

# Up-regulation of kidney NAD(P)H oxidase and calcineurin in SHR: Reversal by lifelong antioxidant supplementation

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## Up-regulation of kidney NAD(P)H oxidase and calcineurin in SHR: Reversal by lifelong antioxidant supplementation.

**Background.** Spontaneously hypertensive rats (SHR) are born normotensive and develop hypertension (HTN) later in life (age 4 to 5 weeks). HTN in SHR is associated with and caused in part, by oxidative stress and renal interstitial inflammation. This study tested the hypothesis that lifelong antioxidant supplementation beginning at prenatal period may delay the onset and reduce the severity of HTN in SHR. The study further sought to explore the effect of diet modification on renal tissue NAD(P)H oxidase and calcineurin abundance.

**Methods.** Pregnant SHR and their offspring were fed either an antioxidant-fortified diet (a chow containing  $\alpha$ -tocopherol 5000 IU/kg, ascorbic acid 500 ppm, selenium 2.76 ppm, and zinc 350 ppm) or regular diet ( $\alpha$ -tocopherol 40 IU/kg, selenium 0.2 ppm, and zinc 70 ppm). Animals were observed for 24 weeks. Wistar-Kyoto rats fed either a regular or antioxidant diet served as control.

**Results.** Onset of HTN was delayed and severity of HTN was reduced in antioxidant-treated compared with untreated SHR. Markers of oxidative stress (i.e., plasma hydrogen peroxide, renal tissue malondialdehyde, and nitrotyrosine abundance) were elevated in untreated but not in antioxidant-treated SHR. gp91<sup>phox</sup> and p22<sup>phox</sup> subunits of NAD(P)H oxidase were markedly elevated in the renal cortex of untreated SHR and partially restored in the treated SHR. Similarly, renal calcineurin A $\alpha$  and B subunits were elevated in untreated SHR and were partially restored in the treated SHR. Antioxidant therapy had no effect on the measured parameters in the WKY control.

**Conclusion.** Lifelong consumption of antioxidant-rich diet ameliorates HTN and oxidative stress in SHR. This is associated with the reduction of superoxide-generating enzyme, NAD(P)H oxidase, and immunoregulatory factor calcineurin. Antioxidant-rich diet appears to attenuate oxidative stress, not only by fortifying antioxidant defense capacity but also by lowering NAD(P)H oxidase, which is a major source of reactive oxygen species.

**Key words:** oxidative stress, hypertension, NAD(P)H oxidase, calcineurin, kidney.

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Spontaneously hypertensive rats (SHR) were originally developed by Okamoto and Aorki [1] from brother-sister inbreeding of Wistar-Kyoto (WKY) rats with a pair of outbred female and male WKY rats, which exhibited mild elevation of arterial pressure. SHR are frequently used as a model of essential hypertension (HTN) in humans. As in humans with essential HTN, the SHR are born normotensive and develop HTN later in life, beginning at 4 to 5 weeks of age. HTN in SHR and various other models of hereditary and acquired HTN is associated with increased production of reactive oxygen species (ROS) [2–24]. ROS can raise arterial pressure via inactivation of nitric oxide, generation of isoprostanes, and cardiovascular remodeling. In fact, amelioration of oxidative stress by a variety of antioxidant regimens has been shown to attenuate HTN in various hypertensive disorders [13–25].

ROS are generated by mitochondria during the cellular respiration and as byproducts of specific oxidases in the cytoplasm. In addition, under pathologic conditions, large quantities of ROS are produced by activated leukocytes, macrophages, and various other cell types. The primary ROS generated in the cell is superoxide. Nicotinamide adenine dinucleotide (phosphate) [NAD(P)H] oxidase, which was originally found in phagocytes, is the major source of superoxide in various other tissues [26]. The enzyme consists of at least five protein subunits, namely gp91<sup>phox</sup> and p22<sup>phox</sup>, which are membrane associated, and p47<sup>phox</sup>, p67<sup>phox</sup> and p40<sup>phox</sup>, which reside in the cytosol of resting cells [27]. Various components of phagocyte NAD(P)H oxidase have also been identified in other cells [26–28], including endothelial cells, vascular smooth muscle cells (VSMCs), and fibroblasts. In addition, all NAD(P)H oxidase subunits have been demonstrated in the rat kidney [29, 30]. NAD(P)H oxidase is up-regulated by various stimuli including shear stress, cyclic strain, and humoral factors such as angiotensin II and proinflammatory cytokines, which can be targeted by specific pharmacologic interventions to ameliorate oxidative stress and HTN [31].

