Enhanced renal sensitivity of the spontaneously hypertensive rat to urotensin II

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Abdel-Razik AE, Balment RJ, Ashton N. Enhanced renal sensitivity of the spontaneously hypertensive rat to urotensin II. Am J Physiol Renal Physiol 295: F1239–F1247, 2008. First published August 13, 2008; doi:10.1152/ajprenal.90374.2008.—Urotensin II (UII) has been implicated widely in cardiovascular disease. The mechanism(s) through which it contributes to elevated blood pressure is unknown, but its emerging role as a regulator of mammalian renal function suggests that the kidney might be involved. The aim of this study was to determine the effect of UII on renal function in the spontaneously hypertensive rat (SHR). UII infusion (6 pmol·min⁻¹·100 g body wt⁻¹) in anesthetized SHR and control Wistar-Kyoto (WKY) rats produced marked reductions in glomerular filtration rate (ΔGFR WKY, n = 7, −0.3 ± 0.1 vs. SHR, n = 7, −0.6 ± 0.1 ml·min⁻¹·100 g body wt⁻¹, P = 0.03), urine flow, and sodium excretion rates, which were greater in SHR by comparison with WKY rats. WKY rats also showed an increase in fractional excretion of sodium (ΔFENa; +0.6 ± 0.1%, P = 0.02) in contrast to SHR in which no such change was observed (ΔFENa, −0.6 ± 0.2%). Blockade of the UII receptor (UT), and thus endogenous UII activity, with urantide evoked an increase in GFR which was greater in SHR (+0.3 ± 0.1) compared with WKY rats (+0.1 ± 0.1 ml·min⁻¹·100 g body wt⁻¹, P = 0.04) and was accompanied by a diuresis and natriuresis. UII and UT mRNA expression were greater in the renal medulla than the cortex of both strains; however, expression levels were up to threefold higher in SHR tissue. SHR are more sensitive than WKY to UII, which acts primarily to lower GFR thus favoring salt retention in this model of hypertension.

UT receptor; kidney; glomerular filtration rate; sodium

The novel peptide hormone urotensin II (UII), originally identified in a neurohemal organ unique to fish (3), is now known to be expressed in many mammalian species, including humans (9) and rats (8). UII binds to a single specific G protein-coupled receptor (2, 18), designated the UT receptor (11), which is expressed abundantly in the nervous (13) and cardiorenal systems (20, 30). The potent vasoconstrictor actions of UII (2) appear to be mediated by Ca²⁺ mobilization through activation of a number of signaling pathways, including Ca²⁺ channels, tyrosine kinase, p38MAPK, and ERK1/2 (26). UII also has a vasodepressor action, particularly in the rat, which is endothelium and nitric oxide dependent (26).

UII has been implicated widely in cardiovascular disease. Plasma (7) and cerebrospinal fluid (31) UII concentrations have been positively correlated with blood pressure in hypertensive patients and single nucleotide polymorphisms of the UII gene have been associated with the development of essential hypertension (35). Elevated plasma or urinary UII concentrations have also been reported in patients with portal hypertension (15), congestive heart failure (22, 27), and renal failure (21, 32). These observations led to the suggestion that UII plays a role in cardiovascular disease. However, the potential underlying mechanisms have not been identified. Infusion of UII into healthy human volunteers had no effect on forearm blood flow in one study (33), whereas dose-dependent reductions in forearm blood flow were reported by another group (4). Measurements of skin microvascular tone showed that UII produced a dose-dependent vasodilator response in normal subjects, whereas vasoconstriction was noted in patients with congestive heart failure (16) or hypertension (29).

In addition to its potent direct vascular actions, UII has an emerging role in the control of mammalian renal function, which may offer an alternative mechanism through which it can contribute to blood pressure regulation. The kidney is the principal site of UII synthesis in humans (23); UII is also secreted by the rat (30) and sheep kidney (6). In common with human kidneys (21), we showed that the rat kidney also expresses the UT receptor on both renal blood vessels and tubular epithelium (30). This pattern of distribution is in agreement with our observations of the profound effects of UII on renal hemodynamic and tubular function in the rat in vivo. UII administration caused a marked reduction in glomerular filtration rate (GFR) which was accompanied by an antidiuresis and antinatriuresis (1, 30). Other groups (24, 36) reported somewhat different effects, perhaps reflecting different doses and/or routes of UII administration, but nonetheless showed that UII can influence both glomerular filtration and tubular sodium reabsorption.

