Angiotensin II Induces Interleukin-6 in Humans Through a Mineralocorticoid Receptor–Dependent Mechanism

James M. Luther, James V. Gainer, Laine J. Murphey, Chang Yu, Douglas E. Vaughan, Jason D. Morrow, Nancy J. Brown

Abstract—This study tested the hypothesis that angiotensin promotes oxidative stress and inflammation in humans via aldosterone and the mineralocorticoid receptor. We measured the effect of intravenous aldosterone (0.7 μg/kg per hour for 10 hours followed by 0.9 μg/kg per hour for 4 hours) and vehicle in a randomized, double-blind crossover study in 11 sodium-restricted normotensive subjects. Aldosterone increased interleukin (IL)-6 (from 4.7±4.9 to 9.4±7.1 pg/mL; F=4.94; P=0.04) but did not affect blood pressure, serum potassium, or high-sensitivity C-reactive protein. We next conducted a randomized, double-blind, placebo-controlled, crossover study to measure the effect of 3-hour infusion of angiotensin II (2 ng/kg per minute) and norepinephrine (30 ng/kg per minute) on separate days after 2 weeks of placebo or spironolactone (50 mg per day) in 14 salt-replete normotensive subjects. Angiotensin II increased blood pressure (increase in systolic pressure: 13.7±7.5 and 15.2±9.4 mm Hg during placebo and spironolactone, respectively; P<0.001 for angiotensin II) and decreased renal plasma flow (~202±73 and ~167±112 mL/min/L.73 kg/m²; P<0.001 for angiotensin II effect) similarly during placebo and spironolactone. Spironolactone enhanced the aldosterone response to angiotensin II (increase of 17.0±10.6 versus 9.0±5.7 ng/dL; P=0.002). Angiotensin II transiently increased free plasma F2-isoprostanes similarly during placebo and spironolactone. Angiotensin II increased serum IL-6 concentrations during placebo (from 1.8±1.1 to 2.4±1.4 pg/mL; F=4.5; P=0.04) but spironolactone prevented this effect (F=6.4; P=0.03 for spironolactone effect). Norepinephrine increased blood pressure and F2-isoprostanes but not aldosterone or IL-6. Aldosterone increases IL-6 in humans. These data suggest that angiotensin II induces IL-6 through a mineralocorticoid receptor–dependent mechanism in humans. In contrast, angiotensin II–induced oxidative stress, as measured by F2-isoprostanes, is mineralocorticoid receptor independent and may be pressor dependent. (Hypertension. 2006;48:1050-1057.)

Key Words: angiotensin II • aldosterone • spironolactone • mineralocorticoid receptor • inflammation • stress • oxidative • IL-6 • F2-isoprostanes

Activation of the renin–angiotensin–aldosterone system (RAAS) is associated with increased risk of atherothrombotic events,1,2 whereas interruption of the RAAS decreases the risk of myocardial infarction and stroke.3–6 Angiotensin (Ang) II induces oxidative stress, activates nuclear factor κB (NF-κB), and induces the expression of inflammatory cytokines and markers such as interleukin (IL)-6 and high-sensitivity C-reactive protein (hsCRP [CRP]).7,8 Inflammation plays a critical role in the pathogenesis of atherosclerosis.9 In epidemiological studies, elevated serum IL-6 and hsCRP predict risk of cardiovascular events and death.10–13 Clinical studies confirm that Ang II induces oxidative stress and inflammation in humans. For example, acute infusion of Ang II increases serum F2-isoprostanes, the product of free radical peroxidation of arachidonic acid and a marker of oxidative stress, in hypertensive individuals.14 Urinary F2-isoprostane concentrations are increased in patients with renovascular hypertension and decrease after revascularization, suggesting that endogenous Ang II also induces oxidative stress.15 The effect of Ang type 1 (AT1) receptor antagonism and angiotensin-converting enzyme (ACE) inhibition on F2-isoprostanes has not been extensively defined in humans; however, AT1 receptor antagonism reduces serum concentrations of the inflammatory markers IL-6 and hsCRP.16–19

Aldosterone also induces oxidative stress and inflammation in animal models.20,21 Studies in vitro and in vivo in animals suggest that Ang II induces oxidative stress and inflammation in part through aldosterone and the mineralocorticoid receptor (MR). Blockade of aldosterone action with either spironolactone or eplerenone inhibits Ang II-induced gene expression in vascular smooth muscle cells and decreases oxidative stress, activation of NF-κB, and inflammation in animals.20–26 This study tests the hypothesis that Ang
II induces oxidative stress and inflammation in humans through an MR-dependent mechanism.

