High plasma concentrations of human urotensin II do not alter local or systemic hemodynamics in man


1. Introduction

Human urotensin II (hUII) is a recently discovered vasoactive peptide hormone that acts as a high affinity ligand for rat G-protein receptor 14 (GPR14) [1–3] and the more recently discovered human receptor [3]. It is the most potent arterial vasoconstrictr yet discovered and has a sustained effect in blood vessels from a variety of species [2,4,5].

Human UII was first isolated in man from subgroups of motor neurones in the spinal cord [1]. Outside the CNS, the kidney has the highest expression of prepro hUII mRNA and, therefore, appears the most likely source of circulating hUII [3]. Its receptor distribution has been mapped using immuno-histochemistry, confirming target binding sites for hUII in cardiovascular tissues including coronary arteries, internal mammary arteries and ventricular cardiomyocytes [2,4]. Thus, it is likely that circulating...
hUII functions as an endocrine hormone. In vitro animal data and studies in rats and non-human primates in vivo, indicate that hUII is a potent vasoconstrictor and influences cardiac function [2,4]. However, conflicting results have been obtained from human blood vessels in vitro [4–6]. Although some studies suggest that hUII is 28- to 50-fold more potent than endothelin-1 (ET-1) [2,4], others show hUII to be a vasodilator [6]. In addition, there is some variability in response to hUII amongst species [7], depending on vessel location and type, and between individual preparations. This is highlighted by some of the in vitro studies having responding and non-responding vessels [5]. Interestingly, in the rat, the activity of hUII is most marked in the region of the proximal aorta, decreasing rapidly further down the arterial tree [4]. Venoconstriction has been found only in some studies in vessels from non-human primates and humans and, in contrast to the effects of ET-1 and norepinephrine, where present, hUII is less potent in veins than arteries [2,4].

To date, there have been no in vivo physiological studies of the actions of hUII in man. On the basis of animal in vivo and the positive human in vitro studies, we hypothesised that hUII would cause arteriolar vasoconstriction in the human forearm, but have little or no effect in veins. We also anticipated that systemic dosing would raise peripheral resistance and, hence, blood pressure. Our aim was to undertake the first human in vivo study with hUII, addressing local responses in human arterial vessels and dorsal hand veins. Subsequently, we explored the effect of higher doses on systemic haemodynamics and plasma hUII concentrations.

2. Methods

These studies were conducted with the approval of the local research ethics committee and the written informed consent of each subject. The investigation conforms to the principles of the Declaration of Helsinki.

2.1. Subjects

Fifteen healthy men, mean age 37±4 years (range 22–53), were recruited from a bank of community volunteers held by the Clinical Research Centre at the Western General Hospital in Edinburgh. Four subjects took part in pilot studies and 11 in the definitive vein and forearm studies. Subjects were asked to fast from midnight before each study, and to abstain from caffeine containing drinks, alcohol and smoking over the preceding 24 h. Subjects’ mean height was 176±3 cm (range 170–180) and mean weight was 80±8 kg (range 63–92).

2.2. Drugs

All drugs were freshly prepared aseptically and dissolved in either saline (0.9% Baxter Healthcare Ltd., Norfolk, UK) or Gelofusine® (Braun Healthcare Ltd., Sheffield, UK). The drugs used were: hUII (Peptide Institute, Osaka, Japan, and SmithKline Beecham, PA, USA), angiotensin II (ANGII; Clinalfa, Laufelfingen, Switzerland), norepinephrine (NE; Abbott Laboratories, Kent, UK), sodium nitroprusside (SNP; David Bull Laboratories, Warwick, UK) and l-N^-monomethylarginine (l-NMMA; Clinalfa).

We confirmed the authentic nature of the hUII from both sources by high performance liquid chromatography and microsequencing (in the laboratory of Drs. S.A. Douglas and E.H. Ohlstein, SmithKline Beecham). We also confirmed the biological activity of the hUII peptides by showing the anticipated responses, and potency, in the rat proximal aorta (data not shown).

