Renal effects of human urotensin-II in rats with experimental congestive heart failure

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Abstract

Background. Urotensin II (U-II) and its receptor GPR-14 are expressed in the kidney and the cardiovascular system of various mammalian species. Recent studies suggested that the U-II/GPR-14 system is upregulated in patients with congestive heart failure (CHF). However, the involvement of the peptide in the alterations of renal function in CHF remains unknown.

Methods. The effects of incremental doses (1.0–100.0 nmol/kg) of human U-II (hU-II) on renal haemodynamic and clearance parameters were assessed in rats with an aorto-caval fistula, an experimental model of CHF, and sham controls. Additionally, the effects of pre-treatment with the nitric oxide (NO) synthase blocker, nitro-L-arginine methyl ester (L-NAME), and the cyclooxygenase inhibitor, indomethacin, on the renal haemodynamic response to hU-II were studied in CHF rats.

Results. hU-II caused a decrease in mean arterial pressure in control and CHF rats. In controls, hU-II did not alter renal blood flow (RBF), and caused a minimal decrease (−12.5%) in renal vascular resistance (RVR). However, in CHF rats, the peptide induced a marked increase in RBF (+28%) and a decrease in RVR (−21.5%). These effects were attenuated by L-NAME, but not by indomethacin. Furthermore, hU-II caused a significant increase (+29%) in glomerular filtration rate (GFR) in CHF rats, whereas GFR tended to decrease in controls. Sodium excretion was not altered in control or in CHF rats in response to hU-II.

Conclusions. hU-II exerts an NO-dependent renal vasodilatation that is more pronounced in rats with CHF. The data further suggest that the U-II/GPR-14 system may be involved in the regulation of renal haemodynamics in CHF.

Keywords: aorto-caval fistula; congestive heart failure; kidney; nitric oxide; rat; renal haemodynamics

Introduction

Urotensin-II (U-II) is a cyclic peptide originally isolated from the caudal neurosecretory organ of the teleost fish [1]. The human isoform was cloned in 1999 and has been identified as the natural ligand for the orphan G protein-coupled receptor GPR-14 [2,3]. The U-II/GPR-14 system is expressed in the central nervous system, the cardiovascular system and in the kidney of various mammalian species, including man [3,4]. Human U-II (hU-II) possesses potent vasoactive properties, although these effects are largely dependent on the species and the vascular bed examined [2,5].

The involvement of the U-II/GPR-14 in the pathogenesis of congestive heart failure (CHF) has recently been suggested, based on the following findings: first, plasma levels of U-II are elevated in patients with CHF, correlating with other markers of the disease, such as the N-terminal brain natriuretic peptide and endothelin-1 (ET-1) [6]. In addition, strong expression of U-II was demonstrated in the myocardium of patients with end-stage CHF, in correlation with the impairment of cardiac function [7]. This suggests that upregulation of the U-II/GPR-14 system could play a part in the cardiac dysfunction associated with CHF.

The kidney plays a major role in the pathophysiology of CHF [8]. Impaired renal function and a reduced glomerular filtration rate (GFR) are considered to be strong independent predictors of mortality in patients with CHF. Several neurohumoral systems with renal
vasoconstrictor as well as vasodilator properties are thought to mediate the alterations in renal function in CHF [8]. In view of the documented vasoactive properties of U-II and the findings that the U-II/GPR-14 system may be upregulated in CHF, it is of interest to examine a possible role for U-II in the regulation of renal function in CHF. In the present study, we have used rats with aorta-caval fistula (ACF) as an experimental model of CHF. Previously, we reported that this model of volume overload CHF is characterized by neuro-hormonal and renal manifestations that closely mimic patients with severe CHF [9–11]. These include activation of the renin–angiotensin–aldosterone and the sympathetic nervous system, a marked decrease in renal blood flow and GFR, and a tendency to salt and water retention. In particular, the alterations in renal haemodynamic and excretory functions resemble those seen in patients with severe CHF. Accordingly, we studied the effects of hU-II on renal haemodynamic and clearance parameters in rats with experimental CHF.

