Effects of truncated angiotensins in humans after double blockade of the renin system

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Plovsing, Ronni R., Christian Wamberg, Niels C. F. Sandgaard, Jane A. Simonsen, Niels-Henrik Holstein-Rathlou, Poul Flemming Høilund-Carlsen, and Peter Bie. Effects of truncated angiotensins in humans after double blockade of the renin system. Am J Physiol Regul Integr Comp Physiol 285: R981–R991, 2003. First published July 17, 2003; 10.1152/ajpregu.00263.2003.—Angiotensins different from ANG II exhibit biological activities, possibly mediated via receptors other than ANG II receptors. We studied the effects of 3-h infusions of ANG III, ANG-(1–7), and ANG IV in doses equimolar to physiological amounts of ANG II (3 pmol·kg⁻¹·min⁻¹), in six men on low-sodium diet (30 mmol/day). The subjects were acutely pretreated with canrenoate and captopril to inhibit aldosterone actions and ANG II. ANG II infusion increased plasma aldosterone to 342 ± 45 pg/ml (45%), plasma aldosterone to 342 ± 8 pg/ml (109%), and blood pressure by 27%. Glomerular filtration rate decreased by 16%. Concomitantly, clearance of endogenous lithium fell by 66%, and fractional proximal reabsorption of sodium increased from 77 to 92%; absolute proximal reabsorption rate of sodium remained constant. ANG II decreased sodium excretion by 70%, potassium excretion by 50%, and urine flow by 80%, whereas urine osmolality increased. ANG III also increased plasma aldosterone markedly (+45%), however, without measurable changes in angiotensin immunoreactivity, glomerular filtration rate, or renal excretion rates. During vehicle infusion, plasma renin activity decreased markedly (~700 to ~200 mIU/l); only ANG II enhanced this decrease. ANG-(1–7) and ANG IV did not change any of the measured variables persistently. It is concluded that 1) ANG III and ANG IV are cleared much faster from plasma than ANG II, 2) ANG II causes hypofiltration, urinary concentration, and sodium and potassium retention at constant plasma concentrations of vasopressin and atrial natriuretic peptide, and 3) a very small increase in the concentration of ANG III, undetectable by usual techniques, may increase aldosterone secretion substantially.

healthy humans; angiotensin peptides; aldosterone secretion; sodium excretion

The RENIN-ANGIOTENSIN-ALDOSTERONE system (RAAS) is the single most important regulator of the sodium homeostasis. This specific role of the RAAS makes it a major determinant of extracellular fluid volume and arterial blood pressure. It has long been recognized that ANG II is the pivotal component of this system. Recent discoveries of other active ANG I fragments have rendered this classic system more complex (23). The distinction between systemic and tissue renin systems, and the fact that ANG II can be formed via non-angiotensin converting enzyme (non-ACE) and nonrenin enzymes, contributes to this complexity (1, 8, 20). Besides ANG II, other peptides formed by the renin system, i.e., ANG III, ANG-(1–7), and ANG IV, may have diverse biological activities on their own. It is generally believed that ANG III exerts its effects via AT1 and AT2 receptors. The effects include vasoconstriction and stimulation of vasopressin release and aldosterone secretion (1, 6). The pressor action of ANG III is commonly assumed to be less pronounced than that of ANG II. However, in organs such as the adrenal gland and brain, ANG III is as potent as ANG II (36). ANG-(1–7) has been shown to act as a vasodilator in many vascular beds, but has little influence on the renal vasculature when given in amounts in which ANG II is efficient. ANG-(1–7) may produce a marked increase in renal sodium and water excretion (11, 14, 16). These effects are distinct from those of ANG II and even often opposite to them. This suggests the involvement of a non-AT1/non-AT2 receptor with a higher affinity for ANG-(1–7) compared with ANG II (1, 8). There is evidence that ANG IV elicits its effects via a specific AT4 receptor. This receptor has been described in many tissues, including human collecting duct cells (4, 7, 15). Due to AT1-receptor stimulation, the actions of ANG IV may mimic some of the effects produced by ANG II. ANG IV is a vasodilator in the renal circulation and a vasoconstrictor in other vascular beds and may play a role in mediating cerebral blood flow and memory (6, 8, 37).

The experiments reported here were designed to study the possible effects of ANG III, ANG-(1–7), and ANG IV. To our knowledge, this is the first human study of truncated angiotensins in humans after double blockade of the renin system.
study addressing the possible actions of exogenous angiotensins during acute inhibition of two components of RAAS, reducing the effects of interference from changes in endogenous ANG II and aldosterone. Angiotensins are powerful inhibitors of renin secretion. Under normal conditions, this inhibition will tend to blunt the effects of exogenous angiotensin, particularly under the present conditions of low-salt diet activating the RAAS. In addition, the antinatriuretic effect of increased levels of aldosterone may complicate the interpretation of the effects of infused angiotensins. Double blockade facilitates the detection of the primary actions of the angiotensins. The use of captopril and canrenoate to block converting enzyme activity and aldosterone receptors, respectively, before infusions of angiotensins, represents a new approach for investigation of the effects of exogenous angiotensins in humans. Peptides were administered at relatively low rates (~3 pmol·kg⁻¹·min⁻¹) to ensure plasma concentrations relevant to physiological concentrations of these peptides.

