Towards an integrated approach on RAAS-blockade
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SODIUM STATUS AND ANGIOTENSIN CONVERTING ENZYME INHIBITION: EFFECTS ON PLASMA ANGIOTENSIN 1-7 IN HEALTHY MAN

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Abstract

ACE inhibitors provide effective intervention for cardiovascular and renal disease. Changes in angiotensin 1-7 (ang 1-7), leading to an altered balance between ang II and ang 1-7 are proposed to be involved in the mechanism of action of ACE inhibition (ACEi). Whether a shift in sodium status affects the changes in ang 1-7 during ACEi is unknown. We therefore studied the effect of a shift in sodium intake on ang 1-7, ang I and ang II during placebo and ACEi.

A double blind placebo-controlled study was conducted in 35 healthy men. The subjects were studied for two two-week periods: enalapril 20 mg/day and placebo. Both periods consisted of a week on high sodium (200 mmol Na+, HS) and a week on low sodium (50 mmol Na+ diet, LS). Angiotensin levels and blood pressure were measured at the end of each week.

During placebo LS intake elicited a threefold rise in ang 1-7 that paralleled the rise in other components of the RAAS. ACEi induced a clear rise in ang 1-7; to the effect that ang 1-7 was highest during combination of ACEi and LS. Consequently, during ACEi LS shifted the balance between ang 1-7 and ang II towards ang 1-7. ACEi reduced the ratio of ang II/ang I on both sodium intakes; this reduction was significantly more reduced during LS, compatible with more effective interference with ACE during LS.

LS enhances the efficacy of ACEi. This enhanced efficacy could be due to the enhanced rise in ang 1-7, the enhanced effect on ang II/ang I ratio or both. These results provide a further rationale for combination of ACEi with low sodium to improve therapeutic benefits of ACEi.
Introduction

ACE inhibitors have provided effective intervention for cardiovascular and renal disease [1;2]. They were originally designed to lower blood pressure by inhibiting the formation of the vasoconstrictor ang II (ang II) from the decapeptide angiotensin I (ang I) by blocking the angiotensin-converting enzyme (ACE). It has become increasingly clear, however, that their mechanism of action is more complex [3].

Changes in smaller cleavage products from ang I, such as the heptapeptide angiotensin 1-7 (ang 1-7), might be involved in the mechanism of action of ACE inhibition (ACEi) [4]. Several studies reported increased ang 1-7 levels during ACEi [5;6]. The increase in ang 1-7 is attributed to both increased formation, by increased availability of ang I as a substrate for neutral endopeptidase and prolylendopeptidase, and decreased breakdown by ACE [7;8].

Ang 1-7 has vasodilator and anti-proliferative properties, and may therefore be a physiological antagonist to ang II [9]. In this line of reasoning, an altered balance between the vasodilator ang 1-7 and the vasoconstrictor ang II has been proposed to be involved in the mechanism of action of ACEi [9].

Sodium status, a main determinant of the activity of the renin-angiotensin aldosterone system (RAAS), is known to consistently modify the effects of ACEi in different clinical conditions, with enhanced efficacy during dietary sodium restriction [10;11]. The mechanisms underlying the increased efficacy of ACEi during sodium restriction are not well elucidated. Animal data suggest better efficacy of the inhibitory effects of ACEi [12], but no such data are available in human. Moreover, the effects of altered sodium status on ang 1-7 concentrations during ACEi have-not been documented so far,

In the present study, therefore, we investigated the effect of a shift in dietary sodium on ang ang I, ang II and ang 1-7 concentrations during placebo and ACEi in 35 healthy subjects

Methods

Subjects

Thirty-five healthy normotensive men (age 26 ± 9 year) were recruited for the study. Normal blood pressure was defined as systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg. All subjects underwent normal routine physical examination. Written informed consent was obtained from each subject after a full explanation of the study. The study protocol was approved by the Ethics Committee of the University Hospital of Groningen.

Study Protocol

The protocol had a double-blind placebo-controlled design, and consisted of two two-week periods, separated by a 6-week washout period, during which the subjects received either placebo or enalapril 20 mg/d capsules. Both periods were divided into a 7-day period on a high sodium diet (HS; aim: 200
mmol Na+/d) and a 7-day period on a low sodium diet (LS; aim: 50 mmol Na+/d). Differences in sodium intake were achieved by replacing sodium-rich products with a low-sodium product of the product group in order to remain isocaloric with a similar balance between protein, carbohydrate and fat. To prevent in advert concurrent changes in dietary habits, the diets were based on the personal food habits of each subject and fitted to the individual caloric need. On day 4 and day 6 of each dietary period, subjects collected 24-hour urine to assess the achievement of a stable sodium balance. Medication was taken before 12 p.m. On day 7 the subjects reported to the research unit at 8:00 a.m. after an overnight fast. An intravenous canula was inserted into the forearm for drawing blood samples. Subjects remained in semi-supine position after a light standardized breakfast in a quiet room for 3 hours to standardize their activities and posture before blood sampling. Blood pressure was measured at 15-minute intervals using a non-invasive device (Dinamap®; GE Medical systems, Milwaukee, WI).

