Aldosterone Administration to Mice Stimulates Macrophage NADPH Oxidase and Increases Atherosclerosis Development

A Possible Role for Angiotensin-Converting Enzyme and the Receptors for Angiotensin II and Aldosterone

Shlomo Keidar, MD; Marielle Kaplan, PhD; Elsa Pavlotzky; Raymond Coleman, PhD; Tony Hayek, MD; Shadi Hamoud, MD; Michael Aviram, DSc

Background—The renin-angiotensin-aldosterone system is involved in the pathogenesis of atherosclerosis, partially because of its pro-oxidative properties. We questioned the effect and mechanisms of action of administration of aldosterone to apolipoprotein E–deficient (E0) mice on their macrophages and aorta oxidative status and the ability of pharmacological agents to block this effect.

Methods and Results—Aldosterone (0.2 to 6 μg · mouse−1 · d−1) was administered to E0 mice alone or in combination with eplerenone (200 mg · kg−1 · d−1), ramipril (5 mg · kg−1 · d−1), or losartan (25 mg · kg−1 · d−1). Mouse aortic atherosclerotic lesion area and macrophage and aortic oxidative status were evaluated. Aldosterone administration enhanced the mouse atherosclerotic lesion area by 32%. Mouse peritoneal macrophages and aortic segments from aldosterone-treated mice exhibited increased superoxide anion formation by up to 155% and 69%, respectively, and this effect was probably mediated by NADPH oxidase activation, because increased translocation of its cytosolic component p47phox to the macrophage plasma membrane was observed. THP-1 macrophages incubated in vitro with aldosterone (10 μmol/L) exhibited a higher capacity to release superoxide ions by 110% and increased ability to oxidize LDL by 74% compared with control cells. Aldosterone administration enhanced mouse peritoneal macrophage ACE activity and mRNA expression by 2.3-fold and 2.4-fold, respectively. Only cotreatment of eplerenone with ramipril or losartan completely blocked the oxidative effects of aldosterone.

Conclusions—Aldosterone administration to E0 mice increased macrophage oxidative stress and atherosclerotic lesion development. Blocking of the mineralocorticoid receptor and inhibition of tissue ACE and/or the angiotensin receptor-1 reduced aldosterone deleterious pro-oxidative and proatherogenic effects. (Circulation. 2004;109:2213-2220.)

Key Words: aldosterone ▪ atherosclerosis ▪ macrophages ▪ angiotensin ▪ angiotensin-converting enzyme

Oxidative stress is involved in the pathogenesis of atherosclerosis, because it promotes lipid peroxidation in LDL and in vascular cells and macrophages.1–4 Under oxidative stress, macrophages generate reactive oxygen species leading to LDL oxidation.5,6 Activation of NADPH oxidase leads to translocation of its p47 component from the cytosol to plasmatic membranes.7 The activated complex in the plasma membrane is responsible for production and release of superoxide anions, which can then lead to cell-mediated oxidation of LDL.3

Aldosterone has an important role in the pathophysiology of heart failure,8,9 and it was recently suggested that aldosterone mediates some of the proatherogenic effects of angiotensin II by amplifying tissue ACE activity.10–14

Rats treated with aldosterone exhibited increased arterial NADPH oxidase activity.15 Hypercholesterolemic rabbits treated with spironolactone showed reduced NADPH oxidase activity compared with placebo-treated rabbits, suggesting a possible involvement of aldosterone in oxidative stress.16 Hypertensive rats given spironolactone exhibited improved vascular changes induced by angiotensin II and reduced oxidative stress.17 We have recently shown that administration of eplerenone (a selective mineralocorticoid receptor blocker) to atherosclerotic mice reduced oxidative stress and attenuated atherogenesis.18

Because ACE inhibitors only transiently suppress the production of aldosterone, a mechanism referred to as “aldosterone escape,” there is a need to block the action of aldosterone through mineralocorticoid receptor blockers.8 The Randomized Aldactone Evaluation Study (RALES) exhibited a significant 30% survival advantage in chronic heart failure patients receiving the aldosterone receptor blocker
spirololactone in addition to standard therapy including diuretics and ACE inhibitors.

