Failure of angiotensin II to suppress plasma renin activity in normotensive subjects with a positive family history of hypertension

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ABSTRACT
The renin–angiotensin system is implicated in the pathophysiology of hypertension. Renin release is regulated by a number of factors, including circulating Ang II (angiotensin II), the so-called short feedback loop. The aim of the present study was to investigate the responsiveness of circulating Ang II on PRA (plasma renin activity) in normotensive subjects with a PFH or NFH (positive or negative family history of hypertension respectively). PRA, renal haemodynamics and urinary sodium excretion were measured during infusion of Ang II without and with pretreatment with the AT1 (Ang II type 1) receptor blocker irbesartan. Normotensive men with a PFH (n = 13) and NFH (n = 10), with a mean age of 38 years, were given on different occasions intravenous Ang II infusions of 0.1, 0.5 and 1.0 ng·kg⁻¹ of body weight·min⁻¹ before and after pretreatment with 150 mg of irbesartan once a day for 5 consecutive days. RPF (renal plasma flow) and GFR (glomerular filtration rate) were also measured. Before Ang II infusion, the PFH and NFH groups did not differ with respect to BP (blood pressure), body mass index, PRA, RBF (renal blood flow) or urinary sodium. There was no difference in BP or renal haemodynamic response to the highest Ang II dose between the groups. PRA declined with the highest Ang II dose (P < 0.01) in subjects with a NFH, but not in subjects with a PFH. After treatment with irbesartan when Ang II had no effect on BP in either group, Ang II also suppressed PRA in subjects with a PFH (P < 0.01), and the difference between the groups at baseline was thus eliminated. In conclusion, these findings indicate that subjects with a PFH have a defective Ang II suppression of PRA, which is corrected by AT1 receptor blockade.

INTRODUCTION
The heredity of essential hypertension is well established based on epidemiological surveys and on analysis of data obtained from twin models [1]. When studying the pathophysiological mechanisms in the development of hypertension it is therefore important to investigate relatives of subjects with hypertension. Such studies have been rewarding and have demonstrated that normotensive first-degree relatives of hypertensives differ from first-degree relatives of normotensives in several ways. Subjects with a PFH (positive family history of hypertension) have a blunted natriuretic response to a saline load [2,3]. The renal tubules show an increased response to insulin [4] and a less pronounced effect of L-arginine in terms of sodium and water reabsorption compared with relatives of normotensives [5]. We have shown previously [6] that these individuals also have an enhanced sensitivity

Key words: angiotensin II, hypertension, plasma renin activity, renal blood flow, renal haemodynamics, sodium excretion.
Abbreviations: Ang II, angiotensin II; AT1, Ang II type 1; ARB, AT1 receptor blocker; AT2, Ang II type 2; BMI, body mass index; BP, blood pressure; DBP, diastolic BP; GFR, glomerular filtration rate; NFH, negative family history of hypertension; PAH, p-aminohippuric acid; PFH, positive family history of hypertension; PRA, plasma renin activity; RBF, renal blood flow; RPF, renal plasma flow; SBP, systolic BP.
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in the renal vasculature to low doses of infused Ang II (angiotensin II). In addition, we have recently observed [7] that the renal vascular sensitivity to Ang II in first-
degree relatives of hypertensives is increased due to a
defective prostaglandin response.

Abnormalities in the renin–angiotensin system may
thus be involved in the pathophysiology of hypertension. These abnormalities may modify renal function in several
ways, i.e. by modulating renal perfusion, GFR (glomer-
ular filtration rate) and tubular sodium reabsorption [8,9].
Circulating Ang II itself exerts a negative feedback on
renin release from the juxtaglomerular apparatus. This
negative feedback control mechanism is also called the
short feedback loop of renin secretion control.

The internal ‘servocontrol’ of renin secretion has been
well documented in humans [10]. In the studies by
Hollenberg and co-workers [11,12], it was demonstrated
that the negative feedback control of renin is abnormal
in a number of hypertensives, a group of patients they
named the non-modulators, because they could not cope
with or handle a salt load normally. They also showed that
treatment with an ACE (angiotensin-converting enzyme)
inhibitor rapidly restored the capacity of the kidney to
handle a salt load and also normalized BP (blood press-
ure) [13]. Therefore the aim of the present study was
to investigate the negative feedback control of renin in
normotensive subjects without or with a PFH and the
effect of AT1 (Ang II type 1) receptor blockade.