Methods
All of the studies were approved by the Vanderbilt University Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained, and subjects underwent a screening history and physical before study.

Study A: Effect of Exogenous Aldosterone on Inflammation
Twelve healthy subjects participated in the randomized, double-blind, crossover study illustrated in Figure 1A. Healthy subjects were studied to minimize the risk of prolonged aldosterone infusion and to avoid confounding medical conditions. Participants without evidence of hypertension or cardiac, pulmonary, renal, or hepatic disease by screening physical and laboratory examination were eligible for the study. Subjects were studied during sodium restriction to minimize the effect of prolonged aldosterone infusion on serum potassium. Subjects were provided a diet containing 10 mmol per day of sodium, 100 mmol per day of potassium, and 2500 mL fluid for 8 days. On the night of day 5 of the diet, subjects were randomly assigned to receive a 14-hour intravenous infusion of aldosterone or vehicle and then crossover to the opposite treatment on the seventh night of the diet after 1 day of washout. The order of aldosterone or vehicle infusion was randomized by the Vanderbilt Investigational Drug Pharmacy. Aldosterone (Clinalfa) was infused in a total volume of 500 mL of 5% dextrose in water starting at 10:00 pm at a rate of 0.7 μg/kg per hour for 10 hours followed by 0.9 μg/kg/hr for 4 hours. The dose of aldosterone was chosen based on previously published pharmacokinetics to yield aldosterone concentrations of 100 ng/dL. Blood pressure and heart rate were measured every 2 hours. Blood was drawn before infusion and at 5 and 12 hours for measurement of aldosterone concentration, potassium, cytokines, and hsCRP.

Study B: Role of Endogenous Aldosterone and the MR in Ang II–Induced Inflammation and Oxidative Stress
Subjects were eligible if they were 18 to 65 years of age, had a body mass index (BMI) ≤30 kg/m², had 3 documented systolic blood pressures ≤135 mm Hg, and had 3 seated diastolic blood pressures ≤85 mm Hg. Subjects with significant medical conditions were excluded from the study.
After screening, subjects were randomly assigned in a double-blind fashion to treatment with either 50 mg of spironolactone daily or a matching placebo daily for 14 days (Figure 1B) and then crossed over to the opposite treatment arm after a 2-week washout period. Spironolactone (Professional Compounding Centers of America) and identical-appearing placebos were prepared by the Vanderbilt Investigational Drug Pharmacy. On day 8, subjects were provided a diet containing 200 mmol per day of sodium, 100 mmol per day of potassium, and 1000 mg per day of calcium for a total of 7 days, while continuing the study drug. Subjects were studied during salt-replete conditions to suppress the endogenous RAAS and minimize the blood pressure effects of spironolactone. On days 11 and 13 of study drug, subjects collected a 24-hour urine sample for determination of urinary creatinine, sodium, and potassium excretion. Subjects reported to the Vanderbilt Clinical Research Center on days 12 and 14 at 7:00 AM after an overnight fast (see Figure 1B). An intravenous catheter was placed in each arm for blood sampling.

On each study day (Figure 1B, inset), a loading dose of parapaminohippurate ([PAH] 8 mg/kg; Merck) was given intravenously, followed by a continuous infusion of 12 mg/min to determine renal plasma flow, as described previously. After 1 hour, subjects were randomly assigned to either Ang II (2 ng/kg/min; Clinalfa) or norepinephrine ([NE] 30 ng/kg per minute; Bedford Laboratories) infusion for 3 hours. After a 2-day washout period, subjects returned on day 14 for the remaining infusion. The doses of Ang II and NE were chosen to produce comparable increases in blood pressure. Blood pressure was measured at 2-minute intervals using an automated oscillometric blood pressure cuff (DINAMAP, GE Healthcare). Five measurements preceding each 30-minute time point were averaged. All of the subjects remained supine during the study. Venous blood was drawn immediately before drug infusion and after 1 and 3 hours on all of the study days for determination of plasma renin activity (PRA), PAH, Ang II, aldosterone, cytokines, oxidized low-density lipoprotein (oxLDL), hsCRP, and F2-isoprostanes.

