NO Synthase Inhibition Increases Aldosterone in Humans


Abstract—To test the hypothesis that NO influences aldosterone production in humans, we examined the effect of N^G-nitro-L-arginine methyl ester (L-NAME) on aldosterone concentrations in the presence and absence of the NO precursor L-arginine (3 g TID) and the angiotensin-converting enzyme inhibitor ramipril (10 mg QD). Ten normal subjects were given L-NAME (66 μg/kg per min for 30 minutes) or vehicle in random order on separate days during placebo and after randomized, double-blind treatment with L-arginine, ramipril, or L-arginine plus ramipril. Infusion of L-NAME significantly increased systolic blood pressure (all P<0.05) and decreased heart rate (all P≤0.02) during all 4 treatment arms. After placebo pretreatment, serum aldosterone was significantly higher during L-NAME infusion than during vehicle (6.6±1.7 versus 3.3±0.5 ng/dL; P=0.045). Combined treatment with L-arginine plus ramipril abolished this effect. There was no effect of L-NAME on plasma renin activity (PRA; P=0.297) or angiotensin II concentrations (P=0.537). However, there was a significant interactive effect of L-NAME and time on serum potassium (P=0.039). There was a significant linear relationship between PRA and aldosterone concentration after vehicle infusion ([aldosterone]=3.9×PRA+1.9; r^2=0.476; P=0.027) and L-NAME infusion ([aldosterone]=7.2×PRA+3.1; r^2=0.457; P=0.032), and the intercepts of these lines were different (P=0.029). There was a significant linear relationship between serum potassium and aldosterone during L-NAME ([aldosterone]=8.2×[potassium]−28.9; r^2=0.609; P=0.008) but not during vehicle (P=0.313). These data suggest that endogenous NO modulates aldosterone synthesis in humans. (Hypertension. 2004;44:739-745.)

Key Words: aldosterone ▪ nitric oxide ▪ angiotensin-converting enzyme ▪ arginine

Endothelial dysfunction, characterized by decreased endothelial NO synthase (NOS) activity, predicts cardiovascular events in patients at risk for coronary artery disease. Studies in animals and humans indicate that the hormone aldosterone impairs NOS activity. For example, in rat models, exogenous aldosterone increases oxidative stress and diminishes endothelium-dependent relaxation to acetylcholine. Increased plasma aldosterone concentrations are associated with decreased arterial compliance in hypertensive individuals. Patients with primary hyperaldosteronism exhibit a greater degree of endothelial dysfunction than do patients with essential hypertension. In patients with congestive heart failure, aldosterone receptor antagonism, but not treatment with amiloride, improves endothelium-dependent vasodilation.

Although studies evidence an effect of aldosterone on endothelial NOS (eNOS) activity in humans, the converse possibility that NOS activity influences aldosterone synthesis has not been studied extensively in humans. In vitro, NO inhibits angiotensin II (Ang II) and adrenocorticotropic hormone (corticotropin [ACTH])-stimulated aldosterone secretion from cultured adrenal glomerulosa cells through a cyclic GMP-independent mechanism. Aldosterone receptor antagonism reverses cardiovascular injury in rats treated with the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME). and some investigators have reported that circulating aldosterone concentrations are increased in this model. In addition, short-term local inhibition of NOS by adrenal arterial infusion of L-NAME increases basal aldosterone secretion in conscious sheep.

The present study tests the hypothesis that NO influences aldosterone production in humans by examining the effect of the potent NOS inhibitor L-NAME on circulating aldosterone concentrations. Because administration of the NOS precursor L-arginine can enhance eNOS function in some populations, we studied the effect of L-NAME in the presence and absence of L-arginine. Because inhibition of NOS increases the activity of the renin-angiotensin–aldosterone system (RAAS), whereas angiotensin-converting enzyme (ACE) inhibition increases eNOS, we also determined the effect of L-NAME on circulating aldosterone concentrations in the presence and absence of the ACE inhibitor ramipril.

