ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 9 & 10, 2005

Forum Original Research Communication

Eplerenone Reduces Oxidative Stress and Enhances eNOS in SHR: Vascular Functional and Structural Consequences

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ABSTRACT

The aim of the present study was to evaluate the effect of the aldosterone receptor antagonist eplerenone on endothelial function, oxidative stress, and structural alterations present in spontaneously hypertensive rats (SHR). To carry out the study, male SHR (18 weeks old) were treated with two doses of eplerenone (30 and 100 mg/kg/day) for 10 weeks. A group of $n = 8$ untreated SHR was used as a control-vehicle group, and a group of Wistar Kyoto rats ($n = 8$) was used as a reference of normotensive conditions. Systolic arterial pressure (SAP) was measured by the tail-cuff method. Endothelium-dependent and -independent relaxations, as well as endothelial nitric oxide synthase (eNOS) and the subunit p22phox of NAD(P)H oxidase mRNA expressions, were studied in aorta from SHR untreated or treated with eplerenone. Media/lumen ratio was also calculated in aortic preparations. In addition, levels of reduced glutathione (GSH), oxidized glutathione (GSSG), and malonyl dialdehyde (MDA) were evaluated in liver homogenates. Treatment with eplerenone reduced ($p < 0.05$) SAP and normalized aortic media/lumen ratio and acetylcholine relaxations. Both doses of the drug enhanced ($p < 0.05$) eNOS and reduced p22phox mRNA expressions. Similarly, eplerenone increased ($p < 0.05$) hepatic GSH/GSSG ratio, and reduced ($p < 0.05$) hepatic MDA levels in a comparable manner. Consequently, it could be concluded that aldosterone participates in the functional and structural vascular alterations of SHR through the diminution of nitric oxide availability and an enhancement of vascular and systemic oxidative stress. *Antioxid. Redox Signal.* 7, 1294–1301.

INTRODUCTION

HYPERTENSION produces functional and structural changes in the arterial wall, which seem to be responsible for most of the vascular complications of this disease (36). Endothelial dysfunction has been proposed as the most important vascular alteration in hypertension, which leads to the development of arteriosclerosis (12, 23, 29). Hypertensive endothelial dysfunction is characterized by reduced endothelium-dependent relaxations, suggesting a reduced availability of nitric oxide (NO) (12, 22, 23, 29). Diminished endothelium NO synthase (eNOS) expression or activity has been proposed as an important mechanism leading to reduced NO availability and endothelial dysfunction associated with different types of hypertension (1, 20, 22, 24, 45). Enhanced vascular production of reactive oxygen species, specifically superoxide anions, has been reported as another important mechanism contributing to hypertensive endothelial dysfunction due to inactivation of NO (25, 26). Moreover, liver has been described as the most important organ involved in the regulation of systemic redox metabolism, due to the key enzymes responsible for reactive oxygen species clearance and the production of the most important antioxidant agent, glutathione (15, 16, 47). In addition, it has been reported that al-

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terations in hepatic antioxidant defense have been associated with endothelial dysfunction, suggesting that changes in liver redox metabolism can be relevant at the vascular level (3, 6, 10). We recently reported that reduced endothelium-dependent relaxations were associated with enhanced malonyl dialdehyde (MDA) levels and reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio in spontaneously hypertensive rats (SHR) (4).

The renin–angiotensin–aldosterone axis plays an important role in the development of vascular disease. Furthermore, angiotensin II has been considered the main active component of the renin–angiotensin–aldosterone axis, due to its participation in most of the alterations underlying the progression and complications of vascular disease. Aldosterone, besides its participation in the renal regulation of Na^+ and K^+, seems to play a larger role than once appreciated in the cardiovascular system (13, 27, 28). Several studies reported the involvement of aldosterone not only in the regulation of vascular tone, but in cardiovascular alterations such as vascular remodeling and fibrosis, left ventricular hypertrophy, congestive heart failure, and cardiac arrhythmias (7, 33, 34, 37, 39). Furthermore, aldosterone has also been shown to be involved in the pathogenesis of hypertension (11, 19, 46). Recent studies demonstrated that aldosterone receptor antagonists were able to reduce blood pressure and to ameliorate endothelial dysfunction in liquorice-induced hypertension, a model of hypertension dependent on 11β-hydroxysteroid dehydrogenase inhibition (31). Furthermore, spironolactone was also able to improve vascular hypertrophy and oxidative stress induced by the angiotensin II, indicating that aldosterone mediates some of angiotensin II-induced vascular effects in hypertension (31, 43). However, it is not known whether aldosterone-receptor antagonists could reduce vascular alterations such as endothelial dysfunction, oxidative stress, and remodeling in a multifactorial model of hypertension as the SHR. In a previous study, we demonstrated that the angiotensin II type 1 (AT_1) receptor antagonist candesartan was able to ameliorate endothelial dysfunction and to reduce vascular and systemic oxidative stress in SHR. Consequently, the present study aimed to evaluate the effect of the aldosterone-receptor antagonist, eplerenone, on the vascular functional and structural alterations present in SHR, as well as to investigate the mechanisms underlying these effects. For this purpose, endothelium-dependent and -independent relaxations, eNOS and p22phox mRNA expressions, and media/lumen ratio were studied in aorta from SHR untreated and treated with eplerenone. In addition, levels of GSH, GSSG, and MDA were evaluated in liver homogenates.