METHODS

Study group

This study was approved by the Ethics Committee of
the Faculty of Medicine, Göteborg University, Göteborg,
Sweden, and informed consent was obtained prior to
the investigation from all subjects. Twenty-three young
normotensive men, mean age 38 years, without (n = 13)
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the investigation from all subjects. Twenty-three young
normotensive men, mean age 38 years, without (n = 13)
or with (n = 10) a PFH were included.

A PFH was defined as a father or mother with at least
5 years of treatment for primary hypertension requiring
three or more antihypertensive drugs, or who was treated
with two antihypertensive drugs and had a DBP (diastolic
BP) above 100 mmHg. Most of the subjects with a PFH
were recruited from the relatives of hypertensive patients
at our outpatient hypertension unit. None of the subjects
with a PFH received any medication or had ever been on
antihypertensive treatment, neither did they have diabetes
mellitus, renal disease or any other chronic disease.

A NFH (negative family history of hypertension) was
defined as both father and mother normotensives. Subjects
with a NFH were matched for age and BMI (body mass index). They were mainly recruited from
relatives of the normotensive patients included in the
Gothenburg Preventive Trial [14]. Three of them were
recruited via advertising in the local daily newspaper.

All parents of the study participants underwent an
interview and a physical examination before their sons
were accepted in the NFH group.

It should be noted that the limited number of subjects
that could be recruited for the present study may some-
what curtail the statistical power.

Study protocol

Before inclusion in the present study, all subjects
underwent a routine physical examination, including BP
measurement. They were told to continue their ordinary
lifestyle and avoid changes in food intake, alcohol con-
sumption and exercise. After on overnight fast, they
arrived at the Research Laboratory at 07.30 hours,
and the experimental procedure was started at approx.
08.00 hours. Throughout the investigation, the patients
remained in a comfortable semirecumbent position
(except when voiding). The two infusion studies of low
doses of Ang II (0.1, 0.5 and 1.0 ng · kg⁻¹ · min⁻¹) were carried out at least 2 weeks apart before and
after treatment with the ARB (AT1 receptor blocker)
irbesartan (150 mg daily for 5 consecutive days).

BP, heart rate and body weight

Indirect BP at rest was measured after 45 min.
Throughout the examination, BP was measured in a semi-
 recumbent position. A rubber cuff, automatically inflated
and deflated, was used. Signals from a microphone placed
over the brachial artery were recorded and BP and heart
rate registered on paper in a mingograph (Automatic
Oscillometric Digital Blood Pressure Monitor; OMRON
model-HEM-705CP).

Body weight was measured with the subjects lightly
dressed, without shoes, using a level balance to the nearest
0.5 kg. Body height was measured to the nearest 0.5 cm.
BMI was calculated as body weight (kg)/height (m²),
and body surface area was calculated as: {[body weight
(kg) + height (cm) – 160]/100} + 1.

Determination of PRA (plasma renin
activity) and plasma concentration of
Ang II and aldosterone

The determinations were made after the 45 min equi-
libration period and at the end of each dose of the
30 min Ang II infusion. RIAs were used to determine
PRA (Renin-RIA bead; Abbot Diagnostics) and plasma
aldosterone (DiaSorin). Plasma Ang II concentration was
assayed according to the methods of Kappelgaard et al.
[15] and Morton and Webb [16]. PRA and Ang II had
within-assay CV (coefficients of variation) of 8.8 %
and 5.1 % respectively.

Renal haemodynamics

GFR was measured as ⁵¹Cr-EDTA clearance and RPF
(renal plasma flow) as PAH (p-aminohippuric acid)
Table 1  Clinical data of normotensive subjects with a PFH and NFH at baseline

Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Subjects with a PFH</th>
<th>Subjects with a NFH</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.0 ± 1.6</td>
<td>38.5 ± 1.0</td>
<td>0.55</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>124 ± 2</td>
<td>124 ± 4</td>
<td>0.19</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78 ± 2</td>
<td>79 ± 2</td>
<td>0.39</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>60 ± 2</td>
<td>59 ± 2</td>
<td>0.73</td>
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<tr>
<td>Weight (kg)</td>
<td>86.8 ± 2.0</td>
<td>89.6 ± 3.6</td>
<td>0.85</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 0.3</td>
<td>26.4 ± 0.3</td>
<td>0.76</td>
</tr>
<tr>
<td>PRA (ng of Al · ml⁻¹ · h⁻¹)</td>
<td>0.77 ± 0.08</td>
<td>1.12 ± 0.23</td>
<td>0.19</td>
</tr>
<tr>
<td>Plasma Ang II (pg/ml)</td>
<td>4.1 ± 0.6</td>
<td>6.2 ± 1.5</td>
<td>0.40</td>
</tr>
<tr>
<td>Plasma aldosterone (pg/ml)</td>
<td>72.3 ± 10.0</td>
<td>86.7 ± 12.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Urinary sodium excretion (µmol/min)</td>
<td>308 ± 25</td>
<td>241 ± 25</td>
<td>0.09</td>
</tr>
</tbody>
</table>