Methods

The study protocol (Figure 1) was approved by the Vanderbilt institutional review board, and subjects gave written informed consent. After screening, subjects were given single-blind placebo for 2 weeks. On the 12th and 14th mornings of placebo, subjects

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739
underwent infusion of L-NAME (Clinalfa AG) or vehicle (normal saline) in randomized order during normal ad libitum sodium intake. At the end of the second infusion day, subjects were randomized to double-blind treatment with L-arginine plus placebo, ramipril plus placebo, or L-arginine plus ramipril for 4 weeks. L-arginine (Professional Compounding Centers of America) was initiated at 750 mg and titrated to 3g TID during 1 week. Ramipril (Monarch Pharmaceuticals) was initiated at 2.5 mg and titrated to 10 mg QD during 1 week. Medications and placebos were prepared by the Vanderbilt Investigational Pharmacy. On the 26th and 28th days of treatment, subjects reported to the Vanderbilt General Clinical Research Center (GCRC) for infusion of either L-NAME or vehicle. After the second infusion, subjects underwent 2 more cycles of 2-week washout, 4-week treatment, and infusion studies until they had completed all active treatment arms.

Subjects reported to the Vanderbilt GCRC in the fasting state at 8:00 AM on infusion days. A catheter was placed in a vein of each arm for drawing blood and administering drugs, and subjects remained supine. One half hour after catheter insertion, oral study medication was given, and 2 mg/kg L-NAME or vehicle was given intravenously in 250 mL during 30 minutes (66 μg/kg per minute) or until systolic blood pressure (SBP) increased 20 mm Hg. L-NAME was stopped early for blood pressure in 2 of 10 subjects (after 15 minutes in a white female and after 20 minutes in a black male). After the first L-NAME infusion, the same dose was used for each L-NAME study day in the same subject. Blood pressure and heart rate were measured every 5 minutes for 4 hours after the start of the infusion using an automated oscillometric cuff (Critikon) and telemetry. Blood was drawn for measurement of ACE activity before, for plasma renin activity (PRA), Ang II, and aldosterone before and 4 hours after the start of infusion.

**Laboratory Analysis**

Blood samples were centrifuged for 20 minutes immediately after blood drawing, and plasma or serum was stored at −80°C until sampling. Analysis methods are available online at http://www.hypertensionaha.org.

**Statistical Analysis**

Data are presented as means±SD in text and tables and means±SEM of the means in figures. The effect of treatment on hemodynamic and endocrine variables was assessed using a general linear model repeated-measures ANOVA in which within-subject variables were presence or absence of L-arginine, presence or absence of ramipril, L-NAME versus vehicle, and time. Percent change in aldosterone from baseline after vehicle and after L-NAME were compared using a paired Student t test. Unless otherwise noted, P values from the ANOVA are presented in the text. P values for post hoc comparisons are presented in tables and figures. A 2-tailed P value ≤0.05 was considered significant. Analyses were performed using SPSS for Windows (version 11.0; SPSS).

**Results**

Ten normal volunteers (5 male, 5 female; 9 white, 1 black) participated in the study. Three women were premenopausal, 1 was postmenopausal and using an estrogen patch, and 1 was postmenopausal and not taking hormone replacement therapy. The mean age was 37.0 years (range 25 to 56 years), and the mean body mass index was 26.1 kg/m² (range 23.1 to 30.0 kg/m²). Table 1 shows baseline blood pressure and RAAS parameters after each treatment arm. There was no effect of L-arginine alone on blood pressure, PRA, ACE activity, Ang II, or aldosterone. Ramipril reduced SBP compared with baseline, and there was no interactive effect of ramipril and L-arginine on SBP. Ramipril significantly decreased serum ACE activity and marginally increased PRA. There was no effect of ramipril on circulating Ang II or aldosterone concentrations. The effects of ramipril were similar in the presence and absence of L-arginine.