**METHODS**

Experiments were performed in six healthy male volunteers 22–28 yr old, weighing 68.3–90.6 kg. Standard clinical examination and laboratory investigations regarding renal and cardiovascular diseases were negative. Each participant gave written, informed consent after having received full explanation of the protocol. The study was approved by the Danish Medicines Agency (J.NR. 2612–1367) and the Regional Ethical Committee (J.NR. 20000090).

**Experimental Protocol**

Investigations were performed after 4 days of standardized food intake designed to provide ~30 mmol NaCl/day. Measuring renal sodium excretion in the last 24-h period preceding the experiments assessed sodium turnover. Experiments were not initiated unless 24-h sodium excretion was <50 mmol (one session was excluded according to this limit). The participants were instructed to fast from 2100 on the day before the experiment. All experiments were performed on separate days with at least 2 wk of recovery between experiments. At 0720, after consumption of a standardized low-salt breakfast (150 g oatmeal, 250 ml milk, and 150 ml tap water), the subject was transported by car to the laboratory. The subject was weighed and afterwards instrumented with two catheters (Venflon 2, 18G, BOC Ohmeda, Halsingborg, Sweden) in the left and right superficial cubital veins, respectively. One catheter was used for blood sampling; the other for injection of the aldosterone antagonist potassium canrenoate (Soldactone, Searle Scandinavia, Malmö, Sweden), infusion of ⁵¹Cr-EDTA (Nycomed Amersham, Buckinghamshire, UK), and infusion of peptide or vehicle. The subject remained in a standard sitting position throughout the day and was allowed to stand only for micturition, which took place during the last minute of every hour.

Hydration was maintained by a combination of oral hypertonic and intravenous isotonic glucose solutions at a total of 200 ml/h, providing ~18 g/h of glucose, to prevent the sensation of hunger. To inhibit the RAAS, 200 mg of potassium canrenoate in 2 ml of 0.9% saline were injected intravenously 3 h before the initiation of the first urine sampling period. One hour before sampling, the subjects received 75 mg of captopril orally (Dumex-Alpharma A/S, Copenhagen, Denmark).

A bolus of ⁵¹Cr-EDTA (0.03 MBq/kg body wt), dissolved in 10 ml of 0.9% saline, was given intravenously 1.5 h before the first urine sampling and was immediately followed by a continuous infusion of ⁵¹Cr-EDTA in isotonic glucose (50 ml/h providing ~1.04 MBq/h) throughout the experiment, generating a plasma activity of 300–500 counts·min⁻¹·ml⁻¹.

**Experimental Series**

Each experiment was divided into five urine sampling periods of 1 h, i.e., one control period, three infusion periods, and a postinfusion period. In each subject, five separate experiments were performed: four peptide infusion experiments and a time control experiment. The latter was identical to the peptide infusion series, except that only vehicle was infused. ANG II, ANG III, ANG-(1–7), or ANG IV was infused at a rate of 3 pmol·kg⁻¹·min⁻¹ dissolved in isotonic glucose (8–13 ml/h) by use of an infusion pump (Perfusor secure FT, B. Braun, Melsungen, Germany). All peptides were purchased from Clinalfa (Läuflfingen, Switzerland).

**Hemodynamics**

Arterial systolic and diastolic pressures and heart rates were recorded noninvasively by the oscillometric method, by using an automatic blood pressure monitor (OMRON 705 CP, OMRON Healthcare Europe, Hoofddorp, Holland). Three consecutive measurements were performed every hour, 15 min before the initiation of the next sampling period. The mean of these values was used to calculate mean arterial blood pressure (MAP = diastolic pressure + one-third pulse pressure).

**Analyses**

Approximately 10 ml of blood was withdrawn before each urine sampling in heparinized tubes for measurements of plasma sodium, potassium, osmolality, and protein and in prechilled polyethylene tubes (Minisorb, Nunc, Roskilde, Denmark) containing aprotinine (Novo, Bagsvaerd, Denmark) and EDTA for measurements of plasma lithium and ⁵¹Cr-EDTA. Additional blood samples for hormone analysis (~20 ml) were collected before, during, and after the infusion period. Blood samples were replaced by equal volumes of isotonic saline.

Urine concentrations of sodium and potassium and osmolality were measured on the day of the experiment, as was the activity of ⁵¹Cr-EDTA. Urine samples for determination of lithium were stored at −18°C until later analysis.

