Differential Effect of Urotensin II on Vascular Tone in Normal Subjects and Patients With Chronic Heart Failure

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Background—Urotensin II (U-II) is a novel vasoactive peptide that also has direct hypertrophic and profibrotic effects on the myocardium. Uproregulated U-II and its receptor has been observed within the heart of patients with chronic heart failure (CHF). Furthermore, plasma levels of U-II have been found to be elevated in some but not all studies in such patients. However, the functional consequences of activation of the U-II system in patients with CHF, assessed by direct administration of exogenous U-II, have not been previously determined.

Methods and Results—We compared the effect of iontophoresed U-II on skin microvascular tone in normal subjects and patients with CHF, assessed with the use of laser Doppler velocimetry. U-II mediated a dose-dependent vasodilator response in normal subjects (baseline, 137.9 ± 52; U-II, 10^{-15} mol/L, 145 ± 134; U-II, 10^{-14} mol/L, 712 ± 179; U-II, 10^{-13} mol/L, 943 ± 139 arbitrary flux units [AFUs], P < 0.0001). In contrast, a dose-dependent vasoconstrictor response was observed in patients with CHF (baseline, 336.1 ± 129; U-II, 10^{-12} mol/L, 317 ± 131; U-II, 10^{-9} mol/L, 129 ± 137; U-II, 10^{-7} mol/L, 22.4 ± 130 AFUs, P < 0.05). Differences in flow between normal subjects and patients with CHF were significant overall (P < 0.001, 2-way ANOVA) and at the U-II 10^{-9} mol/L and U-II 10^{-7} mol/L dose level by Student’s unpaired t test (P < 0.05, P < 0.0001, respectively). In contrast, there was no significant difference between baseline blood flow and any dose of U-II in either group (or between groups) when the opposite polarity was applied.

Conclusions—In addition to direct effects on the myocardium, U-II may contribute to the increased peripheral vascular tone that is characteristic of human CHF. The present observations support the contention that the U-II system may be a potentially important target for pharmacological blockade in the treatment of this condition. (Circulation. 2004;109:1212-1214.)

Key Words: peptides • heart failure • vasculature • ions • endothelium

Urotensin II (U-II) is a somatostatin-like cyclic peptide that has been found to have potent vasoconstrictor actions in some but not all vascular beds in various animal species as well as in humans. U-II also has direct activity within the heart, such as profibrotic, hypertrophic, and inotropic actions. The U-II system (both ligand and receptor) has been found to be upregulated within the heart of patients with chronic heart failure (CHF). Furthermore, plasma levels of U-II have recently been found to be elevated in these patients in comparison to normal subjects in some but not all studies.

The preceding observations raise the possibility that U-II may be contributory to CHF disease progression, both by adverse effects on cardiac remodeling and by an increase in peripheral vascular tone that is characteristic of this condition. To test the latter hypothesis, we undertook a direct comparison of the effect of iontophoresed U-II on skin microvascular tone (measured by laser Doppler velocimetry) between patients with CHF and normal subjects.

Methods

Study Population
Patients with CHF were required to have a left ventricular fractional shortening [LVFS] of < 22% and New York Heart Association functional class [NYHA FC] II-III symptoms. Normal subjects could have no evidence of cardiovascular disease on medical history or physical examination and could not be taking medication known to interfere with the cardiovascular system. Patients with CHF were asked to withhold all medication (except diuretics) for 24 hours before testing.

Study Protocol
U-II was iontophoresed at 10^{-12}, 10^{-11}, and 10^{-10} mol/L (6.94×10^{-14}, 10^{-13}, and 10^{-12} g U-II, respectively, in 0.5-mL wells) in patients with CHF and normal subjects with forearm blood flux of the cutaneous vasculature compared with the diluent Milli-Q water (Aquapore).
Responses to iontophoresis of U-II at these doses with the use of the opposite polarity served as a negative control.

Responses to the endothelium-dependent vasodilator acetylcholine (ACH, 10 mg/mL) and the endothelium-independent vasodilator sodium nitroprusside (SNP, 10 mg/mL) were also assessed in both groups to determine whether skin microvessels respond in a manner similar to resistance vessels, in which these dilators have been previously studied.12

Written informed consent was obtained from all patients, and the study was approved by the Human Ethics Committee of the Alfred Hospital.