The aim of the present study was to characterize the pro-oxidative effects of aldosterone both in vitro and in vivo and to determine the pharmacological agents that block these effects.

Methods

Mouse Protocol

The mouse protocol was approved by the committee for supervision of animal experiments of the Technion Israel Institute of Technology (approval No. IL-066-10-2001/04) and was conducted in accordance with the Israeli law for animal care. Apolipoprotein E-deficient (E<sup>−</sup>) mice develop atherosclerotic lesions on chow diet and are characterized by increased oxidative stress. Aldosterone (0.2, 1, 2, or 6 μg·mouse<sup>−1</sup>·d<sup>−1</sup>) was administered to E<sup>−</sup> mice (8 weeks old) by osmotic minipump implantation (model 1002, Alzet) for 1, 2, or 4 weeks. Aldosterone was prepared in 150 mmol/L saline/EtOH. Alternatively, E<sup>−</sup> mice treated with aldosterone (0.2 or 2 μg·mouse<sup>−1</sup>·d<sup>−1</sup>) were given eplerenone (aldosterone-receptor antagonist, in chow diet, 200 mg·kg<sup>−1</sup>·d<sup>−1</sup>, ramipril (ACE inhibitor, in drinking water, 5 mg·kg<sup>−1</sup>·d<sup>−1</sup>), and losartan (angiotensin receptor 1 antagonist, in drinking water, 25 mg·kg<sup>−1</sup>·d<sup>−1</sup>). The placebo group was given saline-ethanol by osmotic minipumps. Blood, peritoneal macrophages, and aortas were obtained from the mice at the end of the administration period.

Mouse Blood Pressure Measurements

Blood pressure measurements were performed with a tail cuff (5 measurements per mouse) linked to the IITC-229 NIIB System and computer software IITC-31 (Life Science Instruments).

Serum Determinations of ACE Activity and Lipid Profile

Blood was collected from the mouse retro-orbital plexus under ether anesthesia. Serum ACE activity, cholesterol, and triglycerides were determined with commercial kits (Sigma) with a UV microplate reader (PowerWave<sup>®</sup>, Biotech).

Cells

Peritoneal Macrophages

Mouse peritoneal macrophages (MPMs) were harvested from the peritoneum of E<sup>−</sup> mice 4 days after intraperitoneal injection of 3 mL of thioglycolate (24 g/L) in saline. Cells (10<sup>5</sup>/mouse) were cultured in DMEM containing 5% FCS, 100 U penicillin/mL, 100 μg streptomycin/mL, and 2 mmol/L glutamine and used within 3 days.

THP-1

The human monocyte THP-1 cell line was maintained in RPMI 1640 medium supplemented with 2 mmol/L glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% FCS. To induce differentiation, THP-1 cells were cultured for 72 hours in the presence of 100 ng/mL phorbol myristate acetate (PMA).

Cellular Oxidative Stress Determinations

Macrophage Lipid Peroxidation Determination

MPMs were scraped into PBS for sonication at 4°C. Formation of lipid peroxides was assayed with a commercially available kit (CHOD iodide method, Diagnostica Merck).

Macrophage Release of Superoxide Ions

Cells (10<sup>5</sup>/well) were incubated in Hanks’ balanced salt solution containing acetyl cytochrome c (80 μmol/L). Superoxide cellular production was stimulated by addition of PMA (0.5 μg/mL) for 30 minutes. Superoxide dismutase (30 mg/L) was added to control samples. Superoxide release was determined in the medium and expressed as nmol superoxide/mg cell protein, E<sub>550</sub>=21 (mmol/L)/cm.

Macrophage Production of Superoxide Ions by the Lucigenin Enhanced Chemiluminescence Assay

MPMs were plated in 96-well white microplates containing 50 μL of Krebs-Henseleit/HEPES buffer, and data-adapted lucigenin (10 μmol/L; this low concentration of lucigenin is not subject to the artifactual production of superoxide ion observed with higher concentrations of lucigenin<sup>−</sup>) was added. NADPH (100 μmol/L) was added 10 minutes after addition of lucigenin. The chemiluminescence was recorded every 2 minutes in a microplate luminometer at 37°C (Lucy 1, Rosys Anthos). Results are expressed as counts per minute per 10<sup>5</sup> cells.