Urine and plasma sodium and potassium concentrations were measured by flame photometry (IL 943, Instrumentation Laboratories, Milano, Italy), osmolality by freezing-point depression (Advanced Osmometer, model 3D3, Advanced Instruments, Needham Heights, MA), and plasma protein concentration by refractometry (Clinical refractometer T2-NE, Atago, Tokyo, Japan). The activity of ⁵¹Cr-EDTA in plasma and urine was counted in a well scintillation counter for 5,000 s or 100,000 counts. The renal clearance of endogenous lithium (CLi) was used to estimate changes in fluid delivery out of the proximal tubules. Endogenous concentrations of lithium in plasma and urine were measured by flameless atomic absorption spectrometry, according to principles described previously by Leyssac and Christensen (19). The renal CLi and clearance of sodium (CNa) were calculated as the ratio between urinary excretion rate and the mean
plasma concentrations. Filtered load of sodium (FL Na)
was calculated (FL Na = GFR - P Na; where P Na is plasma sodium and GFR is glomerular filtration rate), and endogenous C li
was used for the calculation of distal load of sodium (DL Na =
C li - P Na), absolute proximal tubular sodium reabsorption rate of
sodium [APR Na = (GFR - C li) - P Na], fractional proximal tubular
sodium reabsorption (FFP Na = 1 - (C li / GFR)), absolute
distal tubular reabsorption rate of sodium [ADR Na = (C li
- C li) - P Na], and fractional distal tubular sodium reabsorption
relative compared with DL Na (FDR Na = DLR Na / DL Na).

Plasma hormones. Blood was sampled in a dry syringe, but
transferred within a few seconds to a prechilled tube containing
EDTA and aprotinine, a potent protease inhibitor. There-
after, the samples were placed immediately in an ice-water
bath. Each tube contained ~18 μmol EDTA and 1,800 kIU
aprotinine, providing blood concentrations of EDTA and
aprotinine of ~3 μmol/ml and 300 kIU/ml, respectively. The samples were centrifuged at +4°C, and plasma was stored at
−18°C until later analysis.

Extraction. Before extraction of the angiotensin peptides
and arginine vasopressin (AVP) and atrial natriuretic pep-
tide (ANP), the plasma sample was acidiﬁed by addition of
three volumes of acetic acid (4%) and 0.1% triﬂuoroacetic acid.
Peptides were extracted by use of Sep-Pak C18 columns
(Waters) preconditioned with 4% acetic acid in 96% ethanol,
followed by 100% methanol (5 ml) and distilled water (5 ml).
The samples were applied, and the columns washed with 5
ml of Triton X-100 (0.1%; Sigma). In the ANP
assay, 3 ml of 4% acetic acid in 80% ethanol were added to
the peptide sample. After elution, the samples were evaporated
to dryness under a stream of air in a 25°C water bath over
night (~16 h).

Renin activity. The antibody trapping method of Poulsen
and Jørgensen (24) was used to determine the renin activity.
Plasma sample and ANG I antibody (Ab-3–2008939) were
incubated at 37°C for 3 h. Addition of a cold assay buffer (Tris
buffer, 4°C, 0.5 M, pH = 7.4) stopped the reaction by dilution
and cooling. Incubation with ANG I tracer (125I-ANG I,
Glostrup Hospital, Denmark) took place at 4°C for 16–20 h.
Secondary antibody (Sac-Cel, anti rabbit IgG; IDS, Boldon,
UK) was added, and the tubes were incubated at room tem-
perature for 3 h. After separation by centrifugation for 5 min,
the supernatant was removed, and the radioactivity of the
sediment was counted. The results are reported in milli-
international units per liter of the World Health Organiza-
tion renin activity (human renin, National Institute for
Biological Standards and Control, Herts, UK). Inter- and
intraassay coefﬁcients of variation were 7.4 and 9.0%, respec-
tively. Separate measurements demonstrated that the
present storage procedure, including one freeze-thaw cycle,
did not inﬂuence the results. Samples measured immediately
after separation of plasma showed values indistinguishable
from samples frozen and stored according to the standard
procedure.

Angiotensin immunoreactivity. Plasma peptide concentra-
tion was measured in extracts of plasma by using an anti-
body (Ab-5–030682), as described previously (3). The anti-
body does not recognize ANG I or ANG-(1–7) (cross-reactivity
<10−4 for both), but cross-reacts 100% with ANG III and
ANG IV. Detection limit was 1.0 pg/ml plasma sample, and
mean recoveries of unlabeled peptide added to plasma for
ANG II, ANG III, and ANG IV were 104, 96, and 127%,
respectively. Intra- and interassay coefﬁcients of variation
were 3.9 and 8.4%, respectively. Assuming stable concentra-
tions in plasma of endogenous peptides during peptide infu-
sion, an increase in ANG immunoreactivity (ANG IR) can be
used to determine the metabolic clearance rate of the various
peptides. A signiﬁcant increase in ANG IR was only observed
during infusion of ANG II, and the metabolic clearance rate
of this peptide was calculated from the increase in concen-
tration measured at the end of the infusion.

