Neural and Hypotensive Effects of Angiotensin II Receptor Blockade

Gerard A. Rongen, Steven C. Brooks, Shinn-ichi Ando, Hilmi R. Dajani, Beth L. Abramson, John S. Floras

Abstract—Angiotensin II participates in the neural regulation of the heart and circulation at both central and peripheral sites. To explore the role of endogenous angiotensin II in blood pressure regulation, we conducted a randomized double-blind crossover trial in nine young healthy men (aged 33±3 [mean±SE] years) studied in the absence of salt restriction, comparing the effect of 1 week treatment with the angiotensin II receptor antagonist losartan (100 mg daily) against placebo with respect to the following variables, recorded during supine rest: intra-arterial blood pressure (BP), heart rate (HR), forearm vascular resistance and norepinephrine appearance rate, total body norepinephrine spillover, variability of BP and HR (spectral analysis), and baroreflex sensitivity for HR (gain of the transfer function from systolic BP to HR). Blood pressure was 119±7/66±4 mm Hg (systolic BP/diastolic BP) after 1 week of placebo and 112±6/61±3 mm Hg after 1 week of losartan (P<0.05). Forearm vascular resistance tended to fall, from 42±3±6 9 U on placebo to 32±8±5 0 U with losartan treatment (P=0.07). Losartan had no effect on HR (60±2±3 beats per minute with losartan), total body norepinephrine spillover (3±0.8±3±1 2 nmol/mm), forearm norepinephrine appearance rate (3±8±1 1 versus 5±3±1 1 pmol/100 mL forearm tissue per minute), power in the high- or low-frequency components of the HR variability and BP variability spectra or on baroreflex sensitivity for HR. Endogenous angiotensin II contributes to the maintenance of supine BP in normal subjects, studied in the absence of sodium restriction. The fall in BP caused by losartan is accompanied by a resetting of the baroreflex regulation of HR and sympathetic outflow, but baroreflex sensitivity for heart rate is not altered. Therefore, the reduction in BP observed after short-term angiotensin type 1 receptor antagonism may be achieved through a direct effect on vascular tone rather than through a primary reduction in sympathetic outflow (Hypertension. 1998;31[part 2]:378-383.)

Key Words: baroreceptor reflexes blood pressure heart rate variability humans losartan norepinephrine kinetics sympathetic nervous system

Angiotensin II can modulate the neural regulation of the circulation at both central and peripheral sites, but its role in the control of BP in normotensive subjects remains a subject of debate. Attenuation of angiotensin II formation by converting enzyme and renin inhibitors lowers the BP of patients with essential hypertension, but not of normotensive volunteers studied in the absence of salt restriction.

The development of AT1 receptor antagonists now provides a direct method of defining the role of angiotensin II in the regulation of normal BP. These drugs also lower the BP of patients with essential hypertension, but not of normotensive volunteers studied in the absence of salt restriction.2,3 These observations again suggest that angiotensin II may not be involved in BP regulation in healthy subjects in the absence of salt and/or fluid restriction. However, many of these studies in normotensive volunteers used single or low doses of antagonists or measured BP noninvasively. These limitations make interpretation of any negative observations rather difficult. Therefore, we conducted a double-blind, placebo-controlled crossover trial in normotensive volunteers studied without salt restriction to determine the effects of 1-week treatment with the angiotensin II AT1 receptor antagonist losartan on BP, HR, and FBF. To explore the possible modulation of the autonomic nervous system by endogenous angiotensin II, we quantified forearm and total body NE appearance rate using a radiotracer method. Variability of BP and HR was estimated by spectral analysis and baroreflex sensitivity for HR by the gain of the transfer function from systolic BP to HR. Our findings indicate that endogenous angiotensin II contributes to the maintenance of basal BP in healthy young men.

