Renal haemodynamic and tubular actions of urotensin II in the rat

Alaa E S Abdel-Razik, Ellen J Forty, Richard J Balment and Nick Ashton

Faculty of Life Sciences, University of Manchester, 1.124 Stopford Building, Oxford Road, Manchester M13 9PT, UK

(Correspondence should be addressed to N Ashton; Email: nick.ashton@manchester.ac.uk)

Abstract

Urotensin II (UTS) is a potent vasoactive peptide that was originally identified in teleost fish. Mammalian orthologues of UTS and its receptor (UTSR) have been described in several species, including humans and rats. We have shown previously that bolus injections of UTS caused a decrease in urine flow and sodium excretion rates in parallel with marked reductions in renal blood flow (RBF) and glomerular filtration rate (GFR). The aim of this study was to determine the effect of UTS infusion at a dose that has minimal impact upon renal haemodynamics in order to identify a potential direct tubular action of UTS. Infusion of rat UTS (rUTS) at 0.6 pmol/min per 100 g body weight in male Sprague–Dawley rats, which had no effect on RBF and caused a 30% reduction in GFR, resulted in a significant increase in the fractional excretion of sodium (vehicle 2.3±0.6 versus rUTS 0.6 pmol 4.5±0.6%, P<0.05) and potassium. At the higher dose of 6 pmol/min per 100 g body weight, haemodynamic effects dominated the response. rUTS induced a marked reduction in RBF and GFR (vehicle 1.03±0.06 versus rUTS 6 pmol: 0.31±0.05 ml/min per 100 g body weight, P<0.05) resulting in an anti-diuresis and antinatriuresis, but no change in fractional excretion of sodium or potassium. Uts2d and Uts2r mRNA expression were greater in the renal medulla compared with the cortex. Together, these data support an inhibitory action of Uts2d on renal tubule sodium and potassium reabsorption in the rat, in addition to its previously described renal haemodynamic effects.

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Introduction

Urotensin II (UTS) is a cyclic peptide that was originally isolated from the caudal neurosecretory system, a neuroendocrine system unique to fish (Bern et al. 1985). Subsequently, UTS was identified in amphibia and, more recently, in a number of mammalian species including humans, monkeys, rats and mice (Elshourbagy et al. 2002). A mammalian UTS receptor, designated as UTSR (Douglas & Ohlstein 2000), has also been characterised (Ames et al. 1999) and mapped to a number of locations, including the brain, kidney, adrenal gland, heart, colon and a variety of blood vessels in humans (Matsushita et al. 2001, Totsune et al. 2001). Following the discovery of a mammalian UTS system, much of the initial attention focussed on the potent vasoactive actions of UTS, which appear to be a significant increase in the fractional excretion of sodium (vehicle Matsushita et al. 2001) and rats (Song et al. 2006) at a concentration far exceeding that of plasma. In humans, the renal clearance of UTS is greater than that of creatinine, suggesting that urinary UTS is derived primarily from the kidney (Matsushita et al. 2001). Studies in anaesthetised sheep have revealed an arteriovenous concentration gradient for UTS across the renal circulation (Charles et al. 2005), suggesting that the kidney produces UTS in this species too. UTSRs have also been localised to the mammalian kidney: UTS mRNA has been identified in human (Matsushita et al. 2001), monkey, mouse (Elshourbagy et al. 2002) and rat kidneys (Song et al. 2006). Ligand binding (Disa et al. 2006) and immuno-histochemical localisation studies (Song et al. 2006) have identified the medulla, an exclusively tubular component of the kidney, as the principal site of Utsr expression in the rat kidney. Exposure of LLCPK1 cells, a porcine renal epithelial cell line that expresses UTSR mRNA, to human UTS (hUTS) produced a dose-related increase in intracellular calcium (Matsushita et al. 2003), confirming that UTS activates intracellular signalling processes in epithelial cells.