Materials and methods

Studies were conducted on a local strain of male Wistar rats (Harlan Laboratories, Ltd, Jerusalem), weighing 290–340 g. The rats were kept in individual metabolic cages in a temperature-controlled room, and were fed standard rat chow containing 0.5% NaCl and tap water ad libitum. All experiments were performed according to the guidelines of the committee for the supervision of animal experiments, Technion, IIT.

The experimental model

Heart failure was induced by surgical creation of an arteriovenous fistula between the abdominal aorta and the inferior vena cava. In short, the abdominal aorta and inferior vena cava were exposed through a mid-abdominal incision under pentobarbital anaesthesia [60 mg/kg, intraperitoneally (i.p.)], and an arterio-venous shunt was surgically created in the common wall of the two vessels (side to side, 0.9–1.2 mm o.d.), as previously described from our laboratory [9–11]. Following surgery, the rats were allowed to recover and placed in individual metabolic cages for measurements of daily urinary sodium excretion.

Six to 8 days after the operation, rats with ACF and sham-operated controls were anaesthetized with Inactin (thiobutabarbital sodium, 100 mg/kg i.p.; Sigma Chemicals, St Louis, MO) and placed on a thermoregulated surgical table to maintain their body temperature at 37 °C. After tracheostomy, polyethylene catheters (Portex Ltd, UK) were inserted into the left carotid artery, jugular vein and urinary bladder, for measurements of mean arterial pressure (MAP), infusion of various solutions and urine collections, respectively. A solution of normal saline (0.9% NaCl) was infused at a rate equal to 1.5% of body weight throughout the experiment. The following experimental protocols were then performed.

Effects of hU-II on MAP and renal haemodynamics in control and CHF rats

For measurements of total renal blood flow (RBF), the left renal artery was exposed through a mid-abdominal incision in control (n = 7) and CHF rats (n = 10). An ultrasonic flowprobe (type 1RB) connected to an ultrasonic flowmeter (model T206, Transonic Corp Inc., Ithaca, NY) was placed around the left renal artery, as previously described [11]. Arterial blood pressure was continuously monitored with a pressure transducer (model 156PC05GW; Microswitch, Freepoint, IL) connected to the carotid arterial line. The data of RBF and MAP were continuously recorded by a computerized data acquisition system, using Labtech Notebook® software. Renal vascular resistance (RVR) was calculated on-line by the standard formula (RVR = MAP/ RBF). After surgery and equilibration, baseline measurements were obtained for 30–40 min. Human U-II was then administered intravenously as bolus injections, at three incremental doses (1.0, 10.0 and 100.0 nmol/kg), followed by a 20–30 min recording period after each dose.

To evaluate whether the alterations in haemodynamic parameters were indeed produced by hU-II, measurements of MAP and RBF were performed in additional controls (n = 5) and CHF rats (n = 6). These groups, referred to as time controls, were followed for 130 min and were handled identically to those described in the previous paragraph but without administration of the peptide.

Effects of hU-II on renal clearance parameters

The effects of hU-II on GFR and urinary sodium excretion were determined in additional groups of control (n = 6) and CHF rats (n = 8). The rats were surgically prepared as described in the previous protocol with the exception that the abdominal cavity was not opened. A solution of 2% inulin in normal saline was infused intravenously, at a rate of 1.5 ml/h, throughout the experiment. Following surgery and equilibration, two 30 min control clearance periods were obtained.

hU-II was administered intravenously as bolus injections, at the same incremental doses described in the previous protocol. Two 20 min clearance periods were obtained with each dose. However, only the second 20 min clearance (representing the steady-state period) was used for the calculations. Urine was collected into pre-weighed vials under mineral oil, and urine volume was measured gravimetrically. Blood samples (0.3 ml) were withdrawn into heparinized tubes at the mid-point of each clearance period and separated by centrifugation. Plasma and urine samples were stored at 4 °C until assayed for inulin and electrolytes.

