, we may conclude that arterial pressure does not regulated them. Moreover, the data here presented showed that, in this model, these subunits were regulated by Ang II. However, Ungvari *et al*. demonstrated a different response in aortic rings (*16*), indicating that, depending on the tissue, there are at least two different mechanisms for NAD(P) activation, one mediated by Ang II (heart) and the other by arterial pressure (aortic rings). Nevertheless, in our work, the cardiac hypertrophy generation appears to be independent from the NAD(P)H oxidase component Nox2. Accordingly, it was demonstrated that mice with genetic deficiency in this subunit did not exhibit attenuation of the hypertrophy response to chronic pressure overload (*35*, *36*).

According to found by Maytin *et al*. (*36*), our results demonstrated that the development of hypertrophy appeared to be mainly dependent on the Nox4 isoform in this model of hypertension. Our study also showed that the increase in the expression of NAD(P)H oxidase components Nox4, $p22^{phox}$, and $p47^{phox}$, the enhancement of SOD, and the decrease of CAT activities led to overproduction of H_2O_2. It is important to note that in Coa-Los rats, oxidative stress was diminished and cardiac hypertrophy was prevented. In this regard, these deleterious effects observed in Coa rats could be attributed to the action of Ang II. A different response was obtained in the Coa-Minoxi group: although arterial pressure was diminished, TBARS formation as well as H_2O_2 content were enhanced, indicating that, in this model, cardiac hypertrophy is a result of ROS formation, and arterial pressure *per se* is not implicated in oxidative stress generation. In this context, Coa-Los animals had significantly lower H_2O_2 levels than Coa-Minoxi animals, although the two groups showed similar responses of antioxidant enzymes. This fact could be explained by the reduction of NAD(P)H oxidase protein expression observed in the Coa-Los group, which may have led to a decrease of $O_2^{\cdot -}$ production. In this regard, several authors have demonstrated that $p22^{phox}$ and $p47^{phox}$ expressions reflect the activity of this enzyme (*37*, *38*), and we found a strong correlation between the decreases in $p22^{phox}$ and $p47^{phox}$ expressions and the reduction of H_2O_2 levels in Coa-Los animals. In addition, the development of cardiac hypertrophy was prevented when Coa animals were treated with the antioxidant tempol, reinforcing the suggested link between ROS formation and cardiac hypertrophy.

ROS produced in mammals and other species are faced by the antioxidant defense system (*39*). Studies carried out with animal models of hypertension have shown that ROS abundance, triggered either by increased production or impaired degradation, determine oxidative damage in tissues (*40*, *41*). In this way, one or more moderate episodes of radical stress may increase the power of the defense system by stimulating the expression of the respective enzymes (*41*). Transcription of antioxidant defense enzymes increases in the myocardium from spontaneously hypertensive rats, following oxidative stress development (*42*). In contrast, the activity of antioxidant enzymes may decrease, mainly when the oxidative load overcomes the defense system (*42*). Hypertension changes the activities of antioxidant enzymes in a wide variety of tissues, including myocardium, vascular endothelium, skeletal muscle, liver, kidney, and erythrocytes (*39*). In some cases, different antioxidant enzyme levels and redox statuses were found to depend on the genetic background of the hypertensive animal models used (*43*). However, few studies have shown an association between antioxidant enzyme activity profile and cardiac hypertrophy in a model of renovascular hypertension.

Coa rats treated with losartan or minoxidil showed the same CAT, Gpx, and HO-1 activities with respect to the Sham group. Moreover, both treatments revealed that HO-1 protein abundance was the same as in control Sham values. Considering that losartan and minoxidil showed similar effects on antioxidant enzyme activities, we have concluded that this response is due to a decrease in arterial pressure. For this reason, we propose that antioxidant enzymes (except SOD) as well as HO-1 may be regulated by arterial pressure. These findings indicate that, on the one hand, these enzymes are not involved in the protection against cardiac hypertrophy and, on the other hand, that HO-1 behaves as CAT and Gpx enzymes.

Losartan treatment in the Sham group did not change the activity of antioxidant enzymes (SOD, CAT, and Gpx). However, its administration decreased lipid peroxidation and H_2O_2 formation in the normotensive group, indicating that these results could be due to the antioxidant capacity attributed to AT1 receptor blockers (*40*).

In summary, in hypertensive Coa animals, oxidative stress induction and cardiac hypertrophy were highly dependent on the direct action of Ang II, and are therefore prevented by losartan administration. Moreover, except for SOD, the arterial pressure determines the behavior of antioxidant enzyme activities. In this way, Coa animals reached similar values to the normotensive group when they were treated either with losartan or minoxidil. The findings reported here strongly indicate that the administration of AT1 receptor antagonists in renovascular hypertension therapeutics is highly advisable because it may prevent ROS production and therefore cardiac hypertrophy.

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