Despite the growing body of evidence that shows that the mammalian kidney is a major source of UTS, little is known about the action of UTS on renal sodium and water handling. We have reported that UTS caused an anti-natriuresis and anti-diuresis when administered as an i.v. bolus dose; this was associated with and driven by renal haemodynamic effects leading to a marked reduction in glomerular filtration rate
and withdrawal of blood samples (0.4 ml). A minimal abdominal artery for continuous recording of arterial blood pressure of saline and drugs. A catheter was implanted in the left carotid and the right jugular vein was cannulated for the administration barbital sodium, 110 mg/kg i.p. Sigma–Aldrich Company Ltd) and 12 mg maintained at 37°C, then placed on a continuous i.v. infusion of 154 mM NaCl containing 3H-inulin (0.3 μCi/ml) and PAH (3 mg/h) at 50 μl/min. After a 3-h equilibration period, urine samples were taken every 15 min over a 1-h control period; blood samples were taken once per hour for the measurement of electrolyte and clearance studies as described previously (Song et al. 2006). Animals were prepared for the measurement of UTS. Animals were killed humanely at the end of the clearance experiment for the measurement of Ang II and tissue renin activity. The detection limit was 2 pg/tube.

Measurement of UTS in urine

Urine and plasma sodium and potassium concentrations were measured by atomic absorption spectrophotometry (Solaar S Series, Thermo Elemental (Unicam Ltd), Cambridge, UK). Chloride was measured by electrometric titration (Chloride Analyzer 925, Ciba Corning Diagnostics Ltd, Essex, UK). Osmolality was measured by a freezing point depression osmometer (LH Roebling, Berlin, Germany). PAH concentration was measured by a standard colorimetric assay. 3H-inulin activity was determined using a 1900CA Tri-Carb Liquid Scintillation Analyser (Canberra Industries, Meriden, CT, USA) β-counter.

Materials and Methods

The experiments described below were performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

Renal clearance measurements

Male Sprague–Dawley (SD) rats (250–280 g) were prepared for renal clearance studies as described previously (Song et al. 2006). Rats (n = 7 per group) were anaesthetised (Inactin, thiotubata-barbitral sodium, 110 mg/kg i.p. Sigma–Aldrich Company Ltd) and the right jugular vein was cannulated for the administration of saline and drugs. A catheter was implanted in the left carotid artery for continuous recording of arterial blood pressure (PowerLab 800/s, ADInstruments, Hastings, East Sussex, UK) and withdrawal of blood samples (0-4 ml). A minimal abdominal incision was made, and a urinary bladder catheter inserted for the collection of urine samples. Body temperature was maintained at 37°C by means of a heated table.

Immediately following the cannulation of the right jugular vein, a priming dose of 1 μCi 3H-inulin (Amersham International Ltd) and 12 mg para-aminohippuric acid (PAH, Sigma) in 0-3 ml of 154 mM NaCl was given i.v. Animals were then placed on a continuous i.v. infusion of 154 mM NaCl containing 3H-inulin (0-3 μCi/ml) and PAH (3 mg/h) at 50 μl/min. After a 3-h equilibration period, urine samples were taken every 15 min over a 1-h control period; blood samples were taken once per hour for the measurement of electrolyte and clearance marker concentrations. Animals were then divided into three groups that received either vehicle (154 mM NaCl), rat UTS (rUTS, Peptide Institute, Inc., Osaka, Japan) at 0-6 pmol/min per 100 g body weight or rUTS at 6 pmol/min per 100 g body weight for 1 h. These doses of rUTS were chosen to evoke modest, physiologically relevant changes in plasma UTS concentrations in the absence of marked vasodepressor effects (Song et al. 2006). The infusion was then switched back to saline for a further 1 h. Plasma and urine samples were stored at -80°C until assay of inulin and electrolytes was undertaken. Animals were killed humanely at the end of the experiment by anaesthetic overdose.

