Endothelial function during stimulation of renin-angiotensin system by low-sodium diet in humans

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Omland, Torbjørn, Wendy Johnson, Mary Beth Gordon, and Mark A. Creager. Endothelial function during stimulation of renin-angiotensin system by low-sodium diet in humans. Am J Physiol Heart Circ Physiol 280: H2248–H2254, 2001.—We examined whether physiological stimulation of the endogenous renin-angiotensin system results in impaired endothelium-dependent vasodilation in forearm resistance vessels of healthy subjects and whether this impairment can be prevented by angiotensin II type 1 receptor blockade. A low-sodium diet was administered to 27 volunteers who were randomized to concomitant treatment with losartan (100 mg once daily) or matched placebo in a double-blind fashion. Forearm blood flow was assessed by venous occlusion plethysmography at baseline and after 5 days. Endothelium-dependent and -independent vasodilation was assessed by intra-arterial infusion of methacholine and verapamil, respectively. The low-sodium diet resulted in significantly decreased urine sodium excretion (placebo: 146 ± 64 vs. 10 ± 9 meq/24 h, P < 0.001; losartan: 141 ± 56 vs. 14 ± 14 meq/24 h, P < 0.001) and increased plasma renin activity (placebo: 1.0 ± 0.5 vs. 5.0 ± 2.5 ng·ml⁻¹·h⁻¹, P < 0.001; losartan: 3.8 ± 7.2 vs. 19.1 ± 11.2 ng·ml⁻¹·h⁻¹, P = 0.006) in both the losartan and placebo groups. With the baseline study as the reference, the diet intervention was not associated with any significant change in endothelium-dependent vasodilation to methacholine in either the placebo (P = 0.74) or losartan (P = 0.40) group. We conclude that short-term physiological stimulation of the renin-angiotensin system does not cause clinically significant endothelial dysfunction. Losartan did not influence endothelium-dependent vasodilation in humans with a stimulated renin-angiotensin system.

METHODS

Subjects. The study population included 27 healthy subjects (17 women and 10 men) with a mean age of 31.7 ± 8.3 yr. The study was powered to have an 85% probability to detect a 15% decrease (from 20 to 17 ml·100 ml⁻¹·tissue⁻¹·min⁻¹) in the peak forearm blood flow response to methacholine induced by the low-sodium diet, assuming a SD of 5 ml·100 ml⁻¹·tissue⁻¹·min⁻¹.

All subjects were recruited from the Boston area via advertisements in local newspapers. Subjects were screened using a complete medical history, physical examination, and laboratory analysis. Exclusion criteria for participation in the study included age <18 yr, current tobacco use, essential hypertension, diabetes mellitus, hypercholesterolemia (defined as the 75th percentile for age and sex), evidence of cardiac or pulmonary disease, laboratory evidence of hemato logic, renal, or hepatic dysfunction, and current use of diuretics, vasoactive medications, or nonsteroidal anti-in-
flammary drugs. The study protocol was approved by the Human Research Committee of Brigham and Women’s Hospital, and each subject gave written informed consent.

**Outline of study design.** The study protocol included vascular function testing as well as blood collection for neurohormonal and biochemical analyses on the first day of the study (baseline test). Immediately after testing and blood collection, the subjects were randomized to treatment with losartan (100 mg/day, orally; n = 13) or matched placebo (n = 14) in a double-blind fashion. After the baseline vascular function test, all 27 study subjects received a low-sodium diet (10 meq daily) for a total of 5 days. On the final day of the study (day 6), the study subjects underwent repeat vascular function testing as well as repeat blood collection for neurohormonal and biochemical analyses. Urine collections were performed for 24 h on the day before the vascular studies.

