Effect of urodilatin infusion on renal haemodynamics, tubular function and vasoactive hormones

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1. The renal efficacy of urodilatin in humans has only been partly investigated. It is unknown whether intravenously infused urodilatin has an effect on sodium reabsorption in both the proximal and distal part of the nephron.

2. Twelve healthy subjects participated in this double-blind, placebo-controlled study in a cross-over design. They received, in a randomized order, a short term (60 min) infusion of urodilatin in three different doses (10, 20 and 40 ng min⁻¹ kg⁻¹ of body weight) and placebo. Renal haemodynamics were estimated by clearance technique with radio-active tracers, and proximal tubular handling of sodium was evaluated by lithium clearance.

3. The 20 ng min⁻¹ kg⁻¹ dose increased the urinary sodium excretion and urinary flow rate compared with the effects of placebo. It increased the glomerular filtration rate and decreased the effective renal plasma flow. In addition, the dose increased the lithium clearance compared with placebo, but did not significantly change the fractional excretion of lithium. On the other hand, it markedly decreased the distal fractional reabsorption of sodium. It also had a suppressive effect on renin secretion. The systemic arterial blood pressure was unchanged, but the dose increased the pulse rate and the haematocrit. The highest dose (40 ng min⁻¹ kg⁻¹) induced a wide variation in the natriuretic and diuretic responses, probably due to a blood-pressure-lowering effect.

4. We conclude, that the urodilatin dose of 20 ng min⁻¹ kg⁻¹ of body weight was most efficacious in this short-term infusion study, and that it had potent natriuretic and diuretic qualities, probably due to stimulation of the glomerular filtration rate and inhibition of sodium reabsorption in the distal part of the nephron.

INTRODUCTION

Urodilatin (URO) is a new natriuretic peptide extracted from human urine [1]. It has not been detected in plasma, which suggests that the peptide is probably produced and processed within the kidney. URO is a peptide of 32 amino acids (URO 95–126), an N-terminal extended form of the circulating atrial natriuretic peptide (ANP) (99–126). It is still unclear where and how the exact synthesis of URO takes place, but presumably URO is processed in the distal tubular cells from the same precursor as ANP (99–126) [2–4]. URO may act as a paracrine hormone restricted to the distal part of the nephron. Studies suggest that luminal receptors coupled to guanylate cyclase in the inner medullary collecting ducts are the physiological targets of URO [5, 6]. URO may interact with the amiloride-sensitive sodium channels and inhibit sodium reabsorption by an effect mediated by cyclic GMP (cGMP). Urinary URO excretion is closely associated with the circadian rhythm in renal sodium excretion in humans [7], and increases in parallel with natriuresis after salt ingestion [8] and acute saline infusion [7]. URO, rather than circulating ANP, may be the natriuretic peptide that is involved in the regulation of natriuresis under physiological conditions [9].

Studies suggest that exogenous URO is a more potent natriuretic agent than synthetic ANP under normal and pathological conditions. Infusion of URO into normal dogs and into dogs and rats with heart failure produced considerable natriuresis and diuresis, clearly greater than observed during infusion of equimolar doses of ANP [10–12]. The mechanisms underlying this greater natriuretic potency of URO have not been clarified, but it was considered to be due to the differences in the renal degradation of the peptides. Thus, filtered URO seems to pass the proximal tubules without being degraded, whereas considerable amounts of filtered ANP are cleaved by endopeptidases in the brush border of the proximal tubule before reaching the receptors in the collecting ducts [13]. Exogenous URO also seems to exert more potent renal effects than ANP in healthy men. The excretion of sodium and diuresis produced by bolus injection of 25 µg of URO equalled the effects of 50 µg of ANP [14].

Key words: atrial natriuretic peptide, lithium clearance, sodium, urodilatin.

Abbreviations: Ado, aldosterone; Ang II, angiotensin II; ANP, atrial natriuretic peptide; AVP, arginine-vasopressin; BNP, brain natriuretic peptide; cGMP, cyclic GMP; Cu, sodium clearance; CV, coefficient of variation; DAR, distal absolute reabsorption; DFR, distal fractional reabsorption; FE, fractional excretion; FF, filtration fraction; GFR, glomerular filtration rate; MAP, mean arterial blood pressure; pGMP, plasma cGMP; PRC, plasma renin concentration; PFR, proximal fractional reabsorption; RPF, renal plasma flow; TcGMP, tubular excretion of cyclic GMP; ucGMP, urine cGMP; URO, urodilatin.

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However, in a more recent study neither urine flow nor sodium excretion were different between the URO and the ANP group at a bolus injection dose of 2 pg/kg of body weight [15]. At present, intravenous infusion of URO in a dose-response manner of 2 pg/kg of body weight has not been studied. URO has previously been administered as prolonged infusion to patients with congestive heart failure [16] and to patients following cardiac transplantation [17], but the in vivo mechanisms by which synthetic URO induces natriuresis and diuresis in humans are not clearly established. An increase in glomerular filtration rate (GFR), unaltered renal plasma flow (RPF) and increased fractional excretion of sodium (FE\textsubscript{Na}) have been reported [14]. It is unknown whether intravenously infused URO has an effect on sodium reabsorption in the proximal part of the nephron in humans. A single study of intra-renal infusions of URO in dogs did not demonstrate a change in lithium clearance (CL\textsubscript{Li}), indicating that URO exhibits its renal action not at the proximal tubule site [18].