Iontophoresis and Laser Doppler Velocimetry
Iontophoresis is a noninvasive technique that enables the administration of substances transdermally.13 It involves the direct application of a small electrical current (10% of 1 mA=0.1 mA) such that the vasoactive substance being studied is expelled from the electrode of the same polarity and driven from the skin surface into the superficial cutaneous microcirculation. The vasoactive substance was placed in a polyvinylchloride chamber containing platinum electrodes with an 11-mm diameter and 0.5-mL capacity central reservoir, fixed to the volar aspect of the forearm. Seven wells were placed on the right forearm and 5 on the left forearm for the experiments conducted in the present study.

Laser Doppler velocimetry uses a 633-nm (helium neon) infrared light, which, in conjunction with a Moor laser Doppler imager (Moor Instruments), detects blood flow through frequency shifts of photons caused by moving erythrocytes within the superficial vessels 1 to 2 mm below the surface of the skin. As the resultant Doppler-shifted signals are highly related to flow, velocities in the cutaneous microcirculation accurately and reproducibly measure flux of blood through the vessel and thus vessel tone, as validated by our group previously.13

The 0.1-mA current was applied for 30 seconds to each well, with blood flux data acquisition over the subsequent 6 minutes after cessation of the iontophoretic stimulus. This was achieved by scanning the area of interest 25 times, at 14-second intervals, to determine the area under the perfusion-time curve. Data obtained was expressed as arbitrary flux units (AFUs).

Statistical Analysis
Data are expressed as mean±SEM. U-II responses within groups were analyzed by Student’s unpaired t test. Nonparametric data, for example, gender, were analyzed by χ2 analysis or Mann-Whitney test, as appropriate. A 2-tailed probability value of <0.05 was considered statistically significant.

Results
Normal subjects (n=14) and patients with CHF (n=13) were well matched for most baseline demographic indexes (Table), except that there was a greater percentage of men in the CHF group compared with normal subjects. As expected, plasma N-terminal pro–brain natriuretic peptide levels were significantly higher and serum sodium levels significantly lower in the CHF patient group.

Further demographic data for the CHF group were as follows: CHF disease duration: mean, 4.37 (2.8) years; LVFS, 16.9 (4.3)%; NYHA FC II/III distribution, 83/17%; and causes of idiopathic (51%), ischemic (41%), and drug/alcohol-related (8%). Patients with CHF were receiving treatment with ACE inhibitors or angiotensin receptor block-
ers (92%), β-adrenoceptor blockers (92%), diuretics (76%), digoxin (25%), and spironolactone (8%).

Responses to Urotensin II
There was no significant difference in baseline blood flux between normal subjects and patients with CHF (138±53 versus 336±129 AFUs, P=NS). Responses to increasing doses of iontophoresed U-II in the two groups are shown in the Figure. There was a dose-dependent increase in blood flux (signifying vasodilation) in normal subjects (P<0.001 versus baseline by trend test). In contrast, a dose-dependent vasodilator response to increasing U-II was observed in patients with CHF (P<0.038 versus baseline by trend test). Changes from baseline between the two groups in response to U-II were highly significant by 2-way, repeated-measures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Subjects (n=14)</th>
<th>Patients With CHF (n=13)</th>
<th>P</th>
</tr>
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<tr>
<td>Age, y</td>
<td>50±4</td>
<td>56±4</td>
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<td>Gender, male/female</td>
<td>5/9</td>
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<td>Body mass index, kg/m2</td>
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<td>Systolic blood pressure, mm Hg</td>
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<td>115±4.0</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
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<td>70±4.0</td>
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<td>Heart rate, bpm</td>
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<td>Sodium, mmol/L</td>
<td>143±0.55</td>
<td>141±0.83</td>
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<td>Potassium, mmol/L</td>
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<td>Chloride, mmol/L</td>
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<td>Bicarbonate, mmol/L</td>
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<td>Urea, mmol/L</td>
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<td>7.08±0.36</td>
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<td>Creatinine, mmol/L</td>
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<tr>
<td>NT-proBNP, pg/mL</td>
<td>57.0±28.8</td>
<td>1162±591</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

*P<0.05 for differences in gender, serum sodium, and N-terminal pro–brain natriuretic peptide (NT-proBNP) between groups.

