

Urocortin 2 Infusion in Healthy Humans

Hemodynamic, Neurohormonal, and Renal Responses

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- Objectives** We sought to examine the effects of urocortin (UCN) 2 infusion on hemodynamic status, cardiovascular hormones, and renal function in healthy humans.
- Background** Urocortin 2 is a vasoactive and cardioprotective peptide belonging to the corticotrophin-releasing factor peptide family. Recent reports indicate the urocortins exert important effects beyond the hypothalamo-pituitary-adrenal axis upon cardiovascular and vasohumoral function in health and cardiac disease.
- Methods** We studied 8 healthy unmedicated men on 3 separate occasions 2 to 5 weeks apart. Subjects received placebo, 25- μ g low-dose (LD), and 100- μ g high-dose (HD) of UCN 2 intravenously over the course of 1 h in a single-blind, placebo-controlled, dose-escalation design. Noninvasive hemodynamic indexes, neurohormones, and renal function were measured.
- Results** The administration of UCN 2 dose-dependently increased cardiac output (mean peak increments \pm SEM) (placebo 0.5 ± 0.2 l/min; LD 2.1 ± 0.6 l/min; HD 5.0 ± 0.8 l/min; $p < 0.001$), heart rate (placebo 3.3 ± 1.0 beats/min; LD 8.8 ± 1.8 beats/min; HD 17.8 ± 2.1 beats/min; $p < 0.001$), and left ventricular ejection fraction (placebo $0.6 \pm 1.4\%$; LD $6.6 \pm 1.5\%$; HD $14.1 \pm 0.8\%$; $p < 0.001$) while decreasing systemic vascular resistance (placebo -128 ± 50 dynes-s/cm⁵; LD -407 ± 49 dynes-s/cm⁵; HD -774 ± 133 dynes-s/cm⁵; $p < 0.001$). Activation of plasma renin activity ($p = 0.002$), angiotensin II ($p = 0.001$), and norepinephrine ($p < 0.001$) occurred only with the higher 100- μ g dose. Subtle decreases in urine volume ($p = 0.012$) and natriuresis ($p = 0.001$) were observed.
- Conclusions** Brief intravenous infusions of UCN 2 in healthy humans induced pronounced dose-related increases in cardiac output, heart rate, and left ventricular ejection fraction while decreasing systemic vascular resistance. Subtle renal effects and activation of plasma renin, angiotensin II, and norepinephrine (at high-dose only) were observed. These findings warrant further investigation of the role of UCN 2 in circulatory regulation and its potential therapeutic application in heart disease. (J Am Coll Cardiol 2007;49:461-71) © 2007 by the American College of Cardiology Foundation

Urocortin (UCN) 2 is a recently discovered vasoactive and cardioprotective peptide belonging to the corticotrophin-releasing factor (CRF) peptide family, which has been present throughout vertebrate evolution (1). Recent reports indicate the urocortins exert effects beyond the hypothalamo-pituitary-adrenal axis, directly upon cardiac, vascular, and vasohumoral function in health and cardiac disease (2-7). The CRF family of peptides, including CRF and UCN 1, 2, and 3, act largely through 2 G protein-coupled receptor subtypes, CRF₁ and CRF₂ (8), and acting in part

through Gs-mediated elevation of cyclic adenosine monophosphate, in turn activating cyclic adenosine monophosphate-dependent protein kinase A. Corticotrophin-releasing factor predominantly activates CRF₁ receptors, and urocortin 1 binds to both CRF₁ and CRF₂ receptors, whereas UCNs 2 and 3 are selective agonists for the CRF₂ receptors. CRF₁ receptors are found in the brain, pituitary gland, and gut. There are at least 2 splice variants of the CRF₂ receptors, termed CRF_{2(a)} and CRF_{2(b)}. The CRF_{2(a)} receptor in humans is found in the central nervous system but importantly constitutes the predominant CRF₂ receptor peripherally, particularly in the heart, vasculature, gut, skeletal tissue, and adrenal gland (9,10). In the cardiovascular system, CRF_{2(a)} receptors are found in humans in high concentrations in the left ventricle, intramyocardial blood vessels, and medial layers of internal mammary arteries (11).

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**Abbreviations
and Acronyms****A** = transmitral diastolic flow velocity with atrial contraction**ACTH** = adrenocorticotropic hormone**Am** = diastolic myocardial velocity during atrial contraction**CRF** = corticotrophin-releasing factor**E** = transmitral early diastolic flow velocity**Em** = early diastolic myocardial velocity**SVR** = systemic vascular resistance**UCN** = urocortin

Recently, we have shown in both normal animals and in an ovine model of pacing-induced heart failure that UCN 2 dose-dependently increases cardiac output and reduces both left atrial pressure and systemic vascular resistance (SVR) (3). In intact mice, both wild-type and in the muscle-specific LIM protein-deficient heart failure model, UCN 2 reduced mean atrial pressure and is positively inotropic, chronotropic, and lusitropic (6). In the isolated rat heart, UCN 1, 2, or 3 given before, during, or after myocardial infarction preserves pump function and reduces infarct size (7,12).