The aims of this study were: (1) to determine whether low-dose infusions of synthetic URO in healthy volunteers affect renal function; (2) if they do, to delineate the nephron sites involved, using renal clearance of ingested lithium as a proximal tubular marker; and (3) to investigate the possible contribution of haemodynamics and hormonal factors. The following main parameters were determined during the study period: GFR, effective RPF, filtration fraction (FF), CL\textsubscript{Li}, fractional excretion of lithium (FE\textsubscript{Li}), urinary excretion of sodium (U\textsubscript{Na}), FE\textsubscript{Na}, proximal fractional reabsorption of sodium/water (PFR\textsubscript{waterNa}), proximal absolute reabsorption of sodium (PAR\textsubscript{Na}), distal absolute reabsorption of sodium (DAR\textsubscript{Na}), distal fractional reabsorption of sodium (DFR\textsubscript{Na}), urine flow rate (V), plasma cGMP (pcGMP), urine cGMP (ucGMP) and tubular excretion of cGMP (T\textsubscript{cGMP}), haematocrit, plasma renin concentration (PRC), plasma angiotensin II (Ang II), aldosterone (Aldo), arginine-vasopressin (AVP), ANP, brain natriuretic peptide (BNP), mean arterial blood pressure (MAP) and pulse rate.

**MATERIAL AND METHODS**

**Subjects**

The inclusion criteria were: (1) healthy non-smoking male volunteers; (2) aged between 18 and 50 years; and (3) written informed consent to undergo the study. The exclusion criteria were: (1) clinical and/or laboratory evidence of renal, hepatic, cardiovascular, endocrinological, allergic, infectious or neoplastic disease; (2) history of bladder dysfunction; (3) alcohol abuse; (4) smoking habit; (5) intake of drugs or alcohol within 72 h before the examination; and (6) subjects who did not take the prescribed sodium chloride tablets or the lithium carbonate tablet. The withdrawal criteria after the study were: (1) development of severe adverse events; and (2) a difference of 20% or more in GFR during the two pre-infusion clearance periods. The subjects were recruited by posters at the Medical Faculty of Aarhus University. Twelve healthy male volunteers were recruited. Among them, one was replaced because he developed infectious mononucleosis after the first study day. Twelve subjects completed the study.

The volunteers, aged 20–37 years (mean age 25.4 years) and with a body weight within 65–95 kg (mean weight 78.7 kg), had systemic blood pressure consistently less than 140/90, a normal electrocardiogram and normal routine parameters, including haemoglobin, haematocrit, leucocyte counts, platelets, prothrombin, aspartate aminotransferase, bilirubin, alkaline phosphatase, creatinine, calcium, sodium, potassium, and urine without albumin and glucose.

The study was approved by the local Ethics Committee and the Danish National Board of Health, and it was carried out in accordance with the Declaration of Helsinki.

**Design**

The study was double-blind and placebo-controlled. Each subject was studied on four different days with an interval of 4 weeks between the examinations. Each subject received, in randomized order, intravenous infusion of 10, 20 and 40 ng of URO min\textsuperscript{-1} kg\textsuperscript{-1} of body weight and placebo over a period of 60 min.

**Procedure**

Each subject was instructed to take sodium chloride tablets, 2 g daily, for 3 days before each study day. A 24 h urine was collected and at 22.00 hours before the study day a 300 mg lithium carbonate tablet was given orally. The subjects fasted from midnight. On the study day, 200 ml of water was given orally every 30 min from 8.00 hours. The study day consisted of an equilibration phase of 120 min (9.00 hours to 11.00 hours), the infusion phase of 60 min (11.00 hours to 12.00 hours) and a post-infusion phase of 60 min (12.00 hours to 13.00 hours). Urine was collected in six consecutive 30 min clearance periods from 10.00 hours to 13.00 hours. After the second period at 11.00 hours, synthetic URO (INN: ularitide; Haemopep Pharma, Hannover, Germany) or placebo was infused by an infusion pump with an infusion rate of 0.1 ml min\textsuperscript{-1} kg\textsuperscript{-1}. The subjects were in supine position except during urination. Urine voiding took place in standing position in the first 6 of 48 infusion days. As two subjects developed a vasovagal attack during urination after the last infusion the position was changed to sitting on the bed edge during urination. Urine from the clearance periods was analysed.
for $^{51}$Cr-labelled EDTA and $^{125}$I-labelled hippuran radioactivity, sodium, potassium, lithium, osmolality and cGMP. At the beginning and end of each clearance period, venous blood samples were drawn for determination of $^{51}$Cr-labelled EDTA radioactivity, $^{125}$I-labelled hippuran activity, sodium, potassium, lithium, osmolality and haematocrit. PRC, Ang II, Aldo, AVP, ANP, BNP and cGMP were determined before the start of infusion and at the end of clearance periods 3, 4, 5 and 6. All blood samples were taken in the supine position before urination. After the drawing of a blood sample an equal volume of isotonic saline was given intravenously. Blood pressure and heart rate were measured every 30 min from 8.30 hours to 11.00 hours. During and after infusion the measurements were made every 15 min. The routine parameters were determined before each study day. Sodium, potassium and creatinine were measured in the 24 h urine.