Several recent studies have demonstrated interstitial accumulation of superoxide-producing activated T lymphocytes and macrophages in the SHR kidney [25, 33]. While the contribution of the activated T cells and macrophages to the associated oxidative stress is clear, the mechanism responsible for their activation and accumulation in the renal interstitium is not fully understood. In this regard, Nava et al [25] recently demonstrated significant activation of nuclear transcription factor kappa B (NF $\kappa$ B) in SHR kidney. This event can, in part, contribute to activation and accumulation of inflammatory cells by promoting production of proinflammatory cytokines. Calcineurin and its downstream signal molecule, nuclear factor of activated T cell (NFAT), are essential for production of interleukin-2 (IL-2), which plays an important role in lymphocyte activation and generation of other inflammatory cytokines [34]. Thus, calcineurin may potentially contribute to the previously reported interstitial accumulation of immunocompetent lymphocytes and macrophages in SHR kidney.

The present study tested the hypothesis that lifelong supplementation of an antioxidant-fortified diet beginning at prenatal life may delay the onset and attenuate the severity of HTN. The study further sought to investigate the effect of antioxidant-rich diet on NAD(P)H oxidase and calcineurin abundance in untreated and antioxidant-treated SHR.

## METHODS

### Animal experiments

SHR and control Wistar-Kyoto rats (WKY) were obtained from Charles River Laboratories (Hollister, CA, USA). One-week pregnant rats were fed either a regular rodent laboratory diet containing  $\alpha$ -tocopherol (40 IU/kg), selenium (0.2 ppm), and zinc (70 ppm), or an antioxidant-rich diet prepared by adding  $\alpha$ -tocopherol (5000 IU/kg), ascorbic acid (500 ppm), selenium (2.76 ppm), and zinc (350 ppm) to the regular diet (Purina Mills, Inc., Richmond, IN, USA). The offspring were continued on the respective diets for 24 weeks. WKY rats, fed regular diet, served as control. Only male offspring were included in the present study. To determine the effect of the given antioxidant regimen in the normotensive animals, male WKY rats fed either a regular or antioxidant-fortified diets were included in the study. The animals were housed in a temperature-controlled UC Irvine Vivarium with 12-hour light and dark cycles and were given unrestricted access to food and water throughout the observation period. Twenty-four-hour urine collections were obtained using individual metabolic cages. The protocol employed in this study was approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

At the end of the experiments, animals were anesthetized with intraperitoneal injections of pentobarbital sodium (50 mg/kg). Blood was collected by cardiac puncture, kidneys were removed immediately, snap-frozen in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$  until processed. Urinary protein, serum and urine creatinine concentrations were determined by kits purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Plasma hydrogen peroxide.** Plasma  $\text{H}_2\text{O}_2$  concentration was determined by the quantitative  $\text{H}_2\text{O}_2$  assay kit purchased from OXIS International, Inc. (Portland, OR, USA).

**Tissue malondialdehyde (MDA).** Renal MDA content was determined in kidney supernatants as described by the Buege and Aust [35].

### Blood pressure determinations

Blood pressure was determined by tail-cuff plethysmography (Harvard Apparatus, Inc., Natick, MA, USA) as described previously [36]. Conscious rats were placed on a heated pad in a temperature-controlled quiet room. After 15 minutes rest with the tail placed inside a tail cuff, the cuff was inflated 3 to 4 times to condition the animal to the procedure. Four consecutive measurements were then taken and the average of the recorded measurements was used.

### Western blot analysis

Homogenates (20% w/v) of renal cortex and medulla were prepared in 10 mmol/L HEPES buffer, pH 7.4, containing 320 mmol/L sucrose, 1 mmol/L EDTA, 1 mmol/L DTT, 10  $\mu\text{g}/\text{mL}$  leupeptin, 2  $\mu\text{g}/\text{mL}$  aprotinin, and 1 mmol/L PMSF at 0 to  $4^{\circ}\text{C}$  using a homogenizer. Homogenates were centrifuged at  $9000g$  for 10 minutes at  $4^{\circ}\text{C}$  to remove nuclear fragments and tissue debris without precipitating membrane fragments. A portion of the supernatant was used for the determination of total protein concentration by using a BioRad kit (Hercules, CA, USA).

