Dissociation between the renal effects of angiotensin I and II in sodium-restricted normal subjects

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Abstract

Objective: To determine whether the effects of angiotensin I (AngI) in humans can be explained entirely by its plasmatic conversion to angiotensin II (AngII).

Methods: Ten healthy male volunteers on a sodium-restricted diet were studied on two separate occasions, during which, in random order, AngI or AngII was infused in increasing doses of 0.3, 1 and 3 pmol·kg⁻¹·min⁻¹. Mean arterial pressure (MAP), effective renal plasma flow (ERPF), glomerular filtration rate (GFR), active plasma renin concentration (APRC), AngII, aldosterone (Aldo) and catecholamines were assessed at baseline, after each dose of AngI or AngII and 30 and 60 min after discontinuation of the AngI/AngII infusion.

Results: The rise in plasma AngII was significantly less during AngI infusion as compared to AngII infusion (P < 0.05). Changes in MAP, Aldo and GFR, however, were comparable during both infusions. In the kidney, on the other hand, the decrements in APRC and ERPF during AngII infusion exceeded those during AngI (P < 0.05). After cessation of either infusion, AngII concentrations, MAP, ERPF and Aldo returned to baseline levels within 1 h. Renin, however, was still significantly inhibited at that time (P < 0.05). Catecholamines remained virtually unchanged during all experiments.

Conclusions: Our data show that AngI and AngII have similar effects on blood pressure and Aldo, but they differ in their renal effects. The latter may be due to a low renal capacity to convert AngI. The prolonged inhibition of renin release after cessation of the infusions may be caused by reduced renin mRNA expression or by accumulation of AngII in the kidney. © 1998 Elsevier Science B.V.

Keywords: Man; Angiotensin I; Angiotensin II; Renal hemodynamics; Renin

1. Introduction

Conversion of angiotensin I (AngI) into angiotensin II (AngII) by angiotensin-converting enzyme (ACE) in tissues other than the lung may contribute to circulating levels of AngII [1–3]. Recently, Admiraal and coworkers demonstrated conversion of arterially derived AngI into AngII in the vascular bed of the forearm and leg, while they found only little evidence for conversion in the renal vascular bed [4]. The latter is in keeping with observations in the isolated perfused rat kidney showing that the AngI dose has to be 50 times higher than that of AngII to obtain equipotent vasoconstrictor effects [5]. These data, when added to the fact that AngII is very efficiently extracted from the circulation by the kidney [4,6–9], and possibly sequestered within that organ [10], may lead to the hypothesis that also in human kidneys, the effects of circulating AngI are less prominent than those of circulating AngII. Such a difference in AngI/II effects does not necessarily apply to other vascular beds where, as opposed to the situation in the kidney, the conversion of AngI into AngII may be more pronounced [7]. If on the other hand, all circulating AngI would be converted on a 1:1 molar basis into AngII in the plasma compartment before it

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reaches the renal vascular bed, the functional renal (hemo-
dynamic) responses to AngI should be comparable to those of
AngII.

These two opposing possibilities prompted us to study the
effects of equimolar doses of AngI and AngII on the kidney. Both peptides were infused intravenously in order
to allow conversion of AngI into AngII in plasma. A
second objective of the study was to explore whether, after
cessation of the infusions, the time course of the dissipa-
tion of the AngI/ AngII effects on the kidney was congru-
ent with the time course of the fall in plasma AngII. A
discrepancy between these time courses could point to-
wards accumulation of angiotensins in the kidney.

2. Subjects and methods

Ten healthy normotensive volunteers with a mean age
of 49 (range 24–66) years and a body surface area of
1.76 ± 0.06 m² (mean ± s.e.m.) were included in this
study. Each subject had a medical history taken and under-
went a physical examination. Routine laboratory tests were
carried out before the start of the study. The protocol was
approved by the Hospital Ethics Committee and written
informed consent was obtained from all participants. The
investigation conforms with the principles outlined in the
Declaration of Helsinki.