The effects of UII on renal function in models of hypertension are largely unknown. We reported that the plasma UII concentration in spontaneously hypertensive rats (SHR) is increased (30), and autoradiography suggested that more [¹²⁵I]h-UII binding sites were present in the SHR kidney (10), but a detailed assessment of the effect of UII on the SHR kidney has not been reported. Accordingly, the aim of the current study was to determine the effect of exogenous UII on renal function in the SHR. We also determined the contributions of endogenous UII to renal function by blocking the UT receptor with urantide, a full antagonist of the rat UT receptor (5). Finally, we quantified mRNA expression of UII and UT and localized protein expression by immunohistochemistry in SHR and control Wistar-Kyoto (WKY) rat kidneys.

METHODS

All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and received local ethical approval.

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Animals. Male WKY and SHR (190–250 g, Harlan UK, Belton, UK) were housed under standard conditions (22–24°C with a 12:12-h light:dark cycle) with free access to water and standard chow (Rat & Mouse Standard Diet, Bantin & Kingman, North Humberside, UK).

Effect of UII administration on renal function. Inactin (thiobutabarbital sodium, 100 mg/kg ip)-anesthetized WKY (n = 7 per group) and SHR (n = 7 per group) were prepared for renal clearance measurements (30). Rats were infused via the right jugular vein with 0.154 M NaCl containing 3H inulin (1 μCi/ml) and para-aminomhippuric acid (9 mg/ml) at 50 μl/min for 3 h after which urine samples were collected every 15 min over a 1-h control period. Animals then received either vehicle (0.154 M NaCl) or rat UII (rUII, Peptide Institute, Osaka, Japan) at 0.6 pmol·min⁻¹·100 g body wt⁻¹ or rUII at 6 pmol·min⁻¹·100 g body wt⁻¹ for 1 h, after which all animals were returned to the vehicle alone for a further hour. Blood pressure was recorded throughout the experiment via a catheter implanted in the left carotid artery (PowerLab 800s, ADInstruments, Hastings, East Sussex, UK). Urine and plasma samples were analyzed for Na⁺, K⁺ (atomic absorption spectroscopy, Solaar S Series, Thermo Elemental, Unicam, Cambridge, UK), Cl⁻ (Chloride Analyzer 925, Ciba Corning Diagnostics, Essex, UK), [3H]inulin activity (1900CA Tri-Carb Liquid Scintillation Analyzer, Canberra Industries, Meriden, CT), and PAH (standard colorimetric assay). Hematocrit was measured to allow the calculation of effective renal blood flow (ERBF).

Effect of UII receptor antagonist administration on renal function. A separate group of rats was prepared for renal clearance study. After a 3-h equilibration period, urine samples were collected every 15 min over a 1-h control period. Animals then received either vehicle (0.154 M NaCl; WKY n = 7, SHR n = 7) or a bolus injection (0.2 mg/kg) of the UT receptor antagonist urantide [Pen⁵, DTrp⁷, Orn⁸]hU-II(4-7) (25) (California Peptide Research) followed by a continuous infusion of urantide at a rate 0.02 mg·kg⁻¹·min⁻¹ for 1 h (WKY n = 7, SHR n = 7). All animals then received vehicle alone for 1 h after which rUII (6 pmol·min⁻¹·100 g body wt⁻¹) was added to the infusate for the final 30 min of the experiment to confirm UT receptor blockade.