**Effect of L-NAME on RAAS Parameters**

Table 2 and Figure 2 show the effect of vehicle and L-NAME infusion on endocrine parameters during each treatment arm. PRA decreased significantly from baseline to 1 hour after the start of infusion of either vehicle or L-NAME (effect of time $P=0.027$), but there was no difference between vehicle and L-NAME infusion days ($P=0.297$ for effect of L-NAME). Pretreatment with L-arginine did not affect PRA ($P=0.331$). PRA was significantly greater after either vehicle or L-NAME during the ramipril treatment arms ($P=0.022$ for effect of ramipril). There was no effect of L-NAME infusion ($P=0.537$) or of pretreatment with L-arginine ($P=0.358$) or ramipril.
TABLE 1. Effect of L-Arginine and ACE Inhibition on Baseline Hemodynamic and RAAS Variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>L-Arginine</th>
<th>Ramipril</th>
<th>L-Arginine + Ramipril</th>
<th>P Value (ANOVA)</th>
<th>L-Arginine</th>
<th>Ramipril</th>
<th>L-Arginine×Ramipril</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>109.4±9.8</td>
<td>109.4±12.4</td>
<td>106.6±10.1</td>
<td>105.4±8.9*</td>
<td>0.705</td>
<td>0.037</td>
<td>0.665</td>
<td></td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>64.1±8.3</td>
<td>64.2±10.3</td>
<td>62.1±8.9</td>
<td>62.8±8.1</td>
<td>0.774</td>
<td>0.484</td>
<td>0.289</td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>63.8±8.3</td>
<td>65.3±9.1</td>
<td>65.8±7.8</td>
<td>66.9±9.0</td>
<td>0.614</td>
<td>0.089</td>
<td>0.911</td>
<td></td>
</tr>
<tr>
<td>ACE activity (IU/mL)</td>
<td>27.3±9.8</td>
<td>27.4±9.1</td>
<td>7.0±4.1</td>
<td>8.7±4.2***</td>
<td>0.630</td>
<td>&lt;0.001</td>
<td>0.542</td>
<td></td>
</tr>
<tr>
<td>PRA (ng Ang I/mL per hour)</td>
<td>0.6±0.5</td>
<td>0.6±0.6</td>
<td>1.8±1.5**</td>
<td>4.3±7.5***</td>
<td>0.391</td>
<td>0.052</td>
<td>0.339</td>
<td></td>
</tr>
<tr>
<td>Ang II (pg/mL)</td>
<td>40.7±10.5</td>
<td>42.9±13.8</td>
<td>39.1±11.6</td>
<td>45.0±15.4</td>
<td>0.135</td>
<td>0.942</td>
<td>0.147</td>
<td></td>
</tr>
<tr>
<td>Aldosterone (ng/dL)</td>
<td>6.0±3.1</td>
<td>6.0±3.8</td>
<td>5.1±2.7</td>
<td>5.1±2.6</td>
<td>0.286</td>
<td>0.965</td>
<td>0.932</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001 vs placebo; †P<0.05; ††P<0.01; †††P<0.001 vs L-arginine alone in post hoc comparisons.

For Ang II, pg/mL is equivalent to nmol/L. To convert aldosterone to pmol/L, multiply by 27.74.

(P=0.808) on circulating Ang II concentrations over time. There was also no effect of L-NAME infusion (P=0.662) or of pretreatment with L-arginine (P=0.220) or ramipril (P=0.367) on ACTH.

After placebo treatment for 2 weeks, serum aldosterone decreased with time after vehicle infusion (P=0.002; Figure 2A). This decline in serum aldosterone concentration was abolished by L-NAME (effect of time P=0.224) such that aldosterone concentrations were significantly higher during L-NAME than during vehicle infusion (P=0.045). Specifically, serum aldosterone concentrations were the same (n=1) or higher (n=8) after L-NAME compared with vehicle infusion in 9 of 10 subjects. Aldosterone decreased 31% from baseline after vehicle but increased 3% from baseline after

TABLE 2. Effect of Vehicle or L-NAME on Endocrine Parameters During Each Treatment Arm

