Losartan Blocks Aldosterone and Renal Vascular Responses to Angiotensin II in Humans

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Abstract

In vitro and animal studies have demonstrated that the effect of angiotensin II (Ang II) on aldosterone is mediated through the Ang II type 1 receptor. However, it has been difficult to demonstrate an effect of Ang II type 1 receptor blockade on aldosterone levels in human studies. One possible explanation is that subjects have not been studied under salt-controlled conditions. Therefore, we examined the effects of losartan on the aldosterone and renal plasma flow responses to Ang II infusion in six normotensive subjects under low and high salt conditions. Ang II was infused in graded doses (0.3 to 10 ng/kg per minute) in the presence and absence of losartan (a single 50-mg oral dose). Renal plasma flow was assessed by measurement of para-aminohippurate clearance. Blood pressure, plasma aldosterone levels (low salt conditions only), and para-aminohippurate clearance were measured before and after each Ang II dose. Losartan had no effect on baseline systolic pressure but attenuated the systolic pressure response to exogenous Ang II during both low salt (0.7±1.9 versus 6.7±1.4 mm Hg, P<.001) and high salt (2.0±1.9 versus 12.3±2.1 mm Hg, P=.006) conditions. Under low salt conditions, losartan reduced the baseline plasma aldosterone level from 1135±204 to 558±102 pmol/L (P=.015) and blocked the aldosterone response to Ang II (−49±110 versus +436±83 pmol/L, P=.019). During high salt conditions, losartan had no effect on baseline renal plasma flow but attenuated the renal plasma flow response to Ang II (−90.1±15.1 versus −185.1±2.6 mL/min per 1.73 m², P=.013). These data confirm that losartan lowers both basal and exogenous Ang II–stimulated aldosterone levels under low salt conditions. Losartan does not significantly affect baseline renal plasma flow but does attenuate the renal plasma flow response to exogenous Ang II under high salt conditions.

Key Words: losartan • angiotensin II • receptors, angiotensin • aldosterone • renal circulation

Introduction

Losartan and other specific AT₁ receptor antagonists have been used to delineate the physiological role of Ang II in vivo. In vitro and animal studies have demonstrated that the effects of exogenous Ang II on blood pressure and aldosterone secretion are mediated through the AT₁ receptor. For example, losartan administration antagonizes salt depletion–stimulated increases in aldosterone–synthase mRNA and plasma aldosterone in hypertensive rats. Losartan also attenuates the effect of exogenous Ang II on blood pressure and aldosterone in rats, dogs, and nonhuman primates. In humans, losartan has been shown to block the pressor response to both exogenous Ang I and Ang II; however, it has been difficult to demonstrate a consistent effect of AT₁ receptor blockade on aldosterone levels in normotensive human volunteers. Other possible confounding factors include a limited duration of adrenal AT₁ receptor blockade and inadequate adrenal levels of losartan.

An important possible reason why previous studies have demonstrated little effect of losartan on aldosterone levels is that subjects have not been studied under salt-controlled conditions. Ang II is hypothesized to be the primary...
regulator of aldosterone secretion in response to changes in volume or salt status.\textsuperscript{21, 22} Salt depletion stimulates Ang II-mediated aldosterone secretion.\textsuperscript{23, 24} In addition, the renin status of the subject may influence the effect of losartan on aldosterone levels. Normal- or high-renin subjects may be more sensitive to the effects of AT\textsubscript{1} blockade than low-renin subjects.\textsuperscript{8, 16, 17}