MATERIALS AND METHODS

The study was conducted in 18-week-old SHR following recommendations from the institutional animal care and use committee, according to the guidelines for ethical care of experimental animals of the European Union. Sixteen SHR were treated with the aldosterone receptor antagonist, eplerenone, at two doses, 30 (E30) and 100 (E100) mg/kg/day for 10 weeks. The doses of eplerenone were chosen from pilot studies in SHR where they reduced systolic arterial pressure (SAP) in a dose-dependent manner. Thus, we chose one dose of eplerenone producing a little decrease in SAP (E30), and the other dose reducing SAP to a higher extent (E100), to evaluate the possible contribution of the blood pressure-lowering effect. Eplerenone was given in the food. Every week, food intake was controlled in order to adjust the quantity of drug to obtain the two doses of eplerenone used in the study. An additional group ($n = 8$) of untreated SHR was used as a control-vehicle group, and a group of Wistar Kyoto rats (WKY) ($n = 8$) was used as a reference of normotensive conditions. SAP was measured by a tail-cuff pletysmograph (Narco Bio-Systems, Houston, TX, U.S.A.) at the end of the treatment period as previously described (21, 35).

Aortic morphometry

Aortic segments were fixed in 10% sodium phosphate-buffered formaldehyde, processed, and cut in sections (4 μm). To determine vessel or luminal areas, the cross-sectional area enclosed by the external or internal elastic lamina, respectively, was corrected to a circle by applying the form factor $L^2/_{4\pi}$ to the measurement of the lamina, where L is the length of the lamina. This method was used to avoid miscalculation of vessel and luminal areas, because aortic segments could be deformed during preparation. Media area was obtained by subtracting lumen area from the area encompassed by external elastic lamina. Measurements were made by tracing in digitalized, segmented-colored sections stained with hematoxylin–eosin using a QWIN Leica image analyzer (Leica Imaging Systems Ltd., Cambridge, U.K.) as previously described (18).

Aortic endothelial function

Endothelial function was studied in aortic rings at the end of the treatment period in all animals. The day of the experiment, thoracic aorta was isolated, gently cleaned from surrounding tissue, and placed in oxygenated 95% O_2/5% CO_2 Krebs bicarbonate solution of the following composition (mmol/L): NaCl 118.5, KCl 4.7, $CaCl_2$ 2.8, KH_2PO_4 1.1, $NaHCO_3$ 25.0, and glucose 11.1, at 5°C. Then thoracic aorta was cut transversally in ring segments (2 mm long). Each ring was placed inside a 5-ml heated bath filled with Krebs buffer (37°C) bubbled with 95% O_2/5% CO_2 and suspended between two L-shaped stainless steel hooks. The upper one was attached to a force transducer (FT03, Grass) and coupled to a computerized system (Mc Lab 8E, AD Instruments) for measurement of isometric tension. Rings were allowed to equilibrate for 60–90 min with changes of buffer every 15 min, and with several adjustments of length until baseline tension stabilized at 2 g. In previous studies, we found that 2 g of resting tension is optimal for these types of experiments. When tension was stable, the experiments were initiated by obtaining a reference contractile response to 80 mmol/L KCl. Endothelial function was studied by evaluating relaxations to acetylcholine (ACh; 10^{-11} to 10^{-8} mol/L) in phenylephrine (10^{-6} mol/L) precontracted rings. Endothelium-independent relaxations induced by sodium nitroprusside (SNP; 10^{-10} to 10^{-7} mol/L) were also carried out.