Urine and plasma analysis

Urine and plasma sodium and potassium concentrations were measured by atomic absorption spectrophotometry (Solaar S Series, Thermo Elemental (Unicam Ltd), Cambridge, UK). Chloride was measured by electrometric titration (Chloride Analyzer 925, Ciba Corning Diagnostics Ltd, Essex, UK). Osmolality was measured by a freezing point depression osmometer (LH Roebling, Berlin, Germany). PAH concentration was measured by a standard colorimetric assay. 3H-inulin activity was determined using a 1900CA Tri-Carb Liquid Scintillation Analyser (Canberra Industries, Meriden, CT, USA) β-counter.

Measurement of Ang II and tissue renin activity

Blood and urine samples were collected as described above for the measurement of Ang II. Kidneys were collected from animals at the end of the clearance experiment for the measurement of tissue Ang II and renin activity. The tissue was homogenised on ice in Tris/HCl buffer (pH 7-4, 1 ml buffer per 0-1 g tissue) containing enzymatic inhibitors (1% Triton X-100, 2 mM EDTA, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 0-1 μg/ml phenylmethylsulphonyl fluoride and 0-1 μg/ml bacitracin) and centrifuged at 1000 g for 20 min at 4°C. Prior to the measurement of tissue Ang II, assay buffer containing Captopril (5 ng/mg tissue) was added to each aliquot of homogenised kidney to inhibit Ang II generation in vitro (Sahajpal & Ashton 2005). Ang II was measured by a commercial RIA kit (Euro-Diagnostica AB, Malmo, Sweden). Tissue Ang II concentration is expressed in pmol/g kidney weight. Plasma and urine concentrations are expressed in pmol/l.

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In order to measure tissue renin activity, homogenised kidney samples were diluted in 0.5 M phosphate buffer (pH 6.5) containing 8.8 mM EDTA, 3 mM of 8-hydroxyquinoline sulphate and 5 mM of 2,3-dimercaptopropanol to a final concentration of 1:4000 using a two-step dilution; in the second step, 100 µl renin-free plasma was added. Renal renin activity was measured by a REN-CT2 kit (Cisbio International, Cèze, France) as described previously (Sahajpal & Ashton 2005). The kit includes an angiotensin-converting enzyme inhibitor solution (specific details of its formulation are not provided by the manufacturer) to prevent degradation of angiotensin I during incubation of samples. Renin activity is expressed as µg angiotensin I/g kidney weight per h.

**Quantitative real-time PCR analysis**

SD rats (n = 8) were killed by cervical dislocation before the tissue removal. Kidneys were isolated, divided into the cortex and medulla, frozen in liquid nitrogen and stored at ~80 °C until RNA extraction or protein analysis (see below). Total RNA was extracted from ~50 mg frozen tissue using TRIzol Reagent (Invitrogen Ltd). First-strand cDNA was synthesised using SuperScript II (Invitrogen Ltd) with random primers according to the manufacturer’s instructions. Primers and TaqMan probes (Eurogentec, Southampton, Hampshire, UK) for rUTS and rUTSR (Table 1) were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA) and optimised using standard ABI protocols. Quantitative real-time PCR was carried out using an ABI PRISM 7500 detector (Applied Biosystems). Relative quantitation values were calculated using the 2^−ΔΔCT method as fold changes in the target gene related to the expression of a control sample (brain), taking into account the amplification efficiency of the primers; data were then normalised to two reference genes (β-actin and GAPDH).

**Western blot analysis**

Kidneys (n = 5) were harvested, divided into the cortex and medulla, and homogenised prior to western analysis of UTSR expression. In brief, 50 µg tissue homogenate were solubilised in Laemmli buffer (0.125 M Tris–HCl at pH 6.8, 20% glycerol, 4% SDS, 0.02% bromophenol blue, 10% 2-mercaptoethanol), denatured at 80 °C for 5 min, fractionated using an 14% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane. Non-specific binding was blocked with 3% BSA in TBS/Tween, washed and incubated with a polyclonal rabbit anti-rat UT antibody (1:200, Sigma), washed and incubated with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5000, ab9485, Abcam, Cambridge, UK) and a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5000 Dako UK Ltd, Ely, Cambridgeshire, UK) to confirm that equal amounts of protein were loaded in each lane: UT bands were normalised to the corresponding GAPDH band.