In this study, we aimed to determine the effect of losartan on aldosterone levels in normal- to high-renin normotensive subjects under conditions in which endogenous Ang II levels are increased, namely, salt depletion. Furthermore, we measured the effect of losartan on the aldosterone response to stimulation by exogenous Ang II infusion. Finally, both the aldosterone response to Ang II under salt-depleted conditions and the renovascular response to exogenous Ang II under salt-replete conditions have been used to characterize a group of individuals with normal- to high-renin essential hypertension, called non-modulators.\textsuperscript{25, 26} Non-modulators are resistant to the effects of exogenous Ang II; it is not known whether this resistance reflects increased Ang II with AT\textsubscript{1} receptor downregulation or an intrinsic abnormality of the AT\textsubscript{1} receptor. Depending on the mechanism, differences in the potency of AT\textsubscript{1} receptor blockade may prove useful in distinguishing non-modulators from individuals with modulating hypertension. For this reason, in the present study, we set out to define not only the effect of losartan on the aldosterone response to Ang II under salt-depleted conditions but also the effect of losartan on the renovascular response to Ang II under salt-replete conditions in normal human control subjects.

Methods

Subjects

Six healthy normal- to high-renin normotensive men were studied in a crossover design study (Fig 1) in the outpatient and inpatient divisions of the Vanderbilt University Hospital Clinical Research Center. Subjects underwent an initial history, physical examination, electrocardiogram, and laboratory screen. All had no history or evidence of cardiovascular, endocrine, or renal disease. All were within 20\% of ideal body weight. Subjects were defined as normal- or high-renin normotensive if they had an upright plasma renin activity of at least 2.4 ng/mL per hour when in balance on a diet of 10 mmol sodium per day (day 5, see Fig 1 and Table) as defined by previously established criteria.\textsuperscript{27-30} Subjects with a positive family history of hypertension were excluded. All subjects gave written, informed consent; the study protocol was approved by the Institutional Review Board of Vanderbilt University.

Protocol

Fig 1 outlines the study protocol. Subjects first ingested a xanthine-free diet with 10 mmol sodium, 100 mmol potassium, and 2500 mL fluid per day for 9 days. Subjects collected their urine each day for measurement of sodium, potassium, and creatinine. On the morning of the 5th day, blood for upright plasma renin activity was obtained after 30 minutes of upright posture. Significant increases in plasma renin activity occur within 20 minutes of assumption of upright posture in normal- to high-renin individuals.\textsuperscript{30} No potential subjects were excluded by a low-renin state. The subjects were admitted to the Clinical Research Center on the evening of the 5th day. Urine collections and the diet were continued.

Each salt-depleted subject’s aldosterone and RPF responses to acute Ang II infusion were measured on the morning of the 8th study day. Subjects were kept supine and fasting from after midnight of the night before. At 7 AM, an intravenous catheter was placed in each arm. One catheter was used for drug
infusion and the other for venous sampling. Blood pressure and pulse were measured every 2 minutes throughout the study with an automated blood pressure cuff (Dinamap, Critikon). At 7:30 AM, blood was drawn for baseline PAH and aldosterone levels. At 8 AM, the subject was given an 8 mg/kg loading dose of PAH followed by a constant infusion at 12 mg/min. At 9 AM, the subject was given a continuous infusion of Ang II at successive doses for 45 minutes each. The first three subjects were given doses of 0.3, 1, and 3 ng/kg per minute during both the low salt and high salt diets; to maximize the effect of Ang II, the last three subjects were given doses of 1, 3, and 10 ng/kg per minute. Blood samples for PAH were drawn at 8:45, 8:50, and 8:55 AM and then after each successive dose of Ang II. Blood for aldosterone was drawn before and after each successive dose of Ang II during the low salt phase of the study. The subject remained supine for the duration of the Ang II infusion.

The effect of losartan on the aldosterone and RPF responses to Ang II during low salt conditions was studied on the 9th study day. Subjects were kept supine and fasting from the night before. At 6 AM, the subject was given a 50-mg oral dose of losartan. The acute Ang II infusion was then repeated as outlined above. Thus, 3 hours elapsed between the oral administration of losartan and the start of the Ang II infusion. This is approximately the time required to achieve peak levels of E-3174, the primary pharmacologically active metabolite of losartan.