Aortic eNOS and p22phox mRNA expression

RNA isolation. Aorta was isolated and immediately frozen in liquid nitrogen for molecular biology determinations. Isolation and manipulation of aorta were always performed under sterile conditions. Frozen rat aortas were pulverized in liquid nitrogen, and 100 mg of tissue was homogenized together with 1 ml of TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH, U.S.A.). RNA isolation was performed according to the Chomczynski method (5). RNA was quantified by measurement of optical density at 260 nm with a BioPhotometer (Eppendorf, Germany). RNAs were frozen at −80°C until their usage.

Reverse transcriptase. Five micrograms of total RNA was taken to perform reverse transcription. It was previously heated with 2 μ*M* random hexamer at 70°C for 5 min and quickly chilled on ice. Subsequently, a mixture of 0.7 U of RNase inhibitor, 25 m*M* Tris-HCl (pH 8.3), 37 m*M* KCl, 1.5 m*M* $MgCl_2$, 10 m*M* dithiothreitol, dNTP (0.4 m*M* each), and 2.5 U of Moloney murine leukemia virus reverse transcriptase was added and incubated at 37°C for 60 min, followed by heating at 95°C for 10 min and chilling on ice. Then the mixture was completed with DNase-free water until a final volume of 50 μl.

Multiplex polymerase chain reaction (MPCR). Five microliters of the above cDNA was taken for an MPCR reaction (Maxim Biotech, Inc., San Francisco, CA, U.S.A.). A mixture of MPCR buffer, *Taq* DNA polymerase (2.5 U), and specific MPCR primers for eNOS, p22phox, and glyceraldehyde-3-phosphate dehydrogenase (GADPH) was added. The following time–temperature profile was used to perform MPCR: two cycles of 96°C for 1 min and 58–60°C for 2 min; 28 cycles for amplification of p22phox and GAPDH genes at 94°C for 1 min and 58–60°C for 2 min; one cycle of 70°C for 10 min and a final step of 25°C.

Sequences of the primers were as follows: for eNOS, 5′-TGGCCGTGGAACAACTGGA-3′ (sense) and 5′-TGAGCTGACAGAGTAGTACC-3′ (antisense); for p22phox, 5′-GCTCATCTGTCTGCTGGAGTA-3′ (sense) and 5′-ACGACCTCATCTGTCACTGGA-3′ (antisense); and for GAPDH, 5′-TATGATGACATCAAGAAGGTGG-3′ (sense) and 5′-CACCACCCTGTTGCTGTA-3′ (antisense). Both were designed using rat GenBank and Basic Local Alignment Search Tool (BLAST; NCBI), and then synthesized by TIB MOLBIOL (Germany). MPCR DNA product was fractionated electrophoretically on a 2% agarose gel containing 0.5 mg/ml ethidium bromide. Sizes of the fragments were 352 bp for eNOS, 434 bp for p22phox, and 212 bp for GAPDH. Intensity of the bands was measured using Gel Analysis Software (Syngene, Cambridge, U.K.). Data were normalized with GAPDH intensity data.

Hepatic redox parameters

Livers were isolated, cut in samples, immediately frozen in liquid nitrogen, and stored at −80°C until processing. Liver samples for GSH/GSSG determination were homogenized in 50 m*M* potassium phosphate buffer (pH 7.5) containing 1 m*M* EDTA and 1 mg/ml bovine serum albumin (1 g of liver/2 ml of buffer). Samples for MDA determination were homogenized in 50 m*M* Tris-HCl buffer (pH 7.2) containing 5 m*M* EDTA and 1 m*M* 2-mercaptoethanol (1 g of liver/6 ml of buffer). After centrifugation (20 min, 3,000 rpm), supernatant was separated and redox parameters were measured. All procedures were run at 2–4°C. GSH, GSSG, and MDA levels were measured by spectrophotometric (Hitachi 912 autoanalyzer) assays using commercial kits (Bioxytech GSH/GSSG-412 and Bioxytech LPO-586, Oxis Research). The GSH/GSSG assay uses the thiol-scavenging reagent, 1-methyl-2-vinylpyridinium trifluoromethanesulfonate at a level that rapidly scavenges GSH. The MDA assay is based on the reaction of a chromogenic reagent, *N*-methyl-2-phenylindole, with MDA at 45°C. One molecule of MDA reacts with two molecules of the reagent to yield a stable chromophore with maximal absorbance at 586 nm. Results are expressed per milligram of protein (biuret method, Hitachi 912 autoanalyzer).