Aldosterone. The plasma aldosterone was measured with-
out extraction by using a commercial kit (Cost-A-Count,
Diagnostic Products, Los Angeles, CA). The detection limit
was 11 pg/ml, and intra- and interassay coefﬁcients of vari-
ation were 1.6 and 7.0%, respectively.

Vasopressin. AVP was measured by using an antibody
(AB3096), according to the procedure of Emmeluth et al. (10).
Detection limit was 0.15 pg/ml, and the recovery of unlabeled
AVP averaged 67%. Intra- and interassay coefﬁcients of vari-
ation were 2.8 and 7.3%, respectively.

ANP. ANP was determined, as described earlier (30), with
minor modiﬁcations by use of an antibody (RAS8798) pur-
chased from Peninsula Laboratories Europe, UK. Mean ex-
traction recovery was 73%. Detection limit was 2.0 pg/ml
sample, and intra- and interassay coefﬁcients of variation
were 4.3 and 4.8%, respectively.

Hormone results have not been corrected for incomplete
recovery.

Statistics

Data are presented as means ± SE. The results were
evaluated by one-way ANOVA for repeated measurements
within and between groups. If the results of the ANOVA were
signiﬁcant (P < 0.05), all differences between means were
investigated systematically by Newman-Keuls test. P values
<0.05 were considered to indicate signiﬁcance.

RESULTS

As described above, all subjects participated in the
infusion series and in a full-scale time control series.
Signiﬁcant within-series deviations in mean values
were considered nonspeciﬁc if similar signiﬁcant
changes occurred in the time control series. Conse-
quently, only changes signiﬁcantly different from both
the control period in the morning and the corre-
responding results of the time control series were consid-
ered to be true effects of the peptides.

Sodium Balance

The renal sodium excretion during the last 24-h
period of controlled sodium intake was between 12 ± 4
and 18 ± 6 mmol in the different series. In the hours
preceding the control period, a hefty increase in renal
sodium excretion was observed because of the blockade
of endogenous aldosterone and converting enzyme
inhibition (ACE inhibition, Table 1). In the time control
series, no further increase occurred (Fig. 1).

Hemodynamics

After ACE inhibition, MAP was ~85 mmHg in the
different series of experiments (Table 2). MAP in-
creased markedly (27%) in response to the ANG II
infusion and afterward returned toward control level
in the postinfusion period, although it still remained
signiﬁcantly higher than control level. Infusion of ANG
III elicited a slight increase in MAP (9%), i.e., about
one-third of the increase observed in the ANG II series. During infusion of ANG-(1–7) or ANG IV, no significant changes in blood pressure were seen (Table 2).

Control heart rates in all series were low and constant, ~67 beats/min (Table 2). There were no significant changes in heart rate in any of the series.

**Plasma Composition**

During infusion of ANG II, the plasma sodium concentration decreased slightly but significantly throughout the infusion period (Table 3). A concomitant slight increase in plasma potassium concentration in the first hour of infusion was followed by a decrease in the postinfusion period (Table 3). Plasma sodium and potassium concentrations did not change during infusion of ANG III, ANG-(1–7), or ANG IV (Table 3). Small changes in plasma osmolality and plasma protein concentrations were seen in most series of experiments, including the time control series (Table 3).

**Hormones**

**Renin activity.** In all series, including time control, plasma renin activity (PRA) continuously decreased throughout the experiments, from ~700 mIU/l to a value ~200 mIU/l in the postinfusion period (Table 3). The infusion of ANG II enhanced this response (end-infusion level, ~40% of the corresponding time control level).

**ANG IR.** Infusion of ANG II substantially increased plasma ANG IR from 9 ± 3 pg/ml to a maximum of 53 ± 6 pg/ml in the last infusion period, returning toward control levels in the postinfusion period (Fig. 2). Significant changes were not observed in ANG IR during infusion of ANG III and ANG IV (Fig. 2), even though the antibody cross-reacts 100% with these fragments. As in the time control series, ANG IR remained low and constant throughout these experiments (ANG IR < 17 pg/ml in all series).

**Aldosterone.** Infusion of both ANG II and ANG III markedly increased the plasma aldosterone concentration (Fig. 3). Aldosterone concentrations were elevated to a new level during infusion of ANG III (235 ± 19, 235 ± 22, and 233 ± 21 pg/ml in the first, second, and third infusion period, respectively), whereas infusion of ANG II raised the aldosterone concentration throughout the first and second infusion period, reaching a steady-state concentration in the last two periods (~340 pg/ml). All together, ANG II and ANG III had a striking impact on the aldosterone concentration, raising it by 109 and 45% above control level, respectively, whereas ANG-(1–7) and ANG IV did not affect aldosterone levels at all (Fig. 3).