Both UCN 1 and 2 suppress vasoconstricting and volume-retaining neurohumoral factors and enhance renal function in experimental ovine heart failure (3,4). In addition, UCN 1 activates the adrenocorticotropic hormone (ACTH)-cortisol “stress” response in healthy humans and sheep and in patients and sheep with heart failure—an effect probably mediated by the CRF₁ receptor (4,13–16). Therefore, UCN 1 and 2 appear to have significant effects on cardiovascular function in both normal health and cardiac disease. We hypothesized that, in healthy humans, UCN 2 would induce the hemodynamic effects reported for UCN 1 and 2 without stimulation of ACTH and cortisol, as seen with UCN 1.

We report the first controlled study in healthy human volunteers that examines the effects of UCN 2 on hemodynamic status, echocardiographic parameters, cardiovascular hormones, and renal function.

Methods

Subjects. We studied 8 healthy unmedicated men ages 24 to 58 years (mean \pm SD, 41.1 \pm 11.7 years), weighing 60 to 104 kg (mean 80 \pm 17 kg), with a body mass index 19 to 32 kg/m² (mean 25.6 \pm 4.5 kg/m²), plasma creatinine 0.08 to 0.012 mmol/l (mean 0.098 \pm 0.014 mmol/l), and echocardiographic left ventricular ejection fractions of 58% to 77% (mean 66.5 \pm 7.1%).

Study protocol. Participants gave written informed consent to the study. The protocol was approved by the Ethics Committee of the New Zealand Ministry of Health (Upper South B, Canterbury). Human UCN 2 (the 38 amino acid sequence predicted by Reyes et al. [17]) was provided by Neurocrine Biosciences Inc. (San Diego, California) after manufacture by Bioserv Corporation (San Diego, California). Subjects were studied using a single-blind dose-escalation design, receiving placebo, 25 μ g, and 100 μ g of UCN 2

sequentially with a washout period of 2 to 5 weeks between each dose. Doses were chosen after a 2-person pilot study demonstrated measurable effects with no dose-limiting adverse response to the maximum (100 μ g) dose. On the morning of the third day of a controlled metabolic diet (sodium 120 mmol/day, potassium 100 mmol/day), subjects ate breakfast and presented to the study room by 7:00 AM. A 24-h urine collection was completed at 8:00 AM. The subjects fasted until lunch at 1:00 PM. Participants were weighed, and 10 ml/kg water was given orally at 8:00 AM followed by 200 ml/h between 9:00 AM and 6:00 PM. Subjects were seated throughout the day except when standing to collect urine samples. At 8:15 AM, venous cannulae were placed in each forearm, one for the infusion of UCN 2 or placebo and the other for blood sampling. All subjects received vehicle placebo (dissolved in 1 ml of water then made up to 60 ml in normal saline with 50 ml administered), 25 μ g of UCN 2 (1 mg dissolved in 6.6 ml of water then 0.2 ml of that solution made up to 60 ml in normal saline [0.5 mg/ml] with 50 ml administered), and finally 100 μ g of UCN 2 (1 mg of dissolved in 5 ml of water then 0.6 ml of that solution made up to 60 ml in normal saline [2 μ g/ml] with 50 ml administered) over 1 h, commencing at 9:00 AM.

Venous samples were drawn at 8:30 AM, 9:00 AM, 9:30 AM, 10:00 AM, 11:00 AM, 2:00 PM, and 6:00 PM. Blood was collected into chilled tubes (containing ethylene diamine tetraacetic acid) for hormone samples except cortisol (heparin) and angiotensin II (0.125 mol/l ethylene diamine tetraacetic acid, 0.05 mol/l o-phenanthroline, 2% ethanol, 0.2% neomycin sulfate, and 0.03 mg/ml enalkiren), immediately centrifuged at 4°C, and plasma stored at –80°C before assay for UCN 2, cyclic adenosine monophosphate, cyclic guanosine monophosphate, ACTH, cortisol, plasma renin activity, angiotensin II, aldosterone, arginine vasopressin, N-terminal pro-brain natriuretic peptide, epinephrine, norepinephrine, endothelin 1, adrenomedullin, insulin, and ghrelin, all according to our published methods (15). At the conclusion of infusions, serial sampling was conducted at 10:05, 10:10, 10:15, and 10:20 AM for UCN 2 pharmacokinetics. Generally, for each hormone, all samples from an individual were analyzed in a single assay. Numbers of UCN 2 samples were too great to fit into 1 assay, but samples from the 25- μ g and 100- μ g UCN 2 active phases were assayed together. Intra and interassay coefficients of variation, measured at concentrations similar to those extant during these experiments, were all <18.5% except the interassay coefficient of variation of endothelin-1 at a mean concentration of 1.05 pmol/l (25.15%).