Drugs

Eplerenone was kindly provided by Pharmacia Co. (Peacock, NJ, U.S.A.). Products for morphological analysis were purchased from Merck (Darmstadt, Germany).

All other drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Stock solutions of drugs were prepared in distilled water and diluted to the desired concentration with Krebs buffer immediately before the experiment. Concentrations are expressed as final molar concentration in the organ chamber.

Calculations and statistical analysis

Results are expressed as means ± SEM from eight rats. Relaxation responses are expressed as percent reduction of tension in the phenylephrine-constricted state. Dose-response curves were compared by multivariate analysis of variance for repeated measures using the SPSS 10.0 program (StatSoft Inc., Tulsa, OK, U.S.A.). All other data were analyzed using a one-way analysis of variance, followed by a Newman–Keuls test if differences were noted. The null hypothesis was rejected when the p value was <0.05.

RESULTS

SAP and body weight

SHR presented higher ($p < 0.05$) SAP levels than WKY (190.2 ± 4.2 versus 126.1 ± 1.0 mm Hg, respectively). Treatment with eplerenone reduced ($p < 0.05$) SAP levels (184.6 ± 1.7 mm Hg and 156.3 ± 2.8 mm Hg, E30 and E100, respectively). No differences in body weight were observed among groups (WKY: 427 ± 7 g; SHR: 442 ± 9 g; SHR-E30: 440 ± 8 g; SHR-E100: 430 ± 11 g).

Aortic morphometry

Vessel, media, and lumen areas were larger ($p < 0.05$) in SHR than in WKY, suggesting an outward remodeling in the aorta induced by hypertension. Likewise, the media/lumen

TABLE 1. LUMEN, MEDIA, AND VESSEL AORTIC AREAS AND MEDIA/LUMEN RATIO IN SHR UNTREATED OR TREATED WITH EPLERENONE (30 AND 100 MG/KG/DAY; 10 WEEKS)

Group	*Lumen area (mm^2)*	*Media area (mm^2)*	*Vessel area (mm^2)*	*Media/lumen ratio*
WKY	1.7 ± 0.07	0.69 ± 0.02	2.47 ± 0.10	0.37 ± 0.01
SHR	3.1 ± 0.20*	1.32 ± 0.10*	4.41 ± 0.26*	0.44 ± 0.02*
SHR + E30	2.4 ± 0.09*†	0.95 ± 0.02*†	3.31 ± 0.06*†	0.36 ± 0.01†
SHR + E100	2.5 ± 0.10*†	0.95 ± 0.06*†	3.42 ± 0.10*†	0.37 ± 0.01†

WKY rats were used as normotensive reference.
*$p < 0.05$ versus WKY.
†$p < 0.05$ versus SHR.

ratio was higher in SHR than in WKY. Both doses of eplerenone similarly reduced ($p < 0.05$) vessel, media, and lumen areas, and normalized media /lumen ratio (Table 1, Fig. 1).

Aortic endothelium-dependent and -independent relaxations

Endothelium-dependent relaxations induced by ACh were lower ($p < 0.05$) in SHR than in WKY (Fig. 2). In contrast, endothelium-independent relaxations to SNP were comparable in both SHR and WKY (data not shown). Both doses of eplerenone enhanced ($p < 0.05$) ACh relaxations to values comparable to those observed in WKY, but did not affect relaxations to SNP (Fig. 2).

Aortic eNOS mRNA expression

Aortic eNOS mRNA expression was lower ($p < 0.05$) in SHR than in WKY. Treatment with both doses of eplerenone increased ($p < 0.05$) eNOS mRNA expression to a similar extent (Fig. 3).

Aortic p22phox mRNA expression

Aortic mRNA expression of the subunit p22phox of NAD(P)H oxidase was higher ($p < 0.05$) in SHR than in WKY.