**Methods**

GFR and RPF were measured by a constant infusion clearance technique [19] using $^{51}$Cr-labelled EDTA and $^{125}$I-labelled hippuran as reference substances. A priming dose of $^{51}$Cr-labelled EDTA and $^{125}$I-labelled hippuran at 9.00 hours was followed by continuous intravenous infusions of $^{51}$Cr-labelled EDTA and $^{125}$I-labelled hippuran until the end of the post-infusion phase. Serum radioactivity was kept stable by use of an infusion pump (VIAL Medical, Brezins, France). Serum and urinary concentrations of lithium were measured by atomic absorption spectrophotometry (Perkin-Elmer spectrophotometer, model 3100). Sodium and potassium in serum and urine were determined by an autoanalyser technique by staff at the Department of Clinical Biochemistry, Skejby Hospital, Denmark.

The clearance calculations are based on the formula: clearance of a substance $x$ is: $C_x = C_u(U/C_p)$, where $C_u$ is the concentration of $x$ in urine, $U$ the urinary output and $C_p$ the mean value of the two plasma concentrations of $x$ at the start and end of each clearance period.

Accepting that lithium is solely absorbed in the proximal tubules, and to the same degree as sodium and water, $C_{Li}$ is a measure of end proximal flow of isotonic fluid into the loop of Henle [20]. Using GFR, $C_{Li}$, sodium clearance ($C_{Na}$), $V$ and plasma concentrations of sodium ($P_{Na}$), the following calculations give estimates of segmental renal handling of sodium.

Proximal absolute reabsorption of sodium:

$$\text{PAR}_{Na} = (\text{GFR} - C_{Li})P_{Na}$$

Proximal fractional reabsorption of sodium and water:

$$\text{PFR}_{Na\text{water}} = (1 - C_{Li}/\text{GFR})100\%$$

Distal absolute reabsorption of sodium:

$$\text{DAR}_{Na} = (C_{Li} - C_{Na})P_{Na}$$

Distal fractional reabsorption of sodium:

$$\text{DFR}_{Na} = (1 - C_{Na}/C_{Li})100\%$$

GFR, RPF, $C_{Li}$, $C_{Na}$ and the calculated tubular parameters were all standardized to a body surface of 1.73 m$^2$. $T_{cGMP}$ was calculated using urinary flow rate ($V$), GFR, $pcGMP$ and $ucGMP$:

$$T_{cGMP} = V(ucGMP) - GFR(pcGMP)$$

Ang II in plasma was measured by RIA by a modification of the method described by Kappelgaard et al. [21]. RIA was performed after previous extraction from plasma by Sep-Pak C$\text{I}$$_8$ cartridges (Waters Associates, MA, U.S.A.) which were washed with 20% methanol and water. Elution took place with 100% methanol. The minimum detection level was 2 pmol/l of plasma. The coefficients of variation (CV) were 12% (inter-assay) and 8% (intra-assay). Aldo in plasma was measured by a modification of the method of Rask-Madsen et al. [22]. RIA was performed after extraction from plasma by Sep-Pak C$\text{I}$$_8$ cartridges. The minimum detection level was 42 pmol/l. The CVs were 13% (inter-assay) and 9% (intra-assay).

The quantitative determination of active renin in plasma was measured by a commercial immunoradiometric assay (Nichols Institute, Switzerland). The CVs were 7.4% (inter-assay) and 2.5% (intra-assay). AVP in plasma was measured by a slight modification of the method described by Pedersen et al. [23]. Before the RIA, AVP was extracted from plasma by Sep-Pak C$\text{I}$$_8$ cartridges. The minimum detection level was 0.5 pmol/l of plasma. The CVs were 13% (inter-assay) and 9% (intra-assay). ANP in plasma was determined by RIA as previously described by Thomassen et al. [24]. ANP was extracted from plasma by Sep-Pak C$\text{I}$$_8$ cartridges eluted by 80% ethanol in 4% acetic acid. The minimum detection level was 0.5 pmol/l of plasma. The CVs were 12% (inter-assay) and 10% (intra-assay). There was 100% cross-reactivity with synthetic URO (Bissendorf Pharma, Hannover, Germany). BNP in plasma was measured by a RIA. Immunoreactive BNP was extracted from plasma by use of Sep-Pak C$\text{I}$$_8$ cartridges eluted by 80% ethanol in 4% acetic acid. RIA was performed using a rabbit anti-BNP antibody developed in our laboratory. There was no cross-reactivity with ANP and URO. The sensitivity of the RIA of BNP was 0.49 fmol per tube. The CVs were 6% (intra-assay) and 11% (inter-assay). cGMP in plasma and urine was measured by RIA using a commercial kit (Amersham). Ethanol was used for extraction from plasma. The CVs were 9% (inter-assay) and 6% (intra-assay); the lower detectable limit for pcGMP was 0.3 nmol/l.
The osmolar concentration of serum and urine was determined by an osmometer (Advanced cryometric osmometer, Model 3C2), using freezing-point depression. Blood pressure was determined by a Hawksley random zero sphygomanometer and a semi-automatic digital blood-pressure meter (UA-751; Takeda Medical).