Effects of nitric oxide synthase inhibition and cyclooxygenase blockade on the renal haemodynamic response to hU-II in rats with experimental CHF

Two additional groups of rats with ACF were studied to assess the contribution of the nitric oxide (NO) system and of renal prostaglandin synthesis to the renal haemodynamic effects of the peptide. In the first group, rats with CHF (n = 6) were treated with nitro-l-arginine methyl ester (L-NAME; Sigma Chemicals) added to the drinking water (100 mg/l) for 4–5 days.
before the experiment. On the day of the experiment, rats were anaesthetized by inactin and subjected to the same experimental protocol as described above.

A second group of CHF rats (n = 7) was anesthetized and prepared for haemodynamic measurements as described. The cyclooxygenase blocker indomethacin (Sigma Chemicals) was then administered intravenously as a slow continuous injection (1.0 mg/kg over 30 min, in 0.2 ml of 5% NaHCO3 solution). Previously, we have shown that this dose of indomethacin, administered in a similar protocol, had a natriuretic/diuretic effect in Wistar rats [12]. Incremental doses of hU-II were then administered, and recordings of MAP and renal haemodynamic parameters were obtained, as described.

Chemical analysis
The concentration of inulin in plasma and urine samples was determined by a colorimetric method, as previously described [11]. GFR was equated with the renal clearance of inulin (Cin). The sodium concentration in plasma and urine was determined by flame photometry (model IL 943, Instrumentation Laboratories).

Statistical analysis
One-way analysis of variance (ANOVA) for repeated measures, followed by the Dunnett test, was used for comparison of treatment values with the baseline value in each group. For comparison of the graphs representing control and experimental groups, two-way ANOVA was used. A value of P<0.05 was considered statistically significant. Data are presented as mean±SEM.

Results
Effects of hU-II on renal haemodynamics and MAP in control and CHF rats
The effects of hU-II on renal haemodynamics and MAP in control and CHF rats are summarized in Table 1 and Figure 1. Baseline MAP and RBF were significantly lower in CHF rats compared with control rats (by 25 and 54%, respectively). Similarly, the calculated RVR in CHF rats was higher by ~60% compared with control animals (Table 1).

Administration of hU-II resulted in a dose-related decrease in MAP when injected at the first two doses, both in controls and in CHF rats. The maximal decrease in MAP induced by the peptide in control rats was 25.0 ± 2.2% (mean±SEM), with the peak effect occurring at 10 min after injection.

Table 1. Effects of hU-II on MAP and renal haemodynamics in control and CHF rats

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>RBF (ml/min)</th>
<th>RVR (mmHg/ml/min)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CHF</td>
<td>Control</td>
</tr>
<tr>
<td>Baseline</td>
<td>130.7±2.2</td>
<td>98.06±5.0b</td>
<td>5.9±0.28</td>
</tr>
<tr>
<td>1 nmol/kg</td>
<td>122.5±3.3a</td>
<td>94.04±4.4a,b</td>
<td>5.96±0.33</td>
</tr>
<tr>
<td>10 nmol/kg</td>
<td>118.9±2.96a</td>
<td>92.88±4.8a,b</td>
<td>6.13±0.32</td>
</tr>
<tr>
<td>100 nmol/kg</td>
<td>122.2±3.18a</td>
<td>94.04±4.9a,b</td>
<td>6.12±0.4</td>
</tr>
</tbody>
</table>

Values represent the peak effect with each dose.
For abbreviations, see text.
*Statistically different (P ≤ 0.05) compared with baseline value in the same group.
Statistically different (P ≤ 0.05) compared with control rats.

Fig. 1. Effects of incremental doses of hU-II on MAP, RBF and RVR in control rats and in rats with experimental CHF. Data are expressed as percentage change from baseline values. The lines representing RBF and RVR differed significantly between the control and CHF rats.
rats (from 130.7±2.2 to 118.9±2.96 mmHg) was higher than in CHF rats (from 98.06±5.0 to 92.88 mmHg, Table 1). However, when expressed as percentage change from baseline, there was no significant difference in the magnitude of the hypotensive action of hU-II in control and CHF rats (Figure 1).