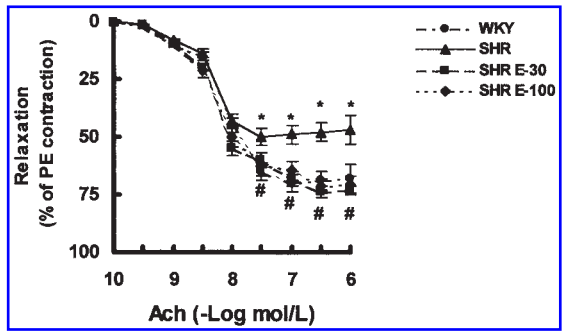

FIG. 2. Relaxations induced by ACh in aortic rings from SHR untreated or treated with eplerenone (30 and 100 mg/kg/day; 10 weeks). WKY rats were used as normotensive reference. *$p < 0.05$ versus WKY; #$p < 0.05$ versus SHR.

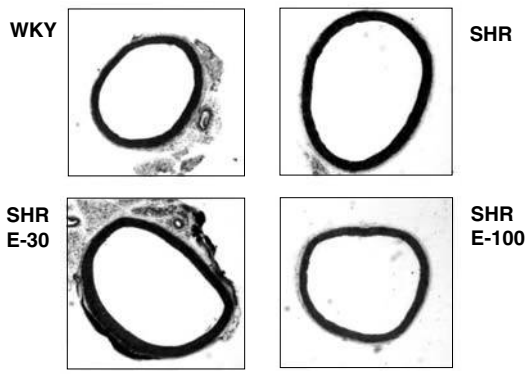

FIG. 1. Representative microphotographs (magnification, ×40) of aorta cross-sections from SHR untreated or treated with eplerenone (30 and 100 mg/kg/day; 10 weeks). WKY rats were used as normotensive reference.

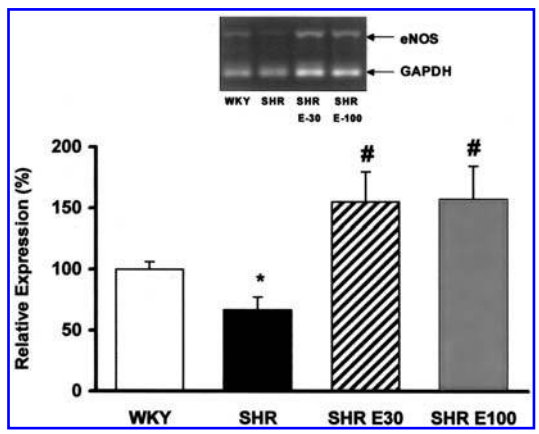

FIG. 3. mRNA eNOS expression in aorta from SHR untreated or treated with eplerenone (30 and 100 mg/kg/day; 10 weeks). WKY rats were used as normotensive reference. *$p < 0.05$ versus WKY; #$p < 0.05$ versus SHR.

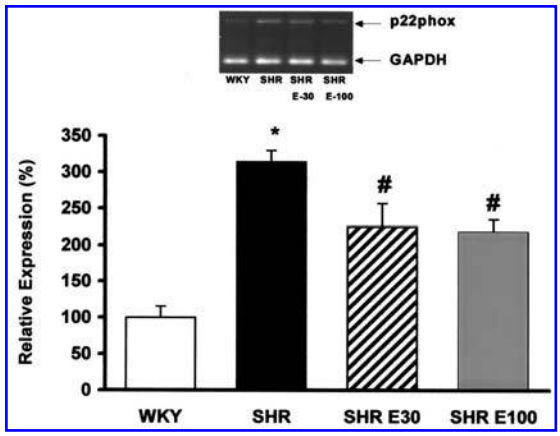

FIG. 4. mRNA p22phox expression in aorta from SHR untreated or treated with eplerenone (30 and 100 mg/kg/day; 10 weeks). WKY rats were used as normotensive reference. *$p < 0.05$ versus WKY; #$p < 0.05$ versus SHR.

Treatment with both doses of eplerenone reduced ($p < 0.05$), although did not normalize, p22phox expression (Fig. 4).

Hepatic redox parameters

GSSG values were higher ($p < 0.05$) in SHR than in WKY. However, GSH values were comparable in both groups. Thus, SHR presented a lower ($p < 0.05$) hepatic GSH/GSSG ratio than WKY. Treatment with both doses of eplerenone similarly decreased ($p < 0.05$) GSSG levels without modifying those of GSH, and consequently, GSH/GSSG ratio increased ($p < 0.05$). MDA levels were higher ($p < 0.05$) in SHR than in WKY, and were reduced ($p < 0.05$) by both doses of eplerenone in a comparable manner (Table 2).