**Statistical analysis**

Twelve subjects completed the study. One was withdrawn after the study because of incomplete emptying of the urinary bladder, demonstrated by a difference of more than 20% in GFR between pre-infusion clearance periods. This left eleven subjects for statistical analysis. The data from the 40 ng group were not included in the statistical calculations, because in this group the renal and hormonal parameters differed considerably due to secondary counteracting mechanisms.

A parametric repeated-measures analysis of variance in conjunction with a paired-samples t-test were used to evaluate differences between clearance periods within doses and differences between doses. A lack of normality in the raw data was overcome by taking natural logarithms of the variables. All the statistical analyses were performed on natural-log-transformed data. The differences between groups were based on the relative changes from baseline. For paired comparison within groups, the data from the clearance periods during the study day were compared with baseline values (average value of the measurements in clearance periods 1 and 2). Clearance periods 1 and 2 are the pre-infusion periods, clearance period 3 (0–30 min) and clearance period 4 (30–60 min) are during infusion and clearance periods 5 (60–90 min) and 6 (90–120 min) are in the post-infusion phase. A P value of 0.05 was the limit of significance. The relative changes (mean values) of the absolute values are selected for presentation in the Results section below.

**RESULTS**

**Renal haemodynamics**

GFR, RPF and FF are shown in Table 1. There was a significant increase in GFR within the 20 ng dose. After 30 min of infusion, the changes (expressed as means) in GFR were significantly different between groups (10 ng, −3%; 20 ng, 5%; placebo, −3%; P < 0.05). RPF decreased significantly during and after infusion of URO. The relative changes of RPF (expressed as means) from pre-infusion levels were significantly different between groups after 60 min (period 4: 10 ng, −13%; 20 ng, −14%; placebo, 0.3%; P < 0.05) and 90 min (period 5: 10 ng, −14%; 20 ng, −22%; placebo, −7%; P < 0.01). There was a significant drug effect on FF within the URO groups. FF increased significantly in period 4 (10 ng, 19%; 20 ng, 25%; placebo, 1%; P < 0.01) and period 5 (10 ng, 19%; 20 ng, 32%; placebo, 3%; P < 0.01).

**Urinary sodium excretion and fractional excretion of sodium**

The urinary sodium excretion \( U_{Na} \) is shown in Table 2 and Fig. 1. \( U_{Na} \) increased significantly within the URO groups. In the placebo group, a significant effect of time was observed. The natriuresis induced by URO (expressed as the mean) was significant in

<table>
<thead>
<tr>
<th>GFR (ml min^{-1} 1.73 m^{-2})</th>
<th>Pre-infusion Periods 1+2 (0 min)</th>
<th>Infusion Period 3 (30 min)</th>
<th>Infusion Period 4 (60 min)</th>
<th>Post-infusion Period 5 (90 min)</th>
<th>Post-infusion Period 6 (120 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>103 (4.6 ± 0.1)</td>
<td>100 (4.6 ± 0.2)</td>
<td>103 (4.6 ± 0.1)</td>
<td>98 (4.5 ± 0.1)</td>
<td>106 (4.7 ± 0.2)</td>
</tr>
<tr>
<td>10 ng</td>
<td>104 (4.6 ± 0.1)</td>
<td>101 (4.6 ± 0.2)</td>
<td>106 (4.7 ± 0.2)</td>
<td>105 (4.6 ± 0.1)</td>
<td>104 (4.6 ± 0.1)</td>
</tr>
<tr>
<td>20 ng</td>
<td>100 (4.6 ± 0.1)</td>
<td>105 (4.7 ± 0.1)**</td>
<td>107 (4.7 ± 0.1)**</td>
<td>103 (4.6 ± 0.1)</td>
<td>98 (4.6 ± 0.1)</td>
</tr>
<tr>
<td>40 ng</td>
<td>103 (4.6 ± 0.1)</td>
<td>106 (4.7 ± 0.1)</td>
<td>101 (4.6 ± 0.2)</td>
<td>97 (4.5 ± 0.1)</td>
<td>103 (4.6 ± 0.1)</td>
</tr>
<tr>
<td>RPF (ml min^{-1} 1.73 m^{-2})</td>
<td>516 (6.2 ± 0.2)</td>
<td>503 (6.2 ± 0.2)</td>
<td>514 (6.2 ± 0.2)</td>
<td>474 (6.1 ± 0.2)</td>
<td>534 (6.3 ± 0.2)</td>
</tr>
<tr>
<td>Placebo</td>
<td>517 (6.2 ± 0.2)</td>
<td>476 (6.1 ± 0.2)**</td>
<td>453 (6.0 ± 0.2)**</td>
<td>444 (6.0 ± 0.2)**</td>
<td>484 (6.2 ± 0.2)**</td>
</tr>
<tr>
<td>10 ng</td>
<td>506 (6.2 ± 0.1)</td>
<td>498 (6.2 ± 0.2)</td>
<td>435 (6.0 ± 0.2)**</td>
<td>406 (6.0 ± 0.2)**</td>
<td>433 (6.1 ± 0.1)**</td>
</tr>
<tr>
<td>20 ng</td>
<td>503 (6.2 ± 0.2)</td>
<td>458 (6.1 ± 0.1)</td>
<td>381 (5.9 ± 0.9)</td>
<td>364 (5.8 ± 0.1)</td>
<td>431 (6.0 ± 0.1)</td>
</tr>
<tr>
<td>FF (%)</td>
<td>20 (3.0 ± 0.1)</td>
<td>20 (3.0 ± 0.1)</td>
<td>20 (3.0 ± 0.1)</td>
<td>21 (3.0 ± 0.1)</td>
<td>20 (3.0 ± 0.1)</td>
</tr>
<tr>
<td>Placebo</td>
<td>20 (3.0 ± 0.1)</td>
<td>22 (3.1 ± 0.1)**</td>
<td>24 (3.2 ± 0.1)**</td>
<td>24 (3.2 ± 0.1)**</td>
<td>22 (3.1 ± 0.1)**</td>
</tr>
<tr>
<td>10 ng</td>
<td>20 (3.0 ± 0.1)</td>
<td>22 (3.1 ± 0.1)**</td>
<td>25 (3.2 ± 0.1)**</td>
<td>26 (3.3 ± 0.1)**</td>
<td>23 (3.1 ± 0.1)**</td>
</tr>
<tr>
<td>20 ng</td>
<td>21 (3.0 ± 0.1)</td>
<td>24 (3.2 ± 0.1)</td>
<td>27 (3.3 ± 0.1)</td>
<td>27 (3.3 ± 0.1)</td>
<td>24 (3.2 ± 0.1)</td>
</tr>
</tbody>
</table>
period 4 (10 ng, 66%; 20 ng, 127%; placebo, 35%; P<0.05) and in period 5 (10 ng, 117%; 20 ng, 146%; placebo, 39%; P<0.05).

