Effects of Urotensin II in Human Arteries and Veins of Varying Caliber

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Background—Urotensin II (UII) is the ligand for the GPR14 receptor and the most potent vasoconstrictor in the cynomolgus monkey. UII also contracts rat thoracic aorta. We studied the effect of human UII (hUII) in human blood vessels.

Methods and Results—Small subcutaneous resistance arteries, internal mammary arteries, saphenous veins, and small subcutaneous veins were studied using standard techniques. Subcutaneous resistance arteries constricted in response to norepinephrine (maximum tension, 2.84±0.38 mN/mm; the concentration required to produce 50% of the maximum response [EC50], 0.52±0.07 μmol/L) and endothelin-1 (maximum tension, 4.19±0.93 mN/mm; EC50, 1.6±0.1 nmol/L). hUII did not contract these arteries, internal mammary arteries, or either type of vein, but it was a potent vasoconstrictor in rat thoracic aorta (maximum tension, 2.36±0.2 mN/mm; EC50, 1.13±0.36 nmol/L).

Conclusions—hUII has no vasoconstrictor action in human arteries and veins of different sizes and vascular beds. Marked species differences in the actions of UII question its importance in human cardiovascular regulation. (Circulation. 2001; 103:1378-1381.)

Key Words peptides n vasoconstriction n arteries n veins

Urotensin II (UII) is a cyclic dodecapeptide, the C-terminal hexapeptide sequence of which is conserved across species, including humans.1 UII is a vasoconstrictor in some species of fish and mammals.2–4 Although sequenced almost 20 years ago, the receptor responsible for its effect on smooth muscle was only identified recently.1 In 1999, Ames et al3 cloned a human G-protein-coupled receptor, similar to the rat GPR14, which selectively bound human UII (hUII). Others quickly confirmed UII as the endogenous ligand for this orphan receptor.5–7 mRNA for GPR14 is widely expressed in human cardiovascular tissues, including the heart, vascular smooth muscle cells, and endothelial cells, as well as in spinal cord and endocrine tissues.5,8,9 Ames et al4 also reported that hUII is the most potent arterial vasoconstrictor recognized in the cynomolgus monkey (≈28 times more potent than endothelin-1 [ET-1]).3 UII also profoundly depressed myocardial contractility by causing coronary and peripheral vasoconstriction.4 UII is found in the atheromatous plaque of diseased human coronary arteries, suggesting a role in human cardiovascular physiology and pathophysiology.4 We studied the effect of UII in human arteries and veins.

Methods

Patients and Vessel Preparation

The study received approval from the Ethics Committee, and volunteers gave written consent.

Small Arteries and Veins

Small subcutaneous resistance (SSR) arteries and veins were obtained from subcutaneous biopsies (1.5±1.5±0.5 cm) that were excised under local anesthesia with 1% lidocaine. Gluteal biopsies were excised from patients with coronary heart disease (CHD), and abdominal wall biopsies were obtained from healthy male volunteers who were not taking regular medication at the time of hernia repair. Isolated, washed vessels were studied on a wire myograph (Danish MyoTech P610).10

Conduit Arteries and Veins

Internal mammary arteries and saphenous veins were obtained from patients undergoing coronary artery surgery or leg amputation and studied using standard methods.11

Pharmacological Protocols

After a normalization procedure and a start-up protocol involving repeated washes with high potassium solution (100 mmol/L KCl), the endothelial viability of vessels was assessed by preconstriction with either norepinephrine (NE; 10 μmol/L) or phenylephrine (PE; 3 μmol/L) followed by vasorelaxation with acetylcholine (10 μmol/L).
The average time in which the vessels were in contact with hUII was 80 minutes.

**Urotensin as a Vasoconstrictor**

Cumulative concentration response curves were constructed with hUII (10^{-12} mol/L to 10^{-6} mol/L), ET-1 (10^{-12} mol/L to 3 \times 10^{-7} mol/L), and NE (10^{-9} mol/L to 10^{-4} mol/L) in SSR arteries. hUII (10^{-10} mol/L to 10^{-6} mol/L) was compared with KCl (100 mmol/L) and PE (3 μmol/L) in internal mammary arteries and with KCl (100 mmol/L), NE (10^{-5} mol/L), and PE (3 μmol/L) in saphenous veins. In small subcutaneous veins, UII (10^{-10} mol/L to 10^{-6} mol/L) was compared with KCl (100 mmol/L) and NE (10^{-5} mol/L).

**Urotensin as a Vasodilator**

A putative vasodilator property of hUII was tested for using SSR arteries and saphenous veins preconstricted with NE (5 μmol/L) or 30 to 60 mmol/L KCl (arteries only).

**Urotensin Integrity Protocol**

The integrity of our hUII solutions and methodology was tested in rat (adult male Wistar) thoracic aortas (n=6).

**Drugs and Solutions**

NE, PE, ET-1, and acetylcholine were purchased from Sigma, and hUII was obtained from Bachem and the Peptide Institute, Inc.

**Data and Statistical Analysis**

The vasoconstriction data are presented as either mN/mm (small vessels) or mN (large vessels). Statistical comparisons of EC_{50} (the concentration required to produce 50% of the maximum response) and maximum responses were performed using Student’s t test (with Bonferroni’s correction). Cumulative concentration response curves were compared using 1-way ANOVA for repeated measures. Unless otherwise specified, results are presented as mean±SEM.

**Results**

**Patients Studied**

Details of the 18 patients studied (all male) are given in the Table. The 7 healthy male volunteers were aged 43 to 79 years.