**Statistical analysis**

Data are presented as the mean ± S.E.M. Statistical analysis was done by two-tailed Student’s t-test, repeated measures ANOVA or one-way ANOVA and Duncan’s test, as appropriate, with significance ascribed at the 5% level (SPSS for Windows, version 13.0, SPSS UK Ltd, Surrey, UK).

**Results**

**Renal clearance measurements**

All measured variables were in a steady state and did not differ between experimental and control groups prior to the infusion of rUTS; mean arterial pressure was unaltered throughout (Fig. 1A) and the plasma concentration of electrolytes and plasma osmolality remained stable and did not differ between groups (Table 2).

Infusion of rUTS at 0.6 pmol/min per 100 g body weight had no effect on effective RBF (ERBF) (Fig. 1B). However, GFR fell significantly (P < 0.05); GFR dropped to 0.63 ± 0.08 ml/min per 100 g body weight over the first 15-min collection period after the start of rUTS infusion compared with 1.03 ± 0.05 ml/min per 100 g body weight in the vehicle-infused group (Fig. 1C). The reduction in GFR was sustained until the end of the experiment, 1 h after rUTS infusion had ceased. Urine flow rate (Fig. 2A) and the urinary excretion of Na⁺ (Fig. 2B), K⁺ and Cl⁻ (data not shown) did not differ from that of the vehicle-treated group. This

### Table 1 Gene-specific primers and probe for urotensin II (UTS), UT receptor (UTSR), β-actin and GAPDH

<table>
<thead>
<tr>
<th>Gene name</th>
<th>TaqMan probe</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>β-actin</td>
<td>CACCATGAAAGATCAAGATCATGGTCTCCTCCT</td>
<td>GACAGGATGCGAGAAGGAGATTACCTG</td>
<td>GAGCCCAAATCCACACAGA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>CTGTCACCTTCACAGCA</td>
<td>CTACCTGAGGACAGGTGTC</td>
<td>CATAAAAGTGGAAGAATGG</td>
</tr>
<tr>
<td>Uts2d</td>
<td>TGACCCGCTTTTCCGAGAAACC</td>
<td>CTGGCCGCAAGTCTCAACAGTA</td>
<td>CGTGTGTCACACGTTGTC</td>
</tr>
<tr>
<td>Uts2r</td>
<td>TGCTCATGTTGCGTTTTCTCCT</td>
<td>GGGCATGTTGGAATGTAT</td>
<td>AGACGTACATGGAGGGCCAG</td>
</tr>
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[www.endocrinology-journals.org](http://www.endocrinology-journals.org)
reflected a significant increase \((P<0.05)\) in the fractional excretion of filtered electrolytes during rUTS infusion. The reduced tubular reabsorption produced maximal fractional excretion rates over the 15-min period immediately after rUTS infusion had ceased (\(\text{Na}^+\): vehicle \(2.3 \pm 0.6\%\) versus rUTS \(0.6\ \text{pmol per 100 g body weight}\) \(4.5 \pm 0.6\%\) \(P<0.05\), \(\text{K}^+\): vehicle \(26.1 \pm 6.3\%\) versus rUTS \(0.6\ \text{pmol per 100 g body weight}\) \(53.4 \pm 5.8\%\), \(\text{Cl}^-\): vehicle \(4.5 \pm 1.2\%\) versus rUTS \(0.6\ \text{pmol per 100 g body weight}\) \(9.8 \pm 1.1\%\)).