Total cellular protein (20  $\mu\text{g}$  each) was electrophoresed in 4%–20% Tris-glycine sodium dodecyl sulphate (SDS) polyacrylamide gels (Novex, San Diego, CA, USA). Proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore Corp., Bedford, MA, USA), blocked in 5% 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 hours, washed 3 times with T-TBS, and incubated with the primary antibody (the antibodies were diluted 1:1000 for p22<sup>phox</sup>, gp91<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup>, calcineurin B, and nitrotyrosine, 1:2000 for calcineurin A $\alpha$ ) for 3 hours at room temperature. The primary antibody for gp91<sup>phox</sup> subunit of NADPH oxidase was purchased from Transduction Labs (Lexington, KY, USA). The p22<sup>phox</sup> polyclonal, p47<sup>phox</sup>, and p67<sup>phox</sup>

**Table 1.** Body weight, urinary creatinine clearance, and protein excretion in WKY rats and SHR groups given regular diet (SHR-R) or antioxidant-fortified diet (SHR-T)

	WKY	SHR-R	SHR-T
Body weight g	341 ± 9.8	345 ± 10.7	327 ± 8.5
Creatinine clearance mL/min	1.23 ± 0.27	1.03 ± 0.20	1.25 ± 0.19
Urinary protein mg/24 h	26.9 ± 4.9	38.6 ± 6.2 <sup>a</sup>	39.9 ± 6.3 <sup>a</sup>

*N* = 6 in each group.

<sup>a</sup>*P* < 0.05 vs. WKY group.

monoclonal antibodies were generously supplied by Dr. Mark T. Quinn, Montana State University, Bozema, MT. Nitrotyrosine (clone 1A6), calcineurin/PP2B A $\alpha$  polyclonal, and calcineurin/PP2B B (clone VA1) were purchased from Upstate (Lake Placid, NY, USA). After washing 5 $\times$  with T-TBS, the blots were incubated with secondary antibody (1:1000; antimouse for gp91<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup>, calcineurin/PP2B B, and nitrotyrosine; antirabbit for p22<sup>phox</sup> and calcineurin/PP2B A $\alpha$ ) conjugated with horseradish peroxidase at room temperature for 2 hours. After washing 5 $\times$  with T-TBS, the membrane was developed using ECL reagent (Amersham Life Science, Inc., Buckinghamshire, UK) and subjected to autoluminography for 1 to 5 minutes. The autoluminographs were scanned with a laser densitometer (Model PD 1211, Molecular Dynamics, Sunnyvale, CA, USA) to determine the relative optical densities of the bands.

### Statistical analysis

All values shown are mean  $\pm$  SE. Comparisons between groups were done using one-way analysis of variance (ANOVA) and least significant difference (LSD) test. Differences were considered statistically significant when *P* < 0.05.

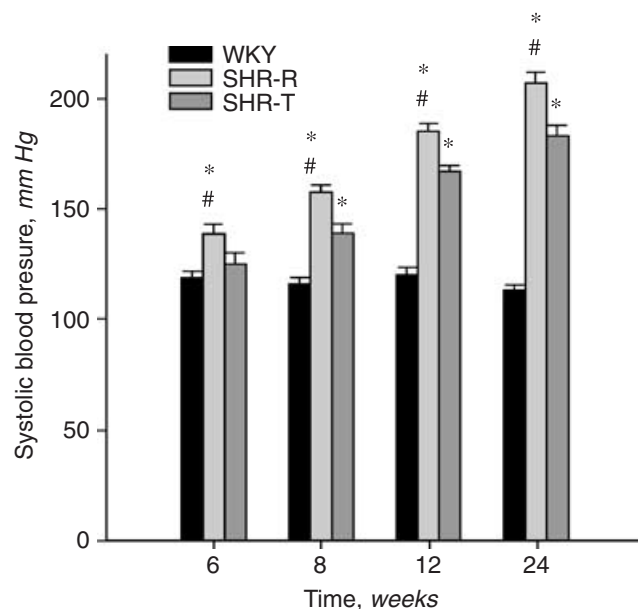
## RESULTS

### General data

Data are summarized in Table 1. There was no significant difference in body weights among the study groups. Mean creatinine clearance in the untreated SHR was insignificantly lower than that found in the WKY and antioxidant-treated SHR groups. Urinary protein excretion was significantly increased in both SHR groups as compared to the WKY control group.

### Arterial pressure data

Data are shown in Figure 1. Significant HTN was present in the untreated SHR but not in the antioxidant-treated SHR at age 6 weeks. Arterial pressure remained virtually unchanged in the WKY group throughout the study period, but rose steadily in the SHR groups. However, arterial pressure values were consistently lower in the antioxidant-treated than in the untreated SHR throughout the study period.



**Fig. 1.** Systolic blood pressure in Wistar Kyoto (WKY) rats and spontaneously hypertensive rat (SHR) groups raised on a regular diet (SHR-R) or antioxidant-fortified diet (SHR-T) obtained at age 6, 8, 12, and 24 weeks. *N* = 6 in each group, *P* < 0.05, \* SHR-R or SHR-T vs. WKY, # SHR-R vs. SHR-T.