**Human SSR Arteries From Patients With CHD**

In SSR arteries (diameter, 249±11 μm), ET-1 produced the most powerful vasoconstriction (n=6; EC_{50}, 1.42±0.84 mmol/L; maximum response, 4.19±0.93 mN/mm; P<0.001 versus hUII), followed by NE (n=7; EC_{50}, 0.52±0.07 μmol/L; maximum response, 2.84±0.38 mN/mm; P<0.01 versus hUII; Figure 1A). In vessels from each of the same subjects (n=8), hUII produced no measurable effect (Figure 1A).

hUII did not cause vasodilation in SSR arteries preconstricted with NE (n=5), KCl 30 mmol/L (n=5), or KCl 60 mmol/L (n=4).

**Human SSR Arteries From Healthy Volunteers**

In case atherosclerosis or concomitant drug therapy explained the lack of effect of hUII, we also studied its effects in healthy volunteer vessels (n=7). hUII had no vasoconstrictor action in
these vessels either, whereas ET-1 had an effect comparable to that in arteries from patients with CHD (Figure 1B).

**Human Skeletal Muscle Small Resistance Arteries**
Small skeletal muscle arteries from 3 patients did not vaso-constrict in response to hUII.

**Human Internal Mammary Arteries**
Although there was a normal response to PE and KCl in internal mammary arteries (n=6), segments of the same vessels did not contract in response to hUII (Figure 1C).

**Studies in Veins**

**Human Saphenous Vein**
Human UII did not constrict saphenous veins (n=8), despite a normal response in these vessels to NE (n=4; maximum response, 5.04±0.86 mN), PE (n=4; maximum response, 3.49±1.28 mN), and KCl (n=8; maximum response, 4.66±0.61 mN; all P<0.001 versus hUII). Similarly, in saphenous veins (n=4) preconstricted with NE, hUII (10⁻⁶ mol/L) did not cause venodilation (data not shown).

**Human Subcutaneous Small Veins**
Small subcutaneous veins (n=6; diameter, 259±42 μm) that vasoconstricted with both NE (maximum response, 1.01±0.20 mN/mm) and KCl (maximum response, 0.79±0.12 mN/mm) during start-up showed no response to hUII (both P<0.05 versus hUII).

**Rat Thoracic Aorta**
The effect of hUII in rat thoracic aortas (n=6) is shown in Figure 2. Potent vasoconstriction was observed, with an EC₅₀ of 1.13±0.36 mmol/L and a maximum effect of 2.36±0.2 mN/mm (at 10⁻⁸ mol/L). This response was not sustained at higher concentrations.

**Discussion**

We demonstrated that hUII has no significant vasoconstrictor or vasodilator action in either human arteries or veins of small or medium caliber. This contrasts with the findings of Ames et al³ in the cynomolgus monkey, where hUII was a powerful vasoconstrictor, suggesting a species difference. Interestingly, although Ames et al³ found hUII did not constrict the abdominal aorta, renal artery, or femoral artery of the rat, it did contract the thoracic aorta, as confirmed by ourselves and others.⁴,⁵,¹²,¹³ This raises the second possibility that the action of UII is confined to vessels from particular beds or of a particular caliber.

Fish UII reportedly causes the contraction of arteries in the rat in a descending order of potency,⁷ a finding supported by a recent study with hUII.¹² The effect is greatest in thoracic aorta, intermediate in abdominal aorta, and least in mesenteric artery. This order matches the intensity of specific UII arterial binding and the presumed density of UII receptors in this species.³ Therefore, in humans, UII may possibly contract much larger conduit arteries than currently studied. The potential role of UII in cardiovascular regulation and dysregulation is less obvious should its actions be confined to large arteries only.

New data, taken in conjunction with our own, help address these questions. MacLean et al¹⁴ showed that UII has no effect in small human pulmonary resistance arteries. However, in 3 of 10 vessels, variable vasoconstriction was shown after the inhibition of nitric oxide synthase. Maguire et al¹³ studied the effects of hUII in a variety of human vessels after the mechanical removal of the endothelium. UII led to a maximum contraction of only 15% of that seen with KCl in coronary arteries, 16% of that in mammary arteries, and 20% of that in radial arteries. Only 6 of 9 coronary arteries and 5 of 7 mammary arteries contracted; only 4 radial arteries were studied. By contrast, the contraction of rat thoracic aorta to UII was 68%. The contractile response to ET-1 (as a percentage of KCl) was much greater than that to UII; it was 84%, 82%, and 57% in human coronary, mammary, and radial arteries, respectively. This suggests that a vasoconstrictor action of UII may be unmasked in larger arteries devoid of endothelium and in smaller ones after nitric oxide synthase inhibition (at least in the pulmonary circulation). However, although this may be true after severe or total endothelial inactivation, the degree of endothelial dysfunction associated with CHD (which our patients had) was insufficient to reveal this action of UII. Indeed, we did not find a different response to UII between arteries from patients with CHD and healthy volunteers, in keeping with a report that the density of UII binding sites is similar between atherosclerotic and healthy vessels.¹³

The lack of effect of UII in veins (also reported in the trout²) is consistent with the observation that the expression of GPR14, at least in the primate, is absent in veins.⁴ Surprisingly, however, Maguire et al¹³ reported saphenous vein constriction with UII, although again only after endothelial removal.

Our study focused on patients with vascular disease. Although this is a limitation, it is in these patients that a pathophysiological role for UII (and the therapeutic potential of antagonists) is proposed.⁴ We also focused on the vascular actions of UII, which could have other cardiovascular effects (eg, on myocardial contractility).

In conclusion, UII has no direct vasoconstrictor or vasodilator action in intact human arteries and veins of different sizes and from different vascular beds. These findings suggest marked species differences in the actions of UII and question the importance of this peptide in cardiovascular regulation.
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References
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