2.3. Forearm blood flow

Studies were performed with subjects resting supine, in a quiet clinical laboratory, maintained at a constant temperature of 22–24°C. The brachial artery of the non-dominant arm was cannulated with a 27-gauge steel needle (Cooper’s Needle Works) under local anesthesia (1% lignocaine; Astra Pharmaceuticals Ltd., Hertfordshire, UK). This was connected to a constant rate infusion pump (Portex, UK). Saline was infused at 1 ml min⁻¹ for a period of 30 min before drug infusion protocols were started to ensure a stable baseline. The total infusion rate was kept constant at 1 ml min⁻¹. Throughout the study FBF was measured simultaneously in both arms by venous occlusion plethysmography [8,9], as previously described [10]. FBF was measured over a 3 min period every 6 min, and the last five recordings of FBF were averaged to determine flow in each arm.

2.4. Hand vein studies

Studies were performed with the subject resting semi-recumbent in a quiet clinical laboratory maintained at a constant temperature between 24 and 26°C. Studies were carried out in accordance with our previously described methodology using a standard displacement technique [11,12]. A 2 cm length of non-branching dorsal hand vein was cannulated in the direction of flow. A tripod was placed 1.5 cm proximal to the cannulation site as described in more detail previously by Aellig [11]. Saline was infused at a rate of 0.25 ml min⁻¹ for 30 min to allow baseline measurements of vein diameter to be made. The total infusion rate was kept constant at 0.25 ml min⁻¹ throughout the study.

2.5. Hemodynamics

Blood pressure (BP) was recorded over the brachial
artery in the non-infused arm using a validated oscillometric sphygmomanometer (HEM 705CP, Omron, Japan) [13]. Cardiac index (CI) was assessed using a validated [14] transthoracic electrical bioimpedance technique (NCCOM3, BoMed, Irvine, CA, USA). Both BP and CI were recorded after each FBF recording was completed. Mean arterial pressure was defined as diastolic pressure plus 1/3 of the pulse pressure. Peripheral vascular resistance (PVR) was calculated as MAP divided by CI and expressed in arbitrary units. Throughout the study continuous electrocardiographic (ECG) monitoring was employed and a full 12-lead ECG recorded at baseline and at the end of the highest UII infusion rate on each study day.

2.6. Plasma urotensin II levels

Plasma hUII concentrations were determined by radioimmunoassay using rabbit anti-flounder UII antibody and hUII iodinated by the iodogen method of Fraker and Speck [15]. The antibody had equal specificity for human and flounder UII, and there was no cross-reactivity in the assay with ET-1, ANGII or somatostatin-14 (Sigma, UK). Before assay, plasma samples were subject to reverse-phase chromatographic purification using Sep-Pak C18 cartridges (Millipore, UK) with acetonitrile solvent. The assay protocol was based on that previously described for flounder UII [16]. Briefly, sample extract was incubated with antibody (38,400 dilution) and 125I hUII at 4°C for 24 h. Following this, the complexes formed were precipitated by the addition of bovine r-globulin (Sigma) and polyethylene glycol (Sigma), and the bound fraction was counted for 10 min in a gamma counter (1275 minigamma, Wallac, Finland). A typical standard curve for the hUII radio-immunoassay is shown in Fig. 1. Also shown is the parallelism of serial dilutions of human plasma extract with the standard curve established for synthetic hUII, confirming the specificity of the assay and its suitability for measurement of plasma hUII. Recovery of hUII in plasma extracts was 63% and intra- and inter-assay coefficients of variation in our laboratory were 7.6 and 13.3%, respectively; the sensitivity of the assay was 1 fmol hUII ml⁻¹ plasma.

2.7. Study protocols

2.7.1. Pilot studies

Human UII (Peptide Institute, Osaka, Japan), diluted in 0.9% saline vehicle, was given intra-arterially on three separate occasions, each in two subjects, at rates of 0.001, 0.003 and 0.01 pmol min⁻¹, 0.03, 0.1 and 0.3 pmol min⁻¹, and 1, 10 and 30 pmol min⁻¹. After 30 min saline run-in, each dose of hUII was given for 20 min.

2.7.2. Study 1: local arterial and systemic hemodynamics

On two occasions, separated by 1 week, each subject received a 30 min infusion of saline and then either hUII (Peptide Institute) or saline in a single-blind, randomised manner. Four subjects received 30 and 100 pmol min⁻¹ hUII, and six subjects 100 and 300 pmol min⁻¹ hUII. Each rate was maintained for a total of 20 min and FBF recorded at 3, 9 and 15 min. After the final FBF recording during saline baseline infusions and each dose increment, systemic hemodynamic measurements were made (heart rate, BP and CI) and 10 ml of venous blood was collected for determination of plasma hUII concentration. In addition, FBF studies were repeated in some of the same subjects. First, we used an alternative batch of hUII (SmithKline Beecham; dose range 0.1, 1, 10, 30 pmol min⁻¹; six subjects). Second, we used an alternative Gelofusine® vehicle with the original hUII (Peptide Institute; dose range 1, 3, 30, 300 pmol min⁻¹; four subjects).