**Blood sampling and analysis**

Blood samples were drawn at 11:00 a.m. in semi-supine position, in pre-chilled tubes and immediately centrifuged at 4°C. Plasma and serum for measurement of aldosterone, plasma renin activity (PRA) and ACE activity was stored at -20°C until analysis. Blood samples for determination of angiotensins were drawn in cold, standard 3ml vacuum tubes containing 5.4 μg K$_3$EDTA and an additional 0.2ml ACE inhibitor cocktail containing 1.704 μg phenantroline, 0.16mg enalaprilat, 1ml ethanol and 4mg neomycin. After centrifugation at 4°C, the plasma was snap-frozen and stored at -80°C until analysis. Plasma concentrations of ang I, ang II and ang 1-7 were measured after SepPak extraction of plasma samples and HPLC separation [13]. Radioimmunoassays of dried collected fractions were used for quantitation of angiotensins using specific antibodies [13]. The antibody for ang 1-7, raised in New Zealand White rabbits, had cross-reactivities with ang I and II of 1.1 and 1.7 %, respectively, and <0.1% and 1.7% with angiotensin 1-4 and angiotensin 2-8, the angiotensins eluting nearest to ang 1-7. Detection limits were 0.5 - 1.0 fmol/tube. Aldosterone was measured with a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma renin activity (PRA) was measured as described previously with a radioimmunoassay

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**Table 1 | Clinical parameters**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>ACE inhibition</th>
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<tbody>
<tr>
<td></td>
<td>High sodium</td>
<td>Low sodium</td>
</tr>
<tr>
<td>Urinary sodium (mmol/day)</td>
<td>219 ± 56</td>
<td>37 ± 23*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>80 ± 10</td>
<td>79 ± 9*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>88 ± 7</td>
<td>85 ± 7*</td>
</tr>
</tbody>
</table>

Data are expressed as mean and SD, * p<0.05 for low vs high sodium (Paired t-test), ** p<0.05 for ACE inhibition vs control (Paired t-test)
that detects the amount of ang I produced per hour in the presence of excess angiotensinogen (nanograms of ang I produced per milliliter of plasma per hour). This assay measures the enzymatic activity of active plasma renin in the presence of an exogenous excess of its substrate [14]. ACE activity was determined as the amount of hippuric acid produced by cleavage of the commonly used substrate Hippuryl-Histidyl-Leucine [15].

Data analysis
Mean values and standard deviations were calculated for normally distributed variables after checking for normality. Medians and quartiles were computed for variables with non-normal distribution. After testing for normality, we used Student paired t-test or Wilcoxon Signed Rank test to compare values between the different periods, each subject being its own control. A value of p<0.05 was considered to be statistically significant.

Results

Clinical parameters
Urinary sodium excretion demonstrated a good dietary compliance at both sodium intakes during placebo as well as during ACEi, as shown in table 1. During placebo, low sodium induced a small but significant reduction in blood pressure. ACEi significantly reduced blood pressure during both sodium intakes. During ACEi, low sodium induced a further reduction in blood pressure. As anticipated, the shift in sodium intake elicited a change in body weight, with higher body weight during high sodium both during placebo and ACEi.

RAAS parameters
ACE activity, PRA and aldosterone levels are shown in table 2. During placebo low sodium intake increased PRA and aldosterone concentration approximately threefold. ACEi significantly reduced serum ACE activity, aldosterone, and elicited a rise in PRA on both sodium intakes. The shift in sodium intake during ACEi elicited significant changes in PRA and aldosterone, with higher levels during the low sodium period. As anticipated, the shift in sodium intake during ACEi did not affect ACE-activity. Angiotensin levels during the different study conditions are shown in figure 1. During placebo

### Table 2 | RAAS parameters

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>ACE inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High sodium</td>
<td>Low sodium</td>
</tr>
<tr>
<td>ACE activity (U/L)</td>
<td>28 (24-35)</td>
<td>31 (25-37)</td>
</tr>
<tr>
<td>PRA (ng ang-I/ml/h)</td>
<td>2.5 (1.6-3.5)</td>
<td>5.9 (4.4-8.1)*</td>
</tr>
<tr>
<td>Aldosterone (ng/L)</td>
<td>43 (24-57)</td>
<td>130 (81-174)*</td>
</tr>
</tbody>
</table>