Methods

Subjects
Nine healthy nonsmoking men were studied. Their average age, weight, and height were 33±3 years, 78±2±1 kg, and 179±2±2.5 cm, respectively. This study protocol was in accordance with our institutional guidelines and approved by our university ethics committee. All subjects provided prior written informed consent.
Study Design
In this double-blind, randomized crossover trial, in the absence of dietary sodium restriction, the effects of 1 week of losartan (100 mg, once daily), were compared against those of 1 week of daily placebo. Each subject attended the laboratory four times, at 08:00 AM, after an overnight fast. The first and third visits were always on the 1st day of either placebo or losartan. In most volunteers, the first dose of the second treatment period was then given 24 hours after the last dose of the first treatment period. The aim of the first and third visits was to ensure that the first dose of each intervention was well tolerated. BP and HR were recorded with subjects in the supine position at 5-minute intervals by an automatic cuff recorder (Physio-Control Lifesat 200). After a 15-minute baseline, the first dose of either placebo or losartan was then given. A light breakfast was prepared, and BP and HR were recorded for an additional 2 hours. Aside from the breakfast period, when the head of the bed was elevated, subjects remained supine, while these measurements were obtained.

The second and fourth visits occurred at the end of each week of treatment. The purpose of these visits was to characterize the hemodynamic and neural responses to 1 week of losartan treatment. Subjects returned to the laboratory after 24 hours of caffeine abstinence. The last dose of placebo or losartan was then given. A deep antecubital vein of the dominant arm was cannulated retrogradely to avoid any potential effects of the intra-arterial cannulation and local anesthetic on the determination of forearm NE kinetics and FBF, venous blood and FBF measurements were acquired from the dominant arm, whereas arterial blood was sampled from the nondominant arm.

Analytical Methods
Blood samples for analysis of plasma NE were collected in prechilled tubes containing EDTA as an anticoagulant. The tubes were centrifuged at 4°C, and the plasma was separated and stored at −70°C. The concentration of NE and [3H]NE in all plasma samples and infusates was determined within 2 months of acquisition according to methods published previously by our group. The intra-assay coefficient of variation for endogenous NE was 1.9% (n=10), and the inter-assay coefficient of variation was 3.0%. The detection limit of the method was about 0.1 nmol/L, and peak area was linear from 0.1 to 50 nmol/L (n=8). Plasma renin activity was measured by the quantitation of generated angiotensin I (RIANEN angiotensin I [3H]munoassay kit, DuPont).

Drugs and Solutions
Trinitium-labeled NE was prepared as described previously by our group. Opaque gelatin capsules (no. 2, TUB Enterprises) containing either 100 mg of losartan (Cozaar, Merck Frosst Canada) or lactulose as the placebo were prepared by our pharmacy. The mean weight of 200 losartan 50-mg tablets was determined, and tablets were then triturated to a fine powder. An equivalent weight of two tablets of losartan was then transferred to these capsules. Patients were randomly assigned by pharmacy services to receive either losartan or placebo in a crossover design. The randomization code remained sealed until all data were collected and analyzed.

Data Collection and Statistical Analysis
During the first and third visits (first dose) BP and HR responses to placebo or losartan were calculated as differences from the average of all values acquired over the 15-minute baseline period. During the second and fourth visits (last dose), all signals (BP, ECG, and FBF) were recorded continuously onto paper and at the same time digitized at a sampling rate of either 200 Hz (BP and FBF) or 1000 Hz (ECG) and stored onto computer. Data-acquisition and analysis in the time and frequency domains were performed using LabVIEW Version 3.1 software (National Instruments). SBP, mean arterial pressure, and DBP for each cardiac cycle occurring during each FBF measurement were averaged to obtain a single value. Forearm vascular resistance (mean arterial pressure/PP, expressed as a resistance unit) was calculated for each flow measurement.