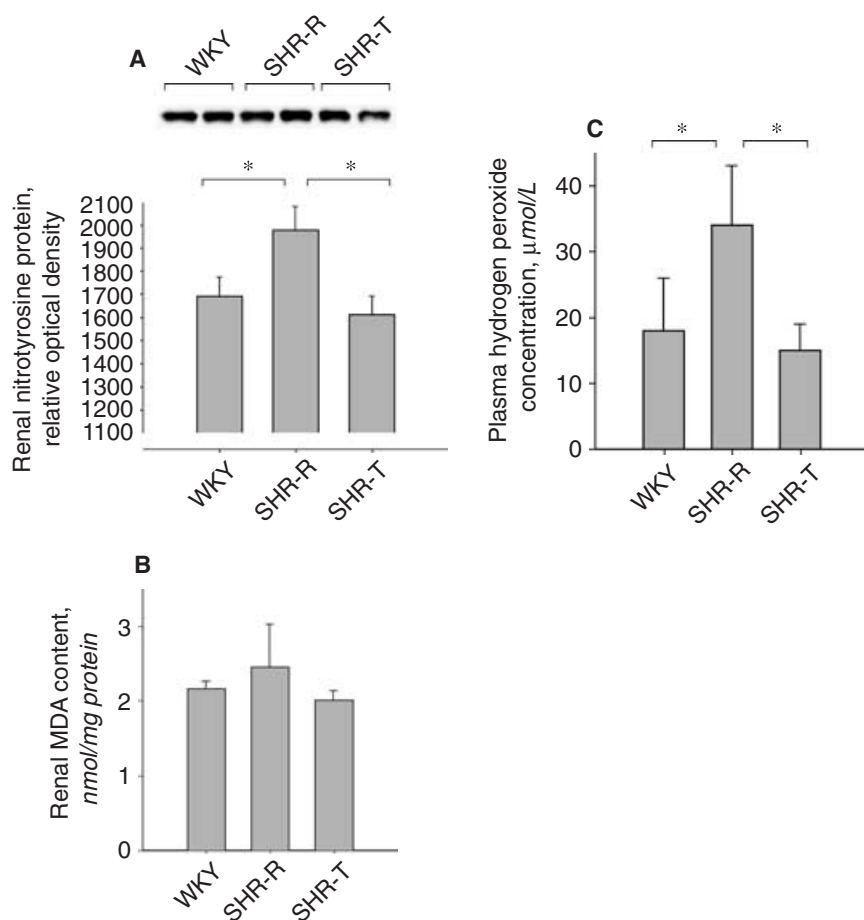
### Markers of oxidative stress

Data are illustrated in Figure 2. The untreated SHR group showed a significant increase in plasma H<sub>2</sub>O<sub>2</sub> concentration as compared to the WKY control rats. Antioxidant therapy prevented the rise in plasma H<sub>2</sub>O<sub>2</sub> in the treated SHR. The untreated SHR exhibited a significant elevation of kidney tissue nitrotyrosine abundance, denoting increased NO inactivation and protein oxidation by ROS. Antioxidant therapy led to normalization of kidney tissue nitrotyrosine content. In addition, malondialdehyde (MDA) content in the kidney tissue was higher in the untreated SHR compared with the WKY and the treated SHR groups. However, the difference did not reach statistical significance.

### Kidney NAD(P)H oxidase

Data are depicted in Figure 3. The untreated SHR exhibited a marked increase in immunodetectable gp91<sup>phox</sup> and p22<sup>phox</sup> subunits of NAD(P)H oxidase in the renal cortex compared with those in the WKY control. The up-regulation of these membrane-associated subunits was partially but significantly attenuated by chronic antioxidant therapy. No significant difference was found in the intracellular p47<sup>phox</sup> and p67<sup>phox</sup> subunits of NAD(P)H oxidase among the study groups.

Protein expression of NAD(P)H oxidase subunits in renal medulla was comparable among the three groups (data not shown).



**Fig. 2.** (A) Renal nitrotyrosine abundance, (B) renal malondialdehyde content, (C) and plasma hydrogen peroxide concentration in Wistar Kyoto (WKY) rats and spontaneously hypertensive rat (SHR) groups raised on a regular diet (SHR-R) or antioxidant-fortified diet (SHR-T).  $N = 6$  in each group, \* $P < 0.05$ .

### Kidney calcineurin

Data are presented in Figure 4. Protein abundance of both calcineurin A $\alpha$  and B subunits was significantly increased in the renal cortex of the untreated SHR compared with those found in the WKY controls. Long-term consumption of antioxidant-fortified diet normalized protein abundance of calcineurin A $\alpha$  subunit, and partially attenuated the up-regulation of calcineurin B subunit.