Plasma sodium (Na⁺), potassium (K⁺), creatinine, glucose, venous bicarbonate, and chloride (Cl[–]) were measured at 9:00 AM, 10:00 AM, 11:00 AM, 12:00 PM, 1:00 PM, 2:00 PM, 3:00 PM, and 6:00 PM, with additional measurement of calcium, magnesium, phosphate, total protein, albumin, aspartate transaminase, alanine transaminase, amylase, creatine ki-

nase, creatine kinase MB fraction, and troponin T at the 9:00 AM and 6:00 PM time points.

After blood sampling, subjects stood to collect urine (9:00 AM, 10:00 AM, 11:00 AM, 2:00 PM, and 6:00 PM) for measurement of volume, cyclic adenosine monophosphate, cyclic guanosine monophosphate, Na^+ , K^+ , and creatinine. Systolic and diastolic blood pressure, heart rate, and pulse oximetry were recorded in duplicate at 8:30 AM and every 15 min from 9:00 to 11:00 AM, then hourly until 6:00 PM with an automatic sphygmomanometer and pulse oximeter (Pro 300 monitor, Dinamap, Wipro GE Healthcare, Bangalore, India). Cardiac output was measured by the thoracic impedance method (Minnesota impedance cardiography, model 304B, Surcom Inc., Minneapolis, Minnesota) every 30 min from 8:30 to 11:00 AM, and then hourly until 6:00 PM.

Echocardiography was performed approximately 1 h before and immediately after infusions with the patient lying in the left lateral position (using standard techniques with a Vivid 3 echocardiogram (General Electric, Fairfield, Connecticut). Left ventricular volume was measured in the 4-chamber view using the modified Simpson's rule. Data were stored digitally for subsequent analysis of left ventricular volumes (diastolic and systolic) and ejection fraction, transmitral early diastolic flow velocity (E), transmitral diastolic flow velocity with atrial contraction (A), deceleration time of the transmitral early diastolic flow velocity, early diastolic myocardial velocity (Em), diastolic myocardial velocity during atrial contraction (Am), and systolic myocardial velocity. Transmitral flow was measured by pulse-wave Doppler at the mitral valve leaflet tips in the 4-chamber view. Tissue Doppler myocardial velocities at the medial mitral valve annulus were measured using the machine presets.

Twelve-lead electrocardiograms (Agilent Pagemwriter 200, Agilent Technologies, Andover, Massachusetts) were recorded at 9:00 AM, 10:00 AM, 2:00 PM, and 6:00 PM. Pulse rate interval, QRS duration, and QT interval both uncorrected and corrected using Fridericia's method ($\text{QT}/\text{RR}^{1/3}$) were assessed.

Human UCN 2 two-site enzyme-linked immunosorbent assay. Urocortin 2 was measured in heparin plasma with a 2-site chemiluminescent enzyme-linked immunosorbent assay using an N-terminal-directed monoclonal antibody for plate coating and a second C-terminal-directed rabbit polyclonal antibody. Mouse antirabbit IgG-alkaline phosphatase conjugate plus CSPD substrate (Applied Biosystems, Foster City, California) were used to generate the chemiluminescent signal. Full details of the assay have been submitted for publication elsewhere. Dilutions of plasma samples were parallel to the standard curve. Mean recovery of UCN 2 added to 6 plasma samples was 107% at 2.43 ng/ml and 100% at 1.21 ng/ml and 0.61 ng/ml. The lower limit of quantitation was 0.3 ng/ml. This was used for pharmacokinetic calculations (as per Food and Drug Administration and International Conference for Harmonisation guidelines). The assay detection limit (upper 95%

confidence interval [CI] for the zero standard) was 0.11 ng/ml ($n = 36$). In studies with multiple samples, valid data can be obtained below the assay limit of quantitation because the number of samples allows valid statistical comparison. We have therefore used all assay data for statistical calculations and reported 95% CI. The intra- and (inter)-assay coefficients of variation ($n = 36$) were 5.0% (5.7%) at 0.68 ng/ml, 2.8% (5.7%) at 1.34 ng/ml, and 2.8% (3.9%) at 2.45 ng/ml.