After completion of period 1 (Figure 1B) and a 2-week washout period, subjects crossed over to the remaining study drug (spironolactone or placebo) and repeated the 2 study days (Figure 1B, period 2). Study drug and infusion order (Ang II or NE) were randomized by the Vanderbilt Investigational Drug Pharmacy. The infusion order was constant for each subject in periods 1 and 2.

**Laboratory Analysis**

All of the blood samples were centrifuged for 20 minutes immediately after blood drawing, and plasma was stored at −80°C until sampling. PAH concentrations were determined by spectrophotometry. PRA was determined by radioimmunoassay (DiaSorin). ACE activity was determined by kinetic analysis (Olympus AU400/AU600, Alpeco Diagnostics). Venous blood was collected in a mixture of protease inhibitors, and Ang II concentrations were determined by radioimmunoassay (Nichols Institute Diagnostics). Aldosterone was determined using a radioimmunoassay using 125I-aldosterone (MP Biomedicals), a primary antibody to aldosterone (National Institute of Diabetes and Digestive and Kidney Diseases National Hormone and Peptide Program, Torrance, CA), and a secondary anti-rabbit γ-globulin antibody (Linco Research, St Charles, MO). Plasma epinephrine and NE concentrations were determined using electrochemical high-pressure liquid chromatography using 3, 4 dihydroxybenzylamine as the internal standard (ESA Inc). Free plasma and urine F2-isoprostanes were determined by negative-ion gas chromatography mass spectrometry, and urinary values were corrected by creatinine concentration. OxLDL was determined using a commercially available ELISA using the specific murine monoclonal antibody, mAb-466 (Mordacia, Uppsala, Sweden).

Human inflammatory cytokines IL-6, IL-1β, IL-10, IL-12p70, IL-8, and tumor necrosis factor (TNF-α) were measured using the cytokmetric bead array Human Inflammation Cytokine kit (BD Biosciences Pharmingen). Prespecified cytokines of interest included on this panel were IL-6 and TNF-α. IL-6 levels below the detectable limit were confirmed by a commercially available ELISA (Amer- sham Bioscience). hsCRP was measured using a commercially available hsCRP ELISA kit (Kalan Biological Ltd).

Urinary sodium and potassium were determined using ion-selective direct potentiometry using monensin for sodium and valinomycin for potassium (VITROS 250 Chemical Analyzer, Ortho-Clinical Diagnostics). Urinary creatinine was determined by a colorimetric assay (VITROS 250 Chemical Analyzer, Ortho-Clinical Diagnostics).

**Statistical Analysis**

Data are presented as mean±SD in text and mean±SEM in the figures unless otherwise specified. Comparisons of baseline values during placebo and spironolactone were made using the Wilcoxon signed-rank test or Wilcoxon rank-sum test, as appropriate. The effect of aldosterone (study A), Ang II, and NE (study B) on blood pressure and biochemical parameters was determined using repeated-measures ANOVA in which the within-subject variables were time and treatment (spironolactone versus placebo), and the between-subjects variables were infuson order and gender. All of the statistical analyses were performed using the statistical package SPSS for Windows (version 14.0, SPSS). A 2-tailed P<0.05 was considered significant.