$\text{FE}_{\text{Na}}$ are summarized in Table 2. $\text{FE}_{\text{Na}}$ changed significantly within each group. The increase of $\text{FE}_{\text{Na}}$ (expressed as mean) differed significantly between groups in period 4 (10 ng, 61%; 20 ng, 110%; placebo, 35%; P<0.05), and in period 5 (10 ng, 114%; 20 ng, 133%; placebo, 45%; P<0.05).

**Urinary flow rate**

The urinary flow rates ($V$) are shown in Table 2 and Fig. 2. There was a significant drug effect after 60 min of infusion within the 20 ng group. $V$ increased markedly following the 20 ng dose (period 4: 10 ng, 13%; 20 ng, 103%; placebo, -14%; P<0.01).

**Lithium excretion**

$C_L$ and fractional excretion of lithium ($\text{FE}_{L}$) are given in Table 2. $C_L$ and $\text{FE}_{L}$ changed significantly over time within the 20 ng group. The significant increase in $C_L$ and $\text{FE}_{L}$ indicates an increase in end-proximal flow of isotonic fluid into the loop of Henle. The increases in $C_L$ were significantly different between groups in the post-infusion phase (period 5: 10 ng, 14%; 20 ng, 18%; placebo, 0.3%; P<0.05). The changes in $\text{FE}_{L}$ from baseline were not significantly different between groups.

**Proximal absolute and fractional reabsorption of sodium**

$\text{PFR}_{\text{water/Na}}$ is shown in Table 3. $\text{PAR}_{\text{Na}}$ did not change significantly within groups or between groups. $\text{PFR}_{\text{Na/water}}$ decreased significantly within the 20 ng group, but this decrease from baseline (period 5: 10 ng, -3.7%; 20 ng, -4.7%; placebo, -1.5%) was not significant compared with the effect of placebo.

**Distal absolute and fractional reabsorption of sodium**

$\text{DFR}_{\text{Na}}$ is shown in Table 3. $\text{DAR}_{\text{Na}}$ did not change significantly within the groups. However, the changes of $\text{DAR}_{\text{Na}}$ differed significantly between groups after 90 min (period 5: 10 ng, 10%; 20 ng,
The secondary messenger cGMP

The plasma and urine concentrations of cGMP are illustrated in Fig. 3. URO stimulated cGMP production in plasma in a significant dose-dependent manner. The net \( \Delta \text{cGMP} \) was markedly elevated after 60 min of infusion (period 4: 10 ng, -3%; 20 ng, -5%; placebo, -1%; \( P = 0.05 \)) and 90 min (period 5: 10 ng, -3%; 20 ng, -5%; placebo, -2%; \( P < 0.01 \)).