rUTS infusion at the higher rate of \(6\ \text{pmol/min per 100 g body weight}\) induced more profound changes in renal hemodynamics including a significant \((P<0.05)\) reduction in ERBF (vehicle \(3.3 \pm 0.7\) versus rUTS \(0.6\ \text{pmol per 100 g body weight}\) \(1.4 \pm 1.1\text{ ml/min per 100 g body weight}\), \(P<0.05\)) in the absence of any change in mean arterial pressure (Fig. 1A). This persisted somewhat after cessation of rUTS infusion, only returning to basal levels 45 min after switching back to vehicle. There was also a more pronounced effect on GFR (Fig. 1C), which was reduced significantly \((P<0.05)\) and reached a nadir 45 min after the start of rUTS infusion (vehicle \(1.03 \pm 0.06\) versus rUTS \(6\ \text{pmol per 100 g body weight}\) \(0.31 \pm 0.05\text{ ml/min per 100 g body weight}\)). GFR had not returned to control levels by the end of the experiment, 1 h after rUTS infusion had ceased (Fig. 1C).

This pattern of greatly reduced filtered load was reflected in the lowered urine flow (Fig. 2A) and electrolyte excretion rates (Fig. 2B). There were significant \((P<0.05)\) reductions in urine flow rate (vehicle \(25.9 \pm 4.1\text{ ml/min per 100 g body weight}\) versus rUTS \(6\ \text{pmol per 100 g body weight}\) \(5.8 \pm 1.8\text{ ml/min per 100 g body weight}\)) and the urinary excretion of \(\text{Na}^+(\text{vehicle }2.6 \pm 0.4\text{ mmol/min per 100 g body weight})\) and \(\text{K}^+(\text{vehicle }1.6 \pm 0.1\text{ mmol/min per 100 g body weight})\) and \(\text{Cl}^-(\text{vehicle }4.7 \pm 0.3\text{ mmol/min per 100 g body weight})\), which reached a nadir 30 min after the infusion of rUTS commenced. These reductions in electrolyte excretion were accompanied by a trend towards higher fractional excretion rates but this did not reach statistical significance (Fig. 2C).

UTS and Ang II concentrations and tissue renin activity

Infusion of rUTS raised the urinary UTS concentration from \(2.8 \pm 10^{-8}\text{ M}\) for the vehicle-infused group to \(3.7 \pm 10^{-8}\text{ M}\) and \(6.4 \pm 10^{-8}\text{ M}\) for rUTS at \(0.6\text{ and }6\ \text{pmol/min per 100 g body weight}\) respectively \((P<0.05)\). Constant infusion of UTS tended to decrease both plasma and renal tissue Ang II concentrations whereas renal tissue renin activity tended to increase, but these differences did not reach statistical significance. However, there was a significant

| Table 2 | Plasma electrolytes and osmolality measured in rats receiving a constant i.v. infusion of either vehicle \((n=7)\) or rUTS at \(0.6\ (n=7)\) or \(6.0\ (n=7)\ \text{pmol/min per 100 g body weight}\) for 1 h. Blood was collected 30 min after the infusate was switched to rUTS. Data are shown as the mean \(\pm\text{S.E.M.}\). Statistical analysis by one-way ANOVA and Duncan’s test revealed no difference between groups |
|-----------------|-----------------|-----------------|
| **Sodium (mmol/l)** | Vehicle | 138 \pm 1.0 | 136 \pm 1.0 | 139 \pm 1.2 |
| **Potassium (mmol/l)** | 4.6 \pm 0.2 | 4.5 \pm 0.2 | 5.2 \pm 0.1 |
| **Chloride (mmol/l)** | 103 \pm 2.9 | 99 \pm 2.8 | 102 \pm 2.7 |
| **Osmolality (mOsm/kg)** | 295 \pm 1.9 | 289 \pm 2.3 | 294 \pm 1.0 |

Figure 1 (A) Mean arterial pressure (MAP), (B) effective renal blood flow (ERBF) and (C) glomerular filtration rate (GFR) during i.v. infusion of vehicle \((n=7\text{ solid line})\) or rUTS at either \(0.6\ (n=7\text{ dotted line})\) or \(6\ \text{pmol/min per 100 g body weight}\) \((n=7\text{ dashed line})\). Data are shown as the mean \(\pm\text{S.E.M.}\) for each 15-min urine collection period. Statistical analysis was by repeated measures ANOVA (see text for details).
A dose-related reduction in the concentration of Ang II in the urine of rats receiving rUTS infusion (Table 3).