In control animals, there was no significant change in RBF with each dose of the peptide (Table 1). The finding that RBF was preserved despite a decrease in MAP could be explained by a minimal, yet statistically significant decrease in RVR from 22.4±0.98 to 19.6±0.9 resistance units (RU), \( P < 0.05 \), with the 10 nmol/kg dose of the peptide (Table 1). This modest change in RVR could be an autoregulatory response and not a direct renal vasodilatory effect of hU-II. In contrast to the mild decrease in RVR in control rats, the peptide caused a significant and prolonged increase in RBF in rats with experimental CHF (from 2.72±0.12 to 3.5±0.32 ml/min, \( P < 0.01 \), +28% from baseline value, Table 1 and Figure 1). In line with these observations, the maximal decrease in RVR induced by hU-II in CHF rats (from 35.9±1.02 to 28.19±1.9 RU, \( P < 0.01 \)) was higher than in controls (−21.5 vs −12.5% from baseline values, respectively). Thus, in contrast to the minimal renal vasodilatory response in control rats, administration of the peptide to rats with CHF was associated with a more pronounced and prolonged effect. These findings indicate that in CHF rats, characterized by increased basal renal vascular tone, hU-II induced a significant and sustained renal vasodilatory effect.

**Time control experiments**

Baseline MAP in time control animals was 128.9±4.9 mmHg and remained unaltered throughout the experiment (129.2±4.6 mmHg, at 120 min). In CHF rats, MAP was stable for the first 80 min of the experiment and then slightly decreased (from 103.3 to 100.4 mmHg, during the last 30 min of the experiment). Similarly, RBF in rats with CHF did not change significantly throughout the observation period (baseline, 1.91±0.25 ml/min; 120 min, 1.81±0.27 ml/min, \( P = \text{NS} \)). These findings indicate that the alterations in MAP observed in control and CHF rats following hU-II administration, as well as the increase in RBF observed in CHF rats, truly reflected the biological effects of the peptide.

**Effects of hU-II on renal clearance parameters**

The changes in GFR and in sodium excretion in response to administration of incremental doses of hU-II, in control rats and in rats with experimental CHF, are shown in Figure 2. As reported previously [9,11], baseline GFR was lower in rats with CHF than in control animals (CHF, 1.5±0.06 ml/min; control, 2.09±0.22 ml/min; \( P < 0.05 \)). In response to the three incremental doses of hU-II, there was an increase in GFR in CHF rats, which became statistically significant with the high dose of the peptide (from 1.5±0.06 to 1.94±0.1 ml/min; \( P < 0.05 \)). In contrast, in control rats, such an effect could not be observed. In fact,
there was a tendency to a decrease in GFR in response to the peptide (Figure 2). This differential response is in line with the alterations observed in RBF and RVR in CHF rats following the administration of hU-II. Thus, these findings suggest that the changes in GFR might be secondary to the renal haemodynamic effects of the peptide.

Baseline UNaV was significantly lower in CHF rats compared with control animals (control, 0.89 ± 0.19 mol/min; CHF, 0.46 ± 0.11 mol/min, P < 0.001). Fractional Na⁺ excretion tended to be lower in CHF rats, although not statistically different from control rats. In response to incremental doses of hU-II, there was no significant change in sodium excretion (expressed as both absolute rates and fractional values), neither in control nor in CHF rats. Thus, acute administration of hU-II did not exert a natriuretic effect in either group.

Effects of NO synthase inhibition and cyclooxygenase blockade on renal haemodynamic response to hU-II in rats with experimental CHF

The current findings indicate that hU-II acts in the kidney primarily as a vasodilator agent, and that this action is augmented in rats with CHF.