Expression of calcineurin subunits in renal medulla was comparable in the three groups (data not shown).

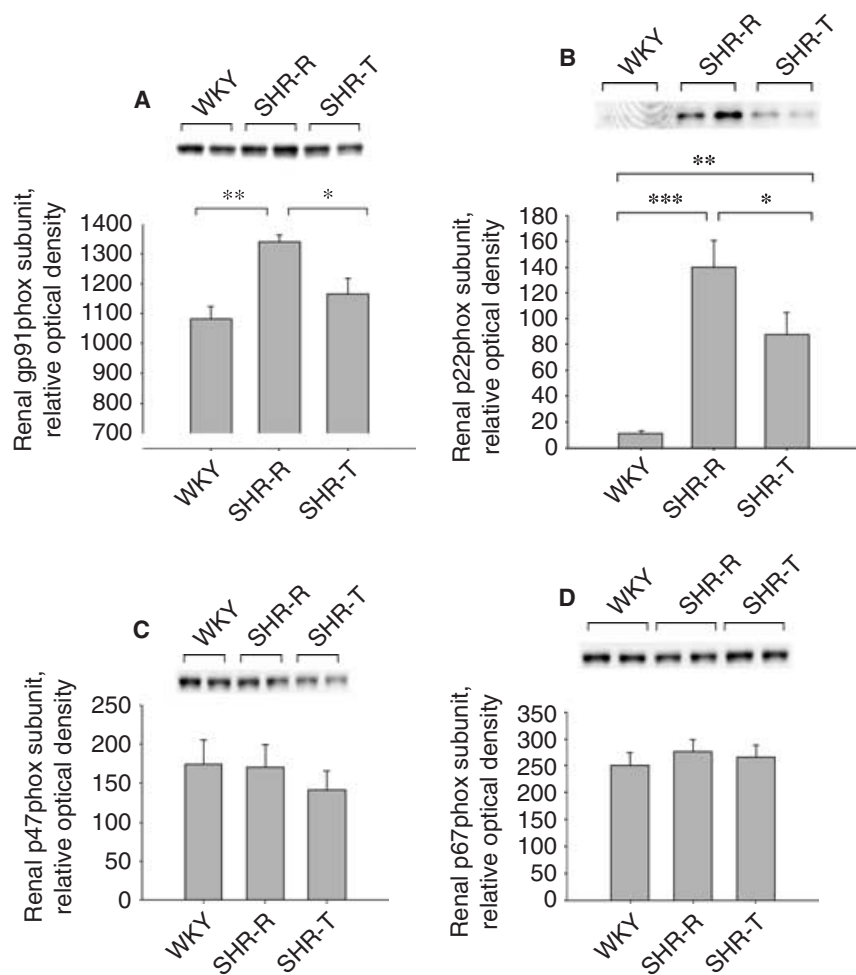
### Effect of antioxidant therapy in WKY rats

Data are shown in Figures 5 and 6. Antioxidant therapy had no effect on either systolic arterial pressure ( $125 \pm 1.5$  vs.  $124 \pm 1.8$  mm Hg), body weight ( $315 \pm 5$  and  $318 \pm 3$  g in untreated and treated groups, respectively), renal tissue nitrotyrosine, gp91<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup>, calcineurin A $\alpha$  or B subunits.

### DISCUSSION

Lifelong consumption of the antioxidant-fortified diet beginning at the prenatal period delayed the onset and

attenuated the severity of HTN in the SHR born in our facility and observed for 6 months. The beneficial effect of lifelong consumption of the antioxidant-rich diet shown here extends the results of short-term administration of the antioxidant drugs, including tempol, lazaroid, and melatonin in the adult SHR [15, 23, 25]. It should be noted that administration of antioxidant agents does not affect blood pressure in normal animals in whom oxidative stress is absent [9, 10, 11, 22, 23]. Together, these observations confirm the contribution of oxidative stress to the pathogenesis and maintenance of HTN in this model. In fact, the SHR group consuming regular diet exhibited significant elevations of plasma hydrogen peroxide, as well as kidney tissue nitrotyrosine and MDA, denoting the presence of oxidative stress. Elevation of nitrotyrosine and lipoperoxides in the SHR kidney observed here is consistent with the results of earlier studies of Bapat et al [37] and Zhou et al [38]. In contrast, the levels of these markers of oxidative stress were nearly normal in the SHR consuming antioxidant-fortified diet. This observation points to the efficacy of the antioxidant mixture ( $\alpha$ -tocopherol, ascorbic acid, zinc, and selenium) employed in the present study. The choice of these nutrients was based on their natural origin, ready availability, low