Determination of Cellular Lipid Peroxidation by DCFH-DA Flow Cytometric Assay

MPMs (2×10<sup>4</sup>) were incubated with 2.5×10<sup>−3</sup> mol/L dichlorofluorescein diacetate (DCFH-DA) for 30 minutes at 37°C. Cellular fluorescence was determined with a flow cytometer (FACS-SCAN, Becton Dickinson). Measurements were taken at 510 to 540 nm after excitation of cells at 488 nm with an argon ion laser.

LDL Oxidation by Macrophages

MPMs were incubated with LDL (100 μg/mL) in RPMI medium (phenol-free) with CuSO<sub>4</sub> (2 μmol/L) for 6 hours, and LDL oxidation was measured in the medium by the thiobarbituric acid-reactive substances assay. Macrophage-mediated oxidation of LDL was calculated by subtraction of LDL oxidation rate without cells from that obtained with macrophages.

Translocation of NADPH Oxidase p47 Cytosolic Component to the Plasma Membrane

Cellular membranes were prepared as previously described, and immunoblot detection of cytosolic NADPH oxidase components was performed. Membranes were analyzed by polyacrylamide gel electrophoresis. Proteins were electrophoretically transferred to nitrocellulose and then blocked with 5% (wt/vol) milk in Tris-buffered saline (pH 7.4). Blots were incubated for 1 hour at room temperature in Tris-buffered saline, 5% (wt/vol) milk containing monoclonal antibody to p47<sub>−</sub> (1:500 (BD-Biosciences Pharmingen)). Immunoblots were incubated with 1 μg/mL peroxidase-conjugated rabbit anti-mouse serum (Sigma) and developed by the enzyme-linked chemiluminescence method.

Macrophage ACE Activity Determination

MPMs were plated into 96-well plates and analyzed for their ACE activity as described for serum ACE activity. Results are expressed as mU/mg cell protein.

ACE mRNA Expression by RT-PCR Analysis

Total RNA was extracted from MPMs with TRI-reagent (Molecular Research Center Inc.). cDNA was generated from 1 μg of total RNA using reverse transcriptase (RT). RT products were subjected to polymerase chain reaction (PCR) amplification. The forward primer for ACE was 5′-TAACCTCGAGTGCGGAAGTG-3′, and the reverse primer was 5′-CCAGCAGGTGCAGTCTTT-3′. The amplification conditions were denaturation (95°C, 1 minute), annealing (10 cycles, 55°C, 45 seconds, and 30 cycles, 50°C, 45 seconds), and extension (72°C, 45 seconds). Similar conditions were used to amplify the housekeeping gene GAPDH using the forward primer 5′-TCCTGCAATGGCGCACATGGG-3′ and the reverse primer 5′-TTGTCCATGGACGTCCTGGCAG-3′. PCR products obtained for ACE and GAPDH were separated on 2% agarose gel.

Aortic Lesion Production of Superoxide Ions

Formation of superoxide ions by vascular aortic vessels was determined by the lucigenin enhanced chemiluminescence assay and the cytochrome reduction assay. The mouse thoracic aortas were rapidly removed and placed into prewarmed Krebs-Henseleit/HEPES buffer. Samples were then
cleaned of excessive fat and adventitial tissue and cut into 3-mm ring segments, and the segments were transferred to a white 96-well microplate containing 50 μL of the same buffer at 37°C. The lucigenin-enhanced luminescence assay was performed as described for the macrophage production of superoxides. Vessels were then dried and weighed. Results are expressed as counts per 10 minutes per gram dry tissue.

The cytochrome reduction assay was performed as described for macrophage release of superoxides. Results are expressed as nmol O₂ per mg dry tissue.