**Drug infusion protocol.** Methacholine chloride, a congener of acetylcholine, was administered via the brachial artery to assess the vasodilatation resulting from endothelium-derived nitric oxide. Forearm blood flow was measured during infusion of increasing doses of methacholine corresponding to 0.3, 1.0, 3.0, and 10.0 µg/min. Verapamil, a nondihydropyridine calcium-channel blocker, was administered via the brachial artery to assess vascular smooth muscle relaxation not dependent on endothelium-derived or exogenous nitric oxide. Forearm blood flow was measured during infusion of increasing doses of verapamil, corresponding to 10, 30, 100, and 300 µg/min. The doses of the intra-arterially administered drugs were aimed at achieving significant changes in forearm blood flow and forearm vascular resistance without causing changes in heart rate or systemic blood pressure. Hemodynamic measurements were performed after infusion of methacholine and verapamil for 3 min at each dose. The infusion rate was 0.4 ml/min.

**Vascular function study.** Each study was conducted in the vascular research laboratory after a light morning meal. Alcohol and caffeine intake were prohibited within 12 h of the study. The room was sound isolated and was kept quiet during the study. Lights were dimmed and temperature was kept constant at 22°C. With the use of local anesthesia and sterile conditions, a 20-gauge polyethylene catheter was inserted into a brachial artery of each subject for determination of systemic blood pressure and infusion of drugs. All of the study subjects rested in the supine position for at least 30 min after catheter placement to establish a stable baseline before data collection.

At the start of each study, physiological saline (0.9% sodium chloride) was infused intra-arterially at a rate of 0.4 ml/min. Baseline measurements of forearm blood flow and systemic blood pressure were repeated every 10 min until stability was obtained. Each study then proceeded with a forearm blood flow dose-response curve to methacholine. After a rest period of at least 30 min to reestablish stable forearm blood flow compared with baseline values, a forearm blood flow dose-response curve to verapamil was obtained. Identical experimental protocols were employed on the baseline and follow-up study.

**Hemodynamic measurements.** Bilateral forearm blood flow was determined by venous occlusion strain-gauge plethysmography (DE Hokanson, Issaquah, WA) using calibrated mercury-in-Silastic strain gauges and was expressed as milliliters per 100 milliliters of tissue per minute. Each arm was supported above the heart level. Target venous occlusion pressure was 35–40 mmHg. Before each forearm blood flow determination, circulation to the hand was stopped by inflating a wrist cuff to suprasystolic pressures. Each forearm blood flow determination comprised at least five separate measurements performed at 10- to 15-s intervals. The direct effect of the vasoactive drugs was determined by measuring blood flow in the infused arm. To rule out systemic effects, contralateral forearm blood flow was monitored during the study. Blood pressure was measured via an indwelling arterial catheter attached to a Gould P23 pressure transducer aligned to an amplifier on a Gould physiological recorder. Forearm vascular resistance was calculated as the ratio of mean blood pressure to forearm blood flow and was expressed as resistance units. Heart rate was calculated from R-R intervals of a simultaneously obtained electrocardiographic signal.

**Neurohormonal and biochemical measurements.** On days 1 and 6, directly before the vascular forearm studies, study subjects were admitted to the Clinical Research Center of Brigham and Women’s Hospital. An intravenous cannula was inserted in a cubital vein and after 30 min of supine rest blood samples were drawn into prechilled plastic tubes for subsequent measurement of plasma norepinephrine and plasma renin activity as well as serum concentrations of sodium, potassium, and creatinine. Urine concentrations of sodium, potassium, and creatinine were determined in 24-h urine samples obtained from the day before the vascular forearm study (excluding the morning urine on the day before, but including the morning urine of the day of the vascular study).

Blood samples for norepinephrine determination were immediately placed on ice whereas samples for plasma renin activity were kept at room temperature until centrifuged (within 20 min of sampling). Plasma samples were snap-frozen and stored at −70°C pending analysis. Plasma norepinephrine was determined by a radioenzymatic assay (12). Plasma renin activity was determined by generation of angiotensin I assessed by radioimmunoassay (3).

**Statistical analysis.** All measurements are given as means ± SD. Comparisons between the losartan and placebo groups were performed using independent samples, two-tailed t-tests, and χ² tests for continuous and categorical variables, respectively. Pre- and postintervention values for continuous variables were compared by paired t-tests. Statistical analyses of the dose-response curves for each drug before and after the low-sodium diet was performed using two-way ANOVA for repeated measures. P < 0.05 was considered significant.