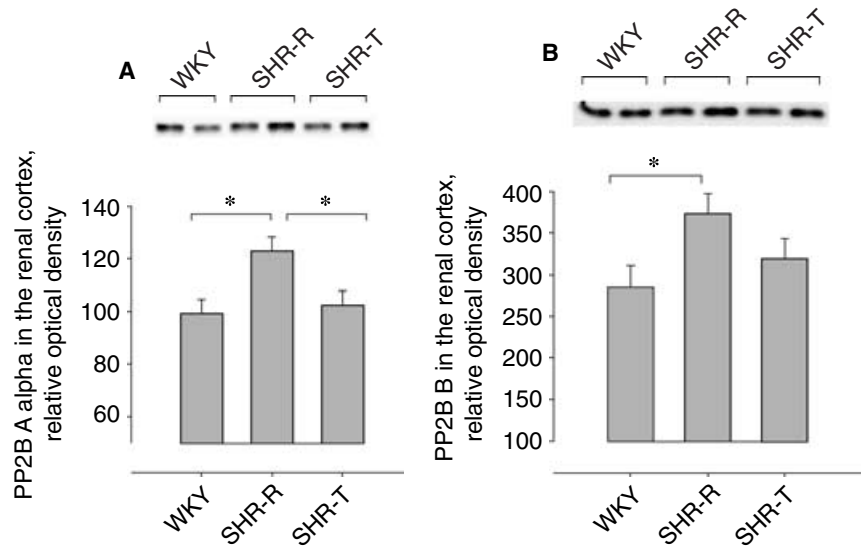
**Fig. 3. Representative Western blots and group data depicting gp91<sup>phox</sup>, p22<sup>phox</sup>, p47<sup>phox</sup>, and p67<sup>phox</sup> subunit of NAD(P)H oxidase in the renal cortex of Wistar Kyoto (WKY) rats and spontaneously hypertensive rat (SHR) groups raised on a regular diet (SHR-R) or antioxidant-fortified diet (SHR-T). *N* = 6 in each group, \**P* < 0.05 and \*\**P* < 0.01, \*\*\**P* < 0.001.**

toxicity, and demonstrated efficacy in various models of HTN [9–11, 39, 40].

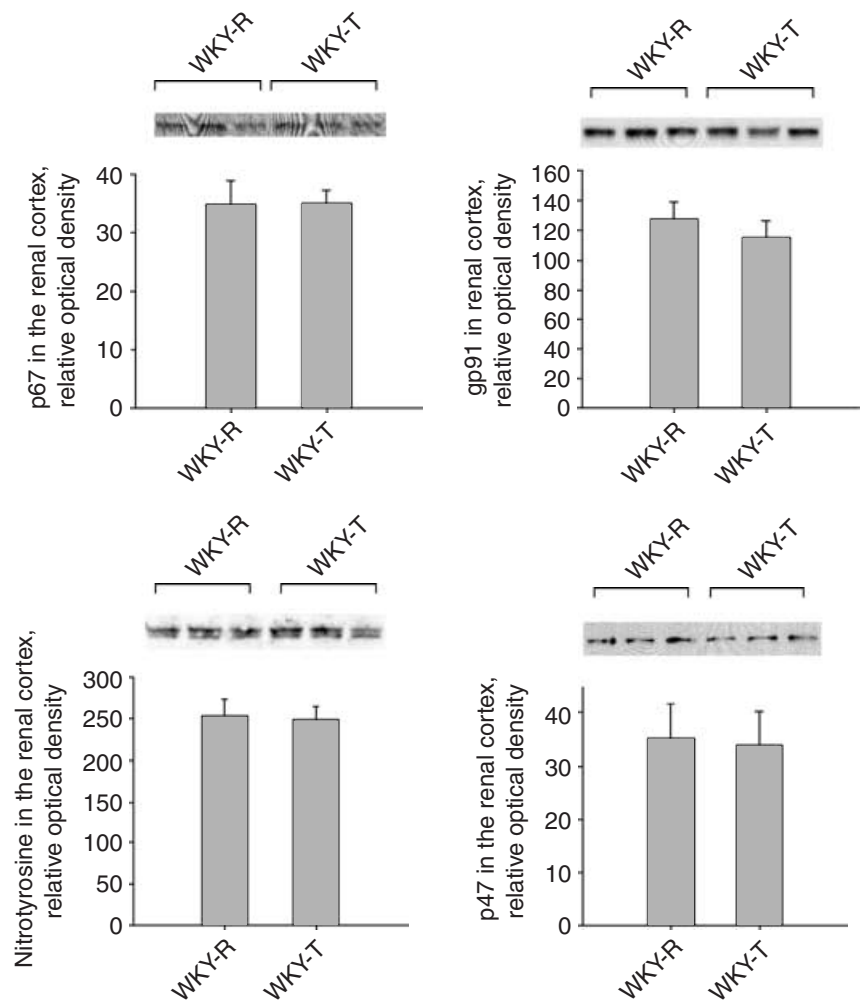
Oxidative stress in the SHR consuming regular diet was accompanied by significant up-regulation of gp91<sup>phox</sup> and p22<sup>phox</sup>, which represent the membrane-associated subunits of NAD(P)H oxidase. The elevation of kidney gp91<sup>phox</sup> and p22<sup>phox</sup> abundance in the untreated SHR was associated with a parallel increase in kidney tissue nitrotyrosine, which is generally viewed as a footprint of superoxide interaction with nitric oxide. The latter observation provides functional evidence for increased superoxide production in the kidney of untreated SHR presumably by NAD(P)H oxidase. These observations are consistent with the results of the elegant studies of Chabrashvili et al [29], who recently demonstrated significant up-regulations of NAD(P)H oxidase and its other isoforms, NOX-1 and NEPHROX, in the SHR kidney. The functional significance of up-regulations of these superoxide-producing enzymes in the kidney is evidence by overabundance of superoxide positive cells in SHR kidney [25]. Amelioration of oxidative stress by lifelong consumption of antioxidant-fortified diet was associated