UII and UT receptor expression. UII and UT receptor mRNA were quantified in the cortex and medulla of kidneys harvested from WKY (n = 6) and SHR (n = 6) using quantitative real-time PCR. Total RNA was extracted using TRIzol Reagent (Invitrogen, Paisley, UK) and first-strand cDNA was synthesized using SuperScript II (Invitrogen) with random primers according to the manufacturer’s instructions. Primers and TaqMan probes (Eurogentec, Southampton, Hampshire, UK) for rat (r) UII (forward CTGGGCGAAGATCTTACACTGTA, reverse CTGGTGTTGCTTTGCCG) and rUT (forward GGCCATGTGGGAATGTAT, reverse AGAGTACATGGGAGGCCGAG) were designed using Primer Express software (Applied Biosystems, Foster City, CA) and optimized using standard ABI protocols. Quantitative real-time PCR was carried out using an ABI PRISM 7000 detector (Applied Biosystems). Relative quantitation values were calculated using the 2⁻ΔΔCT method (19) as fold-changes in the target gene normalized to the reference gene (β-actin) and related to the expression of a control sample.

UT receptor and UII immunoreactivity were localized in paraffin-dehyde-fixed WKY (n = 5) and SHR (n = 5) kidneys. Five-micrometer sections were incubated overnight at 4°C with either a polyclonal rabbit anti-flounder UII antibody (1:1,000 dilution) (34) or a polyclonal rabbit anti-rat UT receptor antibody (1:100 dilution, Santa Cruz Biotechnology), diluted in 0.1% BSA, 0.3% Triton X-100 in PBS. Labeling was identified by application of a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:750 for anti-UII, 1:500 for anti-UT, DakoCyto- mation, Cambridgeshire, UK) before incubation with diaminobenzidine. Negative controls were carried out by preincubating the primary antibody with an excess of immunizing peptide and by omission of either the primary or secondary antibodies.

Statistical analysis. Renal clearance data are presented as means ± SE for the final 15-min urine collection in each hour, corresponding with the maximal effect of rUII or urantide. Statistical analysis was by separate two-way ANOVAs with Tukey’s post hoc test within each strain or independent samples t-test for the renal clearance studies and one-way ANOVA and Tukey’s test for the qPCR data using SPSS for Windows (version 15.0, SPSS UK, Surrey, UK). Significance was ascribed at the 5% level.

RESULTS

Effect of UII administration on renal function. Rat UII infusion rates (0.6 pmol or 6 pmol·min⁻¹·100 g body wt⁻¹) were chosen, on the basis of earlier studies in our laboratory (1, 30), to induce modest, physiologically relevant changes in plasma UII concentrations with no accompanying alterations in systemic blood pressure. This aim was achieved as rUII infusion had no effect on mean arterial pressure (MAP) at either dose in both SHR (P<0.047) and WKY (P<0.41) rats (Fig. 1, A and B). As expected, MAP was significantly higher (P<0.001) in all SHR groups compared with WKY rats.

All measured variables were stable before the onset of rUII administration; upon infusion, rUII induced dose-related changes in both renal hemodynamics and tubular function. Administration of rUII at 0.6 pmol·min⁻¹·100 g body wt⁻¹ had no effect on ERBF in WKY rats (P<0.99; Fig. 1C) but it was associated with a significant reduction in GFR (P<0.001; Fig. 1E). At the higher dose of 6 pmol·min⁻¹·100 g body wt⁻¹, rUII caused significant reductions in both ERBF (P<0.001; Fig. 1C) and GFR (P<0.001; Fig. 1E), the latter persisting until the end of the experiment (P=0.006). SHR were more sensitive to rUII than WKY rats, responding to the lower dose of 0.6 pmol·min⁻¹·100 g body wt⁻¹ with a significant reduction in ERBF (P=0.001; Fig. 1D). However, their response to rUII at the higher dose of 6 pmol·min⁻¹·100 g body wt⁻¹ was comparable with that of WKY rats (P<0.001; Fig. 1D). Rat UII also evoked dose-related reductions in GFR in the SHR (P<0.001; Fig. 1E) which persisted until the end of the experiment (P<0.001; Fig. 1F): at the higher rUII dose the fall in GFR was greater in magnitude in SHR compared with that seen in WKY rats (Table 1; P=0.029).