Plasma concentrations of renin, Ang II, Aldo, AVP, ANP and BNP

Changes in PRC are illustrated in Fig. 4. PRC decreased significantly following low-dose (10 and 20 ng min\(^{-1}\) kg\(^{-1}\)) infusions (period 4: 10 ng, -15%; 20 ng, -16%; placebo, 18%; \( P < 0.05 \), and period 5: 10 ng, -28%; 20 ng, -28%; placebo, 57%), and a rebound in renin secretion was observed after high-dose (period 5: 40 ng, 90%) administration. In addition, Aldo and Ang II decreased following infusion of the 10 and 20 ng min\(^{-1}\) kg\(^{-1}\) doses, but the changes were not significant (Aldo period 5: 10 ng, -23%; 20 ng, -10%; placebo, -9%, and Ang II period 5: 10 ng, -6%; 20 ng, -7%; placebo, +14%). A rebound in plasma Aldo and Ang II secretion was seen in the 40 ng group (Aldo period 5: 40 ng, 51%, and Ang II}

![Image 1](natural log)

**Fig. 1.** Relative changes in urinary sodium excretion (\( U_{\text{Na}} \)) during (30 and 60 min) and after (90 and 120 min) infusion of URO (10, 20, 40 ng min\(^{-1}\) kg\(^{-1}\)) and placebo. Means \( \pm \) SD of the natural-log-transformed data. \* \( P < 0.05 \), significant deviation between groups. Data from the 40 ng group were not included in the statistical comparisons.

![Image 2](natural log)

**Fig. 2.** Relative changes of urinary flow rate (\( V \)) during (30 and 60 min) and after (90 and 120 min) infusion of URO (10, 20, 40 ng min\(^{-1}\) kg\(^{-1}\)) and placebo. Means \( \pm \) SD of the natural-log-transformed data. \* \( P < 0.05 \); ** \( P < 0.01 \), significant deviation between groups. Data from the 40 ng group were not included in the statistical comparisons.

<table>
<thead>
<tr>
<th>Periods</th>
<th>Pre-infusion Mean ( \pm ) SD</th>
<th>Infusion Period 3 (30 min)</th>
<th>Infusion Period 4 (60 min)</th>
<th>Post-infusion Period 5 (90 min)</th>
<th>Post-infusion Period 6 (120 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFRNa(_\text{UONa} ) (%)</td>
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<td></td>
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<tr>
<td>Placebo</td>
<td>73 (4.3 ( \pm ) 0.05)</td>
<td>74 (4.3 ( \pm ) 0.1)</td>
<td>73 (4.3 ( \pm ) 0.1)</td>
<td>73 (4.3 ( \pm ) 0.05)</td>
<td>72 (4.3 ( \pm ) 0.05)</td>
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<tr>
<td>10 ng</td>
<td>74 (4.3 ( \pm ) 0.05)</td>
<td>74 (4.3 ( \pm ) 0.05)</td>
<td>69 (4.2 ( \pm ) 0.1)</td>
<td>69 (4.2 ( \pm ) 0.05)**</td>
<td>70 (4.2 ( \pm ) 0.05)**</td>
</tr>
<tr>
<td>20 ng</td>
<td>72 (4.3 ( \pm ) 0.05)</td>
<td>73 (4.3 ( \pm ) 0.1)</td>
<td>70 (4.2 ( \pm ) 0.1)</td>
<td>75 (4.3 ( \pm ) 0.1)</td>
<td>70 (4.2 ( \pm ) 0.05)</td>
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<tr>
<td>40 ng</td>
<td>72 (4.3 ( \pm ) 0.05)</td>
<td>70 (4.2 ( \pm ) 0.1)</td>
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<tr>
<td>DFNa (%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>94 (4.6 ( \pm ) 0.05)</td>
<td>93 (4.5 ( \pm ) 0.05)</td>
<td>92 (4.5 ( \pm ) 0.05)</td>
<td>93 (4.5 ( \pm ) 0.05)**</td>
<td>93 (4.5 ( \pm ) 0.01)**</td>
</tr>
<tr>
<td>10 ng</td>
<td>94 (4.6 ( \pm ) 0.05)</td>
<td>93 (4.5 ( \pm ) 0.05)**</td>
<td>91 (4.5 ( \pm ) 0.01)**</td>
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<td>20 ng</td>
<td>94 (4.6 ( \pm ) 0.01)</td>
<td>92 (4.5 ( \pm ) 0.05)**</td>
<td>89 (4.4 ( \pm ) 0.05)**</td>
<td>89 (4.4 ( \pm ) 0.05)**</td>
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<td>40 ng</td>
<td>95 (4.6 ( \pm ) 0.01)</td>
<td>92 (4.5 ( \pm ) 0.01)</td>
<td>91 (4.5 ( \pm ) 0.03)</td>
<td>96 (4.6 ( \pm ) 0.01)</td>
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period 5: 40 ng, 117%). AVP secretion increased significantly over time both in the URO groups and placebo, but the changes were not significantly different between groups.

Plasma ANP is illustrated in Fig. 3. ANP showed a significant dose–response curve. The high ANP levels represent plasma URO concentrations, as the ANP antibody used in the ANP RIA cross-reacts almost 100% with URO. The antibody has no cross-reactivity with BNP, but plasma BNP also increased during URO infusions (period 4: 10 ng, 16%; 20 ng, 52%; 40 ng, 75%; placebo, 12%; \( P < 0.01 \)).