**Vasopressin and ANP.** None of the angiotensins influenced the plasma levels of AVP or ANP, both of which remained relatively constant throughout the experiments (Table 3).

**Renal Variables**

The clearance of $^{51}$Cr-EDTA was used as a measure of GFR. Initial values were ~115 ml/min in all series (Table 4). Compared with control levels, significant changes were not observed during infusion of ANG III, ANG-(1–7), and ANG IV. In contrast, GFR decreased consistently in the ANG II series (~16%) and remained decreased in the postinfusion period.

A decrease in $\text{FDR}_{\text{Na-DLNa}}$ was observed in the last two periods in the time control series (Fig. 4). Significant changes in the renal $C_{\text{Li}}$, or in tubular transport of sodium were not seen during infusion of ANG III, ANG-(1–7), and ANG IV (Table 4 and Fig. 4). In contrast, infusion of ANG II caused a marked decrease in $C_{\text{Li}}$ (by ~66%, Table 4). Furthermore, in the ANG II series, a decrease in the $F_{\text{LiNa}}$ and a constant absolute reabsorption of sodium in the proximal tubule led to a
remarkable increase in the fractional reabsorption of sodium in the proximal tubule, reaching ∼92% (compared with 77% in the preinfusion period, Fig. 4). A decrease in the DLNa as well as the absolute distal tubular reabsorption of sodium, together with a constant FDBNa-DLna, were also observed during infusion of ANG II (Fig. 4).

A decrease in urine flow was observed in all series; however, infusion of ANG II had a much higher impact on urine flow than the other peptides, lowering urine flow by ∼70% (Table 4). A tendency toward a decrease in osmolar clearance was seen during infusion of ANG III, ANG-(1–7), and ANG IV, as well as in time control series, whereas the osmolar clearance decreased markedly during infusion of ANG II to ∼60% of control level (Table 4). During infusion of ANG II, urine osmolality increased two- to threefold (Table 4) without any change in plasma vasopressin. Despite a transient increase in urine osmolality in the ANG IV series, urine osmolality values were generally low and almost constant during infusion of ANG III and ANG IV.

During infusion of ANG II, sodium excretion decreased from 88 ± 18 to 25 ± 6 µmol/min and remained suppressed in the recovery period, reaching 63% of the control level (Fig. 1). Infusion of ANG IV induced a significant, but transient, increase in sodium excretion. ANG III and ANG-(1–7) did not change renal sodium excretion, although a tendency toward a diminished sodium excretion was observed during infusion of ANG III (Fig. 1).

Potassium excretion decreased nearly 45% during infusion of ANG II and remained steady in the postinfusion period (Table 4). Infusion of ANG IV, on the other hand, initiated an increase in potassium excretion in the first infusion period, but otherwise did not have an effect on this parameter (Table 4). Potassium excretion did not change in either the ANG III or the ANG-(1–7) series (Table 4).

**DISCUSSION**

The purpose of this study was to compare the cardiovascular and renal effects of angiotensin peptides in healthy humans during acute blockade of endogenous aldosterone and ACE with a combination of previously applied doses of canrenoate (2, 34) and captopril (5, 17). The results support the notions that 1) exogenous ANG II has marked effects on renal excretory function, even in the absence of changes in AVP and ANP, 2) plasma clearances of ANG III and ANG IV are substantially higher than that of ANG II, and 3) ANG III is a potent stimulator of aldosterone secretion, even at increments in plasma concentrations so small that they are undetectable by RIA.

The design of the experiments included standardized conditions with respect to sodium intake. RAAS was antagonized only on the day of the investigation to ensure inhibition of converting enzyme activity and antagonism of endogenously produced aldosterone without significant disruption of fluid and electrolyte balance. Hence the effects of exogenous angiotensins could be examined under minimal interference from the RAAS, thus providing a suitable platform for the study of the possible effects of the infused peptides under normal conditions.

A substantial increase in ANG IR was observed during infusion of ANG II, followed by a return toward control levels after the infusion. The response is very similar to that previously obtained by Krekels et al. (18) in sodium-restricted humans. During stepwise infusion of ANG II, they found an increase in ANG IR reaching ∼56 pg/ml with the highest infusion rate (3 pmol·kg⁻¹·min⁻¹). Plasma levels of ANG II returned to baseline 60 min after discontinuation of the infusion. These effects were elicited with an infusion rate equivalent to the ANG II infusion rate used in the present study. In contrast, we found no significant changes in ANG IR during infusion of ANG III. Under the reasonable assumption of similar clearances of ANG II and ANG III in the brachial circulation, this lack of a measurable increase in ANG IR is surprising. The actions of exogenous substances are mediated via changes in arterial concentrations, which, in the present study, were assessed by forearm venous sampling. In the situation with a forearm arteriovenous
Table 3. Plasma electrolytes, protein and hormone concentrations