clearance using the continuous infusion technique with urine collection as described previously [6]. Briefly, subjects were initially hydrated with tap water (10 ml/kg of body weight) to ensure diuresis. When the urine flow was established, the priming doses of ⁵¹Cr-EDTA and PAH were given. The equilibration period (45 min) started when the subject had emptied the bladder. Thereafter, two 30-min baseline periods followed in which the subject voided at the end of each period. Between the periods, the subjects drank the same volume of water as that of urine passed in the preceding period. These two urine portions were pooled for the renal haemodynamic assessment. Clearance values were expressed per 1.73 m² body surface area. RBF (renal blood flow) was calculated as: PAH clearance/(1 − haematocrit). Haematocrit was measured after centrifugation of blood in a haematocrit centrifuge (Kemila). Filtration fraction was calculated as the ratio of ⁵¹Cr-EDTA clearance/PAH clearance. After completion of the two baseline periods, Ang II infusion was started and three 30-min clearance periods followed, each with a different dose of Ang II (0.1–1.0 ng · kg⁻¹ · min⁻¹).

Statistics

Means and S.E.M. were calculated. Comparisons between the two groups with respect to baseline variables and response variables were performed by Mann–Whitney’s test. Changes within patients were assessed by Wilcoxon’s test.

RESULTS

Baseline data

Clinical data on the subjects are shown in Table 1. There was no difference between the PFH and NFH groups with regard to baseline SBP (systolic BP), DBP, heart rate, BMI, PRA, plasma Ang II concentration, plasma aldosterone concentration or urinary sodium excretion.

PRA and sodium excretion in response to Ang II infusion

As shown in Figure 1, there was a gradual decrease in PRA with increasing doses of Ang II in subjects with a NFH and, with the highest Ang II dose (1.0 ng · kg⁻¹ · min⁻¹), there was a significant fall in PRA compared with baseline (P < 0.01). In subjects with a PFH, there was no gradual decrease in PRA with increasing dose of Ang II and no significant change in PRA from baseline (P = 0.81). The difference in the responses...
to Ang II between subjects with a PFH and NFH reached statistical significance ($P < 0.05$). The slopes of the dose–response curves (PRA at baseline and during the three Ang II doses) for subjects with a NFH and PFH tended to differ, but the difference did not reach statistical significance ($P = 0.09$). Ang II infusions produced similar plasma concentrations of Ang II in both groups (Table 2). Urinary sodium excretion decreased with increasing Ang II doses, but there was no significant difference between the groups (Table 3). Urinary potassium decreased significantly in subjects with a PFH but not with NFH (Table 3).

**Systemic and renal vascular response to Ang II infusion**

In subjects with a NFH and PFH, Ang II caused a small increase in SBP and DBP, but there was no significant difference in the BP response to Ang II between the two groups (Table 3). Heart rate showed a slight, but significant, decrease in subjects with a PFH but not consistently in subjects with a NFH.

Before Ang II infusion, RBF and GFR did not differ in the two groups. The highest Ang II dose (1.0 ng · kg$^{-1}$ of body weight · min$^{-1}$) caused a significant decrease in both RBF and GFR in subjects with a PFH ($P < 0.01$ for both) but, in subjects with a NFH, only RBF showed a decrease ($P < 0.05$). The renal haemodynamic response to Ang II infusion is shown in Figure 3.

**Effect of AT1 receptor blockade**

After treatment with an ARB for 5 days, there was no significant change in SBP in either group, but DBP decreased significantly in both subjects with a PFH ($P < 0.05$) and NFH ($P < 0.01$), and no significant difference between groups was observed (Table 3). Ang II infusion had no significant effect on SBP, but DBP tended to rise in subjects with a NFH. During AT1 receptor blockade before Ang II infusion, PRA increased 10-fold and plasma Ang II increased 3-fold (Table 2), but there was no significant difference between the groups.