Cross-reactivity was determined by measurement of human UCN 1, UCN 3, and CRF at 510, 670, and 500 ng/ml, respectively, all evoking assay responses that were at or less than the limit of quantitation (0.3 ng/ml). Urocortin 1 and CRF responses also were at or less than the 95% CI for the zero standard.

Statistics. Data were analyzed by repeated-measures analysis of variance using SPSS version 11.5 statistical package (SPSS Inc., Chicago, Illinois). The significance reported is the time by dose interaction unless otherwise stated (dose effect). Where significant differences between doses were identified, these were further explored using paired comparisons between doses (i.e., the Fisher least significant difference test). Analyses for study days included measurements assessed from the commencement of infusion to the last sample of the day unless otherwise stated. UCN 2 half-life ($t_{1/2}$), metabolic clearance rate, and volume of distribution were calculated using a 1-compartment model during and after the infusion period (WinNonLin Professional 3.1, Pharsight Corporation, Mountain View, California). Hormonal data were consistently non-normally distributed and were \log_e transformed before analyses, which adequately normalized distributions for parametric analysis. Geometric means and 95% CIs are reported for these measures. Results other than those for hormones are presented as mean \pm SEM. To aid clarity, pooled 95% CIs are displayed for the graphed hormones and pooled SEM for the graphed hemodynamics. A value of $p < 0.05$ was taken to indicate statistical significance.

Results

Preinfusion hormone, biochemical, and hemodynamic variables did not differ between the 3 experimental days (placebo, 25 μg , and 100 μg UCN 2). Hemoglobin decreased during the course of the study period (placebo: 146.7 ± 3.7 g/l; 25 μg : 143.5 ± 4.7 g/l; 100 μg : 138.7 ± 4.7 g/l; $p = 0.004$) secondary to sampling 813 ml of blood over the approximately 6-week course of the study.

Infusion of UCN 2. Plasma UCN 2 concentrations increased in dose-related fashion compared with time-matched placebo concentrations with 25 μg (0.09 [95% CI 0.05 to 0.17] to 1.28 [1.08 to 1.52] ng/ml) and 100 μg infusions (0.09 [95% CI 0.05 to 0.17] to 5.69 [4.48 to 7.23] ng/ml; $p < 0.001$ for both doses). Peak concentrations were achieved at the end of the 1-h infusions. Plasma cyclic

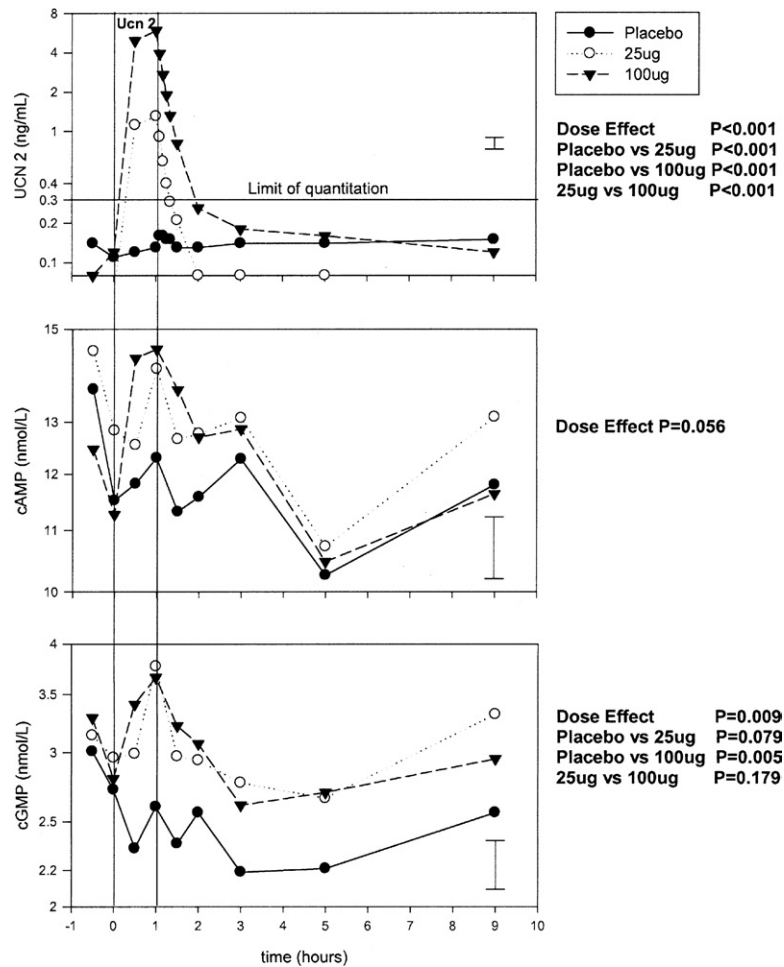


Figure 1 Plasma UCN 2, cAMP, and cGMP Responses to UCN 2 Infusion

Plasma urocortin 2 (UCN 2), cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP) responses (geometric mean and pooled 95% confidence intervals) to UCN 2 infusion in 8 healthy men.

adenosine monophosphate tended to increase ($p = 0.056$), and cyclic guanosine monophosphate clearly was increased ($p = 0.009$) by UCN 2 infusions (Fig. 1).