<table>
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<tr>
<th>Sampling Period, min</th>
<th>Time Control</th>
<th>ANG II Infusion</th>
<th>ANG III Infusion</th>
<th>ANG-(1–7) Infusion</th>
<th>ANG IV Infusion</th>
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<td></td>
<td>Plasma sodium, mmol/l</td>
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<td>7.6 ± 0.1*</td>
<td>7.5 ± 0.2*</td>
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<td>326 ± 65*</td>
<td>417 ± 81*</td>
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<tr>
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<td>204 ± 28*</td>
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<td>Plasma arginine vasopressin, pg/ml</td>
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<td>Plasma atrial natriuretic peptide, pg/ml</td>
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</table>

Values are means ± SE. Infusion of peptide or vehicle was from \( t = 60–240 \). *Significantly different from control level (\( P < 0.05 \)); †significantly different from time control series (\( P < 0.05 \)).

(a-v) difference for ANG III much larger than the corresponding a-v difference for ANG II, the arterial concentration of ANG III might still be substantial, also in a situation where the venous concentrations did not change measurably. The adrenal gland could, under these conditions, be exposed to sizeable arterial concentrations of ANG III not measurable in forearm venous plasma. However, the existence of a difference in forearm a-v extractions for the two peptides adequate to explain the apparent difference in clearance seems very unlikely. However, the possibility, albeit remote, could be addressed by use of sampling of arterial blood. In case of a significant increase in plasma ANG IR, the metabolic clearance rate of the corresponding peptide was calculated. Hence, on the basis of

the assumption of steady-state plasma concentrations of ANG II, metabolic clearance rate was estimated to be \( -65 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) in the ANG II series. However, it is not possible to calculate the metabolic clearance rate for ANG III or ANG IV, because the increases in ANG IR were not significant in the present study. This is valid evidence that the clearances of ANG III and ANG IV are indeed very high, although it does not provide adequate evidence to indicate how high. Nevertheless, the clearances of ANG III and ANG IV are higher to an extent, which eludes quantification by the present methods. These data are, although a difference in species is acknowledged, comparable to an analogous study performed in our laboratory (35). Results of this study were based on similar infusions in the con-
and Christensen (19) have shown that the CL\textsubscript{i} is very
avoids the natriuretic effect of lithium loading. Leyssac
of ANG II (0.5 and 1.5 ng/ml, respectively) to decreases in GFR, sodium and potassium excretion, urine flow, and osmolar clearance; and 3) to increases in aldosterone concentration and urine osmolality. Heart rate and plasma electrolyte, osmolality, and protein concentration were relatively unaffected by peptide infusion. These findings are in accordance with the work of several groups (18, 27, 28). It is, however, not clear why ANG II in the recovery period continued to depress sodium and potassium excretion, GFR, and urine volume and to augment MAP and aldosterone level under conditions in which ANG IR has almost returned to the preinfusion level.

ANG III increased MAP and plasma aldosterone without affecting the renal sodium excretion. The hypertensive effect of ANG III in the present study is in agreement with that found in a previous study on humans by Suzuki et al. (32), who infused ~19 pmol·kg\textsuperscript{-1}·min\textsuperscript{-1}, but the increase in MAP in the present study was obtained with much lower infusion rates of ANG III (3 pmol·kg\textsuperscript{-1}·min\textsuperscript{-1}). Infusion of ANG III did not affect other cardiovascular and renal parameters.

Infusion of ANG II as well as ANG III markedly stimulated aldosterone secretion, which is in accordance with previous findings of other groups (21). It is well established that ANG II is an important mediator of synthesis and secretion of aldosterone, and earlier studies in both dogs and humans suggested that ANG III is a potent stimulator of aldosterone secretion (21, 31, 32, 38). However, the potency of ANG III in this respect, compared with that of ANG II, remains vaguely determined. In a dose-response experiment in sodium-replete dogs, McCaa (21) found that ANG II was twice as effective as ANG III in stimulating aldosterone secretion. In the present study, ANG III markedly increased the aldosterone concentration, even in the absence of a measurable increase in ANG IR. As the actions of the infused peptide must be generated exclusively via increases in the plasma concentration, these data suggest that, per unit increase in plasma aldosterone level under conditions in which ANG IR

oscious dog; it was found from arterial blood samples that the concentrations of ANG III and ANG IV did increase measurably, and that ANG III and ANG IV were metabolized two to four times faster than ANG II.

Usually, the clearance of lithium is measured after oral or intravenous administration of a lithium salt. However, this procedure may affect the tubular transport of sodium and by itself cause natriuresis (13, 18). Recently, it has been shown that an oral test dose of 450 mg LiCO\textsubscript{3} significantly increased the renal sodium excretion compared with subjects not given LiCO\textsubscript{3} (29). The present use of CL\textsubscript{i} as a measure of tubular function avoids the natriuretic effect of lithium loading. Leyssac and Christensen (19) have shown that the CL\textsubscript{i} is very similar to values obtained after an exogenous lithium load. The measurement of CL\textsubscript{i} is, therefore, a valid alternative to the conventional method and completely free from the disadvantage of possibly introducing natriuresis.