Rat UII infusion produced dose-related reductions in urine flow rate in rats of both strains, but only affected urinary sodium excretion in SHR. Thus, at 6 pmol·min⁻¹·100 g body wt⁻¹, rUII infusion resulted in a significant reduction in urine flow rate in WKY rats (P=0.002; Fig. 2A); urine flow was unaffected by the lower dose of rUII (P=0.062). Sodium excretion (UNaV) was not altered by either dose of rUII (0.6 pmol, P=0.59; 6 pmol, P=0.23; Fig. 2C). However, fractional excretion of sodium (FESn) was unchanged at either dose of rUII (0.6 pmol, P=0.99; 6 pmol, P=0.99). The higher dose of rUII had no effect on FESn in WKY rats (P=0.99).

Basal urine flow (WKY 23.0 ± 0.6 vs. SHR 14.2 ± 0.5 μl·min⁻¹·100 g body wt⁻¹, P<0.001; Fig. 2, A and B) and sodium excretion rates (WKY 2.1 ± 0.1 vs. SHR 1.3 ± 0.1 μmol·min⁻¹·100 g body wt⁻¹, P<0.001; Fig. 2, C and D) were lower in SHR compared with WKY rats. Despite this, the higher dose of rUII induced a significantly greater (P<0.048) reduction in urine flow rate by comparison with WKY rats.
(Table 1). Consequently, urine flow rate fell to 5.6 ± 0.8 in rUII (6 pmol)-treated SHR compared with 13.7 ± 0.8 µl·min⁻¹·100 g body wt⁻¹ in vehicle-infused animals (P < 0.001; Fig. 2B) and the sodium excretion rate fell to 0.5 ± 0.1 following rUII (6 pmol) infusion compared with 1.6 ± 0.2 µmol·min⁻¹·100 g body wt⁻¹ in the vehicle group (P < 0.001; Fig. 2D). Neither urine flow (P < 0.001) nor sodium excretion (P < 0.001) had returned to basal levels, 1 h after rUII had ceased. At the lower dose of 0.6 pmol·min⁻¹·100 g body wt⁻¹, rUII infusion had no significant effect on either urine flow (P = 0.063) or sodium excretion rates (P = 0.35). In marked contrast to WKY rats, rUII had no significant effect on FE₅₆ in SHR at either dose (0.6 pmol, P = 0.99; 6 pmol, P = 0.061; Fig. 2F), implying that changes in total sodium excretion rate were driven by alterations in the filtered load of sodium rather than altered tubular handling of sodium in this strain.

The actions of rUII on the renal excretion of potassium and chloride mirrored changes in sodium handling: total excretion

Table 1. Maximal difference between vehicle-infused control animals and rats infused with either rat urotensin II (0.6 or 6 pmol·min⁻¹·100 g body wt⁻¹) or urantide (0.02 mg·min⁻¹·kg body wt⁻¹) for 1 h

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<th>Urotensin II</th>
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<tr>
<td></td>
<td>0.6 pmol·min⁻¹·100 g body wt⁻¹</td>
<td>0.6 pmol·min⁻¹·100 g body wt⁻¹</td>
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<td></td>
<td>6 pmol·min⁻¹·100 g body wt⁻¹</td>
<td>6 pmol·min⁻¹·100 g body wt⁻¹</td>
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<td>WKY (n = 7)</td>
<td>SHR (n = 7)</td>
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<tr>
<td>∆ ERBF, ml·min⁻¹·100 g body wt⁻¹</td>
<td>0.2 ± 0.4</td>
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<td>∆ GFR, ml·min⁻¹·100 g body wt⁻¹</td>
<td>−0.3 ± 0.1</td>
<td>−0.3 ± 0.1</td>
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<tr>
<td>∆ UV, µmol·min⁻¹·100 g body wt⁻¹</td>
<td>−4.1 ± 1.6</td>
<td>−3.3 ± 0.3</td>
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<tr>
<td>∆ U₅₆, µmol·min⁻¹·100 g body wt⁻¹</td>
<td>−0.5 ± 0.4</td>
<td>−0.4 ± 0.2</td>
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<tr>
<td>∆ FE₅₆, %</td>
<td>0.1 ± 0.1</td>
<td>0.03 ± 0.1</td>
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Values are means ± SE. ERBF, effective renal blood flow; GFR, glomerular filtration rate; UV, urine volume rate; U₅₆, urinary sodium excretion rate; FE₅₆, fractional excretion of sodium. Statistical analysis was by independent samples t-test comparing Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats receiving the same drug treatment. *P < 0.05, †P < 0.01, ‡P < 0.001 WKY vs. SHR.
of K⁺ was reduced in both WKY (P = 0.043) and SHR (P < 0.001) at the higher dose of 6 pmol·min⁻¹·100 g body wt⁻¹. Cl⁻ excretion was significantly lower in SHR (P < 0.001) but not in WKY rats (P = 0.052). However, fractional excretion rates were not significantly different (Table 2).