Arterial and venous concentrations of [3H]NE and NE were used for calculations of NE kinetics, as previously described. Total body NE spillover, i.e., the estimated rate of appearance of endogenous NE in arterial plasma, was derived from arterial plasma NE concentration (NEa), the arterial steady-state plasma concentration of [3H]NE ([3H]NEa), and the infusion rate of [3H]NE, according to the equation

\[ \text{NE}_a \text{ (pmol/mL)} \times \left( \frac{\text{Infusion rate (dpn/mm)}^-1}{[3H]\text{NE}_a \text{ (dpn/mL)}} \right) \]

and expressed as pmol/mm. The local forearm NE appearance rate (pmol/100 mL forearm tissue per minute) was estimated from the formula

\[ \text{[PPFX}(\text{NE}_a)^{\text{FPPX}(\text{NE}_a-\text{NE}_a)}^{\text{FPPX}(\text{NE}_a)}{\text{f}_{\text{BEF}}}^{10}) \]

where \( \text{f}_{\text{BEF}} \) is the fractional extraction of the tracer across the forearm, calculated as \([3H]\text{NE}_a - [3H]\text{NE}_a \text{, [3H]NE}_a \text{, and [3H]NEa, are the venous and arterial plasma concentration of [3H]NE, respectively, and FFP is the forearm plasma flow (ml/100 mL forearm per minute), i.e., FBF}(1-\text{hematocrit}) \).

A 10-minute section of the BP and HR acquisition period, free of extrasystoles or movement artifacts, was selected for fast Fourier transformation of R-R-intervals, SBP, and DBP Spectral power was calculated separately across three frequency bands 0 to 0.05 Hz (very low frequency), 0.05 to 0.15 Hz (low frequency), and 0.15 to 0.5 Hz (high frequency). SBP and DBP to HR power cross-spectra were constructed for each of these frequency bands. Power in the cross-spectrum was divided by power in the autospectrum of the BP interval (input) variable to derive gains of these transfer functions as an estimate of the arterial baroreflex control of HR.

Results appear as mean±SE. Effects of the first placebo and losartan dose on BP and HR were compared using an ANOVA for repeated
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Time course of BP and HR responses to the first dose of losartan and placebo. P values indicate the level of significance for the difference between the two curves.

Effect of Initial Dose on BP and HR
Six of these subjects were allocated to placebo first, followed by losartan. At baseline, BP was 114±4/68±4 mm Hg (SBP/DBP) before placebo and 119±3/73±4 mm Hg before losartan (placebo versus losartan; P=0.06 and P=0.05 for SBP and DBP, respectively). Mean arterial pressure before the first dose of losartan was 4.9 mm Hg higher than on the corresponding placebo day in the six subjects who received placebo first, and 4.1 mm Hg higher in the three who received losartan first. Baseline HR was 59±4 and 58±3 beats per minute before placebo and losartan, respectively (NS).

When expressed as changes from baseline and compared against placebo, there was a small but significant reduction in both SBP and DBP and a higher HR after ingestion of losartan (P<0.05 for all variables, see Fig).

Effect of 1-Week Treatment With Losartan on Hemodynamic and Neural Variables
Plasma renin activity was 0.3±0.1 ng/L per second during placebo and 2.3±1.0 ng/L per second on losartan (P<0.05; n=9), demonstrating successful interruption of the negative feedback of angiotensin II on renin release and indicating that the drug was effectively absorbed in all subjects. The low plasma renin activity during placebo treatment confirms that these volunteers were not salt-restricted.

After 1 week losartan reduced BP significantly, by approximately 5 to 7 mm Hg, but had no effect on HR (see Table 1). Losartan had no effect on arterial NE concentrations (1.2±0.3 versus 1.1±0.4 nmol/L on the placebo day; NS), or on total body NE spillover (3.0±0.8 versus 3.3±1.0 nmol/min; NS).