Pharmacokinetics. Pharmacokinetics were consistent with a 1-compartment model. The $t_{1/2}$ for immunoreactive UCN 2 (95% CI) was 10.1 (9.0 to 11.2) min with an metabolic clearance rate of 0.37 (0.31 to 0.43) l/min and a volume of distribution of 5.2 (4.5 to 5.9) l.

Hemodynamics and echocardiography. The administration of UCN 2 induced dose-related increases in cardiac output (maximal increments from preinfusion levels were 0.5 ± 0.2 , 2.1 ± 0.6 , and 5.0 ± 0.8 l/min for placebo, 25 μ g, and 100 μ g, respectively; $p < 0.001$). Corresponding increments in heart rate were 3.3 ± 1.0 , 8.8 ± 1.8 , and 17.8 ± 2.1 beats/min ($p < 0.001$). The administration of UCN 2 decreased diastolic blood pressure (maximal decrements were -4.9 ± 1.2 , -7.4 ± 1.5 , and -14.1 ± 2.1 mm Hg; $p < 0.001$) and mean arterial pressure (-4.3 ± 1.5 , -5.5 ± 1.8 , and -10.2 ± 2.0 mm Hg; $p = 0.01$), but

not systolic blood pressure—with overall increases in pulse pressure of 6.4 ± 0.6 , 10.6 ± 3.0 , and 18.5 ± 2.1 mm Hg ($p < 0.001$). Systemic vascular resistance decreased -128 ± 50 , -407 ± 49 , and -774 ± 133 dynes·s/cm⁵ ($p < 0.001$). Sustained postinfusion decreases in diastolic blood pressure and mean arterial pressure were observed on both active days compared with placebo (analysis of variance analysis by group; diastolic blood pressure: 25 μ g, $p = 0.011$; 100 μ g, $p = 0.034$; mean arterial pressure, 25 μ g, $p = 0.014$; 100 μ g, $p = 0.037$). After a provision of lunch at 1:00 PM (4 h after the commencement of infusions), we observed transitory increases in cardiac output, heart rate, systolic blood pressure, and pulse pressure with decreases in diastolic blood pressure, mean arterial pressure and SVR (Figs. 2 and 3).

Dose-related increases in left ventricular ejection fraction ($p < 0.001$), E ($p = 0.019$), E_m ($p = 0.041$), and systolic myocardial velocity ($p = 0.001$) were observed, whereas end-systolic volume ($p < 0.001$) decreased. No significant