Effect of UII receptor antagonist administration on renal function. All measured variables were stable before infusion of the UT receptor antagonist urantide. Urantide had no effect on MAP in either WKY (P(treatment) = 0.17) or SHR animals (P(treatment) = 0.69): MAP was significantly higher in the latter group of rats (P < 0.001; Fig. 3, A and B). Urantide had no effect on either ERBF (P = 0.98; Fig. 3B) or GFR (P = 0.38; Fig. 3C) in WKY rats. In contrast, SHR responded to urantide administration with large increases in both ERBF (vehicle 5.4 ± 0.1 vs. urantide 6.6 ± 0.2 ml·min⁻¹·100 g body wt⁻¹, P < 0.001; Fig. 3D) and GFR (vehicle 1.0 ± 0.05 vs. urantide 1.3 ± 0.01 ml·min⁻¹·100 g body wt⁻¹, P = 0.001; Fig. 3F). Infusion of rUII over the final 30 min of the experiment caused significant reductions (P < 0.01) in ERBF and GFR in vehicle-infused WKY and SHR, but was ineffective in those rats treated with urantide (Fig. 3, C–F), confirming UT receptor antagonism.

Table 2. Renal handling of potassium and chloride during infusion of rat urotensin II (0.6 or 6 pmol·min⁻¹·100 g body wt⁻¹) in SHR (n = 7 per group) and WKY rats (n = 7 per group)

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<th>WKY</th>
<th>SHY</th>
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<tr>
<td></td>
<td>Vehicle</td>
<td>Urotensin II 0.6 pmol</td>
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<td></td>
<td>0.154 M NaCl</td>
<td>0.6 pmol</td>
</tr>
<tr>
<td>U₁K, μmol·min⁻¹·100 g body wt⁻¹</td>
<td>1.4±0.1</td>
<td>1.1±0.1</td>
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<tr>
<td>FₑK, %</td>
<td>26.4±2.3</td>
<td>30.1±0.9</td>
</tr>
<tr>
<td>U₁Cl, μmol·min⁻¹·100 g body wt⁻¹</td>
<td>4.0±0.3</td>
<td>3.3±0.3</td>
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<tr>
<td>FₑCl, %</td>
<td>3.7±0.3</td>
<td>4.0±0.4</td>
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Values are means ± SE. Statistical analysis was by 2-way ANOVA with Tukey’s post hoc test. U₁X, urinary excretion rate of X; FₑX, fractional excretion of X. *P < 0.05, †P < 0.001 vs. vehicle-infused rat of the same strain.
Basal urine flow and sodium excretion rates were significantly lower \((P < 0.001)\) in SHR compared with WKY rats (Fig. 4, A–D). Urantide infusion had no significant effect on urine flow rate \((P = 0.27;\) Fig. 4A), sodium excretion \((P = 0.82;\) Fig. 4C), or fractional excretion of sodium \((P = 0.99;\) Fig. 4E) in WKY rats. Rat UII infusion over the final 30 min of the experiment had little effect on urine flow rate \((P = 0.19;\) Fig. 4A), sodium excretion \((P = 0.99;\) Fig. 4C), or fractional excretion of sodium \((P = 0.34;\) Fig. 4E) in either group of WKY animals. SHR responded to urantide infusion with a significant increase in urine flow rate \((\text{vehicle } 14.6 \pm 0.9 \text{ vs. urantide } 18.1 \pm 0.9 \text{ µl} \cdot \text{min}^{-1} \cdot \text{100 g body wt}^{-1}, P = 0.023;\) Fig. 4B). Urantide infusion was also associated with an increase in urinary sodium excretion \((\text{vehicle } 1.8 \pm 0.2 \text{ vs. urantide } 2.6 \pm 0.1 \text{ µmol} \cdot \text{min}^{-1} \cdot \text{100 g body wt}^{-1}, P = 0.011;\) Fig. 4D); however, fractional excretion of sodium was not affected \((P = 0.99;\) Fig. 4F). Rat UII infusion over the final 30 min of the experiment resulted in significant decreases in urine flow \((P = 0.005)\) and sodium excretion rates \((P = 0.01)\) in vehicle- but not urantide-treated SHR (Fig. 4, B and D) confirming UT receptor blockade; \(\text{F}E_{\text{Na}}\) was unaffected in either SHR group \((P = 0.99;\) Fig. 4F).