<table>
<thead>
<tr>
<th>Ramipril</th>
<th>L-Arginine</th>
<th>PRA (ng Ang I/mL per hour)</th>
<th>Ang II (pg/mL)</th>
<th>Potassium (mmol/L)</th>
<th>ACTH (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>–</td>
<td>0 0.41±0.29</td>
<td>39.7±12.2</td>
<td>4.00±0.21</td>
<td>51.3±12.0</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>– 0.33±0.24*</td>
<td>41.5±16.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>4 0.34±0.24</td>
<td>43.9±11.2</td>
<td>4.01±0.19</td>
<td>51.1±11.4</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+ 0.89±0.82</td>
<td>41.6±12.7</td>
<td>3.93±0.18</td>
<td>51.3±13.2</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+ 1 0.45±0.40*</td>
<td>41.7±13.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+ 4 0.39±0.33*</td>
<td>38.0±11.4</td>
<td>4.21±0.28*</td>
<td>52.8±13.2</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>0 0.57±0.58</td>
<td>40.3±15.3</td>
<td>3.89±0.25</td>
<td>51.0±13.9</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+ 1 0.43±0.43</td>
<td>37.6±19.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+ 4 0.52±0.39</td>
<td>44.4±16.8</td>
<td>3.94±0.23</td>
<td>52.3±12.4</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>+ 0.73±0.57</td>
<td>45.6±19.0</td>
<td>3.93±0.36</td>
<td>49.3±11.7</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>+ 1 0.52±0.46*</td>
<td>41.8±16.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>+ 4 0.49±0.66*</td>
<td>38.3±14.4</td>
<td>4.08±0.41</td>
<td>51.2±11.9</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>0 1.81±1.38††</td>
<td>39.1±13.2</td>
<td>4.00±0.19</td>
<td>50.4±13.1</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>– 1.20±0.84††</td>
<td>40.0±18.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>4 3.61±6.18††</td>
<td>37.4±17.6</td>
<td>3.94±0.22</td>
<td>52.2±12.2</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>+ 0 1.88±1.72††</td>
<td>39.1±11.1</td>
<td>3.96±0.22</td>
<td>50.9±14.5</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>+ 1 1.24±0.97††</td>
<td>37.3±10.5</td>
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</tr>
<tr>
<td>+</td>
<td>–</td>
<td>+ 4 1.43±1.19†</td>
<td>36.6±13.2</td>
<td>4.06±0.20</td>
<td>51.5±16.4</td>
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<tr>
<td>+</td>
<td>+</td>
<td>0 3.16±4.13††</td>
<td>44.0±18.9</td>
<td>3.96±0.18</td>
<td>48.6±13.1</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+ 1 2.68±4.07†††</td>
<td>37.3±13.4</td>
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<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>– 4 4.89±6.32††</td>
<td>43.4±15.0</td>
<td>3.98±0.24</td>
<td>51.0±11.4</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+ 0 5.40±11.0††</td>
<td>46.0±14.5</td>
<td>4.02±0.21</td>
<td>47.8±12.6</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+ 1 2.60±5.03†††</td>
<td>45.9±17.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+ 4 4.35±7.10†‡</td>
<td>39.0±11.8</td>
<td>4.04±0.14</td>
<td>53.1±12.6</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001 vs 0 hours.
†P<0.05; ††P<0.01 vs during placebo.
‡P<0.05; †††P<0.01 vs during L-arginine alone.

For Ang II, pg/mL is equivalent to nmol/L. To convert ACTH to pmol/L, multiply by 0.2202.
L-NAME \((P=0.034\) by \(t\) test). Pretreatment with L-arginine alone or ramipril alone did not prevent the effect of L-NAME on serum aldosterone concentrations but shortened the duration of the effect. Hence, during the L-arginine arm (Figure 2B), serum aldosterone decreased significantly with time after vehicle \((P=0.024\) but not after L-NAME \((P=0.371\)), and there was a significant effect of L-NAME \((P=0.026\) on aldosterone concentrations. Similarly, during ramipril alone, aldosterone decreased with time after vehicle \((P=0.008\) but not L-NAME \((P=0.157\)), and there was a significant time \(x\) L-NAME effect \((P=0.019\)). However, treatment with combined L-arginine plus ramipril (Figure 2D) abolished the effect of L-NAME on serum aldosterone \((P=0.523\) for effect of L-NAME; \(P=0.017\) for effect of time during L-NAME).