2.1. Protocol

During 1 week, subjects were put on a moderate
sodium-restricted diet of 55 mmol/day, while potassium
intake was fixed at 80 mmol/day. This particular diet was
chosen to allow comparison of responses with earlier data
obtained at the same sodium intake. Compliance with the
diet was checked by measuring sodium output in 24-h
urine collections obtained during the last 3 days of the
study period. Completeness of these urine collections was
inferred from the concurrent creatinine excretion. Subjects
refrained from smoking and alcohol; caffeine and
caffeine-like substances were also forbidden on the study
days. All volunteers were studied after an overnight fast in
the morning of the last 2 days of the dietary period.

On the two study days either AngI or AngII was infused
according to a randomized double-blind study design un-
erwise similar experimental conditions. Studies
started at 08.00 h and subjects remained supine during the
entire session. In both arms, an antecubital vein was
 cannulated with a 20-gauge cannula: the one in the right
arm was connected to a 3-way tap for the infusion of
angiotensins and PAH/inulin (for measuring renal hemo-
dynamics), whereas the one in the left arm was used for
blood sampling. Subjects consumed 200 ml of water every
hour, to ensure diuresis, until the last blood samples had
been drawn. After a 2-h equilibration period, necessary to
reach steady state plasma concentrations of PAH and
inulin, stepwise increasing equimolar doses of either hu-
man AngI or human AngII (Cilinalfa AG, Laufelfingen,
Switzerland) were administered. Infused doses were 0.3, 1
and 3 pmol·kg⁻¹·min⁻¹. Each infusion step was contin-
ued for 30 min to allow renal clearances to reach a new
steady state. Thereafter, the infusion was stopped and
subjects were followed for another hour. The infusion rates
were chosen on the basis of data from the literature and
from earlier experiments performed in our laboratory.

Mean arterial pressure (MAP) and heart rate (HR) were
measured at baseline, after each dose of AngI or AngII and
30 (t = 120) and 60 (t = 150) min after cessation of the
AngI/AngII infusion. At the same time points, blood
samples were drawn for measurement of PAH, inulin,
hematocrit and plasma levels of AngII, active plasma renin
concentration (APRC), aldosterone (Aldo) and catechol-
amines. Blood for neurohumoral assays was sampled in
special tubes which were prechilled if necessary. In the
case of AngII, blood was collected in tubes containing an
inhibitor solution to prevent in vitro generation and degra-
dation of this peptide, and spun immediately in a cooled
centrifuge. Subsequently, the plasma was quickly frozen
in liquid nitrogen. All samples were stored at −80°C until
assay.

2.2. Methods

MAP and HR were measured by a semi-automatic
oscillometric device (Dinamap Vital Signs Monitor 1846,
Critikon, Tampa, FL, USA). Renal hemodynamics, i.e.
effective renal plasma flow (ERPF) and glomerular filtra-
tion rate (GFR) were measured as the clearance of PAH
(p-aminohippurate sodium; MSD, West Point, PA, USA)
and inulin (Inutest, Laevosan Gesellschaft, Linz, Austria),
respectively, using the continuous infusion method [11].
Both variables were corrected for body surface area and
expressed as ml/min per 1.73 m². Effective renal blood
flow (ERBF) was calculated using the formula: ERPF/(1-
hematocrit). Filtration fraction (FF) was calculated as
GFR/ERPF. Renal vascular resistance (RVR) was calcu-
lated according to the formula: (MAP/ERBF)³ 80.000.

Active plasma renin concentration (APRC) was mea-
sured by the IRMA method (Nichols Institute Diagnostics,
Wychen, The Netherlands) with an intra-assay coefficient
d of variation (CV) of 2.9% and an inter-assay CV of 7.6%
when 8.1% levels were measured by means of a spectrophotom-
eter [11,15]. Norepinephrine (intra-assay CV, 4.5%; inter-
assay CV, 8.1%) and epinephrine (intra-assay CV, 4.1%;
inter-assay CV, 8.6%) were assessed by HPLC followed by a sensitive fluorimetric detection method [16]. For each hormone, all samples from the same individual were assayed in a single run.

2.3. Data analysis

Prior to statistical testing, logarithmic or square root transformation of the data was performed, when appropriate. For each individual, the difference in plasma AngII concentrations caused by the AngII infusion and those after the AngI infusion was calculated. Subsequently, we tested, using confidence intervals (CI), whether these differences deviated significantly from zero.