Renal UTS and UTSR expression

The relative mRNA expression levels of Uts2d and Uts2r were determined in the kidney cortex and medulla by real-time qPCR. Uts2d (P<0.01) and Uts2r (P<0.05) mRNA expression levels were significantly greater in the medulla than in the cortex (Fig. 3A and B). Western blot analysis revealed a similar pattern of expression for Uts2r. A band was detected at 60 kDa, corresponding with the glycosylated form of the Uts2r (Boucard et al. 2003), in both the cortex and medulla. Densitometric analysis of the UT band, normalised to the protein loading control GAPDH, showed that UT expression was significantly greater (P<0.05) in the medulla compared with the cortex (Fig. 3B, C & D).

Discussion

This study provides functional evidence that UTS influences tubular sodium and potassium transport, in addition to effects on the renal vasculature. Specifically, rUTS infusion at the lower of the two doses employed (0.6 pmol/min per 100 g body weight) was associated with an increase in the fractional excretion of sodium and potassium, likely reflecting an inhibition of tubular reabsorption. This contrasts with the marked anti-natriuresis and anti-kaliuresis produced with the higher dose rUTS infusion, which were driven by the profound reduction in GFR and thus filtered load of electrolytes.

The pronounced reductions in urine flow and electrolyte excretion rates evoked by rUTS infusion at the higher dose of 6 pmol/min per 100 g body weight mirror the changes seen in GFR and are consistent with our observations following bolus administration of rUTS (Song et al. 2006). Thus, any coincidental tubular action of rUTS was masked by the predominant decrease in filtered load of water and ions. Indeed, when the lower dose of rUTS, which had much less impact upon GFR, was infused a different pattern of response emerged. In these animals, despite the modest fall in filtered load, urine flow rate and urinary sodium excretion did not

Table 3 Plasma, urine and renal tissue angiotensin II (Ang II) concentrations and renal tissue renin activity in rats receiving a constant i.v. infusion of either vehicle (n=7) or rUTS at 0-6 (n=7) or 6-0 (n=7) pmol/min per 100 g body weight. Plasma and urine samples were collected 45 min after commencing rUTS infusion. Data are shown as the mean±S.E.M. Statistical comparisons were by one-way ANOVA and Duncan’s test.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle 0-6 pmol/min per 100 g</th>
<th>Vehicle 6-0 pmol/min per 100 g</th>
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<tbody>
<tr>
<td>Plasma Ang II (pmol/l)</td>
<td>16.6±0.6</td>
<td>15.4±0.6</td>
</tr>
<tr>
<td>Urine Ang II (pmol/l)</td>
<td>49.9±0.9</td>
<td>44.6±1.0</td>
</tr>
<tr>
<td>Renal tissue Ang II (pmol/g kidney weight)</td>
<td>5.8±0.4</td>
<td>5.1±0.4</td>
</tr>
<tr>
<td>Renal tissue rennin activity (μg Ang I/g kidney weight per h)</td>
<td>276.9±21.3</td>
<td>299.2±18.0</td>
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*P<0.05.
Therefore have used bolus and continuous infusions of UTS in the low pmol/100 g body weight range to effect manipulations within a physiologically relevant range. In the present study, this resulted in a twofold increase in urinary UTS concentration at the highest dose employed; a similar increase in urinary UTS concentration has been reported in patients with renal disease (Totsune et al. 2004). Previous clearance studies in rats have injected bolus doses systemically at 1–100 nmol/kg (Ovcharenko et al. 2006) or infused at 2.5–20 ng/kg per min directly into the renal artery (Zhang et al. 2003), which potentially exposed the kidney to supraphysiological doses of the hormone. The heterologous nature of the peptides used in these studies is unlikely to have had a significant impact on the outcome, as the UTSR is unable to distinguish between UTS from a number of species, including humans and rats (Labarrere et al. 2003).