**Histopathology of Aortic Atherosclerotic Lesions**

The mouse heart and entire aorta were rapidly dissected out and immersion-fixed in 3% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer. The aortic arch was dissected from the surrounding fatty tissue, and the first 4 mm of the ascending aorta (beginning with the aortic valves) was removed and cut transversely with razor blades into 4 blocks of 1 mm each. The blocks were trimmed and 1 μm sections cut with a diamond-knife ultramicrotome (LKB). Histomorphometric determinations of lesion size were performed with an Olympus Cue-2 image analysis system with appropriate morphometry software (Olympus Corp).

**Statistics**

Student’s t test (2 tailed) was performed when comparing 2 arrays of data, and ANOVA was used when more than 2 groups were compared. All results are given as mean±SD.

**Results**

**Effect of Aldosterone Administration to E₀ Mice on Their Serum Aldosterone, Lipid Profile, Blood Pressure, and Atherosclerotic Lesion Area**

Administration of aldosterone (2 μg · mouse⁻¹ · d⁻¹) to E₀ mice for 2 weeks significantly increased the mouse serum aldosterone levels by 6.9-fold compared with placebo-treated mice (from 302±144 pg/mL in placebo-treated mice to 2085±309 pg/mL in aldosterone-treated mice). The mouse systolic and diastolic blood pressure increased from 101±4 mm Hg and 76±4 mm Hg, respectively, in placebo-treated mice to 110±5 mm Hg and 88±2 mm Hg, respectively (P<0.01) in aldosterone-treated mice. Administration of aldosterone to mice did not affect their serum cholesterol and triglyceride concentrations (data not shown).

Administration of aldosterone (2 μg · mouse⁻¹ · d⁻¹) to E₀ mice for 4 weeks significantly increased, by 32% (P<0.05), their atherosclerotic lesion area (from 16 002±1059 μm² in placebo-treated mice to 21 407±814 μm² in aldosterone-treated mice).

**Effect of Aldosterone Administration to E₀ Mice on Their Peritoneal Macrophage Oxidative Status**

MPMs were isolated from mice treated with aldosterone (2 μg · mouse⁻¹ · d⁻¹) for 2 weeks and analyzed for their oxidative status. Macrophage peroxide content as measured by the DCFH assay revealed an increase in macrophage peroxides by 91% in aldosterone-treated mice compared with placebo-treated mice (Figure 1A). Macrophages from aldosterone-treated mice also exhibited increased lipid peroxide content, by 162%, compared with placebo-treated mice (Figure 1B). Macrophage superoxide anion release (measured by the cytochrome c assay) in aldosterone-treated mice was significantly increased, by 155%, compared with placebo-treated mice (Figure 1C). Macrophages from aldosterone-treated mice exhibited higher superoxide anion formation, by up to 45%, as measured by lucigenin enhanced chemiluminescence assay, compared with placebo-treated mice (Figure 1D). Finally, the increased macrophage oxidative status was translated into an enhanced ability of cells from aldosterone-treated mice to oxidize LDL, by 35%, compared with placebo-treated mice (Figure 1E).

The effect of aldosterone on MPM superoxide release was dose and time dependent. Aldosterone administration for 2 weeks at dosages of 0.2, 1, and 6 μg · mouse⁻¹ · d⁻¹ led to an increase in macrophage superoxide release by 45%, 111%, 169%, and 162%, respectively, compared with placebo-treated mice (Table, top). When aldosterone was administered for increasing periods of time, ie, 1, 2, or 4 weeks, an increase in macrophage superoxide release of 100%, 169%, and 135%, respectively, was noted compared with placebo-
Dose-Dependent and Time-Dependent Effect of Aldosterone on Macrophage Superoxide Ion Release

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Time of treatment (wk) for aldosterone at 2 µg·mouse⁻¹·d⁻¹

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E0 mice were given increasing concentrations of aldosterone (0.2, 1, 2, or 6 µg·mouse⁻¹·d⁻¹) for 2 weeks or with aldosterone at 2 µg·mouse⁻¹·d⁻¹ for increasing periods of time (1, 2, and 4 weeks). Peritoneal macrophages were then analyzed for their cytochrome c-derived superoxide ions compared with placebo-treated mice. n=6, *P<0.01 vs 0.