Oxygen saturation in deep antecubital venous blood was 50.7±2.6% in subjects receiving placebo and 54.6±2.8% with losartan (NS), indicating that the relative contribution of venous blood originating from skin and muscle did not differ between the two study days. Forearm vascular resistance tended to fall from 42.3±6.9 U on the placebo day, to 32.8±5.0 U on the losartan day (P=0.07). Forearm NE appearance rate was not affected significantly by losartan (3.8±1.1 versus 5.3±1.1 pmol/100 ml forearm tissue per minute on the placebo and losartan days, respectively (P=0.14).

One subject was excluded from power spectrum analysis because of frequently occurring extrasystoles, which precluded the selection of a stable recording period of sufficient duration. Losartan had no effect on the variability of HR or BP in the remaining eight subjects (Table 2). Gains of the transfer functions between the BP and HR spectra were also similar on the placebo and losartan days (Table 3).

| TABLE 1. Hemodynamic and Noradrenergic Effects of 1-Week Treatment with Losartan |
|------------------|------------------|------------------|
| Variable         | Placebo          | Losartan         |
| Systolic blood pressure, mm Hg | 119±2±6.7       | 112±1±5.7       |
| Mean arterial blood pressure, mm Hg | 86±0±5.1       | 80±1±4.6       |
| Diastolic blood pressure, mm Hg | 65±9±3.8       | 60±8±3.4       |
| Heart rate, bpm | 59±7±2.9       | 58±8±2.1       |
| Forearm blood flow, ml/100 ml forearm tissue per minute | 25±0.3         | 29±0.3         |
| Forearm vascular resistance, U | 42±3±6.9       | 32±8±5.0†      |
| Forearm norepinephrine appearance rate, pmol/100 ml forearm per minute | 3.8±1.1        | 5.3±1.1        |
| Arterial norepinephrine concentration, nmol/l | 11±0.4         | 12±0.3         |
| Total body norepinephrine spillover, nmol/min | 3.0±0.8        | 3.3±1.0        |

n=9, mean±SE

*Statistically significant difference between placebo and losartan (P<0.05)
†0.05<P<0.10 for comparison between placebo and losartan

| TABLE 2. Power Spectrum Analysis of BP and HR |
|------------------|------------------|------------------|
| Frequency Band   | Placebo          | Losartan         |
|                  | HR (ms²)         | DBP (mm Hg²)     | SBP (mm Hg²)     |
| VLF              | 3633±7±1135.5    | 3.9±0.9          | 52±1±13          | 48±2±8          |
| LF               | 3672±4±1575.7    | 4.1±0.8          | 61±1±11          | 66±5±0          |
| HF               | 2615±7±1036.6    | 11±0.3           | 23±0±6           | 24±0±3          |
| LF/HF            | 21±0.7           | 4.8±0.8          | 37±0.9           | 33±0±6          |

n=8, mean±SE. VLF indicates very-low-frequency band (0 to 0.05 Hz), LF, low-frequency band (0.05 to 0.15), and HF, high-frequency band (0.15 to 0.5 Hz). Losartan had no significant effect on any of these variables.
Discussion

The purpose of this study was to determine whether endogenous angiotensin II participates in the maintenance of supine BP in healthy young men. Our objectives were, first, to determine the effect of angiotensin II AT₁ receptor blockade on the BP of healthy volunteers studied without dietary salt restriction and, second, to determine whether any change in BP was accompanied by alterations in the neural regulation of the heart or peripheral circulation or of forearm vascular tone. The radionuclide technique, which permits simultaneous estimation of forearm and total body NE release, was used to quantify the sympathoneural action of losartan.