Haematocrit

The changes in haematocrits are shown in Fig. 5. The haematocrit decreased over time in the placebo
Twenty-four hour urinary sodium excretion and body weight

The sodium content of the 24 h urine (means±SD) did not vary significantly between groups (10 ng group, 198±37 mmol/24 h; 20 ng group, 210±64 mmol/24 h; 40 ng group, 192±75 mmol/24 h; and placebo, 202±74 mmol/24 h). In each group, the body weight (medians and ranges) decreased significantly (P<0.01) during the study (10 ng group, −1.2 kg (−1.7 to −0.7 kg); 20 ng group, −1.2 kg (−1.6 to −0.9 kg); 40 ng group, −1.1 kg (−1.5 to −0.6 kg); and placebo, −1.0 kg (−1.4 to −0.8 kg)). Between groups, there was no significant difference in body weight loss.

Adverse effects

No change was observed in any of the routine laboratory parameters.

Two subjects (No. 5 and No. 6) suddenly developed symptoms of a vasovagal attack during urination in standing position after the end infusion of the highest dose. In Trendelenburg position, they recovered quickly without treatment and without any sequelae. A third subject (No. 11) developed transitory dizziness and nausea during urination in sitting position 30 min after infusion of the highest dose. The systemic MAP and pulse rate remained stable.

DISCUSSION

The dose–response effect of intravenously infused URO has not so far been studied in healthy men. Previously, a clear dose-dependent natriuretic and diuretic effect has been found after bolus injection of URO [14]. We found, in the present double-blind placebo-controlled and randomized cross-over study in healthy men, that the dose of 20 ng min⁻¹ kg⁻¹ effectively induced a marked increase in urinary sodium excretion and urinary flow rate, probably due to increased fluid delivery from the end-proximal tubules in addition to diminished sodium reabsorption in the distal part of the nephron. The dose increased the GFR and decreased the effective RPF. It had a suppressive effect on the renin–aldosterone system and increased AVP secretion. It did not affect systemic arterial blood pressure, but it increased the pulse rate and the haematocrit. The 40 ng min⁻¹ kg⁻¹ dose did not cause any additional natriuresis or diuresis. This was probably due to a concomitant blood-pressure-lowering effect that increased the pulse rate and stimulated the secretion of salt/water regulating hormones including the renin–angiotensin–aldosterone system and AVP. There was a clear dose-dependent increase in the plasma and urine concentrations of the second messenger cGMP, which may indicate that the moderate renal effects in the 40 ng group are not likely to be
related to saturation of the URO receptors in the medullary collecting ducts.

The increase in GFR following the 20 ng dose is consistent with the findings observed after bolus injections of URO in healthy men [14]. The lack of effect on GFR in the 10 ng group may be explained by the lower dose. Similar dose-dependent effects have been observed following ANP administration. ANP in supraphysiological doses has been found to increase GFR, whereas inconsistent data on the effect of ANP on GFR have been reported from low-dose ANP infusion studies [25–27]. The decrease in effective RPF differs from the unaffected RPF observed after bolus injections of URO [14]. The different doses and route of administration of URO may explain the conflicting data. The changes in renal haemodynamics are explained by dilatation of the afferent arterioles and constriction of the efferent arterioles, which increases glomerular hydrostatic pressure [28].

We used the lithium clearance method in an attempt to delineate the site(s) of action of URO on renal tubular sodium handling in humans. It is assumed that $C_{Li}$ is a quantitative measure of end proximal fluid delivery to the thin descending limb of Henle's loop. Most studies support the view that the lithium clearance method is the most reliable non-invasive technique available to estimate changes in proximal reabsorption of sodium and water in humans [29]. A prerequisite of the method relies on the fact that no significant lithium reabsorption occurs in tubular segments distal to the pars recta of the proximal tubules. An amiloride-sensitive distal tubular reabsorption of lithium has been reported in sodium-depleted animals, while conflicting data exist about active lithium reabsorption in salt restricted men [29]. Most probably, the lithium method can be used with confidence in humans on normal or high sodium intake. As our subjects were on an unrestricted diet supplemented with sodium chloride tablets it is unlikely that reabsorption of lithium in the distal part of the nephron would have occurred. A lithium tablet in a dose of 750 mg may have a small natriuretic effect, but the dose (300 mg) we used does not affect sodium excretion.