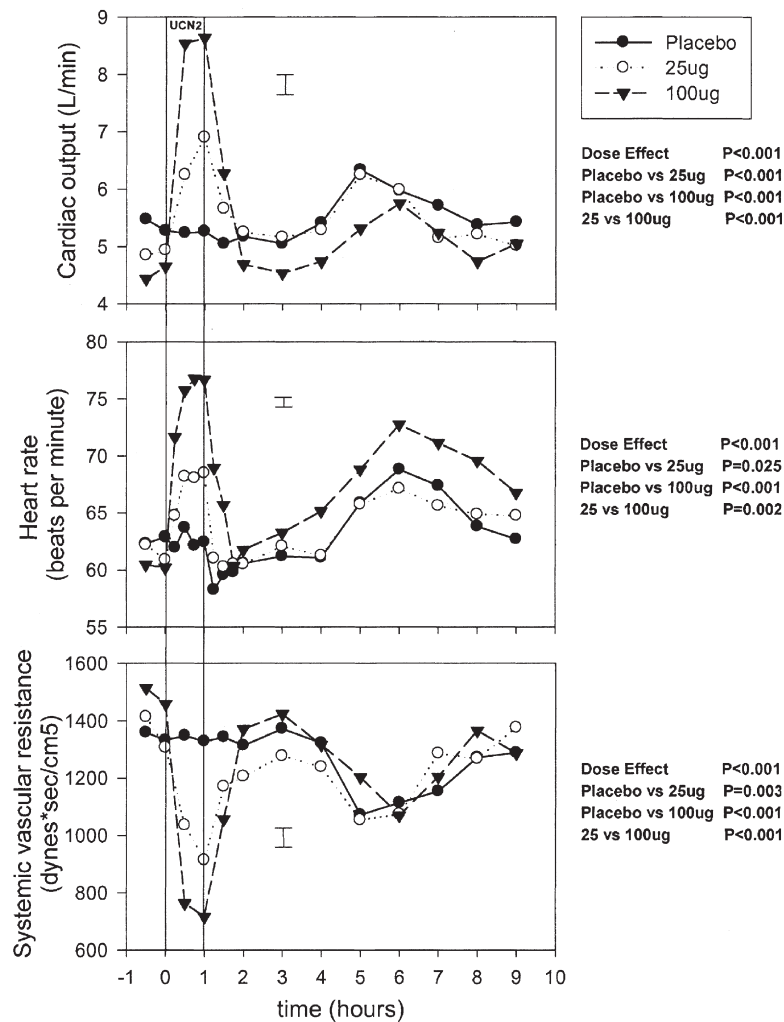


Figure 2 Cardiac Output, Heart Rate, and Systemic Vascular Resistance Responses to UCN 2 Infusion

Cardiac output, heart rate, and systemic vascular resistance responses (mean and pooled standard errors) to uroctin (UCN) 2 infusion in 8 healthy men. Lunch was given after the 4-h observations.

changes were observed in end-diastolic volume ($p = 0.808$), deceleration time ($p = 0.743$), or E/E_m ($p = 0.808$), whereas A ($p = 0.054$) and A_m ($p = 0.062$) tended to increase (Figs. 4 and 5).

Neurohormones. The 100 μg dose of UCN 2 induced significant increases in plasma renin activity ($p < 0.001$), angiotensin II ($p = 0.002$), norepinephrine ($p < 0.001$), cyclic guanosine monophosphate ($p = 0.005$), and blunted the increase in epinephrine observed compared with placebo ($p = 0.034$). Aldosterone also increased during the 2 h from commencement of infusion ($p = 0.031$) (Fig. 6). A sustained postinfusion increase in cyclic guanosine monophosphate was observed on both active days compared with placebo (analysis of variance analysis by group; 25 μg , $p = 0.023$; 100 μg , $p = 0.042$). Neurohormonal spikes were all within normal ranges except for UCN 2, where the normal range is unknown,

and plasma renin activity and angiotensin II where the normal ranges are 0.4 to 2.3 nmol/l/h and 6 to 26 pmol/l, respectively. Adrenocorticotrophic hormone, cortisol, insulin, ghrelin, N-terminal pro-brain natriuretic peptide, arginine vasopressin, endothelin 1, or adrenomedullin were not significantly altered by either dose of UCN 2 (results not shown).

Plasma biochemistry. The infusion of UCN 2 did not alter plasma Na^+ , K^+ , creatinine, glucose, venous bicarbonate, Cl^- , calcium, magnesium, phosphate, total protein, albumin, aspartate transaminase, alanine transaminase, amylase, creatine kinase, creatine kinase-MB, or troponin T (results not shown).

Urinalysis. The administration of UCN 2 induced subtle but significant dose-related decreases in urinary volume ($p = 0.012$), excretion of sodium ($p = 0.001$), potassium ($p < 0.001$), and creatinine ($p = 0.024$) (Fig. 7).