The effects of urantide on renal potassium and chloride handling reflected the strain-dependent changes in sodium handling. Urantide infusion had no effect on either total or fractional excretion of potassium \((U_{K^+}, P = 0.99; \text{FE}_{K^+}, P = 0.99)\) and chloride \((U_{Cl^-}, P = 0.92; \text{FE}_{Cl^-}, P = 0.99)\) in WKY. In SHR, urantide infusion was associated with a significant increase in potassium \((P < 0.001)\) excretion, but no change in total chloride \((P = 0.22)\) or the fractional excretion of either ion \((\text{FE}_{K^+}, P = 0.54; \text{FE}_{Cl^-}, P = 0.93;\) Table 3).

**UII and UT receptor expression.** Two-way ANOVA showed that renal UII mRNA expression was significantly higher \((P_{\text{region}} < 0.001)\) in the medulla compared with the cortex. Comparing strains, UII mRNA was significantly higher in the medulla of SHR kidneys than that in WKY rats \((P < 0.001;\) Fig. 5); however, there was no difference between SHR and WKY cortices \((P = 0.81)\). Positive immunostaining for UII was seen in the proximal tubules, cortical collecting ducts, and inner medullary collecting ducts of both WKY and SHR (Fig. 6). UT receptor mRNA expression was also greater \((P_{\text{region}} < 0.001)\) in the medulla compared with the cortex of both strains (Fig. 5). UT mRNA expression was significantly greater in the medulla of SHR kidneys compared with that of WKY rats \((P < 0.001);\) SHR cortex UT expression did not differ from that in WKY rats \((P = 0.59)\). UT receptor immunostaining was observed primarily in the thin limbs of Henle’s loop and the inner medullary collecting ducts (Fig. 6).

**DISCUSSION**

The SHR kidney appears to be much more sensitive to exogenous rUII than the WKY kidney. Infusion of rUII in-
duced profound reductions in urinary water and electrolyte excretion in the SHR, whereas WKY rats only showed modest falls in urinary output. These changes in urinary excretion by the SHR appear to be driven in large part by a reduction in filtered load, as rUII caused a 60% fall in GFR at the higher rate of infusion (6 pmol/min/100 g body wt/1); this compares with a 30% reduction in WKY animals. We reported previously that the basal plasma UII concentration of SHR is greater than that of WKY rats (30), so it is possible that the marked reduction in GFR seen in SHR reflects, in part, a higher circulating concentration of UII in the rUII-infused animals compared with WKY rats. However, blood pressure was not affected in either strain and while rUII evoked a fall in ERBF in both SHR and WKY, the magnitude was similar in both strains. These observations suggest that the predominant effect of exogenous rUII on the SHR is to reduce GFR.

Table 3. Renal handling of potassium and chloride during infusion of urantide (0.02 mg·min⁻¹·kg body wt⁻¹) in SHR (n = 7 per group) and WKY rats (n = 7 per group)