To avoid the statistical problems related to the analysis of serial measurements [17], we first applied summary statistics to assess overall differences in the other variables between the AngI and AngII infusion regimens. To this end, we calculated, for each individual, the area under the curve (AUC) for each response variable during both infusions and then compared the average AUCs from both infusion regimens. In addition, changes induced by each infusion were assessed by repeated measures analysis of variance and analysis of covariance taking the type of infusion regimens. In addition, changes induced by each infusion were assessed by repeated measures analysis of variance and analysis of covariance taking the type of infusion as the covariate. To answer the main study question, the period from \( t = 0 \) until \( t = 90 \), coinciding with the angiotensin infusions, was analyzed separately. To determine whether after cessation of the AngI/AngII infusion variables had returned to baseline, we tested whether at the end of the experiment (\( t = 150 \)) changes in variables differed significantly from zero.

Results are expressed as means ± s.e.m. A \( P \)-value of less than 0.05 was considered significant.

3. Results

On the fifth day of sodium restriction, all subjects were in sodium balance. Sodium excretion averaged 53 ± 6 mmol/24 h, while potassium excretion averaged 58 ± 8 mmol/24 h. Baseline data on the two experimental days were comparable (Table 1). During infusion of AngI, plasma levels of AngII rose to 17 ± 3 pmol/l with the 0.3 pmol·kg\(^{-1}\)·min\(^{-1}\) dose, to 20 ± 2 pmol/l with the 1.0 pmol·kg\(^{-1}\)·min\(^{-1}\) dose and to 35 ± 4 pmol/l with the 3.0 pmol·kg\(^{-1}\)·min\(^{-1}\) dose. During the AngII infusion, corresponding levels were 16 ± 2, 28 ± 4 and 54 ± 9 pmol/l, respectively. The increment in AngII levels was significantly greater during the AngII than during the AngI infusion; with the highest infusion rates levels rose by 40 ± 9 pmol/l during AngII as opposed to only 21 ± 4 pmol/l during AngI infusion (\( P < 0.05 \)). Sixty minutes after discontinuation of both infusions, plasma AngII had returned again to baseline (14 ± 2 pmol/l after both infusions).

### Table 1

<table>
<thead>
<tr>
<th>Hemodynamic and hormonal data at baseline</th>
<th>AngI infusion</th>
<th>AngII infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>90 ± 3</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>65 ± 3</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>ERPF (ml/min·1.73 m(^2))</td>
<td>431 ± 54</td>
<td>423 ± 58</td>
</tr>
<tr>
<td>GFR (ml/min·1.73 m(^2))</td>
<td>101 ± 10</td>
<td>102 ± 10</td>
</tr>
<tr>
<td>FF (%)</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>RVR (units)</td>
<td>11639 ± 1728</td>
<td>11923 ± 1735</td>
</tr>
<tr>
<td>APRC (mU/l)</td>
<td>24 ± 2</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Ang II (pmol/l)</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Aldo (pmol/l)</td>
<td>209 ± 36</td>
<td>254 ± 44</td>
</tr>
</tbody>
</table>

MAP = mean arterial pressure; HR = heart rate; ERPF = effective renal plasma flow; GFR = glomerular filtration rate; FF = filtration fraction; RVR = renal vascular resistance; APRC = active plasma renin concentration; AngII = angiotensin II; Aldo = aldosterone. Data expressed as mean ± s.e.m.

3.1. Blood pressure and heart rate

Both angiotensin infusions increased MAP to the same extent: to 105 ± 6 mmHg during the highest dose of AngI and to 102 ± 6 mmHg during the highest dose of AngII. The changes in MAP during both infusions are depicted in Fig. 1. No statistically significant differences could be detected for the responses in MAP between the two infusions. At the end of both experiments, MAP no longer differed from baseline levels. HR remained virtually unchanged during all experiments.

3.2. Renal responses

With the highest infusion rates, ERPF fell to 387 ± 41 ml/min·1.73 m\(^2\) during AngI and to 338 ± 36 ml/min·1.73 m\(^2\) during AngII. Changes in ERPF are shown in Fig. 2. AngII caused a significantly greater fall in ERPF than AngI (\( P < 0.05 \)) which was most pronounced at the highest infusion rates (17 ± 4 vs. 7 ± 4%; \( P < 0.05 \)). Sixty minutes after either angiotensin infusion, ERPF no longer differed from baseline.