Immediately after completion of the Ang II infusion on day 9, subjects were switched to a xanthine-free diet of 200 mmol sodium, 100 mmol potassium, and 2500 mL fluid per day for 6 days. Daily urine collections were continued. On the 12th study day, the RPF response to intravenous Ang II infusion was measured. Loading doses and maintenance infusions of PAH were given as before. On day 14, measurement of the RPF response to acute Ang II infusion was repeated in the presence of losartan.

Analytic Methods

Urinary electrolytes were measured by flame photometry. All blood samples were collected on ice, spun, and frozen until the time of assay. Plasma renin activity was measured by radioimmunoassay for Ang I. Aldosterone was measured with a radioimmunoassay kit from Diagnostic Products Corp. RPF was measured by determination of PAH clearance from plasma. When corrected for body surface area, PAH clearance reflects about 90% of RPF for the plasma concentrations of PAH achieved during this study. Plasma PAH levels were measured by spectrophotometric autoanalyzer techniques. PAH clearance was determined as previously described. The coefficient of variation for PAH clearance in our laboratory is 3.91 ± 3.53% (SD). RVR was calculated as the ratio of mean arterial pressure to RPF.

Statistical Analysis

Results are presented as mean ± SE. Statistical analysis was performed with the SPSS Advanced Statistics program (SPSS Inc). The angiotensin dose–response curves in the presence and absence of losartan were compared by two–way ANOVA with repeated measures in which the within–subject variables were Ang II dose, the presence or absence of losartan, and salt condition. Comparisons between treatment arms at specific Ang II doses were made with a two–tailed, paired Student’s t test. Probability values reported are from the t tests unless indicated otherwise. The criterion for significance was a value of P < .05.

Results

The upright plasma renin activity of the subjects in the salt–depleted state was 7.61 ± 1.88 ng Ang I/mL per hour. Baseline characteristics of the subjects on each study day before initiation of Ang II infusion are shown in the Table. Losartan had no effect on baseline systolic pressure, diastolic pressure, or RPF in either the salt–depleted or salt–replete state. Although the relatively small sample size of this study may have limited our ability to detect a significant effect of losartan on baseline RPF under salt–depleted conditions, our findings were similar to those of two previous larger studies. Under salt–depleted conditions, losartan significantly lowered baseline aldosterone level from 1135 ± 204 to 558 ± 102 pmol/L (P = .015). In the absence of losartan, exogenous Ang II significantly increased blood pressure (Fig 2). The blood pressure response to exogenous Ang II was significantly greater in the salt–replete state than in the salt–depleted state (ANOVA, P = .002). Losartan administration dramatically attenuated the pressor response to Ang II in both
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The aldosterone response to Ang II infusion was measured in the salt-depleted state. After an initial decrease in aldosterone, Ang II caused a significant rise in aldosterone compared with baseline. The increment change in aldosterone was similar to that reported for salt-depleted normotensive subjects in other studies. The addition of losartan dramatically attenuated the aldosterone response to exogenous Ang II (Fig 3, ANOVA, \( P < .001 \)).