**Results**

**Inflammatory Response to Exogenous Aldosterone**

Eleven subjects completed the study protocol (4 women, 4 blacks, and 7 whites). An additional subject was discontinued because she had mild hypokalemia (3.5 mmol/L) during aldosterone infusion. The mean age was 35±9 years, BMI was 27.5±5.0 kg/m², systolic blood pressure (SBP) was 122±12 mm Hg, diastolic blood pressure (DBP) was 78±10 mm Hg, and total cholesterol was 191±38 mg/dL. Plasma aldosterone concentrations increased from 26±25 to 108±46 ng/dL during aldosterone infusion (P=0.005) and returned to baseline within 2 hours after cessation. In contrast, aldosterone concentrations tended to decrease during vehicle infusion (27±9 versus 21±1.4; P=0.07; n=4 for this
measurement only). Blood pressure, heart rate, and serum potassium were unchanged during either aldosterone or vehicle in these sodium-restricted subjects. Urinary potassium excretion was similar (72.4 ± 26.6 and 66.6 ± 18.4 mmol/24 hours during vehicle and aldosterone, respectively; P = 0.77), whereas urinary sodium excretion tended to decrease during aldosterone infusion (9.9 ± 8.5 versus 16.2 ± 12.8 mmol/24 hours during vehicle; P = 0.09).

Plasma IL-6 concentrations increased from 4.7 ± 4.9 to 9.4 ± 7.1 pg/mL during aldosterone infusion (Figure 2; F = 4.95; P = 0.04) but not during vehicle (4.4 ± 2.3 to 5.6 ± 3.7 pg/mL; P = 0.13; P = 0.02 for infusion × time interaction). However, there was no effect of aldosterone on IL-1β (P = 0.56), IL-8 (P = 0.59), TNF-α (P = 0.84), or the anti-inflammatory cytokine IL-10 (P = 0.56). There was a significant effect of aldosterone on IL-12p70 concentrations (Figure 2; P = 0.02 for infusion × time interaction). There was no effect of aldosterone on hsCRP (P = 0.66).

**Vasoconstrictor and Endocrine Responses to Ang II and Norepinephrine in the Presence and Absence of Spironolactone**

Fourteen subjects completed the study protocol (8 women, 5 blacks and 9 whites). The mean age was 33.4 ± 10.9 years, BMI was 24.5 ± 4.4 kg/m², SBP was 118.1 ± 9.0 mm Hg, DBP was 69.4 ± 9.4 mm Hg, and total cholesterol was 175.6 ± 42.7 mg/dL. An additional 6 subjects enrolled but did not complete the entire protocol for the following reasons: urticaria during spironolactone (1), chest discomfort during NE infusion without evidence of ischemia by ECG or cardiac enzymes (1), nephrolithiasis (1), subject noncompliance (1), and scheduling conflict (2).

Ang II increased SBP and DBP (Figure 3; increase in SBP 13.7 ± 7.5 and 15.2 ± 9.4 mm Hg during placebo and spironolactone, respectively; P = 0.001 for both). NE significantly and similarly increased SBP and DBP (increase in SBP: 10.6 ± 8.5 and 11.8 ± 8.8 mm Hg during placebo and spironolactone, respectively; P < 0.001 for each). The pressor responses to NE and Ang II were statistically similar (P = 0.29).

Spironolactone did not alter the pressor response to either Ang II (P = 0.27) or NE (P = 0.60). Ang II (–202 ± 73 mL/min/1.73 kg²; P < 0.001 for Ang II effect) and NE (–243 ± 136 mL/min/1.73 kg²; P < 0.001 for NE effect) significantly and similarly decreased renal plasma flow. Spironolactone did not alter the renal vasoconstrictor response (P = 0.28 for Ang II; P = 0.76 for NE). Spironolactone significantly increased baseline PRA and aldosterone (Table) but did not alter baseline potassium, ACE activity, or Ang II concentrations (43 ± 16 versus 45 ± 20 pg/mL, placebo versus spironolactone; P = 0.63). PRA decreased significantly during Ang II infusion with both placebo (change of –0.25 ± 0.37 ng/mL per hour; F = 4.51; P = 0.03) and spironolactone (–0.51 ± 0.59 ng/mL per hour; F = 8.51; P = 0.008 for Ang II effect). NE infusion did not affect PRA (change: 0.12 ± 0.24 ng/mL per hour, P = 0.21 for NE effect during placebo; 0.31 ± 0.86 ng/mL per hour, P = 0.29 for NE effect during spironolactone). Order of drug administration (placebo or spironolactone) did not affect serum potassium, PRA, or aldosterone on the days of study.