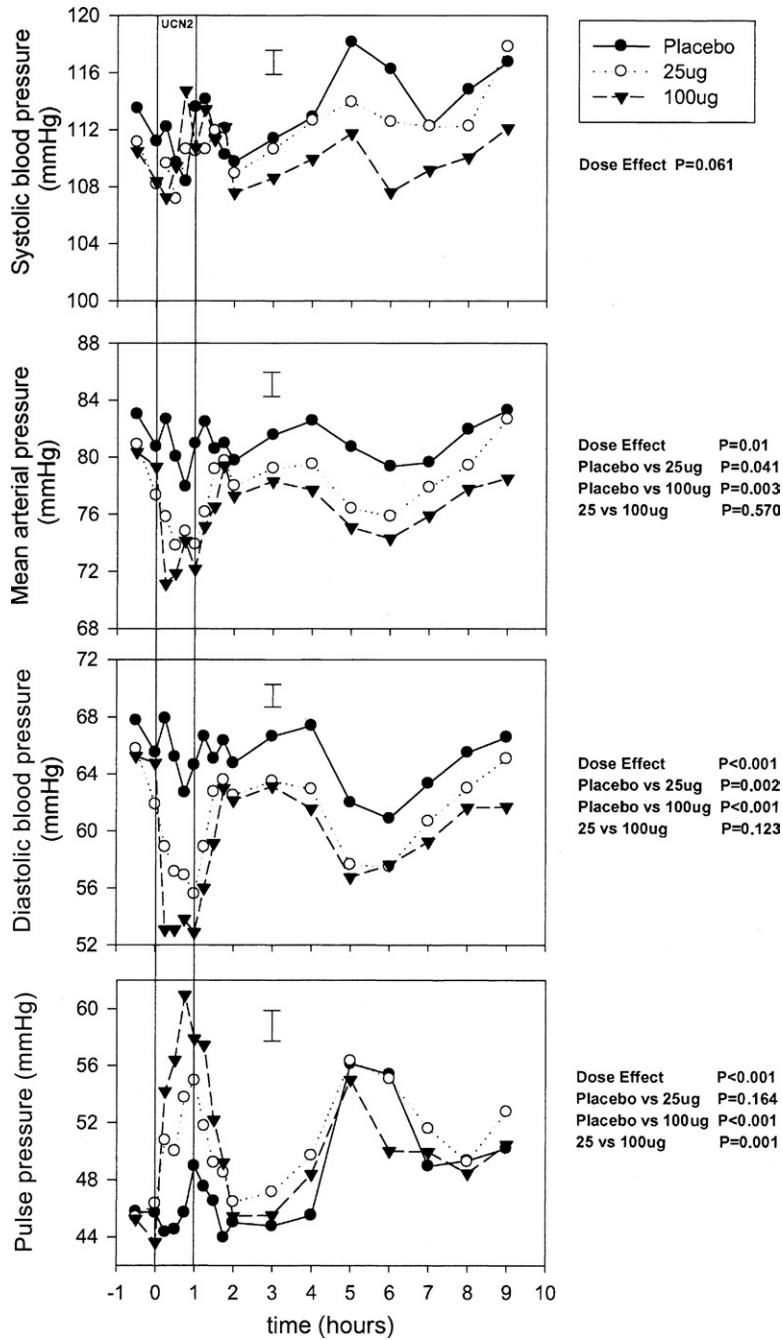


Figure 3 Systolic, Mean Arterial, and Diastolic Blood Pressure and Pulse Pressure Responses to UCN 2 Infusion

Systolic, mean arterial, and diastolic blood pressure and pulse pressure responses (mean and pooled standard errors) to urocortin (UCN) 2 infusion in 8 healthy men. Lunch was given after the 4-h observations.

Electrocardiogram. The administration of UCN 2 did not change pulse rate interval, QRS duration, QT interval, or corrected QT interval.

Observed events. All volunteers experienced flushing during active infusions of UCN 2, which subsided within an hour of the end of infusions. Four subjects had the sensation of increased heart rate during active infusions. In addition,

four subjects had mild and transient hypokalemia (nadir 3.3 mmol/l) on active days after eating lunch, coinciding with postprandial increases in insulin, which also was seen in 1 person on the placebo day (nadir 3.4 mmol/l). Two subjects felt dizzy upon intravenous needle insertion, 1 of who fainted subsequently (at the end of high-dose infusion). Among these water-loaded subjects, 2 exhib-

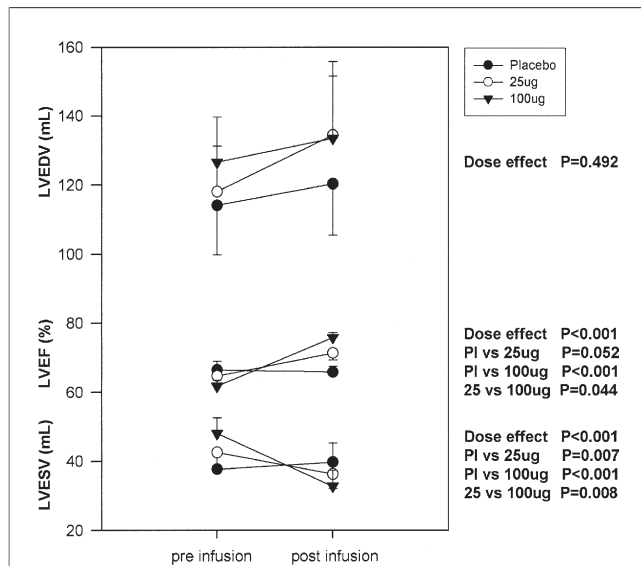


Figure 4 LVEDV, LVESV, and LVEF Responses to UCN 2 Infusion

Left ventricular end-diastolic volume (LVEDV), end-systolic volume (LVESV), and ejection fraction (LVEF) responses (mean ± SEM) to urocortin (UCN) 2 infusion in 8 healthy men.

ited mild and transient hyponatremia on active days (nadir 133 mmol/l) also seen in 2 on placebo days (nadir 133 mmol/l). One volunteer had mild headache, 1 had anorexia in the evening after a high-dose infusion, and 1 had mild transient hyperamylasemia recorded at 6:00 PM (38 U/l at 9:00 AM to 96 U/l [normal range 8 to 53 U/l]) on the day of high-dose infusion with resolution when retested 3 days later.