When compared with placebo, the first dose of losartan caused a modest but significant reduction in SBP and DBP and a significant, presumably baroreflex-mediated, increase in HR. In contrast, after 1 week of losartan, significant reductions in SBP and DBP of greater magnitude were not accompanied by any detectable changes in HR, total body spillover, or forearm NE appearance rate within this sample size of nine subjects. These findings indicate first that endogenous angiotensin II contributes to the maintenance of supine BP in normal subjects, studied in the absence of sodium restriction and second that losartan reset the arterial baroreflex regulation of HR and sympathetic outflow to the lower prevailing level of BP. This concept is supported by experimental data. Forearm NE appearance rate was not reduced by losartan (and indeed tended to rise), yet there was a trend toward lower forearm vascular resistance, indicating that the vasodilator action of losartan is not mediated by attenuation of NE release from sympathetic nerve endings.

The gain of the transfer function relating BP to pulse interval as assessed by spectral analysis was unaltered, indicating that this resetting is not accompanied by any change in baroreflex sensitivity for HR. The differing HR responses on the 1st and 7th day suggest that such resetting does not occur fully after the first dose of losartan. This dissociation may be related to the time course over which orally administered losartan or its active metabolite binds to angiotensin AT₁ receptors at sites involved in cardiovascular regulation both within and outside the blood-brain barrier. It could be argued that this aspect of our observations should be interpreted cautiously, because the true magnitude of differences between responses to the first dose of placebo and losartan may have been obscured by a carryover effect of losartan in a few subjects, as there was no fixed washout period between the two parts of this study. However, this would seem unlikely in that BP did not rise over time in placebo-treated patients (Fig), the number of subjects in whom a carryover effect might be present was small (three of nine), the differences in baseline values on the placebo and losartan days were independent of the order in which these capsules were administered, and the half-life in healthy volunteers of losartan (1.3 to 2.2 hours) and its rapidly appearing active metabolite (4.4 to 6.4 hours) are substantially less than 24 hours.

Taken together, these observations indicate that the hypertensive effect of AT₁ receptor blockade in healthy normotensive subjects is mediated through a tonic action on vascular tone in skeletal muscle and in other hemodynamically significant vascular beds, rather than a primary reduction in sympathetic tone. An effect of losartan on salt and water excretion in these salt-repleted subjects is unlikely. A reduction in vascular tone may be achieved by blockade of AT₁ receptors on smooth-muscle cells that cause vasoconstriction, by antagonism of AT₁ receptors on endothelial cells that release contracting factors, such as endothelin-1, when stimulated, or by inhibition of angiotensin II-induced formation of vascular superoxide in the vascular wall. This could subsequently deactivate nitric oxide, a substance with a tonic vasodilator action. The latter possibility is supported by an animal study in which the coronary vasodilator effect of losartan could be partially blocked by the nitric oxide synthase inhibitor N⁵-nitro-L-arginine-methyl ester.

This demonstration of a significant effect of specific blockade of the renin-angiotensin system on BP in healthy volunteers, studied in the absence of salt restriction, contrasts with previous studies of losartan in healthy volunteers, in which BP fell only when the renin-angiotensin system was activated by sodium depletion. Several characteristics of our experiment may account for this. In contrast to previous groups, we recorded BP invasively, thereby reducing possible measurement errors. We administered 100 mg of losartan, a dose shown to inhibit the forearm vasoconstrictor response to intra-arterially infused angiotensin II. It is not known whether a 50-mg dose, as given in previous studies, also abolishes this response to angiotensin.

AT₁ receptors are present within the central nervous system, sympathetic ganglia, and sympathetic nerve endings. Depending on the experimental preparation and species studied, the