Dietary sodium modifies the aldosterone and renovascular responses to Ang II in opposite directions. Thus, whereas salt depletion accentuates the aldosterone response to Ang II, the renovascular response to Ang II is greater in the salt-replete state. As illustrated in Fig 4, RPF, as measured by PAH clearance, decreased significantly in response to exogenous Ang II (ANOVA, \( P < .001 \)), and this decrease was greater in the salt-replete than in the salt-depleted state (ANOVA, \( P < .05 \)). The magnitude of the decrease in RPF was similar to that reported in previous studies of normotensive subjects. RVR (Fig 5) increased significantly in response to exogenous Ang II (ANOVA, \( P < .001 \)). This response was also greater during salt repletion (ANOVA, \( P < .05 \)). Losartan significantly attenuated but did not abolish the RPF and RVR responses to Ang II (ANOVA, \( P < .02 \)). The magnitude of this losartan effect was similar under both salt conditions.
Both the adrenal response to Ang II in the salt-depleted state and the renovascular response to Ang II in the salt-replete state have been used to characterize non-modulators. Both the aldosterone and renovascular responses to Ang II are highly reproducible within subjects. Moreover, the increment in aldosterone in subjects ingesting a diet of 10 mmol sodium has been reported to correlate significantly with the decrement in PAH clearance in the same subjects ingesting a diet of 200 mmol sodium. Fig 6 illustrates the correlation between the change in PAH clearance in the salt-replete state and the change in aldosterone in the salt-depleted state. In the absence of losartan, there was a highly significant correlation between the aldosterone and renovascular responses to exogenous Ang II ($P < .005$). The addition of losartan abolished this relationship. These data may reflect the fact that although not significant, losartan tended to have a greater effect on the aldosterone response to Ang II than on the renovascular response. For example, at the highest doses of Ang II (3 and 10 ng/kg per minute), losartan blocked the aldosterone response to ~11% of baseline, whereas losartan decreased the renovascular response to 40% of baseline ($P = .089$ for the effect of losartan on the aldosterone response in the salt-depleted state versus the effect of losartan on the renovascular response in the salt-replete state).
Discussion

This study is the first to examine the effect of acute administration of the specific AT₁ receptor antagonist losartan on aldosterone under salt-depleted conditions and in response to exogenous Ang II. Losartan significantly decreased baseline plasma aldosterone levels and completely blocked the effect of Ang II on aldosterone.

Despite the fact that in vitro and animal studies indicate that the AT₁ receptor mediates the steroidogenic effect of Ang II, it has been difficult to demonstrate a significant effect of AT₁ receptor blockade on aldosterone secretion in previous studies of normotensive humans. This apparent lack of effect of losartan on aldosterone levels has been attributed to a confounding diurnal variation in aldosterone levels. In prior studies, aldosterone levels decreased significantly after acute losartan administration but decreased to a similar magnitude under control conditions. Such a diurnal fall in aldosterone levels was suggested in the present study by the finding that on the control day, plasma aldosterone levels appeared to decrease at the lowest Ang II dose before increasing in response to higher doses. Interestingly, this initial fall was not seen in the presence of losartan.

One striking difference between the present study and prior studies in normotensive subjects that failed to show an effect of losartan on aldosterone is the fact that in the present study, subjects were studied while in balance on a diet of 10 mmol sodium per day. Although factors regulating aldosterone synthesis include Ang II, corticotropin, and potassium, Ang II is the primary stimulant of aldosterone secretion in response to changes in salt or volume status. Conditions of salt depletion increase the adrenal sensitivity to exogenous Ang II. Among the prior studies, only Burnier et al and Tsunoda et al controlled for sodium intake, studying subjects ingesting diets of 50 mmol and 10 g (435 mmol) sodium per day, respectively. In the present study, because the aldosterone response to losartan was measured only during salt depletion, we cannot exclude the possibility that losartan also lowers aldosterone during high salt intake.

Although the majority of investigators have found no aldosterone response to acute administration of losartan, four groups have reported a significant decrease in plasma aldosterone after chronic losartan administration, although two of the four studies were not controlled. One of the four groups also reported an acute effect of 100 mg losartan, but not 25 mg, on plasma aldosterone. Interestingly, in addition to studying chronic doses of losartan, all four of these investigations studied hypertensive individuals. Hypertensive subjects may be more sensitive to the aldosterone-lowering effects of losartan than normotensive subjects. Normal- or high-renin hypertensive subjects have previously been shown to have an enhanced aldosterone response to exogenous Ang II compared with normotensive subjects. An increased sensitivity to losartan has been demonstrated in spontaneously hypertensive rats compared with normotensive Wistar-Kyoto rats. Hypertensive individuals may also have greater adrenal AT₁ receptor sensitivity to AT₁ blockade. Comparison of the aldosterone response to losartan between hypertensive and normotensive subjects in salt-depleted and salt-replete conditions may help elucidate this possible difference. Gottlieb et al reported a significant decrease in plasma aldosterone after acute administration of losartan in individuals with congestive heart failure. These subjects may have increased sensitivity to AT₁ receptor blockade resulting from the activation of the renin–angiotensin axis observed in heart failure.