2.7.3. Study 2: local arterial hemodynamics with inhibition of endothelial mediators

Five of the subjects who took part in Study 1 underwent a further study involving a “nitric oxide clamp” [17]. First, saline was infused for 30 min, and followed by l-NMMA infused intra-arterially at 4 μmol min⁻¹ to block endogenous nitric oxide (NO) generation [18]. FBF was then restored to within ±10% of baseline by the co-infusion of SNP, an endothelium-independent NO donor (mean dose 0.6 nmol min⁻¹, range 0.3–1.0). To produce a simultaneous inhibition of prostanoid production, each subject received 600 mg aspirin dissolved in 200 ml of water 30 min before the study. At this dose, aspirin inhibits
bradykinin-stimulated endothelial prostacyclin generation and platelet thromboxane production [19], but has no direct effect on BP or basal vascular tone. Once FBF had returned to basal levels, hUII was co-infused at 1, 10 and 100 pmol min\(^{-1}\), each rate for 20 min. FBF and systemic haemodynamics were recorded as for Study 1.

2.7.4. Study 3: venous tone
Six subjects made two visits, separated by 1 week. Each received a 30 min infusion of saline into a selected dorsal hand vein followed by either L-NMMA (100 nmol min\(^{-1}\)) or saline in a single-blind, randomised manner for 5 min. hUII was then co-infused at 3, 30 and 300 pmol min\(^{-1}\), each rate for 20 min. Saline was then infused for 10 min, followed by ANGII (25 ng min\(^{-1}\)) for 3 min then saline for a further 10 min and finally NE (8 ng min\(^{-1}\)) for 3 min to assess the integrity of the vein. Hand vein diameter was measured every 5 min after a 10 min baseline saline infusion. The total infusion rate was kept at 0.25 ml min\(^{-1}\).

2.8. Statistical analysis
All results are expressed as mean±S.E.M. Data for FBF have been expressed as percent change from baseline of the FBF ratio (derived from infused arm value divided by non-infused arm value). Repeated measures ANOVA was used to identify differences in FBF response between hUII and saline, hUII concentrations during placebo and drug infusion and in the vein studies between presence and absence of hUII and L-NMMA co-infusion. For single comparisons, data were analysed using paired Student’s t-tests. Results were considered significant at \(P<0.05\).

3. Results
All subjects were symptom free throughout each study. Baseline FBF, heart rate, CI, BP, plasma hUII concentrations and vein diameter were similar on the different study days and there was no significant difference in the basal FBF between the infused and non-infused arms. Neither continuous single-lead ECG monitoring, nor the full 12-lead ECGs, revealed any changes during the three studies.

3.1. Pilot studies
There was no significant change in FBF in either arm, or systemic hemodynamics, during infusion of saline or hUII (data not shown).

3.2. Study 1
Baseline values for the non-infused and infused FBF were as follows: 3.5±0.9 and 4.5±2 ml 100 ml tissue\(^{-1}\) min\(^{-1}\), respectively, for Fig. 2A and 2.9±0.4 and 2.9±0.7 ml 100 ml tissue\(^{-1}\) min\(^{-1}\) for Fig. 2B. There was no significant change in FBF ratio during infusion of saline or hUII in either of the dose ranging studies (Fig. 2A and B). There was no significant change in systemic hemodynamics during infusion of hUII at any dose (Table 1A and B). However, there was a substantial and significant increase in circulating plasma hUII concentrations during hUII infusion (Fig. 3A and B). Studies with hUII diluted in Gelofusine\(^\text{TM}\) rather than saline, and UII from an alternative supplier (SmithKline Beecham), similarly did not change FBF or systemic hemodynamics (data not shown).