**RESULTS**

**Baseline characteristics.** The losartan and placebo groups were well matched with regard to age, sex, and weight. The average age was 31 ± 7 yr in the losartan group and 32 ± 10 yr in the placebo group (P = 0.85). The losartan group included nine men and four women, whereas the placebo group consisted of eight men and six women (P = 0.53). The average weight was 66 ± 8 kg in the losartan group and 70 ± 11 kg in the placebo group (P = 0.31). Serum total and high-density lipoprotein cholesterol levels did not differ significantly between the two groups (losartan vs. placebo: 161 ± 22 vs. 177 ± 26 mg/dl; P = 0.09, and 57 ± 19 vs. 52 ± 12 mg/dl; P = 0.45, respectively), whereas serum low-density lipoprotein cholesterol levels were significantly higher in the placebo than in the losartan group (102 ± 23 vs. 84 ± 19 mg/dl; P = 0.03). As detailed in Table 1, systolic blood pressure was also significantly higher in the placebo than in the losartan group (122 ± 13 vs.
111 ± 10 mmHg; \( P = 0.02 \)) whereas diastolic blood pressure and heart rate did not differ significantly between the groups. The two groups were well matched with regard to baseline plasma renin activity and plasma norepinephrine and epinephrine concentrations as well as to baseline serum and urine electrolyte values (Table 1).

**Effect of diet and losartan on hemodynamic and biochemical variables.** The effect of the low-sodium diet on various hemodynamic and biochemical variables in the losartan and placebo groups is summarized in Table 1. In both the placebo and losartan groups, the diet intervention resulted in significantly decreased urine sodium excretion (placebo: 146 ± 64 vs. 10 ± 9 meq/24 h, \( P < 0.001 \); losartan: 141 ± 56 vs. 14 ± 14 meq/24 h, \( P < 0.001 \)) and increased plasma renin activity (placebo: 1.0 ± 0.5 vs. 5.0 ± 2.5 ng·ml\(^{-1} \)·h\(^{-1} \), \( P < 0.001 \); losartan: 3.8 ± 7.2 vs. 19.1 ± 11.2 ng·ml\(^{-1} \)·h\(^{-1} \), \( P = 0.006 \)). In both groups the weight of the study subjects was significantly reduced during the intervention period (placebo: 66 ± 8 vs. 64 ± 8 kg, \( P < 0.001 \); losartan: 70 ± 11 vs. 68 ± 11 kg, \( P < 0.001 \)). Whereas systolic blood pressure remained unchanged in the placebo group (122 ± 13 vs. 120 ± 15 mmHg; \( P = 0.58 \)), a significant decrease occurred in the losartan group (111 ± 10 vs. 106 ± 10 mmHg; \( P = 0.03 \)). No significant change in diastolic blood pressure was observed in either group. Heart rate tended to increase in the placebo group (66 ± 10 vs. 71 ± 14 beats/min; \( P = 0.06 \)) and increased significantly in the losartan group (59 ± 8 vs. 63 ± 12 beats/min; \( P = 0.02 \)).

The effect of treatment with losartan compared with placebo on various hemodynamic and biochemical variables during the low-sodium diet intervention is summarized in Table 2. Although both systolic and diastolic blood pressure tended to decrease more in the losartan than in the placebo group, the differences between the two treatment groups were not significant (Table 2). Similarly, although the weight reduction during the low-sodium diet intervention tended to be more pronounced in the losartan than in the placebo group, the difference between groups was only borderline significant (\( P = 0.09 \)). No significant between-group differences were observed for changes in heart rate, plasma catecholamines, serum electrolytes, or urine excretion of sodium, potassium, or creatinine (Table 2). The increase in plasma renin activity during the intervention period was significantly greater in the losartan than in the placebo group (\( P = 0.02 \)) likely reflecting the loss of feedback inhibition caused by angiotensin II blockade as well as sodium depletion in the losartan group. In contrast, the increase in serum creatinine levels was significantly greater in the placebo than in the losartan group (\( P = 0.02 \)).