Despite the decrease in GFR and the associated parallel decrease in the FL\textsubscript{Na}, the absolute proximal reabsorption of sodium remained unchanged during infusion of ANG II, as the fractional reabsorption of sodium in the proximal tubule increased substantially. These data are comparable to those obtained by Schou et al. (29), indicating that relatively low infusion rates of ANG II (0.5 and 1.5 ng·kg\textsuperscript{-1}·min\textsuperscript{-1}, respectively) prevent the increase in the end-proximal delivery of tubular fluid otherwise induced by water immersion. In the present study, ANG II seems to have no measurable effect on the distal fractional tubular sodium reabsorption, leaving aldosterone as a possible mediator of the effects of ANG II in the distal parts of the nephron. The antinatriuretic effect observed in the present study is most likely due to a direct effect on the proximal tubule in which fractional sodium reabsorption was elevated to the remarkably high level of 92%.

Infusion of ANG III, ANG-(1–7), and ANG IV did not affect the renal CL\textsubscript{i} or the renal handling of sodium.

The observed pattern of effects during infusion of ANG II was expected. The ANG IR showed steady state of ~55 pg/ml, and this led 1) to a 20-mmHg increase in MAP; 2) to decreases in GFR, sodium and potassium excretion, urine flow, and osmolar clearance; and 3) to increases in aldosterone concentration and urine osmolality. Heart rate and plasma electrolyte, osmolality, and protein concentration were relatively unaffected by peptide infusion. These findings are in accordance with the work of several groups (18, 27, 28). It is, however, not clear why ANG II in the recovery period continued to depress sodium and potassium excretion, GFR, and urine volume and to augment MAP and aldosterone level under conditions in which ANG IR has almost returned to the preinfusion level.

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![Fig. 2. Plasma angiotensin immunoreactivity (ANG IR). Values are means ± SE. Dotted line, control series; ■, ANG II series; ▲, ANG III series; ●, ANG IV series. *Significantly different from preinfusion value by ANOVA (P < 0.05) and Newman-Keuls test. †Significantly different from corresponding values in time control series by ANOVA (P < 0.05) and Newman-Keuls test.](http://ajpregu.physiology.org/)

![Fig. 3. Plasma aldosterone concentration. Values are means ± SE. Dotted line, control series; ■, ANG II series; ▲, ANG III series; ●, ANG-(1–7) series; ○, ANG IV series. *Significantly different from preinfusion value by ANOVA (P < 0.05) and Newman-Keuls test. †Significantly different from corresponding values in time control series by ANOVA (P < 0.05) and Newman-Keuls test.](http://ajpregu.physiology.org/)
concentration, ANG III is much more potent than ANG II in stimulating aldosterone secretion.

Hollenberg et al. (17) have shown that administration of captopril in healthy humans, on normal or restricted sodium diet, increases PRA and decreases plasma ANG II levels. A similar increase in PRA has been reported in humans on unrestricted sodium intake after inhibition of converting enzyme by captopril (33). However, these studies are difficult to compare with the present work due to differences in experimental design. In the present study, a high renin activity was observed in the control period in all series, that is, 2 h after the administration of captopril. The mean of these control values was ~700 mIU/l. This is at least one order of magnitude higher than the renin values obtained in a comparable human study performed in our laboratory, where mean PRA control values were ~80 mIU/l (25). This study was identical to the present study with respect to sodium intake, but the subjects received no pharmacological pretreatment; hence, this value is a valid estimate of the untreated value of PRA in our study, indicating a large increase in renin activity after the administration of captopril. The increase seems, at least in part, to be due to the withdrawal of the short-loop feedback suppression by ANG II on renin secretion. Changes in blood pressure might also be significant in this context, although the difference in mean blood pressure, compared with the study by Rasmussen et al. (25), was only 5–8 mmHg in the preinfusion period. One might speculate that the decrease in MAP after ACE inhibition only to a minor extent is involved in the observed increase in PRA. Under conditions with pressures in the physiological range, ANG II is probably a very powerful regulator of renin secretion. During ACE inhibition, elimination of the short-loop feedback suppression by ANG II has great impact on renin secretion, a response that may be completely reversed by infusion of ANG II (22). Pressure-dependent renin release is, as shown by Reinhardt and Seeliger (26), another major regulator of