Change in AFUs from baseline after iontophoresis of U-II onto skin microvasculature in normal subjects (closed bars) and patients with CHF (open bars). U-II iontophoresis resulted in dose-dependent vasodilation in normal subjects and constriction in patients with CHF, with differences in responses between the two study groups significant at the 10−9 and 10−7 U-II mol/L (M) dose levels.
ANOVA (P<0.001) and significant when compared at the 10^{-9} and 10^{-7} mol/L U-II levels by Student’s unpaired t test (P<0.05, P<0.0001, respectively, Figure). In contrast, there was no significant difference between baseline blood flux and any dose of U-II in either group (or between groups) when the opposite polarity was applied (negative control). Indeed, there was minimal difference between groups when differences from baseline were directly compared (difference between patients with CHF and normal subjects in U-II at 10^{-12} mol/L= –7, U-II 10^{-9} mol/L = –28, U-II 10^{-7} mol/L = 55 AFUs, P = NS at any dose).

**Responses to ACh and SNP**

There was a nonsignificant 31% reduction in vasodilator responsiveness to 10 mg/mL ACh in patients with CHF compared with normal subjects (4233±709 versus 3222±558 AFUs) but no difference in responses to SNP 10 mg/mL between the two groups (3121±386 versus 2911±390 AFUs, P = NS).

**Discussion**

The present study has observed, for the first time, that U-II mediates differential responses between normal subjects and patients with CHF in skin microcirculation, with dose-dependent dilation to U-II in normal subjects and dose-dependent constriction in patients with CHF. Clear-cut differences in response to U-II were therefore observed between the two groups, with relative vasoconstriction to U-II in patients with CHF compared with normal subjects. It is difficult to determine what concentration of U-II actually reaches the effector (vascular smooth muscle) cell to exert these differential effects. However, in the present study, differences in vascular responses began to be observed at extremely low doses of cutaneously administered U-II. Plasma levels of U-II in patients with CHF have been measured in the nanomolar and picogram-per-milliliter{\textsuperscript{7,8}} ranges. The present data would therefore suggest that these levels may in fact be sufficient to contribute to the increased vascular tone observed in patients with CHF. Nevertheless, there appears to be at present large variations in measured levels of U-II as well as poor characterization and validation of assay methodologies{\textsuperscript{9,10}}; therefore, the above conclusions may be somewhat speculative.

It was of interest that U-II exerted clear-cut, dose-dependent vasodilator effects in normal subjects. This finding contrasts with constrictor{\textsuperscript{2}} or neutral{\textsuperscript{4}} responses to exogenous infusion of U-II in resistance vessels of normal subjects, as observed in earlier studies. Widespread variability in vascular responsiveness to U-II across species and vascular beds (and even within the same vascular bed) is characteristic of this peptide.{\textsuperscript{15}} Indeed, dose-dependent vasodilation to U-II has been observed in humans in human pulmonary arteries, as assessed in an organ bath preparation.{\textsuperscript{16}}

Laser Doppler velocimetry was used in the present study to evaluate vascular tone in patients with CHF, as it was believed that with the U-II system upregulated in these patients, a noninvasive approach was considered ethically appropriate. Specifically, systemic doses of U-II have previously been observed to cause cardiovascular collapse, at least in primates.{\textsuperscript{1}} Patients with CHF have endothelial dysfunction, which has been repeatedly demonstrated in the forearm resistance vasculature.{\textsuperscript{12}} In the present study, responses to endothelium-dependent and endothelium-independent vasodilators appeared to be similar in skin microvessels of patients with CHF to those previously described in resistance vessels. It was of interest that responses to the endothelium-independent vasodilator SNP were similar in normal subjects and patients with CHF. This finding is important in the context of the U-II responses in the two study groups because it makes the influence of edema in patients with CHF limiting diffusion of U-II onto skin microvessels appear to be an extremely unlikely explanation for the differential U-II responses observed.

In conclusion, the present data suggests that the vasoactive peptide U-II may contribute to peripheral vasoconstriction in human CHF. Therefore, antagonism of the U-II system may be of benefit in reversing increased vascular tone in these patients as well as potentially attenuating the adverse cardiac effects of activation of this peptide. U-II may represent an important, novel target for therapeutic blockade in the pharmacological treatment of heart failure.

**Acknowledgments**

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**References**

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