DISCUSSION

The present results demonstrate that the aldosterone receptor antagonist eplerenone normalized endothelium-dependent relaxations in SHR and reduced the aortic media/lumen ratio. These effects were associated with an enhancement of eNOS mRNA expression and diminution of vascular and systemic oxidative stress. All these effects even occurred with minimal reductions of SAP.

Aldosterone is now recognized to participate in a number of different pathophysiological effects in the cardiovascular system (7, 13, 28, 33, 34, 37, 39, 46). Moreover, several reports have demonstrated that the aldosterone receptor antagonist, eplerenone, exerts cardiovascular protection under different experimental and clinical situations (2). The present results show that eplerenone normalized ACh-induced relaxations in SHR, suggesting an important participation of aldosterone in endothelial dysfunction associated with this model of hypertension. Amelioration of ACh-induced relaxations with aldosterone receptor antagonists was previously observed in other types of experimental hypertension in rats, such as angiotensin II- and liquorice-induced hypertension, as well as in experimental atherosclerosis in rabbits (31, 32, 43). Previous studies support the concept that aldosterone seems to affect endothelial function. This is suggested by the reduced endothelium-dependent relaxations found in patients with primary hyperaldosteronism, and the ability of aldosterone to attenuate endothelium-dependent vasodilatation to ACh in healthy humans (9, 41). The participation of aldosterone in endothelial dysfunction is also supported by the observation that blockade of the aldosterone receptor significantly improved vasodilatory response to ACh in patients with heart failure (8).

According to numerous previous studies (1, 12, 20, 22–26, 29, 45), the improvement of ACh-induced relaxations produced by eplerenone treatment in SHR suggests an increase in NO availability. The molecular mechanisms whereby eplerenone ameliorated endothelium-dependent relaxations in SHR were investigated in the present study through the aortic mRNA expression of eNOS and the subunit p22phox of NAD(P)H oxidase. The results show an increase in eNOS mRNA expression in SHR treated with both doses of eplerenone, which could account for an enhanced NO availability and contribute to explain the enhanced endothelium-dependent relaxations. Previous studies reporting increased NO bioactivity by aldosterone receptor antagonists support the present finding and led to the proposal that aldosterone could down-regulate the NO system by a reduction of eNOS gene expression (8, 31, 43). Also supporting this concept are the results by Ikeda *et al.* (14), who reported that aldosterone inhibited NO synthesis induced by interleukin-1β in rat vascular smooth muscle cells. Another mechanism accounting for the normalization of ACh-induced relaxations observed in SHR treated with eplerenone seems to be related to a reduction of oxidative stress and subsequent increase of NO avail-

TABLE 2. GSH, GSSG, GSH/GSSG RATIO, AND MDA HEPATIC LEVELS IN SHR UNTREATED OR TREATED WITH EPLERENONE (30 AND 100 MG/KG/DAY; 10 WEEKS)

Group	*GSH (U/g of protein)*	*GSSG (U/g of protein)*	*GSH/GSSG ratio*	*MDA (μmol/g of protein)*
WKY	29.4 ± 0.9	0.72 ± 0.01	40.4 ± 6.7	0.424 ± 0.014
SHR	32.3 ± 1.9	1.3 ± 0.06*	27.4 ± 2.3*	0.564 ± 0.044*
SHR + E30	33.3 ± 1.9	0.62 ± 0.01*†	53.1 ± 4.0*†	0.456 ± 0.024*†
SHR + E100	31.1 ± 1.3	0.67 ± 0.02*†	54.0 ± 8.0*†	0.411 ± 0.046*†

WKY rats were used as normotensive reference.
*$p < 0.05$ versus WKY.
†$p < 0.05$ versus SHR.

ability. The present results showed that elevated aortic p22phox mRNA expression observed in SHR was reduced by both doses of eplerenone. This suggests that the enhanced aortic superoxide anion production in SHR could depend, at least partially, on aldosterone stimulation of NAD(P)H oxidase. The effect of aldosterone receptor antagonists on superoxide anion production was previously reported in angiotensin II-induced hypertension in rats and in atherosclerotic rabbits (32, 43). Both studies reported that reduction of elevated NAD(P)H oxidase accounted for the diminution of superoxide anion production produced by aldosterone receptor antagonists in both experimental models.