**Effect of diet and losartan on forearm vascular function.** In the losartan group, basal forearm blood flow averaged 1.89 ± 0.64 ml·100 ml\(^{-1} \)·time\(^{-1} \) in the preintervention study and 1.51 ± 0.44 ml·100 ml\(^{-1} \)·time\(^{-1} \) in the postintervention study (\( P = 0.08 \)). In the losartan group, basal forearm vascular resistance averaged 43.7 ± 14.9 U in the preintervention study and 48.5 ± 17.7 U in the postintervention study.

### Table 1. Effect of low-sodium diet on hemodynamic and biochemical variables

<table>
<thead>
<tr>
<th></th>
<th>Prediet</th>
<th>Postdiet</th>
<th>( P ) Value</th>
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<tbody>
<tr>
<td></td>
<td>Losartan</td>
<td>Placebo</td>
<td>Losartan</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66 ± 8</td>
<td>70 ± 11</td>
<td>64 ± 8</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>111 ± 10</td>
<td>122 ± 13</td>
<td>106 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>64 ± 8</td>
<td>70 ± 7</td>
<td>61 ± 8</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>59 ± 8</td>
<td>66 ± 10</td>
<td>63 ± 12</td>
</tr>
<tr>
<td>Plasma renin activity, ng·ml(^{-1} )·h(^{-1} )</td>
<td>3.8 ± 7.2</td>
<td>1.0 ± 0.5</td>
<td>19.2 ± 11.2</td>
</tr>
<tr>
<td>Plasma norepinephrine, pg/ml</td>
<td>262 ± 197</td>
<td>234 ± 247</td>
<td>308 ± 128</td>
</tr>
<tr>
<td>Plasma epinephrine, pg/ml</td>
<td>114 ± 318</td>
<td>98 ± 251</td>
<td>40 ± 22</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>0.81 ± 0.10</td>
<td>0.77 ± 0.22</td>
<td>0.85 ± 0.15</td>
</tr>
<tr>
<td>Serum sodium, mmol/l</td>
<td>141 ± 3</td>
<td>142.0 ± 2.2</td>
<td>140 ± 2</td>
</tr>
<tr>
<td>Serum potassium, mmol/l</td>
<td>4.0 ± 0.4</td>
<td>4.0 ± 0.3</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Urine creatinine, mg/24 h</td>
<td>1,224 ± 306</td>
<td>1,247 ± 468</td>
<td>1,219 ± 379</td>
</tr>
<tr>
<td>Urine sodium, meq/24 h</td>
<td>141 ± 56</td>
<td>146 ± 64</td>
<td>14 ± 14</td>
</tr>
<tr>
<td>Urine potassium, meq/24 h</td>
<td>60 ± 30</td>
<td>55 ± 31</td>
<td>55 ± 21</td>
</tr>
</tbody>
</table>

Values are means ± SD.