The increase in fractional excretion of sodium and potassium evoked by rUTS in the present study, and by hUTS in Zhang’s experiments (Zhang et al. 2003), suggests that UTS acts to inhibit renal tubular epithelial Na⁺ and K⁺ transport. A similar inhibitory action on Na⁺ and Cl⁻ transport has been observed in epithelial cells of the skin and opercular membrane of fish (Loretz et al. 1981, Marshall & Bern 1981). The precise tubular site at which UTS exerts this inhibitory action in the rat kidney is not yet known, nor is it clear whether UTS has a direct effect on epithelial transport or works through an intermediary. However, radio-ligand binding studies (Disa et al. 2006) coupled with the UTSR mRNA and protein expression data reported herein place the majority of UT receptors in the renal medulla. This is consistent with immuno-localisation of the UTS receptor, which has identified the thin ascending limbs of the loop of Henle and the inner medullary collecting duct as likely sites (Song et al. 2006). Unfortunately, these parts of the nephron are not readily accessible to micropuncture, so direct measurements of sodium flux cannot be made in vivo.

It is worthy of note that the UT antibody that we used for our western blots recognised a band at 60 kDa, whereas the predicted molecular mass of the UTS receptor is 43 kDa. Similar observations have been reported previously for UTS expression in rat UTS receptor-transfected COS-7 cells (Boucard et al. 2003) and in rat heart (Boussete et al. 2006) in which a 60 kDa glycosylated form of the receptor was identified, but no signal was seen at 43 kDa. Confirmation of the nature of the 60 kDa band was provided following deglycosylation which resulted in a shift from 60 to 42 kDa (Tootsune et al. 2003), which potentially explained the thin ascending limbs of the loop of Henle and the inner medullary collecting duct as likely sites (Song et al. 2006). Unfortunately, these parts of the nephron are not readily accessible to micropuncture, so direct measurements of sodium flux cannot be made in vivo.

Other groups have reported a variety of renal responses to UTS infusion in the rat. Zhang et al. (2003) observed an increase in fractional sodium excretion following intra-renal infusion of hUTS, but this was seen against a rather different background of diuresis and natriuresis accompanied by no change in GFR. By contrast, Ovcharenko et al. (2006) observed no change in sodium excretion or fractional excretion of sodium in response to bolus injections of hUTS in Wistar rats. In the latter study, hUTS produced a modest reduction in RBF, no change in GFR, yet mean arterial pressure fell by 30 mmHg. Some of the differences between earlier studies may be due to the varying doses of UTS employed. We measured circulating plasma UTS in the SD rat in the low pmol range (Song et al. 2006) and
close proximity to UTSR expression (Song et al. 2006). This suggests that the kidney may be the principal site of origin of the immuno-reactive UTS measured in rat urine and also lends support to the notion that UTS acts to inhibit electrolyte transport by the renal tubule, independent of any actions on renal haemodynamics.

Systemic administration of rUTS produced dose-related reductions in both GFR and RBF; however, the effect on the former was greater in magnitude. These observations are consistent with Gardiner et al. report of a modest reduction in RBF in conscious SD rats following either bolus injection (Gardiner et al. 2001) or 6-h infusion (Gardiner et al. 2006) of hUTS. The effects of hUTS on RBF were blocked by indomethacin and attenuated by L-NAME (Gardiner et al. 2006) implying that cyclooxygenase products and nitric oxide were involved in the response to hUTS. A similar nitric oxide-dependent vasodilatation was observed when small renal arteries (250–300 μm internal diameter) were exposed to hUTS in vitro (Zhang et al. 2003). However, the actions of UTS on smaller glomerular arterioles remain to be determined. On the basis of our observations, namely modest reductions in RBF accompanied by a marked reduction in GFR, it seems likely that UTS was acting predominantly at the efferent arteriole. Afferent arteriolar constriction tends to reduce RBF and GFR to a similar degree, whereas efferent arteriolar dilatation has a greater effect on GFR (Dworkin & Brenner 1996), which is consistent with the observed actions of rUTS in our hands.