### Table 3. Gains of the Transfer Functions between BP and HR

<table>
<thead>
<tr>
<th>Frequency Band</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Losartan</td>
</tr>
<tr>
<td>VLF (0-0.05 Hz)</td>
<td>11.4 ± 1.8</td>
<td>10.4 ± 1.7</td>
</tr>
<tr>
<td>(0.24 ± 0.05)</td>
<td>(0.22 ± 0.03)</td>
<td>(0.32 ± 0.05)</td>
</tr>
<tr>
<td>LF (0.05-0.15 Hz)</td>
<td>22.4 ± 4.8</td>
<td>19.6 ± 3.4</td>
</tr>
<tr>
<td>(0.72 ± 0.05)</td>
<td>(0.70 ± 0.05)</td>
<td>(0.72 ± 0.04)</td>
</tr>
<tr>
<td>HF (0.15-0.5 Hz)</td>
<td>21.6 ± 5.1</td>
<td>17.9 ± 4.0</td>
</tr>
<tr>
<td>(0.45 ± 0.03)</td>
<td>(0.44 ± 0.04)</td>
<td>(0.68 ± 0.07)</td>
</tr>
</tbody>
</table>

n = 8, mean ± SE. The values in parentheses indicate the mean values for coherence between power spectra of BP and HR across the entire frequency band.
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central nervous system administration of angiotensin II will increase sympathetic outflow and inhibit vagal nerve firing. Application of angiotensin II into sympathetic ganglia increases the firing rate of postganglionic nerves,29 infusion of angiotensin II raises muscle sympathetic nerve activity in humans,29 and stimulation of prejunctional angiotensin II receptors on sympathetic nerve endings facilitates NE release.30 Despite this ubiquitous involvement of central and peripheral AT1 receptors in the neural control of the heart and circulation,1 losartan had no effect on total body NE spillover, forearm NE release, or frequency domain estimates of parasympathetic and sympathetic neural modulation of splanchnic discharge (although longer recording periods may be needed to detect any potential effect of AT1 receptor antagonism on the very-low-frequency components of HR and BP' variability). Nor was the gain of baroreflex sensitivity for HR, which reflects primarily the parasympathetic efferent limb of the arterial baroreflex, affected by this intervention.

On reviewing the literature on interactions between angiotensin II, the sympathetic nervous system, and baroreceptor reflexes in the regulation of BP, Reid3 concluded that these potential sympathoexcitatory actions do not contribute significantly to the pressor response to angiotensin II. The overall lack of any impact of losartan on these neural variables in these healthy men studied supine under conditions of routine unrestricted sodium intake may be related to their low plasma renin activity, as documented on the placebo day. The effects of endogenous angiotensin II on vascular tone may assume greater importance with respect to BP and HR regulation than angiotensin-autonomic nervous system interactions under conditions, such as those, when the renin-angiotensin system, the sympathetic nervous system, or both are relatively quiescent, and arterial baroreflex regulation of HR is intact. In contrast, interactions between endogenous angiotensin II and sympathetic and parasympathetic outflow are more readily observed in disease states in which both the renin-angiotensin and the adrenergic nervous systems are activated, such as accelerated hypertension31 and congestive heart failure.32

The lack of HR response to a fall in BP after long-term treatment with angiotensin-converting enzyme inhibition has been attributed to a decrease in baroreflex sensitivity, a resetting of the baroreceptor reflex control of HR, or a combination of these two effects.1 Our results would suggest that AT1 receptor blockade resets the baroreflex control of HR without altering the gain of the relationship between changes in systolic BP and pulse interval.

The principal limitation to this study is that we did not compare responses to losartan with those of a direct vasodilator, free of any modulating influence on autonomic outflow (eg, hydralazine), to determine whether the magnitude of the resetting of the baroreceptor control of HR, and sympathetic nervous system activity over time is more pronounced (and HR, and noradrenergic responses attenuated) when BP is lowered by means of AT1 receptor blockade.

This study demonstrates that angiotensin II receptor subtype 1 blockade can reduce BP in healthy young men, studied in the absence of sodium restriction. This observation indicates that angiotensin II contributes to the maintenance of supine BP in normal subjects. This modest hypotensive action occurred without changes in HR or total body NE spillover, suggesting that the arterial baroreflex may have reset with time to the lower prevailing level of BP. In the absence of corresponding reductions in sympathetic outflow, such resetting cannot be considered the primary mechanism by which losartan exerts its hypotensive action in healthy humans.

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