The beneficial effect of eplerenone on systemic oxidative stress is supported by the observed reduction of hepatic MDA levels and reduction of GSSG levels. Similarly, we recently reported that treatment with candesartan decreased hepatic MDA levels and enhanced GSH/GSSG in SHR (4). As in the present study, the amelioration of the hepatic redox system was associated with a reduction of vascular p22phox and enhancement of ACh relaxations in SHR. Consequently, the results suggest that aldosterone, together with other agents such as angiotensin II, stimulates oxidative stress at both the vascular and systemic level. Thus, it could be hypothesized that the effects of eplerenone on oxidative stress are due to two mechanisms operating in the same direction: (a) the reduction of aortic p22phox expression, and consequently aortic superoxide anion production, and (b) an enhancement of hepatic antioxidant defense, as suggested by the increase in GSH/GSSG ratio.

The results also show that treatment of SHR with eplerenone normalized the aortic media/lumen ratio, suggesting an involvement of aldosterone in arterial remodeling associated with hypertension. Supporting this finding are the results from Park and Schiffrin (30) showing increased media/lumen ratio in mesenteric arteries of aldosterone-infused rats. Recently, Virdis *et al.* demonstrated the participation of aldosterone in the development of structural remodeling of resistance arteries in angiotensin II-induced hypertension in rats (43). The mechanisms through which aldosterone causes vascular remodeling are the subject of ongoing investigation. Potential mechanisms accounting for this effect would include endothelial dysfunction, increased sodium influx into vascular smooth muscle cells, hypertrophy of vascular smooth muscle cells, generation of reactive oxygen species, inhibition of norepinephrine uptake, stimulation of transforming growth factor-β, and potentiation of angiotensin II on medial growth through AT_1 receptors (38, 42). Furthermore, in pigs submitted to coronary angioplasty, eplerenone treatment attenuated constrictive remodeling by mechanisms involving reduction of collagen accumulation (44). Therefore, aldosterone seems to be importantly involved in structural vascular alterations associated with hypertension and other pathological vascular alterations.

The diminution of hemodynamic overload due to arterial pressure reduction, although it could not be ruled out, does not seem to be the main mechanism accounting for the observed amelioration of vascular alterations in eplerenone-treated SHR. This is based on the fact that treatment with 30 mg/kg/day slightly reduced SAP and presented similar functional, structural, and molecular effects as the dose of 100 mg/kg/day, which highly reduced SAP. Thus, it could be postulated that aldosterone participates, along with other factors, in the functional and structural vascular alterations of SHR through the diminution of NO availability and an enhancement of vascular and systemic oxidative stress.

Finally, it is important to mention that the results obtained in the present study are comparable to those obtained in SHR treated with AT_1 receptor antagonists (4). Interactions between angiotensin II and aldosterone seems to have important pathophysiological and therapeutic implications in cardiovascular alterations associated with hypertension and heart failure (8, 17, 31, 40, 43). The present results further support this concept and suggest that angiotensin II and aldosterone share common actions and participate in a crucial manner in endothelial dysfunction, oxidative stress, and vascular hypertrophy associated with hypertension.

In view of the present results, it can be concluded that endogenous aldosterone seems to increase vascular and systemic oxidative stress and to reduce NO availability in SHR. Moreover, the results further support the participation of aldosterone in the vascular functional and structural alterations associated with hypertension.

ACKNOWLEDGMENTS

This work was supported by grants from Comisión Interministerial de Ciencia y Tecnología (SAF 2004-01884; BFI 2001-1324), Fondo de Investigaciones Sanitarias (FIS 01/0088-02), Plan Regional de Investigación de la Comunidad Autónoma de Madrid (08.4/0002.1/2003), and Pharmacia Co. N. de las Heras was paid with a Grant from Red Cardiovascular del FIS (C03/01). We also thank Mrs. Blanca Martínez and Mr. Antonio Carmona for their technical assistance.

ABBREVIATIONS

ACh, acetylcholine; AT_1, angiotensin type 1 receptor; E30 and E100, 30 and 100 eplerenone mg/kg/day, respectively; eNOS, endothelial nitric oxide synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malonyl dialdehyde; MPCR, multiplex polymerase chain reaction; NO, nitric oxide; SAP, systolic arterial pressure; SHR, spontaneously hypertensive rats; SNP, sodium nitroprusside; WKY, Wistar Kyoto rats.

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Received for publication January 14, 2005; accepted March 7, 2005.

This article has been cited by:

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