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<th>WKY</th>
<th>SHR</th>
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<td>Vehicle</td>
<td>Urantide</td>
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<tr>
<td>U₆K,V, μmol·min⁻¹·100 g body wt⁻¹</td>
<td>1.1±0.1</td>
<td>1.2±0.2</td>
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<tr>
<td>FEK, %</td>
<td>20.2±1.8</td>
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<tr>
<td>U₆Cl,V, μmol·min⁻¹·100 g body wt⁻¹</td>
<td>4.0±0.2</td>
<td>4.4±0.2</td>
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<tr>
<td>FECl, %</td>
<td>3.4±0.2</td>
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Values are means ± SE. Statistical analysis was by 2-way ANOVA with Tukey’s post hoc test. *P < 0.001 vs. vehicle-infused rat of the same strain.
These observations contrast with two recent studies which failed to block the renal effects of exogenous UII by urantide (28) and vascular (14) actions of UII in the SHR by urantide treatment. These observations are consistent with Disa et al.'s (10) report that binding of $[^{125}\text{I}]h$UII to renal membranes was 70–80% lower in WKY rats compared with SD animals, suggesting that the sensitivity of Wistar-derived rats to UII may be lower than that of SD rats. This may explain, at least in part, why the basal plasma UII concentration was 10-fold higher in WKY compared with SD rats (30). The renal response of SHR to rUII appears to fall midway between that of SD and WKY rats: infusion at 6 pmol·min$^{-1}$·100 g body wt$^{-1}$ before the effects on GFR were minimized enough to reveal the tubular effect (1). Indeed, in our earlier study (1), infusion of rUII at 6 pmol·min$^{-1}$·100 g body wt$^{-1}$ in SD rats resulted in a 70% reduction in GFR which contrasts with the more modest 30% reduction in GFR seen in WKY rats in the current study. SD rats also exhibited a more pronounced antidiuresis and antinatriuresis than similarly treated WKY rats. These observations are consistent with the findings of UII in SD rats (1, 30) and the SHR (current study) and our experience of the long-lasting effects of UII on renal function in SD rats (1, 30). The renal tissue UT mRNA are elevated in SHR compared with WKY (28) and vascular (14) actions of UII in the SHR by urantide administration and refute the associated suggestion that there may be two functional sites for UII in the rat.

It is interesting to note that the increase in FE$_{\text{Na}}$ by WKY rats was observed when rUII was infused at 6 pmol·min$^{-1}$·100 g body wt$^{-1}$; in SD rats we had to reduce the dose of rUII to 0.6 pmol·min$^{-1}$·100 g body wt$^{-1}$ before the effects on GFR were minimized enough to reveal the tubular effect (1). Indeed, in our earlier study (1), infusion of rUII at 6 pmol·min$^{-1}$·100 g body wt$^{-1}$ in SD rats resulted in a 70% reduction in GFR which contrasts with the more modest 30% reduction in GFR seen in WKY rats in the current study. SD rats also exhibited a more pronounced antidiuresis and antinatriuresis than similarly treated WKY rats. These observations are consistent with the findings of UII in SD rats (1, 30) and the SHR (current study) and our experience of the long-lasting effects of UII on renal function in SD rats (1, 30). The renal tissue UT mRNA are elevated in SHR compared with WKY (28) and vascular (14) actions of UII in the SHR by urantide administration and refute the associated suggestion that there may be two functional sites for UII in the rat.

Although glomerular hemodynamic responses, especially changes in GFR, appear to dominate the response to rUII administration, we reported recently that rUII also has a tubular action (1). By reducing the dose of rUII such that changes in GFR were minimized, we were able to observe significant increases in the fractional excretion of sodium and potassium in the SD rat. In the current study, rUII infusion in WKY rats resulted in a significant increase in the FE$_{\text{Na}}$ suggesting that rUII was acting on the renal tubule in this strain, too. This observation is consistent with the qPCR data which show that the UT receptor is much more abundant in the medulla than the cortex. No such change in fractional excretion was observed in the SHR, despite the threefold higher expression of UT mRNA in the SHR medulla. It is likely that the profound influence of rUII on GFR in the SHR group, which led to an antidiuresis and antinatriuresis, masked any potential further tubular action. These observations contrast with a recent report in which UII infusion over a similar dose range was associated with a reduction in FE$_{\text{Na}}$ in the SHR (28). Unusually, the effects of UII on GFR and urine flow were transient in this study, returning to baseline 30 min after UII infusion had commenced. The transient nature of the response contrasts with our experience of the long-lasting effects of UII on renal function in SD rats (1, 30) and the SHR (current study) and does not appear to be consistent with the notion that UII binding to the UT receptor is pseudo-irreversible ($K_d$ ~0.1 nM) (2).