### Table 2. Change in prediet and postdiet values for hemodynamic and biochemical variables during a low-sodium diet

<table>
<thead>
<tr>
<th></th>
<th>Losartan</th>
<th>Placebo</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>-2.3 ± 1.1</td>
<td>-1.6 ± 0.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>-5.0 ± 7.3</td>
<td>-1.8 ± 11.9</td>
<td>0.41</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>-2.8 ± 5.8</td>
<td>1.6 ± 8.9</td>
<td>0.14</td>
</tr>
<tr>
<td>Basal forearm blood flow, ml/100 ml</td>
<td>-0.4 ± 0.7</td>
<td>-0.2 ± 0.6</td>
<td>0.49</td>
</tr>
<tr>
<td>Forearm vascular resistance, U</td>
<td>4.8 ± 17.8</td>
<td>0.0 ± 2.2</td>
<td>0.57</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>4.0 ± 5.3</td>
<td>0.3 ± 9.8</td>
<td>0.44</td>
</tr>
<tr>
<td>Plasma renin activity, ng·ml(^{-1} )·h(^{-1} )</td>
<td>15 ± 16</td>
<td>4 ± 2</td>
<td>0.02</td>
</tr>
<tr>
<td>Plasma norepinephrine, pg/ml</td>
<td>46 ± 195</td>
<td>1.2 ± 253</td>
<td>0.62</td>
</tr>
<tr>
<td>Plasma epinephrine, pg/ml</td>
<td>-7.4 ± 306</td>
<td>0.6 ± 303</td>
<td>0.90</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>0.04 ± 0.10</td>
<td>0.15 ± 0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum sodium, mmol/l</td>
<td>-0.8 ± 2.7</td>
<td>-1.5 ± 1.5</td>
<td>0.42</td>
</tr>
<tr>
<td>Serum potassium, mmol/l</td>
<td>0.09 ± 0.20</td>
<td>0.02 ± 0.40</td>
<td>0.37</td>
</tr>
<tr>
<td>Urine creatinine, mg/24 h</td>
<td>-4.5 ± 340</td>
<td>-9.6 ± 470</td>
<td>0.57</td>
</tr>
<tr>
<td>Urine sodium, meq/24 h</td>
<td>-127 ± 566</td>
<td>0 ± 66</td>
<td>0.71</td>
</tr>
<tr>
<td>Urine potassium, meq/24 h</td>
<td>-3.2 ± 34</td>
<td>-1.8 ± 28</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Values are means ± SD.
(\(P = 0.35\)). In the placebo group, basal forearm blood flow averaged 2.14 ± 0.77 ml·100 ml\(^{-1}\) tissue·min\(^{-1}\) and 1.95 ± 0.52 ml·100 ml\(^{-1}\) tissue·min\(^{-1}\) in the pre- and postintervention studies, respectively (\(P = 0.29\)). In the placebo group, basal forearm vascular resistance averaged 43.2 ± 20.9 and 43.0 ± 2.7 U in the pre- and postintervention studies, respectively (\(P = 0.97\)). There was no significant difference between the losartan and placebo groups with regard to the change in basal forearm blood flow or forearm vascular resistance during the diet-intervention period (each \(P = 0.49\); see Table 2).

Intra-arterial infusion of methacholine caused a dose-dependent and significant increase in forearm blood flow and a decrease in forearm vascular resistance during the baseline study. Likewise during the follow-up study (i.e., after the diet intervention), intra-arterial infusion of methacholine resulted in a dose-dependent and significant increase in forearm blood flow and a decrease in forearm vascular resistance in patients randomized to both losartan and placebo (Fig. 1, A and B). With the baseline study dose-response curve as the reference, no significant change in the forearm blood flow response to methacholine was observed in either the losartan (\(P = 0.40\)) or the placebo (\(P = 0.74\)) group during the follow-up study. Moreover, no significant change in forearm vascular resistance was observed in either the losartan (\(P = 0.38\)) or placebo (\(P = 0.98\)) group between the baseline and follow-up methacholine infusion.

The intra-arterial infusion of incremental doses of verapamil also resulted in a dose-dependent and significant increase in forearm blood flow and a decrease in forearm vascular resistance during both the baseline (preintervention) and the follow-up (postintervention) studies. In the placebo group, no significant difference (\(P = 0.42\)) in the forearm blood flow response to verapamil was evident between the preintervention and postintervention studies (Fig. 2A). In contrast, in the losartan group, the forearm blood flow response during the postintervention study was significantly attenuated compared with the response to verapamil infusion during the preintervention study (\(P = 0.01\)). However, when adjusting for the baseline forearm blood flow in the follow-up study by calculating the percentage increase in forearm blood flow (as compared with baseline) during verapamil infusion, no difference was observed between the prelosartan and the postlosartan studies (\(P = 0.13\)). Moreover, no significant difference in forearm vascular resistance changes between the preintervention and the postintervention studies was observed for either the placebo (\(P = 0.64\)) or the losartan group (\(P = 0.63\)) during the verapamil infusion studies.