![Fig. 1. Changes in mean arterial pressure (MAP) during infusion of angiotensin I or II.](image-url)
Fig. 1. Changes in effective renal plasma flow (ERPF) during infusion of angiotensin I or II.

GFR fell slightly during both infusions, but responses to AngI and to AngII were comparable (data not shown). Changes in FF during AngII infusion tended to exceed those due to AngI, but the difference between the two infusions just failed to reach statistical significance ($P < 0.05$). At $t = 150$, FF was not different from baseline on either infusion day.

The increment in RVR amounted $4377 \pm 969$ dynes $\cdot$ s/cm$^5$ when AngII was infused vs. $2845 \pm 584$ dynes $\cdot$ s/cm$^5$ during the administration of AngI. This numerical difference in response was, however, not statistically significant. In addition, RVR was not different from baseline 60 min after cessation of the AngI/AngII infusion (data not shown).

3.3. Hormonal responses

Hormonal data, including those for catecholamines, were entirely comparable at baseline. Responses of APRC during the two infusions are shown in Fig. 3. APRC fell during both angiotensin infusions but, on average, the reduction in APRC caused by the AngII infusion was significantly greater than that due to AngI ($33 \pm 5$ vs. $21 \pm 3\%$ with the highest infusion rates; $P < 0.05$). After the 3.0 picomolar dose, APRC amounted to $19 \pm 2$ mU/l when AngI had been infused versus $17 \pm 1$ mU/l during the AngII infusion. At the end of the experiment ($t = 150$), APRC was still significantly suppressed after both substances ($P < 0.05$). At that time, APRC levels were $20 \pm 1$ and $21 \pm 3$ mU/l respectively.

Aldo concentrations rose to the same extent during the AngI and AngII infusions to a maximum of $550 \pm 76$ pmol/l (AngI) and $613 \pm 115$ pmol/l (AngII). In relative terms, Aldo increased by $222 \pm 65$ and $201 \pm 93\%$, respectively. While this represented a significant change from baseline on both study days, there was no difference between the two infusions. Aldo levels fell again to baseline after discontinuation of the infusions.

Catecholamines did not change significantly during either angiotensin infusion.

3.4. Results of the regression analysis

When the data were subjected to regression analysis, essentially the same results were obtained. Thus, changes in MAP and Aldo did not differ between the two infusions, while the effects on ERPF and APRC were significantly greater for AngII than for AngI.

4. Discussion

In the present study we compared the systemic and renal effects of equimolar doses of AngI and AngII. As the functional responses to both angiotensins may be influenced by dietary sodium, we studied these effects in subjects, who were in balance on a low sodium diet. Moreover, we infused Ile AngI and -II, which are exact replicates of the endogenous peptides. To minimize carry-over effects, the different infusions were given in random order and on separate days.

We anticipated that if, on a molar basis, there was a 1:1 conversion of AngI into AngII in the plasma compartment, the hemodynamic and humoral effects of the two peptides should be similar. If, on the other hand, the effects of AngI would be less prominent than those of AngII, there would likely be insufficient conversion, either in plasma or at the tissue level. In particular, this would be relevant for the kidney where conversion of arterially delivered AngI is thought to be very low, if existent at all [4].

One important result of the present study is that plasma AngII rose significantly less during AngI than during AngII infusion. Our data, therefore, could point to incomplete conversion of AngI into AngII, at least in the plasma compartment.
compartment. However, given the $K_m$ of ACE [18], it is unlikely that saturation of ACE was responsible for the lesser increment in AngII levels after the AngI infusion. An alternative explanation for our findings could be that part of the infused AngI was rapidly metabolized by plasma angiotensinases, thus leaving less AngI available for conversion. Although we cannot exclude this possibility, there are some striking features in our data that make us think otherwise. Indeed, the rises in blood pressure and in plasma aldosterone, two well-known biological sequelae of an increase in AngII, were remarkably similar during the AngI and the AngII infusion. This suggests that the vascular wall and the adrenal cortex, as two of the major target tissues for the renin–angiotensin system, were facing similar AngII concentrations on both occasions. The lesser increment in plasma AngII concentrations after the AngI infusion, could now be explained by tissue uptake of AngI and local conversion of this peptide into AngII. Since the rise in AngII was approximately 20% less during AngI than during AngII infusion, we would have to assume that about one-fifth of the administered AngI was converted at the tissue level. This fraction could even be higher if, after local conversion, the formed AngII would be released into the circulation.