Data are expressed as median and quartile range, * p<0.05 for low vs high sodium (Wilcoxon Signed Rank Test), ** p<0.05 for ACE inhibition vs control (Wilcoxon Signed Rank Test)
ang I, ang 1-7, and ang II concentrations were approximately twofold higher during the low sodium intake. ACEi elicited a significant rise in ang I and ang 1-7 on both sodium intakes. ACEi reduced ang II levels during low sodium only. Data are expressed as median and quartile range, * p<0.05 for low vs high sodium (Wilcoxon Signed Rank Test), ** p<0.05 for ACE inhibition vs control (Wilcoxon Signed Rank Test).

Figure 1 | Angiotensins: effect of sodium intake during control and ACE inhibition
Angiotensins were measured in 35 healthy men during high (200 mmol/day) and low (50 mmol/day) dietary sodium intake both with placebo and ACE inhibition. During control ang I, ang 1-7 and ang II concentrations were approximately twofold higher during the low sodium intake. ACEi elicited a significant rise in ang I and ang 1-7 on both sodium intakes. ACEi reduced ang II levels during low sodium only. Data are expressed as median and quartile range, * p<0.05 for low vs high sodium (Wilcoxon Signed Rank Test), ** p<0.05 for ACE inhibition vs control (Wilcoxon Signed Rank Test).

ang I, ang 1-7, and ang II concentrations were approximately twofold higher during the low sodium intake. ACEi elicited a significant rise in ang I and ang 1-7 on both sodium intakes. ACEi reduced ang II levels during low sodium only. During ACEi the shift in sodium intake induced significant changes in both ang I and ang 1-7, with significantly higher levels during low sodium (both p<0.05). The resulting balance between ang 1-7 and ang II is shown in figure 2 as the ratio of ang 1-7 to ang II. ACEi elicited a rise in the ang 1-7/ang II ratio during both sodium intakes. During ACEi, low sodium elicited a further rise in the ratio, towards ang 1-7. The balance between ang II and ang I, a marker for ACE activity, is shown in figure 2 as ratio of ang II to ang I. ACEi elicited a fall in the ang II/ang I ratio during both sodium intakes. During ACEi, low sodium elicited a further fall in the ratio.
Discussion

This study documents for the first time plasma ang 1-7 levels during different sodium intakes in man on both placebo and ACEi. Low sodium intake was associated with a significantly higher ang 1-7 concentration during placebo and during ACEi, with the highest ang 1-7 levels during the combination of ACEi with low sodium. Consequently, during ACEi low sodium shifted the balance between ang 1-7 and ang II towards ang 1-7. Moreover, this study is the first to show that the reduction of the ang II/ang I ratio during ACEi is more pronounced during low sodium, suggesting that the conversion of ang I is more effectively reduced by ACEi during low sodium than during high sodium.

Experimental studies reported increased plasma ang 1-7 concentrations during ACEi in dogs [16], spontaneously hypertensive rats [17;18], Wistar rats [12], Sprague-Dawley rats [6] and transgenic mice (REN-2) [19]. However, less data is available on the effects of ACEi in man. In essential hypertension a two-fold increase in ang 1-7 was reported after the daily captopril dose during maintenance therapy. However, after seven months ang 1-7 concentrations were not increased and even decreased compared to baseline [5]. Furthermore, lisinopril 20mg/d and omapatrilat 40mg/d had no significant effect on plasma ang 1-7 concentration in salt-sensitive hypertensive patients [20]. The discrepancies between the various studies may be related to differences in species, disease model, timing and dose of ACEi, or to characteristics of the study population. Our present data add to the previous studies by demonstrating that dietary sodium can be relevant to the rise in ang 1-7 during ACEi, with a clear-cut rise during low sodium, but only a modest, non-significant, rise during high sodium, indicating that differences in sodium status may contribute to differences between studies as well. The effect of sodium intake is either not uniform. For example, in rat studies from our lab as well as others no such

Figure 2 | Ratio of Ang 1-7 / Ang II and Ang II / Ang I
Data are expressed as median and quartile range. ACEi elicited a rise in the ang 1-7/ang II ratio during both sodium intakes, with the lowest ratio during low sodium. ACEi elicited a fall in the ang II/ang I ratio during both sodium intakes, with the lowest ratio during low sodium. * p<0.05 for low vs high sodium (Wilcoxon Signed Rank Test), **p<0.05 for ACE inhibition vs control (Wilcoxon Signed Rank Test)
effect was observed \[12;21\] so differences in species and/or protocol may overrule the effects of sodium intake.