To elucidate further the mechanism of this effect, studies were repeated with CHF rats pre-treated either with L-NAME, the NO synthase (NOS) blocker, or with the cyclooxygenase inhibitor indomethacin. Chronic NO blockade in CHF rats was associated with a significant increase in baseline MAP (CHF + L-NAME, 132.1 ± 6.86 mmHg; CHF, 98.06 ± 5.0 mmHg, P < 0.001) and RVR (CHF + L-NAME, 43.4 ± 3.15 RU; CHF, 35.9 ± 1.02 RU, P < 0.01). Baseline RBF in the L-NAME-treated rats did not differ from that in CHF rats with an intact NO system (3.1 ± 0.24 vs. 2.72 ± 0.12 ml/min, P = NS). Figure 3 summarizes the effects of incremental doses of hU-II on MAP and renal haemodynamics in the L-NAME- and the indomethacin treated CHF groups compared with non-treated CHF rats. As shown, the hypotensive effect of hU-II was of similar magnitude in the three groups, although it tended to be more pronounced in the L-NAME- and indomethacin-treated groups. However, the decrease in RVR and the increase in RBF induced by hU-II in CHF rats were significantly attenuated by pre-treatment with L-NAME. Thus, chronic NOS inhibition was able to block, in part, the renal vasodilatory effect of hU-II. In contrast, pre-treatment with indomethacin did not significantly affect the renal vasodilatory response to hU-II in rats with experimental CHF. Thus, the lines representing the changes in RBF and RVR in response to hU-II administration did not differ between the groups of CHF rats pre-treated or not with indomethacin (Figure 3). Also, infusion of indomethacin for 30 min was not associated with a significant change in baseline MAP or RVR in CHF rats (data not shown).

Discussion

The findings of the present study provide novel information on the renal effects of hU-II in rats and their altered regulation in experimental CHF. Our data indicate that in the kidney hU-II acts primarily as a vasodilator. Moreover, the renal vasodilatory properties of the peptide are augmented in rats with experimental CHF, apparently by an NO-dependent mechanism. Finally, acute administration of hU-II increased GFR only in rats with CHF, but did not alter urinary sodium excretion, neither in control nor in CHF rats.

Studies in various mammalian species including humans demonstrated that the mRNAs of both preproU-II and its receptor, GPR-14, are expressed in the kidney and in the cardiovascular system [2,4].
In the human kidney, immunoreactive staining for U-II was detected in the epithelial cells of the tubules, mostly in the distal tubule, with moderate staining in the endothelial cells of the renal capillaries [13]. Moreover, several recent studies suggested that the hU-II/GPR-14 system is upregulated in patients with CHF [6,7,14]. However, the pathophysiological significance of these findings, in particular the role of U-II in the regulation of the altered renal function in CHF, has not been established.

In the original study by Ames et al. [2], hU-II induced a potent vasoconstrictor effect on isolated arteries from non-human primates that was an order of magnitude greater than that of ET-1. Since then, hU-II has been considered as the most potent mammalian vasoconstrictor identified so far [2,5]. However, careful analysis of the literature reveals that this general notion may be unjustified, and that U-II may exert both vasoconstrictor and vasodilatory effects. These actions depend largely on the animal species as well as on the vascular bed examined [5]. In the rat, the predominant cardiovascular actions of U-II are hyperaemic vasodilation in the mesenteric and hindquarter vascular beds, associated with hypotension and dose-dependent tachycardia [15]. Gibson [16] showed that U-II at low concentrations caused relaxation of noradrenaline-pre-contracted aortic strips of rats in an endothelium-dependent manner. Likewise, in the isolated perfused rat heart, U-II elicited a sustained coronary vasodilation through factors such as cyclooxygenase products and NO [17]. These findings may suggest that although the direct effect of U-II on large vessels is contraction, U-II also relaxes blood vessels, apparently by the release of vasodilators from endothelium.

The kidney appears to be an additional organ where hU-II may affect vascular tone. Recently, Zhang and co-workers [18] reported that continuous infusion of hU-II into the renal artery of normal anaesthetized rats produced a dose-dependent increase in RBF, GFR and urinary sodium excretion, and that these effects were produced a dose-dependent increase in RBF, GFR and hU-II into the renal artery of normal anaesthetized rats. However, the pathophysiological significance of these findings, in particular the role of U-II in the regulation of the altered renal function in CHF, has not been established.

In their study, hU-II exerted a modest renal vasodilatation in spontaneously hypertensive (SHR) rats but not in SD rats [19].