As UTS has been shown to act synergistically with Ang II to induce contraction of the rat thoracic aorta (Wang et al. 2007, Lamarre & Tallarida 2008), we measured plasma, urine and renal tissue Ang II concentrations and renal tissue renin activity in rats receiving a continuous infusion of rUTS to determine whether the actions of rUTS were influenced by Ang II. Neither plasma nor tissue Ang II concentrations nor renal tissue renin activity differed significantly at either rUTS infusion rate. This appears to be at odds with the reduction in GFR observed upon rUTS infusion, as one might predict a compensatory increase in renin secretion and thus intra-renal Ang II generation following such a profound drop in GFR (Ito & Abe 1997). If, as we propose, UTS acts at the efferent arteriole to cause dilatation and a reduction in post-glomerular resistance, autoregulatory processes should act to increase glomerular capillary hydrostatic pressure to restore GFR. This did not occur over the 1-h rUTS infusion period; indeed, GFR remained lower than that in vehicle-infused rats once the infusate had been switched back to saline. Similar sustained responses beyond the period of rUTS infusion were also observed for a number of renal variables in this study and in our earlier report (Song et al. 2006). This is consistent with the very slow UTS receptor dissociation rate (Kd~0.1 nM (Ames et al. 1999)) which has lead to the suggestion that UTS binding is ‘pseudo-irreversible’ (Douglas et al. 2004).

In contrast to the lack of change in plasma and tissue Ang II concentrations, UTS infusion was associated with a dose-related reduction in urinary Ang II concentration. As circulating Ang II is filtered by the glomerulus, the dose-related reduction in urinary Ang II concentration may simply reflect the rUTS-induced decrease in GFR and hence the amount of filtered Ang II entering the tubular fluid. Ang II is also generated within the tubular fluid itself. Little or no angiotensinogen passes through the glomerular filtration barrier, however, proximal tubules have been reported to secrete angiotensinogen into the tubular fluid (Rohrwasser et al. 1999). In conjunction with renin, which is both filtered and secreted by connecting tubule cells (Rohrwasser et al. 1999), and angiotensin-converting enzyme, which is expressed abundantly throughout the tubule and on renal endothelial cells (Casarini et al. 1997), secretion of angiotensinogen may lead to Ang II formation within the tubular fluid. Thus, it is possible that rUTS may have interacted with the intra-luminal generation of Ang II. However, in view of the lack of change in renal tissue Ang II content, is seems likely that a reduction in filtered Ang II was primarily responsible for the observed reduction in urinary Ang II concentration.

In summary, this study provides evidence that rUTS has not only haemodynamic but also tubular actions on the rat kidney, when infused at a non-depressor, physiologically relevant dose. The haemodynamic effects predominate at higher doses, causing a profound reduction in GFR that is accompanied by an antidiuresis and anti-natriuresis. When a lower infusion rate of rUTS was employed, a tubular action to reduce electrolyte reabsorption became apparent through an increase in fractional excretion of sodium and potassium. Whether this was a direct effect on tubular transport or was mediated via a secondary mechanism is not clear from the available data. However, immuno-localisation showed that the UTS receptor is expressed in the thin ascending limb of the loop of Henlé and the inner medullary collecting duct (Song et al. 2006), consistent with the greater UTSR mRNA and protein expression observed in the medulla compared with the cortex, which suggests that UTS may indeed have a direct action on tubular electrolyte transport. The role of endogenous UTS in the day-to-day regulation of renal function remains to be determined, but this study adds further weight to the suggestion that UTS is one of a number of intra-renal peptides that contributes to the control of the kidney. In view of the reported increases in plasma or urinary UTS concentrations in hypertension (Cheung et al. 2004), congestive heart failure (Russell et al. 2003) and renal disease (Matsushita et al. 2001), the data presented herein provide more evidence to support the notion that UTS may have a role in cardiorenal disease.

Declaration of interest

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References


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