3.3. Study 2
Baseline values for non-infused and infused FBF were 3.1±0.3 and 3.2±0.5 ml 100 ml tissue\(^{-1}\) min\(^{-1}\), respectively. Infusion of L-NMMA resulted in a significant reduction in the FBF ratio (1±0.1 at baseline compared with 0.6±0.1; \(P=0.01\) Student’s t-test) (Fig. 4). Co-infusion of SNP (mean dose 0.6 nmol min\(^{-1}\), range 0.3 to 1.0)
Table 1
Systemic hemodynamics during UII infusion in Study 1

<table>
<thead>
<tr>
<th></th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>HR (min⁻¹)</th>
<th>CI (l min⁻¹ m⁻²)</th>
<th>PVR (arbitrary units)</th>
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<tbody>
<tr>
<td>(A)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Saline</td>
<td>136±5</td>
<td>79±3</td>
<td>98±4</td>
<td>63±2</td>
<td>3.4±0.2</td>
<td>29.4±1.9</td>
</tr>
<tr>
<td>hUII 30 pmol min⁻¹</td>
<td>131±9</td>
<td>81±1</td>
<td>98±3</td>
<td>65±3</td>
<td>3.4±0.1</td>
<td>28.8±1.7</td>
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<td>hUII 100 pmol min⁻¹</td>
<td>133±7</td>
<td>80±2</td>
<td>98±3</td>
<td>68±4</td>
<td>3.3±0.1</td>
<td>29.7±1.0</td>
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<tr>
<td>Saline</td>
<td>137±8</td>
<td>82±4</td>
<td>100±5</td>
<td>66±3</td>
<td>3.2±0.1</td>
<td>31.4±1.3</td>
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<tr>
<td>(B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>124±6</td>
<td>75±6</td>
<td>91±6</td>
<td>63±4</td>
<td>3.7±0.2</td>
<td>24.9±2.5</td>
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<tr>
<td>hUII 100 pmol min⁻¹</td>
<td>124±7</td>
<td>77±5</td>
<td>93±5</td>
<td>61±4</td>
<td>3.7±0.2</td>
<td>25.2±2.3</td>
</tr>
<tr>
<td>hUII 300 pmol min⁻¹</td>
<td>122±6</td>
<td>77±5</td>
<td>92±5</td>
<td>60±3</td>
<td>3.8±0.2</td>
<td>25.6±2.8</td>
</tr>
<tr>
<td>Saline</td>
<td>128±5</td>
<td>77±4</td>
<td>94±4</td>
<td>62±3</td>
<td>3.7±0.2</td>
<td>26.2±2.2</td>
</tr>
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</table>

Results are expressed as mean value±S.E.M. (n=4). SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; CI, cardiac index; PVR, peripheral vascular resistance.

returned the FBF ratio to baseline (1±0.1 at baseline compared with 0.8±0.1, P=0.7 Student’s t-test). There was no significant change in the FBF ratio, or FBF in either arm, during co-infusion of hUII following l-NMMA and SNP (FBF ratio, P=0.3). Human UII infusion did not significantly alter heart rate, systolic or diastolic blood pressure (P=0.8, P=0.8 and P=0.3, respectively, ANOVA).

3.4. Study 3

Increasing doses of hUII had no significant effect on hand vein diameter compared with baseline (P=0.9, ANOVA) (Table 2). During co-infusion of hUII and l-NMMA, there was also no significant change in hand vein diameter (P=0.8, ANOVA). In contrast, ANGII and NE both induced a substantial venoconstriction (see Table 2). The response to ANGII and NE was slightly higher during l-NMMA co-administration, but these differences from the response with l-NMMA were not significant (P=0.8 and P=0.2, respectively, Student’s t-test).
Peripheral vascular tone. Defined. In conclusion, we have found no evidence of local hUII does not play an important role in regulating the physiological role of hUII in man to be more clearly in FBF at only 5 pmol min\(^{-1}\) [21,22], and suggests that antagonists in vivo can address this issue, and should allow contrasts markedly with the local vascular responses in hUII on human large arteries merits further investigation.