Peak Ang concentrations of 78 ± 21 pg/mL (n = 8 for this measurement only; P = 0.01 for peak versus baseline) were achieved during Ang II infusion. Peak concentrations of norepinephrine were 850 ± 240 pg/mL during NE infusion (P = 0.001 for peak versus baseline). Spironolactone did not affect Ang II, epinephrine, or NE concentrations at baseline or during infusion. Ang II increased plasma aldosterone concentration (Figure 4; increase of 9.0 ± 5.7 ng/dL), and spironolactone significantly enhanced this response (increase of 17.0 ± 10.6 ng/dL). NE did not change aldosterone concentrations compared with baseline (increase of 1.2 ± 2.5 ng/dL, P = 0.37 during placebo; 0.9 ± 2.5 ng/dL, P = 0.09 during spironolactone).

**Effect of Spironolactone on Baseline Characteristics (Study B)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (n = 14)</th>
<th>Spironolactone (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>108 ± 7</td>
<td>108 ± 9</td>
<td>0.94</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>61 ± 7</td>
<td>61 ± 8</td>
<td>0.66</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>60 ± 10</td>
<td>61 ± 10</td>
<td>0.41</td>
</tr>
<tr>
<td>PRA, ng/mL per hour</td>
<td>0.44 ± 0.5</td>
<td>0.72 ± 0.6</td>
<td>0.048</td>
</tr>
<tr>
<td>ACE, III/L</td>
<td>28.0 ± 9.5</td>
<td>29.1 ± 9.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Ang II, pg/mL (n = 8)</td>
<td>43.2 ± 16</td>
<td>45.2 ± 20</td>
<td>0.61</td>
</tr>
<tr>
<td>Aldosterone, ng/dL</td>
<td>8.0 ± 3.5</td>
<td>10.8 ± 4.6</td>
<td>0.006</td>
</tr>
<tr>
<td>NE, pg/mL</td>
<td>257 ± 121</td>
<td>228 ± 117</td>
<td>0.55</td>
</tr>
<tr>
<td>Epinephrine, pg/mL</td>
<td>28 ± 19</td>
<td>25 ± 22</td>
<td>0.27</td>
</tr>
<tr>
<td>Serum potassium, mmol/dL</td>
<td>4.2 ± 0.23</td>
<td>4.3 ± 0.40</td>
<td>0.43</td>
</tr>
<tr>
<td>Urine sodium excretion, mmol/24 h</td>
<td>143 ± 42</td>
<td>143 ± 42</td>
<td>0.78</td>
</tr>
<tr>
<td>Urine potassium excretion, mmol/24 h</td>
<td>51 ± 14</td>
<td>50 ± 16</td>
<td>0.64</td>
</tr>
<tr>
<td>Urine sodium/potassium ratio</td>
<td>2.9 ± 0.7</td>
<td>3.0 ± 0.8</td>
<td>0.73</td>
</tr>
</tbody>
</table>
Inflammatory and Oxidative Stress Responses to Ang II and NE in the Presence and Absence of Spironolactone

Baseline IL-6 concentrations were similar during spironolactone and placebo treatment (P = 0.87) but significantly lower (1.9 ± 0.9 versus 5.6 ± 3.7 pg/mL; P < 0.001) in these salt-replete subjects compared with in the salt-deplete subjects in study A. Aldosterone concentrations were also significantly lower in salt-replete subjects (8.0 ± 3.5 versus 26.0 ± 25 ng/dL; P = 0.009). Ang II significantly increased IL-6 from 1.8 ± 1.1 to 2.4 ± 1.4 pg/mL during placebo (Figure 5; F = 4.5; P = 0.04) but not during spironolactone (from 1.8 ± 1.0 to 1.7 ± 0.8 pg/mL; P = 0.22). Spironolactone significantly altered the IL-6 response to Ang II (F = 6.4; P = 0.03 for effect of spironolactone). After controlling for treatment, there was a highly significant relationship between serum aldosterone concentration and IL-6 concentration in studies A and B (IL-6 [pg/mL] = 0.07 × aldosterone [ng/dL] + 1.24; R² = 0.43; P < 0.001). There was no effect of Ang II on IL-1β (P = 0.34), IL-8 (P = 0.92), IL-12p70 (P = 0.94), TNF-α (P = 0.27), or IL-10 (P = 0.40). NE did not affect IL-6 concentrations (P = 0.62 for NE; P = 0.45 for spironolactone; P = 0.94 for effect of spironolactone) or the concentrations of any other cytokine (all P > 0.17). Ang II (P = 0.23), NE (P = 0.61), and spironolactone (P = 0.90) did not alter hsCRP concentrations. In 9 subjects who were randomly assigned to Ang II and then NE, there was no change in CRP at 48 hours after Ang II. Both Ang II (P = 0.002) and NE (P = 0.04) significantly increased free plasma F₂-isoprostanes at 1 hour (Figure 6), but F₂-isoprostanes returned to baseline by 3 hours. Spironolactone did not significantly alter the F₂-isoprostane response to either Ang II or NE.