Consequent to the effects of sodium intake during ACEi, in our study the effect of ACEi on the balance between ang 1-7 and ang II was more readily apparent during low sodium. This may well contribute to the efficacy of ACEi during low sodium. It cannot be derived from our data, however, whether the rise in ang 1-7 during ACEi and low sodium indeed contributes to the effects of ACEi as conclusive proof would require blockade of ang 1-7, which is not feasible in man. In SHR such evidence was obtained by Iyer et al, by acute blockade of ang 1-7 by antibodies during ACEi, which abolished the blood pressure response \[22\]. Based on studies in different animal models, with different states of activity of the renin-angiotensin system, these authors argue that the impact of depressor systems like ang 1-7 is influenced by the state of activity of the renin-angiotensin system, with a larger impact when the system is activated \[9;21\]. Our current data are in line with this concept.

The ang II/ang I ratio is suggested to be a sensitive indicator of the effects of ACE inhibition on angiotensin peptide metabolism and a method to assess the effectiveness of inhibition of ACE conversion \[23\]. Our data point towards more effective inhibition of ACE conversion by ACEi during low sodium compared to high sodium. This is in line with our prior studies in rats, where high sodium diet blunted the inhibitory effects of ACEi on the vascular conversion of angI, measured as the in vitro responses of isolated aortic rings to ang I and ang II \([12]\). Better efficacy of inhibitory activity of ACEi during low sodium therefore, could be a mechanism underlying the better efficacy on clinical parameters. The mechanism underlying the effect of sodium status on the efficacy of inhibiting ang I conversion cannot be concluded from our data. The decrease in plasma ACE activity was similar during both sodium intakes. Data by our lab as well as others \[12;24;25\] suggest however that high sodium can lead to an increase in tissue ACE, which could be involved in the current observations.

In addition, the dietary sodium restriction during ACEi causes a further activation of the RAAS, as shown by a sixfold increase in PRA and twofold increase in ang I. The higher ang I levels can be converted to the vasodilator ang 1-7. The increased rise in ang 1-7 could further be promoted by a decrease in ang 1-7 breakdown, due to effective ACEi, eliciting a shift towards ang 1-7 during the combination of ACEi and low sodium. This shift towards ang 1-7 may contribute to the enhanced therapeutic benefit of this class of drugs during low sodium as well.

It has been argued that the balance between ang 1-7 and ang II may not only be relevant to the effects of ACEi, but also in blood pressure regulation as such \[9\], as supported by an inverse relationship between urinary ang 1-7 concentrations and blood pressure in normotensive and hypertensive subjects \[26\]. No correlation between plasma ang 1-7 concentrations and blood pressure was detected in our study, which had however, a low power to detect such an association. The impact of urinary ang 1-7, moreover, may not be equivalent to that of plasma ang 1-7.
Low sodium diet not only enhances the effects of ACEi, but also those of AT1-receptor antagonists. It is not known however, whether low sodium during AT1-receptor blockade enhances ang 1-7 levels similar to the effects observed here during ACEi. In this respect the recent finding that a homologue of ACE, ACE2, which converts ang II to ang 1-7, may be of interest, as AT1-receptor blockade is associated with high levels of ang II [27]. In rats with myocardial infarction Ishiyama et al. [27] found a strong relation between up regulation of ACE2 and concentrations of ang I, ang 1-7 and ang II during AT1-receptor blockade.

In healthy subjects, sodium restriction induces RAAS activation within 3 days, with concurrent sodium balance. We used a time-frame of 7 days, as this is sufficient to re-establish steady-state as to sodium balance and circulatory hormones [28]. Steady-state was established by sodium-excretion and body weight. However, it should be noted that the duration of our study was limited. Although it is common practice to study effects of sodium intake after a short-term, it remains unknown whether the observed hormonal changes will be sustained during long-term. To assess the eventual significance of our findings to the long term treatment of hypertension, obviously studies in patients on long term treatment would be needed.

In conclusion, we identified two possible mechanisms underlying the better efficacy of ACEi during low sodium. First, the effect of ACEi on ang II/ang I ratio was significantly more pronounced during low sodium, suggesting a larger efficacy of the inhibition of ACE as such. Moreover, during ACEi the level of ang 1-7 was further increased by low sodium intake. Our data show that the level of this vasodepressor peptide is accessible to therapeutic manipulation by restricting dietary sodium intake. Our data further support the therapeutic benefit of combining ACEi with low sodium, and provide a plausible mechanism underlying the enhanced efficacy on clinical parameters.
References

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