In the present study, in contrast to the negligible renal vasodilatory effect in control rats, the peptide produced a prominent and prolonged decrease in RVR associated with a significant increase in RBF and GFR in rats with experimental CHF. Thus, under these conditions of increased baseline renal vascular tone, hU-II has the ability to act as a potent vasodilator in the kidney. Furthermore, our findings suggest that this increase in renal perfusion is dependent in part on NO production. In that respect, our study fully supports the finding of Zhang and co-workers [18] on the importance of NO in the mediation of hU-II-induced renal vasodilatation. Previous studies from our laboratory showed that in rats with ACF, the expression of endothelial NOS (eNOS) and cyclooxygenase II in the kidney is increased, in particular in the renal medulla [10,20]. In the present study, we therefore evaluated the effects of pre-treatment with L-NAME and indomethacin, the blockers of NO and cyclooxygenase respectively, on the renal response to hU-II in CHF rats. Our findings demonstrate that the renal vasodilatory effect of the peptide was partially blocked by chronic L-NAME treatment, but not inhibited by indomethacin. It is possible that the contribution of prostanoid vs NO-mediated vasodilatation may differ in various vascular beds. Thus, in the kidney, hU-II-induced vasodilatation may be primarily NO dependent. However, since L-NAME did not completely block this vasodilatory effect, it appears that other mechanisms may also be involved. Whether release of other vasodilatory agents, such as endothelium-derived hyperpolarizing factor (EDHF), may participate, as well as their relative role, requires further investigation.

Recently, Clozel et al. [21] reported that ACT-058362, a selective U-II receptor antagonist, was highly effective in preventing post-ischaemic renal vasoconstriction in an experimental model of renal ischaemia–reperfusion in the rat. This might suggest that U-II acts as an endogenous vasoconstrictor in the kidney, mediating the abnormal renal vasoconstriction after ischaemia. It is noteworthy, however, that such an effect of the drug could be observed only after induction of ischaemia but not in sham-operated control rats. Based on their findings, the authors concluded that U-II does not participate in the control of renal blood flow under physiological conditions [21].

The findings of the present study show a differential effect of the peptide on GFR in control and CHF rats. Thus, in control animals, hU-II tended to cause a decrease in GFR, in particular with the high dose. The decrease in GFR induced by the peptide could be secondary to the decrease in MAP with a concomitant decline in glomerular hydrostatic capillary pressure. In contrast, in rats with CHF, in which baseline values of GFR were significantly lower than in controls, there was a significant increase in GFR in response to the
Urotensin-II in experimental CHF

peptide. The observed increase in GFR could be related to the significant and prolonged increase in RBF elicited by hU-II in rats with CHF. This could serve as an effective mechanism for the preservation of renal haemodynamics and GFR in CHF. It is possible that the renal U-II/GPR-14 may act as an additional counter-regulatory system to the renal vasoconstrictors that are responsible for the decrease in RBF and GFR in CHF. However, this interpretation is based on findings from acute experiments, and therefore needs further validation by long-term studies.

Notwithstanding the action of hU-II on renal haemodynamics, our findings indicate that short-term administration of the peptide did not influence sodium handling by the kidney, neither in normal controls nor in CHF rats. The lack of an effect on urinary sodium excretion could suggest that the peptide did not alter tubular epithelial sodium transport directly. Alternatively, this might be related to the hypotensive effect of hU-II that could counteract a direct inhibitory action of the peptide on tubular sodium reabsorption. Since the direct effects of hU-II on tubular sodium transport were not evaluated in the present study, additional studies will be required to elucidate the potential actions of hU-II on electrolyte transport in the nephron. Interestingly, Loretz et al. [22] demonstrated that in the teleost urinary bladder, U-II actually stimulated sodium transport, lending further support to the notion that the biological effects of U-II are highly species dependent.

In summary, the findings of the present study suggest that the U-II/GPR-14 system may participate in the control of renal haemodynamics in the rat. This regulation may be significantly altered in rats with experimental CHF, which could contribute to the adaptive changes in renal function and renal haemodynamic response in CHF.

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Conflict of interest statement. None declared.

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