The present study is the first to examine the effect of losartan on the aldosterone response to continuous infusion of exogenous Ang II. The aldosterone response to Ang II has been used to characterize patients with essential hypertension, with a normal response defined as an increase in aldosterone of at least 15 ng/dL in response to a continuous infusion of 3 ng/kg per minute Ang II during salt depletion. All six subjects studied exhibited a normal response to Ang II. Christen et al and Munafo et al have measured the effect of losartan on the pressor response to boluses of Ang I and Ang II, respectively. Although plasma aldosterone levels were measured sequentially after losartan in these studies, the aldosterone response to Ang I or Ang II boluses was not specifically ascertained.
The aldosterone response to exogenous Ang II during salt-depleted conditions and the RPF response to Ang II under salt-replete conditions have been used to define a subset of individuals with normal- or high-renin hypertension. These non-modulators exhibit decreased sensitivity to exogenous Ang II, and this decreased sensitivity may reflect an abnormality, either intrinsic or related to desensitization, in the AT\textsubscript{1} receptor. Since comparison of the effects of AT\textsubscript{1} blockade with losartan may prove useful in distinguishing normotensive individuals and modulators from non-modulators, we measured the effect of losartan on the RPF response to Ang II infusion. Similar to the findings of Burnier et al.,\textsuperscript{3,36} we found no significant difference in baseline RPF after AT\textsubscript{1} receptor blockade in normotensive subjects. Also as reported previously, the renal vasoconstrictor response to exogenous Ang II was greater during salt repletion than during salt depletion.\textsuperscript{27} Losartan significantly attenuated the renal constrictor response to Ang II, and the shifts in the Ang II-RPF and Ang II-RVR dose–response curves were similar under low and high salt conditions. In contrast to the aldosterone response to Ang II during salt depletion, losartan did not completely block the renal vasoconstrictor response to Ang II during salt repletion. Although one possible mechanism for this differential effect of AT\textsubscript{1} receptor blockade on the aldosterone and renal vasoconstrictor responses to exogenous Ang II could be differences in plasma concentrations of losartan and its active metabolite under low and high salt conditions, the lack of an effect of salt balance on the effect of AT\textsubscript{1} receptor blockade of the pressor response to Ang II does not support this possibility. Differences in the renal and adrenal predominance of AT\textsubscript{1} receptor subtypes may also underlie the differences in AT\textsubscript{1} receptor blockade. In rats, the AT\textsubscript{1A} receptor is the predominant subtype expressed in the kidney. In contrast, the AT\textsubscript{1B} subtype is predominant in the rat adrenal gland.\textsuperscript{40,41} There may be differences in the degree of receptor blockade achieved with losartan between subtypes, although human data are thus far lacking. Further studies are needed to characterize the relative potency of AT\textsubscript{1} receptor antagonists for blocking the adrenal and renal vascular responses to Ang II.

In summary, this study demonstrates an unequivocal effect of acute losartan administration on Ang II–stimulated aldosterone secretion under salt-depleted conditions. Losartan attenuates the renal vasoconstrictor response to Ang II. In future studies, the potency of losartan in blocking the adrenal and renal hemodynamic responses to exogenous Ang II may be used to further characterize subgroups of individuals with essential hypertension.

**Selected Abbreviations and Acronyms**

Ang I, II = angiotensin I, II  
AT\textsubscript{1} = angiotensin type 1 (receptor)  
PAH = para-aminohippurate  
RPF = renal plasma flow  
RVR = renal vascular resistance

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**Footnotes**

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