Our study further shows discordant renal responses to AngI and AngII. In fact, the fall in ERPF during infusion of AngI was about twice as great as that during AngII infusion. In addition, AngI was less effective than AngII in suppressing renin. If our hypothesis about tissue uptake of AngI and local conversion is correct, the renal data point towards some disturbance of this mechanism in the kidney. Enhanced intrarenal degradation of AngI (after uptake of this peptide) or a reduced capacity of the kidney to convert AngI or both could all explain these findings. Indeed, the group of Schalekamp has repeatedly shown that the kidney is not an important conversion site of arterially derived AngI, but mainly metabolizes this peptide [4,7,19]. Experiments in isolated perfused rat kidneys have also provided evidence that renal conversion of AngI is only limited [5]. Data from experimental animals further suggest that higher renin levels, such as may occur during sodium restriction, are associated with reduced ACE activity in the kidney [20]. If such a mechanism is also operative in man, this may have precluded renal conversion of AngI even more.

At first sight, our data seem to be at variance with those of Vos and associates who could not establish a difference between the effects of AngI and AngII on the kidney [21]. However, these investigators did not infuse equimolar amounts of AngI and AngII, but rather amounts that would yield comparable plasma levels of AngII. When one closely examines their data, one can see that with 4 pM AngI they did obtain lesser effects than with 4 pM AngII. Moreover, in their study subjects used a high sodium diet and a high dose of enalapril, both of which could have increased the sensitivity of the renal vasculature to angiotensin II.

If there is, indeed, only minimal conversion of AngI into AngII within the kidney, the fall in ERPF and in renin which were seen during the AngI infusion in the present study are most likely due to the effect of AngII that was formed in plasma and/or at extrarenal tissue sites before it reached the kidney.

As far as blood pressure, aldosterone and ERPF are concerned, variables had returned to baseline 1 h after the infusions had been stopped and plasma AngII had fallen again to preinfusion levels. Thus, with respect to these variables no dissociation between plasma levels of AngII and its physiological effects were seen. However, with respect to renin, such a dissociation did occur as renin was still suppressed 1 h after cessation of both angiotensin infusions. Although we did not include time-control experiments in the present study, in previous experiments employing placebo infusions we have not found such a decline in renin levels [22]. It is likely, therefore, that there was persistent suppression of renin after the angiotensins had been discontinued. The fall in renin may be attributed to short-loop feedback suppression by AngII [23–25]. Apparently, this suppressing effect on renin is longer-lasting than the other effects of AngII. A possible explanation for this discrepancy may be that AngII has switched off renin mRNA expression and that the latter will not restore as quickly as, for instance, renal blood flow. Alternatively, it may be that AngII has accumulated within the kidney [6,10] to cause sustained suppression of the juxtaglomerular apparatus. Further studies are necessary to discriminate between these possibilities.

Taken together, our data suggest that the effects of AngI on blood pressure and aldosterone release may be brought about by AngII that is, at least partly, formed from the AngI at tissue sites. Consequently, the effects of exogenous AngI on blood pressure and aldosterone are comparable to those of equimolar doses of exogenous AngII. However, due to a low capacity of the kidney to convert arterially delivered AngI, the renal effects of AngI are less prominent than those of AngII. It is likely that the effects of AngI on the kidney require the formation of AngII at extrarenal tissue sites.

While most effects of both angiotensins subside soon after their administration is discontinued, suppression of renin release through the short feedback loop is long-lasting. The latter may be due to reduced renin mRNA expression or to accumulation of AngII, which has been taken up from the circulation, in renal tissue. Although our hypotheses need to be tested in a more formal way, the data from the present study are at least consistent with the view that peripheral tissues, and in particular the kidney, are capable of regulating their own local production of AngII or uptake of this peptide from the circulation. Nevertheless, one should bear in mind that our data still do not allow to draw definitive conclusions about the (lack of) intrarenal conversion of AngI into AngII as it remains possible that AngI was degraded before it could reach the site of conversion.
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References