During the placebo arm, there was a significant linear relationship between PRA and aldosterone concentration after vehicle infusion \((\text{aldosterone}=3.9\text{PRA}+19; \ r^2=0.476; \ P=0.027\)) and L-NAME infusion \((\text{aldosterone}=7.2\text{PRA}+3.1; \ r^2=0.457; \ P=0.032\); Figure 3A). Although the slopes of these lines were not statistically different, the intercepts were significantly different \((P=0.029\). There was no statistical relationship between Ang II and PRA or between Ang II and aldosterone concentrations after either vehicle or L-NAME \((P>0.90\).

Effect of L-NAME Infusion on Hemodynamic Parameters

During the placebo treatment arm, L-NAME caused a significant increase in SBP compared with vehicle \((P=0.012; \text{Figure 2.}\) Effect of L-NAME or vehicle on aldosterone during treatment with placebo (A), L-arginine (B), ramipril (C), or combination L-arginine and ramipril (D). *\(P<0.05\); **\(P<0.01\) vs baseline. †\(P<0.05\) vs vehicle. To convert aldosterone to pmol/L, multiply by 27.74.

There was no effect of L-NAME infusion \((P=0.301\) of pretreatment with L-arginine \((P=0.461\) or ramipril \((P=0.679\) on serum sodium concentrations (data not shown). During the placebo treatment arm, there was a borderline effect of time \((P=0.053\) and significant interactive effect of time by L-NAME \((P=0.039\) on serum potassium (Table 2). This was not seen during the L-arginine, ramipril, or L-arginine plus ramipril treatment arms. To determine whether increased serum potassium could account for the effect of L-NAME on serum aldosterone, we examined the relationship between serum potassium and aldosterone concentrations drawn 4 hours after the initiation of vehicle or L-NAME infusion during the placebo treatment arm. As illustrated in Figure 3B, there was a significant linear relationship between serum potassium and aldosterone during L-NAME \((\text{aldosterone}=8.2\cdot\text{potassium}−28.9; \ r^2=0.609; \ P=0.008\) but not during placebo \((P=0.313\). The slope of the relationship between serum potassium and aldosterone concentrations during L-NAME was significantly different from the slope of the relationship during vehicle \((P=0.007\).

Figure 3. Relationship between PRA and aldosterone (left) and serum potassium and aldosterone (right). There was a significant linear relationship between PRA and aldosterone concentration after vehicle infusion \((r^2=0.476; \ P=0.027\)) and L-NAME infusion \((r^2=0.457; \ P=0.032\). The intercepts of these 2 lines were significantly different \((P=0.029\). There was a significant linear relationship between serum potassium and aldosterone during L-NAME \((r^2=0.609; \ P=0.008\) but not vehicle \((P=0.313\), and the slopes of the 2 lines were different \((P=0.007\). To convert aldosterone to pmol/L, multiply by 27.74.

During the placebo treatment arm, there was a significant linear relationship between PRA and aldosterone concentration after vehicle infusion \((\text{aldosterone}=3.9\text{PRA}+19; \ r^2=0.476; \ P=0.027\) and L-NAME infusion \((\text{aldosterone}=7.2\text{PRA}+3.1; \ r^2=0.457; \ P=0.032\); Figure 3A). Although the slopes of these lines were not statistically different, the intercepts were significantly different \((P=0.029\). There was no statistical relationship between Ang II and PRA or between Ang II and aldosterone concentrations after either vehicle or L-NAME \((P>0.90\).
The change in SBP after L-NAME remained significantly greater than that after vehicle until 165 minutes after initiation (135 minutes after discontinuation) of L-NAME infusion. However, there was no carryover effect between treatment days (data not shown).