**DISCUSSION**

The novel information obtained from this study is that physiological stimulation of the renin-angiotensin system by ingestion of a low-sodium diet does not cause impairment of endothelium-dependent vasodilation in humans. A second finding is that administration of the AT\(_1\)-receptor blocker losartan does not appear to significantly affect endothelium-dependent vasodilation in humans with a stimulated renin-angiotensin sys-
vascular superoxide production is dose dependent, and that the receptor-mediated effect of angiotensin II on reproduce the results from the animal experiments is for 5 days. One potential explanation for the failure to enous renin-angiotensin system by a low-sodium diet observed after physiological stimulation of the endog-

The original hypothesis of our study was based on results of experiments performed both in cell culture and animal models. In 1994, Griengl and colleagues (5) published data showing that cultured vascular smooth muscle cells exposed to angiotensin II for 4 h produced nearly three times as much superoxide as did control cells. Moreover, exposure to angiotensin II caused a large increase in both NADH and NADPH oxidase activity. The activation of these enzymes could be blocked by the AT1-receptor antagonist losartan which suggests a receptor-mediated process. In 1996, Rajagopalan and colleagues (15) reported that subcu-
taneous infusion of angiotensin II in rats (0.7 mg·kg\(^{-1}\)·day\(^{-1}\)) was associated with increases in blood pressure and vascular superoxide anion production. Infusion with norepinephrine was also associated with an increase in blood pressure but not vascular superoxide production. In vascular ring experiments, infusion of angiotensin II but not norepinephrine was as-

hemodynamic response was present in organoid culture experiments of mouse aortas exposed to levels of angiotensin II above 10 nM (4). Interestingly, in human aortic smooth muscle cells, angiotensin II moderately increased the transcriptional rate but markedly increased extracellular superoxide dismutase gene expression and activity in mice. The increased mRNA expression was prevented by the ad-

As demonstrated by the present data, however, no change in endothelium-dependent vasodilatation was observed after physiological stimulation of the endog-

one renin-angiotensin system might cause endothelial dysfunction in humans and that this im-

pairment might be corrected by the concomitant ad-

administration of an angiotensin II receptor antagonist.

As demonstrated by the present data, however, no change in endothelium-dependent vasodilatation was observed after physiological stimulation of the endog-

ous renin-angiotensin system by a low-sodium diet for 5 days. One potential explanation for the failure to reproduce the results from the animal experiments is that the receptor-mediated effect of angiotensin II on vascular superoxide production is dose dependent, and that physiological stimulation of the renin-angiotensin system results in concentrations of angiotensin II inadequate to elicit the response induced by pharmaco-

logical doses. We did not measure circulating levels of angiotensin II in the current study, nor were plasma levels measured in the animal experiments, which makes direct comparison of the circulating concentra-
tions difficult. However, the plasma renin activity data strongly suggest that the circulating renin-angiotensin system indeed was markedly stimulated by the low-
sodium diet, thereby questioning the physiological rele-

vance of the results from the animal studies using pharmacological doses of angiotensin II.

A second potential explanation for the discrepant results is that the duration and intensity of renin-

angiotensin system stimulation required to induce increased superoxide anion production and subsequent endothelial dysfunction may differ between species. We cannot exclude the possibility that long-term (i.e., >5 days) physiological stimulation of the renin-angio-
tensin system in humans would have resulted in im-

pairment of endothelium-dependent vasodilation. Thus intervention with an angiotensin II receptor blocking agent in a diseased population or in patients with established endothelial dysfunction secondary to long-term renin-angiotensin system activation may have yielded a different result as was suggested by a recent report (14). It is also conceivable that even more intense stimulation of the renin-angiotensin system than that obtained by a low-sodium diet is required for the development of endothelial dysfunction in humans.