During AT1 receptor blockade, there was a gradual decrease in PRA in both groups with increasing doses of Ang II (Figure 1B). With the highest Ang II dose, there was a significant fall in PRA in both subjects with a PFH ($P < 0.01$) and NFH ($P < 0.05$), but there were no differences in the responses of renal sodium and RBF to Ang II infusion between subjects with a PFH and NFH (Figures 2B and 3B).

**DISCUSSION**

The present study demonstrates that normotensive subjects with a PFH have a blunted suppression of PRA by Ang II (the short feedback loop). This abnormality was reversed by treatment for 5 days with a selective ARB and is probably due to a failure of Ang II to suppress renin release. It may indicate that the negative feedback control of renin release by Ang II is an important factor involved
Table 3 Effects of Ang II-infusion on BP, heart rate and urinary sodium and potassium excretion without (− ARB) and with (+ ARB) irbesartan in subjects with a PFH and NFH

Values are means ± S.E.M. Ang II was infused at 1.0 ng·kg⁻¹ of body weight·min⁻¹. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with baseline. †P < 0.05 and ††P < 0.01 compared with − ARB at baseline.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects with a PFH (n = 13)</th>
<th>Subjects with a NFH (n = 10)</th>
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<tbody>
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<td>SBP (mmHg)</td>
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<tr>
<td>Baseline</td>
<td>124 ± 2</td>
<td>120 ± 3</td>
</tr>
<tr>
<td>Ang II</td>
<td>132 ± 2**</td>
<td>119 ± 2</td>
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<tr>
<td>DBP (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>78 ± 2</td>
<td>75 ± 2†</td>
</tr>
<tr>
<td>Ang II</td>
<td>88 ± 2**</td>
<td>78 ± 2</td>
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<tr>
<td>Heart rate (beats/min)</td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>60 ± 2</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>Ang II</td>
<td>57 ± 2**</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>Urinary sodium excretion (µmol/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>308 ± 25</td>
<td>285 ± 25</td>
</tr>
<tr>
<td>Ang II</td>
<td>159 ± 18***</td>
<td>259 ± 18</td>
</tr>
<tr>
<td>Urinary potassium excretion (µmol/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>111 ± 9</td>
<td>118 ± 9</td>
</tr>
<tr>
<td>Ang II</td>
<td>64 ± 5**</td>
<td>90 ± 7**</td>
</tr>
</tbody>
</table>

Figure 2 Effect on the urinary sodium excretion response to Ang II infusion before (A) and during (B) AT1 receptor blockade with irbesartan in normotensive subjects with a PFH (■) and NFH (□)

Values are means ± S.E.M. *P < 0.05 and ***P < 0.01.

in the pathophysiology of primary hypertension. The present study has, however, a small sample size that would limit the ability to detect significant changes between groups for PRA and Ang II levels. The calculated power to assess a difference between the groups with respect to Ang II suppression of PRA was 51%. Further studies intending to verify the finding of a difference in PRA suppression will need a sample size which is 1.8 times larger than the present one in order to achieve a power of 80%.

The failure of Ang II to suppress PRA in normotensives with a PFH could not be explained by different Ang II levels or by a different BP response during Ang II infusions. Ang II infusions produced similar plasma Ang II concentrations, which were within the physiological range. The groups had the same SBP and DBP before Ang II infusion and the BP responses to Ang II did not differ between the groups. With respect to sodium balance, basal urinary sodium excretion did not differ, but a 24-h urine collection was not performed in the subjects before the study. Sodium excretion fell in a parallel fashion with increasing Ang II doses. It should also be noted that the defect could be beyond the stage of renin release.

Treatment for 5 days with 150 mg of the ARB irbesartan caused a marked increase in PRA and plasma Ang II in both PFH and NFH groups. This dose did not
influence SBP, but there was a significant fall in DBP in both groups. It also blocked the effects of Ang II on BP, although there was a tendency to increase DBP. There were no effects on renal tubular sodium reabsorption or on adrenal cortical aldosterone secretion. However, renal vascular AT1 receptors were not fully blocked, since the highest Ang II dose caused a decline in renal blood flow, similar in both groups. The elevated plasma Ang II concentration rose during Ang II infusion, but with no significant difference between the groups.

The dose of irbesartan (150 mg once a day) was chosen as it does not influence BP significantly in healthy subjects, but completely blocks BP responses to infused Ang II [18]. The drug was administered for 5 consecutive days to ensure steady-state conditions.