with significant reductions of gp91<sup>phox</sup> and p22<sup>phox</sup> abundance in the renal cortex of antioxidant-treated compared to the untreated SHR. Accordingly, in addition to direct scavenging of ROS, antioxidant regimen employed here appears to limit production of ROS by lowering NAD(P)H oxidase abundance in the kidney. The latter finding is consistent with the recent report by Chen et al [19], who found significant reduction of kidney NAD(P)H oxidase activity by administration of vitamins E and C in stroke-prone SHR. The authors attributed this phenomenon to inhibition or interruption of complex formation of NAD(P)H oxidase subunits [19]. The antioxidant-treated SHR used in the present study showed a significant reduction in the immunodetectable gp91<sup>phox</sup> and p22<sup>phox</sup> abundance, which points to quantitative modification of renal NAD(P)H oxidase by antioxidant supplementation. Thus, alleviation of oxidative stress appears to limit both activation and expression of this enzyme.

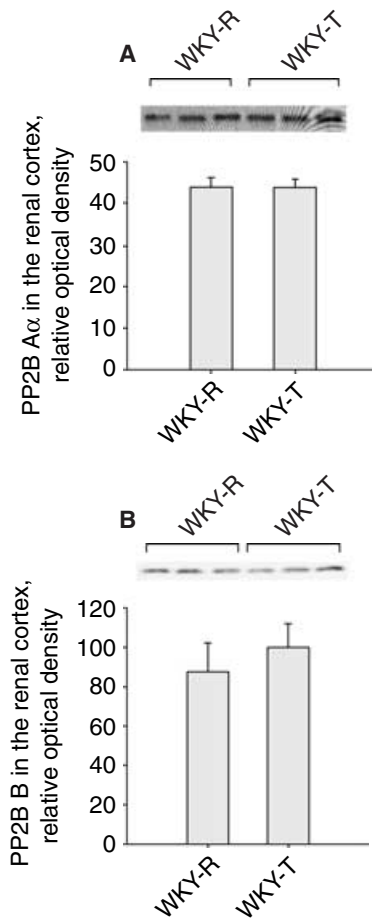
Oxidative stress can up-regulate NAD(P)H oxidase expression and promote inflammation via ROS-mediated activation of NFκB and the resultant generation of



**Fig. 4.** Representative Western blots and group data depicting A $\alpha$  and B subunit of calcineurin in the renal cortex of Wistar Kyoto (WKY) rats and spontaneously hypertensive rat (SHR) groups raised on a regular diet (SHR-R) or antioxidant-fortified diet (SHR-T). *N* = 6 in each group, \**P* < 0.05.



**Fig. 5.** Representative Western blots and group data depicting gp91<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup>, and nitrotyrosine abundance in the renal cortex of the Wistar Kyoto (WKY) rats fed a regular (WKY-R) or antioxidant-fortified (WKY-T) diet. *N* = 6 in each group, no significant differences were found.



**Fig. 6.** Representative Western blots and group data depicting protein abundance of calcineurin subunits (PP2BA  $\alpha$  and PP2BB) in renal cortex of the Wistar Kyoto (WKY) rats fed a regular (WKY-R) or antioxidant-fortified (WKY-T) diet.  $N = 6$  in each group, no significant differences were found.

proinflammatory cytokines. In fact, Nava et al [25] have recently shown marked activation of NF $\kappa$ B and its attenuation by antioxidant therapy in the SHR kidney. Therefore, the reduction in NAD(P)H oxidase abundance in our antioxidant-treated SHR may be due to diminished NF $\kappa$ B activity.