It is interesting to note that the increase in FE$_{\text{Na}}$ by WKY rats was observed when rUII was infused at 6 pmol·min$^{-1}$·100 g body wt$^{-1}$; in SD rats we had to reduce the dose of rUII to 0.6 pmol·min$^{-1}$·100 g body wt$^{-1}$ before the effects on GFR were minimized enough to reveal the tubular effect (1). Indeed, in our earlier study (1), infusion of rUII at 6 pmol·min$^{-1}$·100 g body wt$^{-1}$ in SD rats resulted in a 70% reduction in GFR which contrasts with the more modest 30% reduction in GFR seen in WKY rats in the current study. SD rats also exhibited a more pronounced antidiuresis and antinatriuresis than similarly treated WKY rats. These observations are consistent with the findings of UII in SD rats (1, 30) and the SHR (current study) and our experience of the long-lasting effects of UII on renal function in SD rats (1, 30). The renal tissue UT mRNA are elevated in SHR compared with both WKY and SD rats. Clearly, the Wistar-derived SHR is much more sensitive to rUII and has greater UT receptor expression than its control WKY counterpart. Whether similar differences occur between SD-derived hypertensive models, such as the Dahl salt-sensitive rat and normotensive SD rats, remains to be determined.

Urantide, which is a full antagonist of the rat UT receptor (5), was infused in a second experiment to identify the contribution of endogenous UII to the regulation of renal function in WKY and SHR. We showed previously that an infusion rate of 0.02 mg·kg$^{-1}$·min$^{-1}$ was effective in blocking the hypertensive action of intravenous rUII injection in SD rats (30); this was confirmed in a pilot study in WKY and SHR (data not shown). The effectiveness of UT blockade was also confirmed in our current clearance experiments by the administration of urantide over the final 30 min of the experiment. In naive rats, rUII evoked changes in renal function similar to those seen in the first experiment, whereas those animals which received urantide did not respond to rUII, confirming the efficacy of the receptor antagonist under these conditions. These observations contrast with two recent studies which failed to block the renal (28) and vascular (14) actions of UII in the SHR by urantide administration and refute the associated suggestion that there may be two functional sites for UII in the rat.

The predominant effects of endogenous UII blockade in WKY and SHR were hemodynamic. In accordance with their enhanced sensitivity to exogenous rUII, urantide evoked greater increases in RBF and GFR in SHR compared with WKY rats. This was not driven by a change in systemic pressure, which remained stable in both groups of rats through-
out the experiment. Interestingly, fractional excretion of electrolytes was not altered in either strain, suggesting that endogenous UII may have had little influence on tubular function in these animals. This is rather surprising, given the high UT and UII mRNA expression levels in the medullas of WKY and SHR. It seems unlikely that the blockade of UT receptors was functionally incomplete as subsequent infusion of rUII was ineffective. One possible explanation is that other homeostatic mechanisms may have compensated any change in tubular function. Nonetheless, these data show that endogenous UII has a tonic influence on renal function in both SHR and WKY rats and that its impact is greater in the former.

The role of UII in the control of blood pressure in SHR remains unclear. Central pressor (17) and peripheral depressor (14) responses to UII are enhanced in the SHR. Similarly, the UII system is upregulated in the SHR kidney. In contrast to WKY and SD rats in which UII appears to inhibit tubular sodium reabsorption, UII’s predominant action in the SHR is to reduce GFR and the filtered load of water and ions. The impact of this effect on blood pressure regulation remains to be determined. Short-term UT receptor blockade had no effect on systemic blood pressure in the SHR. This may be explained in part by the pseudo-irreversible binding characteristics of UII to the UT receptor, which results in relatively low spare receptor capacity. Alternatively, long-term UT receptor antagonism may be required if UII contributes to blood pressure control through the regulation of extracellular fluid volume. The therapeutic potential of the UII system as a drug target for the control of hypertension has yet to be fully realized. The data presented in this study show that UII is upregulated in hypertension, but whether this is an underlying cause of elevated blood pressure or a compensatory mechanism is not yet known and clearly warrants further study.

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