The renal response to natriuretic peptides is well known to be dependent on baseline sodium status [30]. In our sodium-loaded subjects, the 24 h urinary sodium excretion and $F_{ENa}$ at baseline were almost the same before the four different study days. Hence, it is not likely that the baseline sodium status would be responsible for the observed differences in the natriuretic response. Our results may indicate an intra-renal mechanism being the cause for the natriuresis. $C_{Li}$ increased markedly in the 20 ng group. However, the absolute proximal tubular reabsorption rate of sodium was almost unchanged, indicating that URO is not having a direct inhibitory effect on proximal tubular cell function. As $F_{ENa}$ did not differ significantly between groups, the increase in $C_{Li}$ seems to be due to an increase in GFR. The results resemble changes in $C_{Li}$ and $F_{ENa}$ obtained in some human infusion studies with ANP [26,27]. In the distal part of the nephron, the $DFR_{Na}$ decreased significantly in response to the 20 ng dose and the $DAR_{Na}$ increased. The increase in $DAR_{Na}$ is probably a result of the increased distal fluid delivery. The action of URO on distal handling of sodium resembles the mechanism of ANP [27]. A possible direct effect of URO or ANP on the distal segments is in accordance with localization of receptors for the natriuretic peptides along the medullary collecting ducts [31]. Haemodynamic changes, with either increasing peritubular capillary hydrostatic pressure [32] or an increase in medullary blood flow with a wash out of the medullary concentration gradient [33], have been proposed as possible indirect tubular mechanisms responsible for the natriuretic effect of ANP. Renal perfusion pressure may also influence the natriuretic response to URO [34]. In the present study, where the effective RPF decreased, efferent arteriolar vasoconstriction might have diminished the natriuretic and diuretic response to URO because of increased renal vascular resistance and reduced hydraulic pressure in the peritubular capillaries.

The increase in cGMP secretion in plasma and urine reflects the action of URO on guanylate cyclase-coupled receptors in the vessels and the kidneys. The significant increase in $T_{cGMP}$ indicates a net secretion of cGMP from the kidney after 60 min of URO infusion. Perhaps exogenous URO stimulates both luminal and basolateral receptors, increasing the intracellular cGMP level [35]. The second messenger may reduce apical Na+-channel activity either by binding directly to the amiloride-sensitive channel or by stimulating cGMP-dependent protein kinase (G-kinase) [36].

The interaction of the natriuretic peptides with the renin–angiotensin–aldosterone system is complex. The endocrine responses to ANP seem to be dose dependent. A low dose of ANP may reveal a suppressive effect on renin release, Ang II [37] and Aldo concentration [38], whereas during administration of ANP in higher doses no change or even increase in the hormone concentrations is observed. This may be explained by the blood-pressure-lowering effect of high doses of ANP [39]. Bolus injections of URO in healthy men [14] and prolonged infusion of URO in patients with congestive heart failure [16] did not alter plasma renin activity or Aldo concentration, whereas the hormones decreased in response to URO infusion in dogs [10]. In the present study, the decreases in plasma renin and Aldo concentration in the low-dose groups (10 and 20 ng·min⁻¹·kg⁻¹) may suggest an inhibitory effect of URO, while the rebounds in plasma renin, Ang II and Aldo in the 40 ng group are likely to be due to the systemic blood-pressure-lowering effect of high-dose URO. AVP concentration increased during infusion of URO and placebo, probably due to a decrease in plasma volume and a change in

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blood pressure following administration of the high dose. Intravascular volume contraction is possibly promoted by a rapid egression of fluid from the intravascular compartment into the interstitial compartment [40]. Our data, showing an increase in the haematocrits, are consistent with a rapid shift of fluid, and this haemodynamic event may oppose the natriuretic action of the peptide.

The elevated levels of plasma BNP may be due to excess amounts of exogenous URO in plasma. Endogenous BNP is metabolized by specific clearance receptors, which also remove endogenous ANP and synthetic URO from the bloodstream [41], and by endopeptidases [42]. A saturation of the clearance receptors by URO may increase the plasma level of BNP and prolong the half-life of the peptide. Whether the increased level of BNP has contributed to the natriuretic and diuretic effect of URO is unknown.

The gradual decline in systemic blood pressure following the 40 ng dose may be associated with a decrease in cardiac output caused by a fall in preload [43]. The decrease in venous return may be due to a direct effect of the peptide on the vascular tone in the venous system and to an egression of fluid from the intravascular compartment to the interstitial compartment [44-46]. A sudden onset of symptomatic hypotension and bradycardia has been reported during or after continuous infusion of natriuretic peptides [27, 47]. These symptoms may reflect inhibition of adrenergic sympathetic activity. The sudden breakdown of cardiovascular compensatory mechanisms may occur in subjects who have no increase in heart rate in response to a gradual decline in blood pressure [48]. In the present study, the two subjects who developed a vasovagal attack had a slight increase in pulse rate before the onset of symptoms. One had a vasovagal attack 30 min after discontinuation of URO, at a time when the ANP/URO concentration had declined to the pre-infusion level while pcGMP was still enhanced. As cGMP is the second messenger mediating the biological effects of the natriuretic peptides, the delayed onset of the hypotensive action may be a result of prolonged intracellular production of cGMP.

In summary, we have demonstrated that a URO dose of 20 ng min⁻¹ kg⁻¹ was effective in healthy men. The natriuresis and diuresis were probably results of increased GFR and diminished sodium reabsorption in the distal part of the nephron. In addition, the 20 ng dose suppressed the renin–aldosterone system. Incrementing the dose to 40 ng min⁻¹ kg⁻¹ induced a wide variation in the natriuretic and diuretic responses, probably due to a fall in the systemic blood pressure and a concomitant activation of the renin–angiotensin–aldosterone system and AVP. Further investigations are needed to define whether low-dose infusions of URO have a beneficial effect in treatment of acute renal failure and sodium/water retaining states, such as cirrhosis of the liver, congestive heart failure and nephrotic syndrome.

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