Treatment with l-arginine (Figure 4B) blunted the early increase in SBP in response to L-NAME (P = 0.047 for the comparison of SBP during the first 30 minutes of L-NAME in the presence of l-arginine versus placebo); however, L-NAME significantly increased SBP compared with vehicle during l-arginine (P < 0.001). Treatment with ramipril alone or in combination with l-arginine (Figure 4C and 4D) did not affect the early increase in SBP in response to L-NAME but significantly shortened the duration of the L-NAME effect such that increase in SBP from 2 to 3 hours after initiation of L-NAME was significantly smaller during ramipril (P = 0.017) or l-arginine plus ramipril (P = 0.019) compared with placebo. Nevertheless, the change in SBP after L-NAME was significantly greater than that after vehicle during ramipril alone (P = 0.012) and l-arginine plus ramipril (P = 0.044).

There was no effect of vehicle on heart rate during any study arm (P > 0.175 for each vehicle infusion). Heart rate decreased significantly in response to L-NAME (P = 0.010 versus vehicle), l-arginine (from 65.9 ± 12.1 to 51.0 ± 5.4 bpm; P = 0.020 versus vehicle), ramipril (from 65.7 ± 9.8 to 52.7 ± 9.1 bpm; P < 0.001 versus vehicle), and l-arginine plus ramipril (71.5 ± 12.5 to 55.4 ± 9.0 bpm; P < 0.001 versus vehicle). The effect of L-NAME on heart rate persisted for the same duration as the blood pressure effect.

Discussion

NO modulates Ang II and ACTH-induced adrenal aldosterone synthesis in vitro and in animal models.7-9 Aldosterone contributes to the deleterious cardiovascular effects of NOS inhibition in animal models.10,11 Although several studies indicate that increased aldosterone concentrations cause endothelial dysfunction in patients with hypertension and congestive heart failure,4,5 the current study is the first to demonstrate that endogenous NO modulates aldosterone concentrations in humans.

In this study, NOS inhibition using L-NAME exerted a pressor effect similar to that reported previously,20,21 indicating that endogenous NO contributes to basal vascular tone. As in previous studies, the blood pressure effect persisted after discontinuation of L-NAME, consistent with conversion of L-NAME to a long-acting active metabolite L-N^G-nitro-l-arginine.22 L-NAME decreased heart rate, most likely because of stimulation of the baroreflex. Interestingly, although 1 month of pretreatment with oral l-arginine attenuated the peak blood pressure response to L-NAME, it did not abolish this pressor response. This differs from the effect of intravenous l-arginine administration20 on the pressor response to L-NAME and may reflect issues of the bioavailability of oral l-arginine. As reported previously, interruption of the RAAS partially attenuated the late pressor response to L-NAME.23

Although increased blood pressure during L-NAME would be expected to suppress the RAAS, aldosterone concentrations were higher during L-NAME than during vehicle. To investigate the mechanism whereby NOS inhibition increased plasma aldosterone concentrations relative to vehicle, we measured the effect of L-NAME on the aldosterone secretagogues Ang II, ACTH, and potassium. L-NAME could affect circulating aldosterone concentrations by increasing 1 of these secretagogues or by altering the sensitivity of the adrenal glomerulosa to their effects. In this regard, NOS inhibition has been reported to increase renin activity12 and to enhance Ang II type 1 receptor expression.12,18 In the present study, there was no effect of L-NAME on PRA or Ang II concentrations. Whereas there was no relationship between Ang II concentrations and either PRA or aldosterone, indi-
cating the limitation of circulating Ang II as a measure of local Ang II concentrations. L-NAME enhanced the relationship between aldosterone and PRA. Together, these findings suggest that NOS inhibition enhances adrenal sensitivity to activation of the RAAS.