A third possibility is that the efficiency of physiolog-

ical mechanisms counteracting the stimulatory effect of angiotensin II on superoxide production may differ between species. Recently, Fukai and colleagues (4) reported that angiotensin II infusion was associated with a marked increase in extracellular superoxide dismutase gene expression and activity in mice. The increased mRNA expression was prevented by the ad-

ministration of losartan. Similar results were observed in organoid culture experiments of mouse aortas exposed to pharmacological doses of angiotensin II (100 nM) (4). Interestingly, in human aortic smooth muscle cells, angiotensin II moderately increased the trans-

criptional rate but markedly increased extracellular superoxide dismutase mRNA stability, which suggests that in humans the stimulatory effect of angiotensin II on superoxide production may be effectively balanced by increased extracellular superoxide dismutase activity (4). Our findings are compatible with the hypothesis that this counterregulatory mechanism is operative in healthy subjects. However, we do not discount the possibility that sustained activation of the renin-angio-
tensin system and increased oxidative stress may be coupled with an attenuation of the increased transcrip-
tion as well as stabilization of extracellular superoxide dismutase RNA and may disrupt this balance.

It is notable that our results concur with a previous study by Stein and co-workers (16), who compared the forearm vascular response to incremental doses of methacholine in a small group (n = 7) of healthy male
subjects receiving a diet with a low- and high-sodium content for 5 days. In vascular function studies performed after the diet-intervention periods (i.e., 4 wk apart), no difference in the forearm blood flow response to methacholine was observed. However, due to the limited sample size, a β-error can hardly be excluded in that study. Moreover, the fact that no baseline study (before the diet intervention) was performed as well as the long time period between the two studies make the interpretation of the results even more difficult. To provide a more definite answer to the question whether a low-sodium diet affects endothelial-dependent vasodilation, in the current study we therefore included a substantially greater number of subjects to reduce the probability of a β-error and performed vascular function studies both before and after the low-sodium intervention.

The choice of losartan, a specific AT1-receptor blocker, rather than an ACE inhibitor in our study merits some comments. As noted above, clinical trials in humans have demonstrated that the ACE inhibitor quinapril reverses endothelial dysfunction in both peripheral conduit vessels (1) and epicardial coronary arteries (10) of patients with coronary artery disease. Interestingly, in a direct comparison of two ACE inhibitors, quinapril and enalapril, and one AT1-receptor blocker, losartan, only quinapril resulted in improved flow-mediated vasodilation as assessed by brachial artery ultrasound (1). In another study of patients with mild atherosclerosis (13), the ACE inhibitor enalapril seemed to abolish abnormal flow-mediated epicardial vasomotion in part by increasing endogenous bradykinin activity. Accordingly, we cannot exclude the possibility that in our study a different result would have been obtained if we had chosen to intervene with an ACE inhibitor rather than with losartan. However, the objective of our study was not to compare the effects of ACE inhibitors and AT1-receptor blockers but to elucidate the importance of angiotensin II in the development of impaired endothelium-dependent vasodilation in humans. In this context, the use of a specific receptor blocker was a more appropriate pharmacological tool than a substance with numerous non-angiotensin II-mediated actions that might affect vascular reactivity.

The study subjects in the current investigation were not randomly selected from the general population but were recruited by advertisements in local newspapers. Although it is possible that these subjects may have some characteristics that would differ from a randomly drawn sample (e.g., a high proportion of the subjects were students), we do not believe that these differences or the fact that small patient samples by chance can vary from the population average have introduced major bias in our study. In contrast to large-scale clinical trials where the generalizability issue is of major importance, in physiological studies like the current one, the main focus is on ascertaining that the study subjects recruited indeed are healthy. We believe that the thorough screening procedure and the fact that the study population was relatively young make it unlikely that our study subjects have asymptomatic vascular disease. Consequently, we believe that our results can be extrapolated to other young presumably healthy populations.

In summary, we have demonstrated that physiological stimulation of the renin-angiotensin system by ingestion of a low-sodium diet for 5 days does not cause impaired endothelium-dependent vasodilation in humans. Moreover, AT1-receptor blockade with losartan does not significantly affect endothelial function in healthy human subjects with a stimulated renin-angiotensin system. Our findings suggest that the pathophysiological significance of angiotensin-mediated superoxide production via stimulation of vascular NADH/NADPH-dependent oxidases may vary between species, but do not rule out the possibility that this mechanism may be of consequence in pathophysiological states characterized by increased oxidative stress in humans.

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