Recent studies have demonstrated the presence of infiltrating immunocompetent lymphocytes and macrophages in the renal interstitium of SHR [25, 33]. Moreover, the infiltrating cells were found to be an important source of angiotensin II and superoxide [25, 33]. By promoting local angiotensin II production and oxidative stress, the inflammatory cells present in the renal interstitium can contribute to hypertension in SHR. This supposition is supported by several elegant studies [32, 41–43] demonstrating amelioration of HTN and renal interstitial inflammatory infiltration in response to administration of the immunosuppressive agent, mycophenolate mofetil, in SHR and several other models of HTN [32, 40–42]. The

role of immune system in the pathogenesis of HTN is further enforced by the earlier studies of Svendsen [44], who demonstrated that athymic nude mice were resistant to the salt-dependent phase of DOCA salt-induced HTN. Renal interstitial infiltration of T-lymphocytes and macrophages in SHR is associated with and caused by at least in part, activation of NF $\kappa$ B [25] and the resultant generation of proinflammatory cytokines.

Calcineurin, a  $Ca^{2+}$ -calmodulin-activated serine/threonine phosphatase, plays an important role in numerous cellular events involving  $Ca^{2+}$ -calmodulin signal transduction pathways [34]. For instance, dephosphorylation of NFAT by calcineurin results in activation and translocation of NFAT from cytoplasm to nucleus, where it binds to its DNA binding site to initiate transcription and eventual production of interleukin-2 (IL-2) [45–47]. IL-2 produced in this manner binds to IL-2 receptor to generate signals required for completion of lymphocyte activation cascade, cell proliferation, and generation of other proinflammatory cytokines [45–47]. Given the critical role of calcineurin in T-cell activation and inflammatory cascade, it is conceivable that accumulation of activated T cells and macrophages in SHR kidney may be, in part, mediated by calcineurin. The present study showed marked up-regulation of both calcineurin A $\alpha$  (the catalytic subunit) and calcineurin B (the regulatory subunit) abundance in the renal cortex of untreated SHR. It is of interest that chronic inhibition of calcineurin by either cyclosporine or FK506 commonly results in HTN and oxidative stress in humans and experimental animals [48–51]. Thus, the role, if any, of the observed up-regulation of renal calcineurin in the pathogenesis of HTN and oxidative stress in SHR is uncertain and awaits investigation. Interestingly, up-regulation of calcineurin expression in renal cortex was partially ameliorated by long-term antioxidant supplementation. This observation suggests that oxidative stress may be involved in regulation of calcineurin in this organ. Partial restoration of renal calcineurin expression with antioxidant supplementation was accompanied by a parallel reduction of NAD(P)H oxidase abundance in the treated SHR. This observation points to possible interdependent regulation of these enzymes.

In contrast to the SHR, which showed significant reductions in blood pressure, kidney tissue nitrotyrosine, NAD(P)H oxidase, and calcineurin abundance, the measured parameters were unaffected by antioxidant therapy in the WKY rats. This observation is consistent with the earlier studies and suggests that in the absence of oxidative stress, antioxidant supplementation, above that contained in the regular diet, has no additional impact on the measured parameters. Despite reduction in arterial pressure, proteinuria was not attenuated in the antioxidant-treated SHR. The authors wish to point out that while arterial pressure was significantly reduced, it was not

normalized by antioxidant therapy, and significant, albeit less severe HTN existed in the treated animals. Moreover, while urinary protein excretion was significantly higher in SHR than in WKY group, it was relatively mild. More severe proteinuria and progressive decline in glomerular filtration rate occurs at a much later stage in SHR [52]. Therefore, long-term studies are needed to discern whether or not antioxidant therapy can affect development of severe proteinuria and retard development of renal insufficiency in the aged SHR.

## CONCLUSION

Lifelong consumption of antioxidant-rich diet ameliorates HTN and oxidative stress in SHR. This is associated with the reduction of NAD(P)H oxidase. Thus, antioxidant-rich diet appears to attenuate oxidative stress, not only by fortifying antioxidant defense capacity but also by lowering NAD(P)H oxidase and hence, ROS-generating capacity. Calcineurin abundance is increased in the renal cortex and declines with antioxidant therapy in SHR. The role of the observed alterations of the renal cortical calcineurin abundance in the pathogenesis of hypertension and its response to antioxidant therapy is presently unknown and awaits future investigation.

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