Effect of Aldosterone on Macrophage Superoxide Ion Release

To determine whether the pro-oxidative effects of aldosterone demonstrated in vivo are not only linked to its ability to raise blood pressure, we analyzed the effect of aldosterone on oxidative stress in vitro. THP-1 monocytes that were induced to differentiate into macrophages by PMA were incubated with aldosterone (10 µmol/L) for 18 hours. After aldosterone treatment, the ability of the macrophages to release superoxides was increased by 110% (Figure 3A), and this translated into an increased macrophage-mediated LDL oxidation by 74% (Figure 3B).

Effect of Aldosterone on Macrophage Superoxide Ion Release

Administration of aldosterone to E0 mice led to an activation of the cellular NADPH oxidase as measured by several parameters. Macrophages isolated from aldosterone-treated mice exhibited higher NADPH-induced macrophage superoxide formation by up to 70%, compared with macrophages isolated from placebo-treated mice (Figure 2A).

Activation of NADPH oxidase requires the translocation of the cytosolic component p47phox to the plasma membrane. Macrophages from aldosterone-treated mice exhibited higher translocation of p47phox (measured by Western blot analysis) in the absence or presence of the stimulator PMA by 3.24-fold and 3.74-fold, respectively, compared with macrophages derived from placebo-treated mice (Figure 2B).

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Effect of Aldosterone Administration to E0 Mice on Their Aortic Lesion Superoxide Production

Aortic segments from aldosterone-treated mice (2 µg·mouse⁻¹·d⁻¹ for 2 weeks) revealed a 69% increase in superoxide production as measured by lucigenin-derived chemiluminescence compared with aortic segments from placebo-treated mice (from 23.5±2.8 counts per 10 minutes per g dry tissue in placebo-treated mice to 39.6±3.0 counts per 10 minutes per g tissue in aldosterone-treated mice). Similarly, aortic superoxide release measured by the superoxide dismutase inhibitable reduction of cytochrome c revealed a 55% increase in aortic segments from aldosterone-treated mice compared with placebo-treated mice (from 1.34±0.2 nmol/mg tissue in placebo-treated mice to 2.07±0.5 nmol/mg tissue in placebo-treated mice).

Effect of Aldosterone on Macrophage ACE Expression (mRNA and Activity)

To determine whether upregulation of ACE is involved in the pro-oxidative effects of aldosterone, we analyzed ACE mRNA expression and activity in MPMs isolated from E0 mice that were treated with aldosterone (2 µg·mouse⁻¹·d⁻¹ for 2 weeks). MPMs isolated from aldosterone-treated mice...
To determine the possible involvement of the mineralocorticoid receptor in the pro-oxidative effects of aldosterone, E0 mice were given eplerenone (200 mg · kg\(^{-1} \cdot d^{-1}\)) together with aldosterone (2 µg · mouse\(^{-1} \cdot d^{-1}\)). Administration of eplerenone to aldosterone-treated mice attenuated the aldosterone stimulation of macrophage superoxide ion release by 58% (Figure 4A). Similarly, eplerenone administration to aldosterone-treated mice lowered aldosterone stimulatory effect on macrophage peroxide content by 45% compared with placebo-treated mice, as measured by cellular DCF fluorescence (mean fluorescence values of 922±91, 1730±120, or 1361±108 were determined in macrophages from mice treated with placebo, aldosterone, or aldosterone+eplerenone, respectively). Similar results were demonstrated in aortic lesions obtained from the treated mice, because eplerenone administration to aldosterone-treated mice decreased aldosterone stimulation of aortic segments superoxide ion production by 62% (Figure 4B). To determine whether the failure of eplerenone to abolish the pro-oxidative effects of aldosterone was caused by administration of high doses of aldosterone, we next treated mice with a low aldosterone dose (0.2 µg · kg\(^{-1} \cdot d^{-1}\), which represents 1/10 of initial dosage) and with eplerenone (200 mg · kg\(^{-1} \cdot d^{-1}\)). Under such conditions, eplerenone was not able to totally block the pro-oxidative effect of aldosterone: macrophage superoxide release was 40±4, 75±7, and 59±4 nmol/mg cell protein in control, aldosterone-treated, and aldosterone+eplerenone-treated mice, respectively.