Several studies have been performed relating to renal function in young normotensive subjects with a PFH. Bianchi et al. [19] reported that young people with a PFH had increased RBF, normal cardiac output and slightly increased GFR, whereas Van Hooft et al. [20] in a similar group of normotensive subjects with a PFH found a lower RBF. A common finding in both studies was a decreased PRA in these young people at risk of hypertension. In the present study and in previous studies from our group [4], the two hereditary subgroups did not differ significantly from each other with respect to RBF or GFR, but the group with a predisposition to hypertension tended to be higher in both parameters. As in the above-mentioned studies, we found slightly lower levels of PRA in subjects with a PFH, but the difference did not reach statistical significance.

The mechanism behind the tendency for decreased PRA in normotensives with a PFH is not known. Small differences in BP could cause a lower PRA through the pressure-dependent renin release in hypertension-prone individuals [17]. In the Dutch study [20] and the present study, the renal vasoconstriction due to enhanced sensitivity to Ang II or other vasoconstrictors may have caused decreased sodium excretion [21]. The interpretation of the data from the study by Bianchi et al. [19] indicated that some abnormality in kidney function, due to alteration of tubular cell membrane transport, caused increased renal tubular sodium reabsorption, which may have preceded the rise in BP. Decreased sodium excretion would cause sodium and fluid retention, increase extracellular volume and, subsequently, give rise to an elevated BP. We have shown in a previous study [7] that subjects with a PFH have an impaired prostaglandin control of the renovascular sensitivity to Ang II. This defect control mechanism may also influence the regulation of renin release. In our previous study [7], 3 days of indomethacin treatment, with a dose that caused a 40% decrease in urinary PGE2 (prostaglandin E2) excretion, gave a baseline PRA reduction of 40% in both hereditary subgroups. When the Ang II infusion was given during indomethacin treatment, the already low PRA could be significantly reduced further in the subjects with a PFH [7]. These results are compatible with a different pattern of prostanoid response to Ang II infusion in subjects without and with a PFH.

The results of the present study extend previous observations made in hypertensives by Hollenberg et al. [11]. These workers reported an abnormal pattern of Ang II response in terms of suppression of renin release in what they called non-modulators, who comprise approx. 50% of patients with primary hypertension. The non-modulators were characterized by an inability to modulate normally the responsiveness of the renal vasculature and the adrenal gland to Ang II at different levels of sodium intake. When non-modulators were given a short-term saline infusion or sustained shifts in dietary salt intake, the response differed from that in normal subjects and from modulators. They excreted the salt load more slowly and were prone to salt-sensitive hypertension. The defective Ang II-mediated renin suppression is, in fact, one of the characteristic features of non-modulation in hypertensives and may thus be one of the mechanisms leading to high BP [12].

The high prevalence of non-modulation of renin release in patients with high BP has suggested this trait to be an important hereditary factor in primary hypertension [22]. In the present study on normotensive subjects, there was also a non-modulation of renin release in the group with a PFH. This subgroup may form ‘true non-modulators’ and the fact that irbesartan so promptly reversed the negative feedback control mechanism is compatible with an elevated intrarenal Ang II concentration in this group of subjects. In addition, the fact that non-hypertensive subjects with a PFH in a previous study [23] exhibited an attenuated PRA response to
hyperinsulinemia also suggests an impaired regulation of the renin release in hypertension-prone subjects.

Ichihara et al. [24] showed that both AT1 and AT2 (Ang II type 2) receptors reside in rat juxtaglomerular cells, and that AT1 receptor stimulation caused a diminished renin secretion, whereas AT2 receptor stimulation decreased active renin through inhibition of pro-renin processing. Such results may explain how Ang II infusion during AT1 receptor blockade decreased PRA due to AT2 receptor stimulation. Other investigations suggest that signalling through the AT2 receptor may inhibit renin release via cGMP and protein kinase GII [25,26]. Therefore we hypothesize that it is the action of Ang II on the unblocked AT2 receptor that allows the PRA to respond to Ang II.

In conclusion, we have shown that normotensive subjects with a PHF have a defective Ang II suppression of PRA. Treatment for 5 days with the ARB irbesartan diminished renin secretion, whereas AT2 receptor stimulation caused a diminished renin secretion, whereas AT1 receptor stimulation caused a diminished renin secretion, whereas AT2 receptor stimulation caused a diminished renin secretion, whereas AT1 receptor stimulation caused a diminished renin secretion, whereas AT2 receptor stimulation caused a diminished renin secretion.

ACKNOWLEDGMENTS

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Plasma renin and family history of hypertension 317

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