Table 4. Renal variables

<table>
<thead>
<tr>
<th>Sampling Period, min</th>
<th>Time Control</th>
<th>ANG II Infusion</th>
<th>ANG III Infusion</th>
<th>ANG-(1–7) Infusion</th>
<th>ANG IV Infusion</th>
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<td>51Cr-EDTA clearance, ml/min</td>
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<td>116 ± 5</td>
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<td>103 ± 3†</td>
<td>108 ± 5</td>
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<td>97 ± 4†</td>
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<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2*</td>
<td>2.1 ± 0.2</td>
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<td>65.9 ± 12.7</td>
<td>66.4 ± 10.7*</td>
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<td>120–180</td>
<td>51.0 ± 8.1</td>
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<td>45.4 ± 6.9</td>
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<td>32.7 ± 4.3*</td>
<td>48.4 ± 8.9</td>
<td>52.9 ± 11.1</td>
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<td>27 ± 3</td>
<td>23 ± 3</td>
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<tr>
<td>60–120</td>
<td>29 ± 4</td>
<td>10 ± 1*†</td>
<td>16 ± 2†</td>
<td>22 ± 2</td>
<td>31 ± 5</td>
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<td>120–180</td>
<td>26 ± 4</td>
<td>8 ± 1*†</td>
<td>18 ± 1</td>
<td>19 ± 2</td>
<td>31 ± 8</td>
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<tr>
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<td>9 ± 2*‡</td>
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<td>18 ± 1</td>
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<td>240–300</td>
<td>22 ± 2</td>
<td>17 ± 1</td>
<td>20 ± 3</td>
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<td>23 ± 5</td>
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</table>

Values are means ± SE. Infusion of peptide or vehicle was from 60–240. *Significantly different from control level (P < 0.05); †significantly different from time control series (P < 0.05).
PRA, but in the present study it seems that much larger changes in MAP are required to account for the large increase in PRA. The large decrease in PRA throughout the day (e.g., from 707 to 204 mIU/l) was most likely preceded by an even larger increase in renin activity (~80 to ~700 mIU/l), as a result of the administration of captopril. The large decrease in renin activity during all of the experiments could indicate a relative shortage of renin in the juxtaglomerular cells after a persisting, powerful stimulus for renin secretion following captopril. Infusion of ANG II augmented the decrement in renin activity. This response may be attributed to the short-loop feedback suppression by ANG II on renin secretion or to a direct effect of the increase in arterial pressure.

It has been shown that infusion of ANG III in humans on a normal sodium diet significantly decreases PRA (32), although this response was achieved with rather high infusion rates of ANG III (20 ng·kg⁻¹·min⁻¹, ~19 pmol·kg⁻¹·min⁻¹) and, consequently, a markedly elevated blood pressure. In our study, infusion of ANG III did not significantly alter the decrease in renin activity compared with time control series. Infusion of relatively large amounts of ANG III seems capable of reducing renin secretion, but whether this response is related to a direct effect on the juxtaglomerular cells (short-loop feedback suppression of ANG III) or to the increase in blood pressure remains to be elucidated.

Injection of ANG II and ANG III into cerebral ventricles in animals has marked dipsogenic and pressor effects, induces salt appetite, and stimulates the release of vasopressin from the pituitary gland (1, 36). Zini et al. (39) have shown that the action of ANG II on vasopressin secretion depends on the prior conversion of ANG II to ANG III. These studies were performed in mice, after intracerebroventricular injections of ANG II or ANG III, together with inhibitors of aminopeptidase A and N. Thus, regarding the release of vasopressin, there is now evidence that ANG III is the main effector peptide. In the present study, intravenous infusions of both ANG II and ANG III failed to produce changes in vasopressin levels. Even in the absence of changes in vasopressin levels, a remarkable urinary concentration was still observed during infusion of ANG II. This phenomenon might be due to the decrease in tubular flow, i.e., GFR and Cₐₗ. The lack of increase in vasopressin might well be due to the fact that much higher plasma concentrations of ANG II and ANG III are needed to stimulate release of vasopressin.

**Perspectives**

The exact mechanisms involved in the onset and continuation of essential hypertension are still not determined. An activity above normal of the RAAS and functional abnormalities involving the kidney have been implicated in the development of this as well as other cardiovascular diseases (9, 12, 17), but still very little is known about the pathophysiology underlying essential hypertension.

The present data represent an extension of several other reports, confirming that the RAAS is much more complicated than previously thought. The recent discovery of other biologically active angiotensins and specific receptors distinct from the AT₁ and AT₂ receptors sets the stage for further investigation regarding the involvement and interaction of the RAAS in physiological as well as pathophysiological conditions. The recent work by Zini et al. (39) showed that conversion of ANG II to ANG III was required to stimulate vasopressin secretion in mice. In the present study, ANG
III seems to be more potent than ANG II in stimulating aldosterone secretion, raising the question of specific receptors for ANG III. A specific ANG III receptor has, to our knowledge, never been established. The discovery of such a receptor may yield new possibilities with regard to explanation of aldosterone control, normal control of sodium homeostasis, pathophysiological mechanism of, for example, hypertensive diseases, and pharmacological intervention.

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DISCLOSURES

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