After cotreatment of the mice with aldosterone (2 µg · mouse\(^{-1} \cdot d^{-1}\))+ramipril or with aldosterone+losartan, the stimulatory effect of aldosterone on macrophage superoxide ion release was inhibited by 70% or by 75%, respectively (Figure 4A). Treatment of the mice with aldosterone+ramipril+losartan reduced the stimulatory effect of aldosterone on macrophage superoxide ion release by 77% (Figure 4A). Similarly, in aortic segments, treatment of the mice with aldosterone+ramipril or with aldosterone+losartan inhibited the stimulatory effect of aldosterone on superoxide ion production by 69% or 70%, respectively, whereas cotreatment of mice with aldosterone, ramipril, and losartan reduced the aldosterone effect on aortic superoxide ion production by 71% (Figure 4B).

Administration of eplerenone or ramipril or losartan to aldosterone-treated mice did not affect their blood pressure compared with mice treated with aldosterone only. Systolic blood pressure was 110±5, 109±2, 112±1, and 110±3 mm Hg in aldosterone-treated mice, aldosterone+eplerenone-treated mice, aldosterone+losartan-treated mice, and aldosterone+ramipril+losartan-treated mice, respectively. Diastolic blood pressure was 88±2, 86±4, 87±2, and 87±2 mm Hg in aldosterone-treated mice, aldosterone+eplerenone-treated mice, aldosterone+losartan-treated mice, and aldosterone+ramipril+losartan-treated mice, respectively.

Because blocking of the mineralocorticoid receptor, or ACE inhibition, or blocking of the AT1 receptor did not completely abolish the pro-oxidative effects of aldosterone, we analyzed the effect of a combined treatment of eplerenone with ramipril or with losartan in the aldosterone-treated mice. Administration of aldosterone to E0 mice together with eplerenone+ramipril or eplerenone+losartan to mice treated with aldosterone completely blocked the aldosterone enhancing effect on macrophage superoxide ion release to levels obtained in the placebo-treated mice (Figure 4A). Similar results were obtained when studying the mouse aortic segments (Figure 4B).

**Discussion**

In the present study, we have presented strong evidence for the pro-oxidative properties of aldosterone in macrophages
and in aortic segments in atherosclerotic E0 mice. This effect is mediated in part by the mineralocorticoid receptor and by the angiotensin receptor-1 (AT1). Furthermore, we demonstrated that aldosterone upregulated macrophage ACE expression and activity. Only combined treatment by eplerenone with ramipril or losartan to the mice totally reversed the aldosterone pro-oxidative effects in the mouse macrophages and aorta.

Aldosterone, which plays an important role in the pathophysiology of heart failure, has been attributed with proinflammatory and pro-oxidative properties. In the present study, we have shown a specific pro-oxidative effect of aldosterone on macrophages and on vascular cells present in aortic segments. This effect was documented by analyses of various oxidation parameters, including the DCFH assay, lipid peroxides content, and the ability of the cells to release superoxides, thus indicating that NADPH oxidase activation is involved in the pro-oxidative effects of aldosterone. The ability of aldosterone to induce oxidative stress in arterial cells in general and in macrophages in particular could be linked to the involvement of aldosterone in cardiovascular diseases. “Oxidized macrophages” exhibit increased proatherogenic properties, such as their ability to induce LDL oxidation, Ox-LDL uptake, and macrophage foam cell formation. Indeed, macrophages isolated from aldosterone-treated mice had increased ability to oxidize LDL compared with placebo-treated mice. Increased atherosclerosis lesion size observed in aldosterone-treated mice could have resulted in part from the increased macrophage oxidative stress. In addition, the enhancing effect of aldosterone on the mouse blood pressure could also independently contribute to its proatherosclerotic effects.