To further elucidate the mechanism whereby NOS inhibition increased plasma aldosterone concentrations in humans, we determined whether interruption of the RAAS with an ACE inhibitor prevented the effect of NOS inhibition on aldosterone. Ramipril significantly decreased ACE activity and increased PRA, indicating that adequate ACE inhibition was achieved; ramipril did not reduce baseline aldosterone or Ang II, consistent with escape.24,25 The finding that ACE inhibition was necessary but not sufficient to prevent the effects of L-NAME on circulating aldosterone concentrations suggests that although NOS inhibition may increase aldosterone in part through an angiotensin-dependent pathway, additional RAAS-independent pathways may be involved. There was no effect of L-NAME on ACTH concentrations, compatible with data from a previous study in normal men.26 However, as has been reported in sheep,15 L-NAME significantly increased serum potassium concentrations. A similar increase in serum potassium has been noted in eNOS-deficient animals and may reflect changes in renal potassium transport.27

Given that potassium acts as a potent secretagogue for aldosterone, increased serum potassium during L-NAME could have contributed to the effect of NOS inhibition on circulating aldosterone concentrations after placebo treatment. However, several findings suggest that increased serum potassium is not the primary mechanism underlying increased aldosterone during L-NAME. For example, L-NAME increased aldosterone concentrations even when there was no effect on potassium (eg, during L-arginine alone). Conversely, ACE inhibition attenuated L-NAME–induced increases in aldosterone, although ACE inhibition does not alter potassium-stimulated aldosterone secretion.28 In addition, just as L-NAME shifted the relationship between aldosterone and PRA, L-NAME also significantly enhanced the relationship between aldosterone and serum potassium. Thus, NOS inhibition appears to increase adrenal responsiveness to multiple secretagogues. These findings are compatible with in vitro data indicating that NO inhibits Ang II–7,9 ACTH–7 25-hydroxycholesterol,8 and progesterone–8 stimulated aldosterone synthesis through a cyclic GMP-independent pathway and that NO has a direct inhibitory effect on the cytochrome P450s involved in aldosterone synthesis, probably by binding to the heme group of the cytochromes.8 Removal of this tonic inhibitory effect during L-NAME would then enhance the response to many stimuli.

The results of the current study contrast data from Davidson and Struthers,17 who reported that the NOS inhibitor Nω-monomethyl-L-arginine (L-NMMA) did not affect Ang II-stimulated aldosterone synthesis, although baseline aldosterone concentrations tended to be increased during L-NMMA. One possible explanation for the discrepancy between these 2 studies relates to the relative potencies of L-NMMA and L-NAME. Whereas the effects of L-NMMA are limited by its degradation to L-arginine, L-NAME is not degraded to L-arginine.29 Indeed, on the basis of studies comparing the pressor effects of the 2 NOS inhibitors,20 the dose of L-NAME administered in the present study is equivalent to a dose of L-NMMA of 12 mg/kg 4-fold higher than the dose administered in the study by Davidson and Struthers.

Finally, despite the fact that acute L-NAME administration increased aldosterone concentrations in this study, chronic administration of the NO precursor L-arginine and ACE inhibition, which has been shown to improve endothelial function,19 did not decrease basal aldosterone. There are several explanations for this observation. First, we studied individuals under conditions of low baseline activity of the RAAS. Second, we studied normal individuals without risk factors for endothelial dysfunction, in whom chronic L-arginine16 or ACE inhibition30 might be expected to have little effect on endogenous NOS.

Perspectives
Endothelial dysfunction predicts cardiovascular events in patients at risk for coronary artery disease.1 Studies indicate that the hormone aldosterone impairs NOS activity. This is the first study to demonstrate that endogenous NO regulates aldosterone synthesis in humans. Given that aldosterone causes cardiovascular injury and fibrosis,10,31 this study suggests yet another mechanism whereby deficient NO synthesis predisposes to cardiovascular disease. Although the present study was conducted in individuals without risk factors for endothelial dysfunction, further studies are needed to explore whether pharmacological strategies to increase endogenous NO in patients with existing endothelial dysfunction alter aldosterone concentrations.

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NO Synthase Inhibition Increases Aldosterone in Humans

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