There was no effect of spironolactone (P = 0.45), Ang II (P = 0.45), or NE (P = 0.79) on urinary F₂-isoprostane concentrations. Likewise, there was no effect of spironolactone (46.8 ± 17 versus 47.4 ± 17 U/L; P = 0.88), Ang II (P = 0.42), or NE (P = 0.50) on oxLDL concentrations.

Discussion

Studies demonstrating that AT₁ receptor antagonism reduces biomarkers of inflammation indicate that endogenous Ang II causes inflammation in humans. This study provides evidence in vivo in humans that exogenous aldosterone increases circulating IL-6 concentrations and that MR antagonism attenuates Ang II-induced increases in IL-6, suggesting that endogenous aldosterone contributes to the proinflammatory effects of Ang II in humans.

Aldosterone can act through classical genomic mechanisms involving binding to the MR, translocation of the agonist-receptor complex to the nucleus, and induction of transcription or through rapid nongenomic effects, which are not blocked by inhibitors of transcription. In animal models, aldosterone induces inflammation through a genomic mechanism involving increased expression and activation of NF-κB. Two lines of evidence suggest that aldosterone induces inflammation through a genomic mechanism in humans as well. First, the time course of induction of IL-6 concentrations by aldosterone was compatible with an effect
on expression. Moreover, spironolactone prevented the proinflammatory effect of Ang II, although MR antagonism can block some nongenomic effects of aldosterone.34

Both aldosterone and Ang II increased IL-6 concentrations without inducing concentrations of IL-1\(\beta\), IL-8, IL-10, or TNF-\(\alpha\). This pattern is consistent with the pattern of increased IL-6 concentrations without a concomitant rise in IL-1 or TNF-\(\alpha\) after vaccination with Salmonella typhi in normal volunteers.38 Interestingly, there was an interactive effect of aldosterone and time on IL-12p70 concentrations. IL-12 promotes T-cell recruitment in atherosclerotic plaque,39 and ACE inhibition decreases production of IL-12 by human peripheral mononuclear cells.40

IL-6 serves as a major stimulus to hepatic CRP expression, and costimulation by IL-1 is necessary for maximal response.41 Given that IL-1 and IL-6 exert synergistic effects on CRP expression, the lack of effect of aldosterone and Ang II on IL-1\(\beta\) could account for the lack of effect of Ang II on CRP, despite the increase in IL-6. Alternatively, aldosterone and Ang II may not have increased CRP because of the short duration of the infusions and the short half-life of IL-6, as elevations in IL-6 typically precede elevations in hsCRP by 48 hours after inflammatory stimuli, such as infection,42 surgery,43 and cardiopulmonary bypass.44