In isolated human arteries in vitro, Maguire et al. [4] demonstrated that hUII receptors are present in vascular smooth muscle layers. In addition, they showed a positive response to hUII where the potency of hUII in coronary, mammary and radial arteries was 50-fold greater than ET-1. However, there were differences in the characteristics of the responses. The maximal responses to ET-1 were consistently greater than those to hUII, and 30% of the arteries failed to respond to hUII, whereas all responded to ET-1. Recently, Hillier et al. [20] examined a wide range of human arteries and veins of differing calibre in vitro, and found no effect of hUII. The reason for this is not yet clear. Ames et al. performed a detailed in vivo study of the systemic hemodynamic response to hUII in non-human primates [2]. At lower systemic doses of hUII, Ames observed positive inotropism, whereas at higher doses hUII induced ischaemic myocardial dysfunction and extreme rises in peripheral resistance. On the basis of early human in vitro and the in vivo cynomolgus monkey data, we hypothesised that hUII would cause constriction of resistance vessels of the human forearm and coronary blood pressure. Based on a FBF of 50 ml min\(^{-1}\) and an infusion rate of 300 pmol min\(^{-1}\), the estimated local plasma concentration of hUII in the infused arm in our study would be 6 nmol l\(^{-1}\), similar to those causing vasoconstriction in human in vitro studies [4]. Nevertheless, we found no effect of hUII at 300 pmol min\(^{-1}\) for 20 min in either the brachial artery or dorsal hand vein. This contrasts markedly with the local vascular responses in humans to other paracrine and endocrine mediators, such as ET-1 and ANGII, both of which cause \(\sim 40\%\) reduction in FBF at only 5 pmol min\(^{-1}\) [21,22], and suggests that hUII does not play an important role in regulating peripheral vascular tone.

Indeed, during intra-arterial infusion of ANGII, a 10-fold increase in plasma concentrations of ANGII in the non-infused arm caused mean arterial pressure to rise by \(\sim 15\) mmHg [23], whereas 30-fold increases in plasma hUII had no effect.

In any negative study it is important to consider the possibility that a real effect on arteriolar tone was missed. This is particularly important given the variability in the responses of isolated human vessels to hUII [4,6,7,20]. This is unlikely to be the case because there was no suggestion of groups of responders and non-responders from the 15 subjects who received hUII over a wide range of doses. In addition, brachial infusion studies are an extremely powerful tool for detecting vasoactive responses, usually requiring no more than six subjects to have a high degree of confidence in showing statistically significant effects [9].

Previously, Gibson found that fish urotensin II caused endothelium dependent vasodilatation at low dose, prior to vasoconstriction, in rat aortic tissue [24]. This raised the possibility that hUII may induce activation of NOS and subsequent release of NO. To date, only one in vitro study has studied the influence of NOS on responses to hUII [5], using \(\text{L-}\)nitro-arginine methylester (L-NMMA) to inhibit NOS in isolated pulmonary vessels [5]. L-NMMA increased maximal responses but not potency of hUII in rat main pulmonary artery. L-NMMA also enhanced maximal responses to hUII in human pulmonary arteries, though only three of 10 vessels responded to hUII, and then only with very variable contractions. In the current studies, NO and prostanoid production were inhibited, using standard techniques. Even so, we were unable to unmask vasoconstriction to hUII in either human resistance or capacitance vessels in vivo.

The lack of response of resistance vessels in vivo may be due to low receptor density or poor coupling to signal transduction mechanisms at this site, perhaps as part of inter-species variation. The proximal aorta seems to be most sensitive to hUII and it is possible that subtle effects on large arteries are caused by hUII but not detected using routine hemodynamic assessment. The in vivo effects of hUII on human large arteries merits further investigation. A possible alternative explanation for the lack of effects of hUII is high receptor occupancy. Studies with hUII antagonists in vivo can address this issue, and should allow the physiological role of hUII in man to be more clearly defined. In conclusion, we have found no evidence of local or systemic hemodynamic effects of hUII in vivo despite

### Table 2

<table>
<thead>
<tr>
<th>l-NMMA</th>
<th>UII (pmol min(^{-1}))</th>
<th>Saline</th>
<th>ANGII (25 ng min(^{-1}))</th>
<th>Saline</th>
<th>NE (8 ng min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>30</td>
<td>300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>0.6±6.9</td>
<td>0.8±5.5</td>
<td>0.8±4.4</td>
<td>0.7±2.4</td>
<td>59.4±8.5***</td>
</tr>
<tr>
<td>With</td>
<td>-5.2±3.2</td>
<td>-4.2±3.9</td>
<td>-5.3±5.0</td>
<td>-8.5±4.6</td>
<td>64.2±15.3**</td>
</tr>
</tbody>
</table>

Values represent mean percentage change in hand vein diameter±S.E.M. (n=6), **P<0.01, ***P<0.001.
infusion of hUII at doses that increase plasma concentrations 30-fold.

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References