Aldosterone’s pro-oxidative effects on macrophages could be related to activation of NADPH oxidase. We have shown that treatment of mice with aldosterone led to the translocation of the cytosolic p47phox to the plasma membrane. This effect could be seen even in the absence of PMA, the potent activator of NADPH oxidase, suggesting that aldosterone treatment induced NADPH oxidase activation. These results were reinforced by measurement of macrophage superoxide production by use of the lucigenin chemiluminescence assay, showing that after addition of NADPH to aldosterone-treated mice.
macrophages, a greater aldosterone stimulatory effect over placebo-treated mice was obtained.

Increased blood pressure in aldosterone-treated mice could be at least partially involved in the pro-oxidative effect and proatherogenic effects of aldosterone shown in the present study. However, aldosterone’s ability to directly increase oxidative stress in vitro suggests that aldosterone’s pro-oxidative effects are not only linked to its ability to raise blood pressure. Moreover, it was previously shown that increased blood pressure does not necessarily lead to increased oxidative stress, as illustrated by the lack of effect of norepinephrine-induced hypertension on oxidative stress in rats.34

Involvement of the mineralocorticoid receptor in the pro-oxidative effect of aldosterone was shown both in macrophages and in aortic segments. The mineralocorticoid receptor is specific to aldosterone and has been shown to mediate most of its effects.8 Recently, results of the EPHESUS trial have shown that administration of eplerenone to heart failure patients treated with ACE inhibitors and/or angiotensin receptor blockers reduces morbidity and mortality.39 However, combined treatment with aldosterone and eplerenone only partially reduced the pro-oxidative properties of aldosterone both in macrophages and in aortic segments, even at low dosages of aldosterone. These results suggest that alternative pathways, independent of the mineralocorticoid receptor, are involved in the pro-oxidative effects of aldosterone.

We have thus extended the search for additional mechanisms related to the enhancing effect of aldosterone on oxidative stress. Although aldosterone is located downstream of angiotensin II in the renin-angiotensin-aldosterone system, several lines of evidence suggest interrelationships between angiotensin II and aldosterone cascades,10,11 specifically by upregulating ACE mRNA expression.12–14 Moreover, administration of ACE inhibitors, which substantially reduce angiotensin II plasma levels, only partially decrease plasma aldosterone concentrations, a phenomenon referred as “aldosterone escape.”15 In the present study, we have clearly shown that aldosterone upregulated macrophage ACE mRNA expression and activity in macrophages, whereas circulating ACE activity was not affected. The selective effect of aldosterone on tissue ACE, versus circulating ACE, further demonstrates differences in the regulation pathways of tissue and circulating ACE. The physiological importance of ACE expression upregulation by aldosterone is questionable, because aldosterone infusion completely abolishes plasma renin activity.53 However, the upregulating effect of aldosterone on ACE activity and expression could still be of importance, because in addition to the ability of ACE to hydrolyze angiotensin I to angiotensin II, it is also involved in several additional systems, such as bradykinin degradation and modulation of nitric oxide production. The ability of aldosterone to upregulate macrophage ACE is in line with the ability of the ACE inhibitor ramipril to antagonize the pro-oxidative effects of aldosterone, as shown in macrophages and in intact aortic segments. However, we have shown that although ACE inhibitors and AT1 antagonists reduce the pro-oxidative effects of aldosterone, this reduction was only partial. These results were recently reinforced by a study showing that cotreatment with eplerenone and enalapril was more effective in reducing left ventricular mass and systolic blood pressure that eplerenone alone.34

In the present study, a total blockade of the pro-oxidative effects of aldosterone on macrophages and aortic segments was obtained only when the mice were given a combined treatment with a mineralocorticoid receptor antagonist, ACE inhibitor, or AT1 antagonist. In addition to the importance of these findings for understanding the mechanisms of action of the pro-oxidative characteristics of aldosterone, these findings could have important clinical implications regarding the treatment of cardiac patients. A combined therapy of mineralocorticoid receptor antagonists together with ACE inhibitors and AT1 antagonists could result in optimal treatment of such patients.

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