Aldosterone increased IL-6, and spironolactone decreased the IL-6 response to Ang II through a pressure-independent mechanism. Likewise, increasing blood pressure alone was insufficient to stimulate IL-6 concentrations during infusion of NE. In addition to increasing expression and activation of NF-kB, aldosterone can enhance the response to Ang II by increasing AT\(_1\) receptor expression and sensitivity.25,45–48 However, the lack of spironolactone effect on Ang II-induced, AT\(_1\) receptor-mediated vasoconstriction does not support an effect of endogenous aldosterone on AT\(_1\) receptor expression or sensitivity. To the contrary, MR antagonism enhanced Ang II-stimulated aldosterone secretion. The mechanism of the increased aldosterone response to Ang II during spironolactone is not known. Potassium and vascular AT\(_1\) receptor-mediated responses were similar in the presence and absence of spironolactone. However, other groups have reported increased basal concentrations of PRA and aldosterone during MR antagonism in humans.49 Although spironolactone did not detectably alter plasma Ang II concentrations, elevated PRA could have increased tissue Ang II and stimulated aldosterone synthase activity or expression. Silvestre et al50 reported increased cardiac expression of aldosterone synthase in rats treated with an MR antagonist, and Ang II is a known regulator of aldosterone synthase gene expression.

Studies in rat models of hypertension suggest that Ang II induces inflammation via the MR-dependent formation of reactive oxygen species.21 However, in contrast to the effect of Ang II on IL-6, Ang II induced a transient increase in plasma F\(_2\)-isoprostanes in normal subjects, which was both MR-independent and nonspecific, as NE similarly increased F\(_2\)-isoprostanes. Although Ang II and NE did not increase oXLDL, F\(_2\)-isoprostanes seem to be more sensitive than other biomarkers of oxidative stress.51 The transient elevation of F\(_2\)-isoprostanes after Ang II has been described in cell culture and ex vivo and may suggest the induction of compensatory antioxidant mechanisms, such as increased expression of extracellular superoxide dismutase.52,53 That Ang II and NE both increased concentrations of F\(_2\)-isoprostanes concurs with the observations of Aizawa et al,54 who reported that both Ang II and NE increased free plasma F\(_2\)-isoprostanes in rats, but conflicts with data from other studies.55 Nevertheless, the lack of effect of NE on PRA or aldosterone suggests that NE increased F\(_2\)-isoprostanes independently of activation of the RAAS.

A few study limitations warrant mention. We studied healthy subjects in whom IL-6 concentrations were only
modestly increased by aldosterone or Ang II. On the other hand, the magnitude of increase in IL-6 was similar to that observed after 
S typhii vaccine, which has been shown to induce endothelial dysfunction in normal subjects. In addition, we did not determine the effect of increased endogenous aldosterone on inflammatory cytokines in the absence of exogenous Ang II. Thus, although baseline aldosterone and IL-6 concentrations were increased in the subjects studied during low salt intake compared with those studied during high salt intake, we did not measure the effect of MR antagonism on circulating IL-6 concentrations during sodium restriction. However, we have reported previously that spironolactone decreases circulating concentrations of the inflammatory biomarker plasminogen activator inhibitor 1 in hypertensive individuals treated with a thiazide diuretic, suggesting that decreasing endogenous aldosterone may in fact decrease inflammation.

**Perspectives**

Clinical studies implicate inflammation in the progression of cardiovascular disease, and Ang receptor blockade or converting enzyme inhibition reduce markers of inflammation. This study provides evidence that exogenous and endogenous aldosterone increase the inflammatory cytokine IL-6 in humans. Inflammation plays a critical role in atherosclerosis, and MR antagonists may have a role in attenuating inflammation in humans.

**Sources of Funding**

This work was supported by National Institutes of Health grants RR000095, HL077389, GM 007569, DK048831, CA077839, ES013125, GM015431, and HL067308.

**Disclosures**

J.D.M. is a consultant for Unilever Ltd, United Kingdom. None of the remaining authors report any conflicts.

**References**


44. Bruins P, te Velthuis H, Yazdanbakhsh AP, Jansen PG, van Harevelt FW, de Beaumont EM, Wildevar CU, Eijjsen L, Trouwborst A, Hack CE. Activation of the complement system during and after cardiopulmo-
Angiotensin II Induces Interleukin-6 in Humans Through a Mineralocorticoid Receptor–Dependent Mechanism

James M. Luther, James V. Gainer, Laine J. Murphey, Chang Yu, Douglas E. Vaughan, Jason D. Morrow and Nancy J. Brown

Hypertension. 2006;48:1050-1057; originally published online October 16, 2006;
doi: 10.1161/01.HYP.0000248135.97380.76

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/48/6/1050

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/