Discussion

We provide the first report on the biological effects of UCN 2 infused in healthy humans. Brief intravenous infusions of 25 and 100 µg of UCN 2 markedly increased measured plasma UCN 2, and pharmacokinetic measurements indicated a half-life of 10.1 min with a volume of distribution of 5.2 l. The administration of UCN 2 had clear effects on hemodynamic status with more subtle neurohormonal and renal actions.

Hemodynamic status. The impressive dose-related increase in cardiac output is in large part the result of decreased after-load through vasodilation (as evidenced by the large decreases in SVR and the flushing in all subjects at both doses) with some contribution from increased heart rate. Whether the increase in heart rate reflects a direct effect of UCN 2 or is purely baroreflex-mediated is unknown. Increased cardiac output counterbalanced the effect of the vasodilatory response on systolic pressures, the net result being minimal change in systolic blood pressure compared with clear decreases in diastolic blood pressure. The echocardiographic findings are consistent with augmented left ventricular systolic

function with dose-related decreases in end-systolic volume and increases in ejection fraction and systolic mitral valve tissue velocity. Comment on inotropism must be reserved because loading conditions and heart rate were not fixed; however, UCN 2 has a positive inotropic effect in the isolated mouse heart as does UCN 1 in sheep (2,6). Direct effects on myocardial relaxation were not measured, but the dose-related increase in Em would be

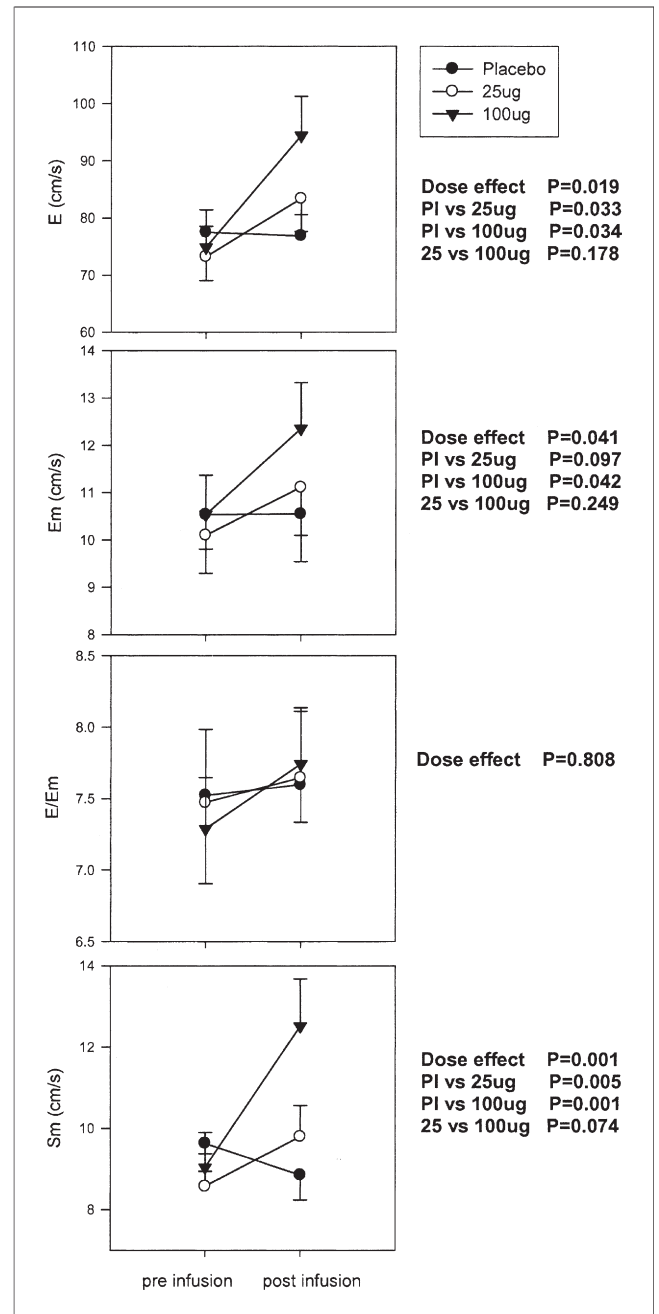


Figure 5 E, Em, E/Em, and Sm Mitral Valve Tissue Velocity Responses to UCN 2 Infusion

Transmitral early diastolic flow velocity (E), early diastolic myocardial tissue velocity at the medial mitral valve annulus (Em), E/Em, and systolic myocardial (Sm) mitral valve tissue velocity responses (mean ± SEM) to urocortin (UCN) 2 infusion in 8 healthy men.

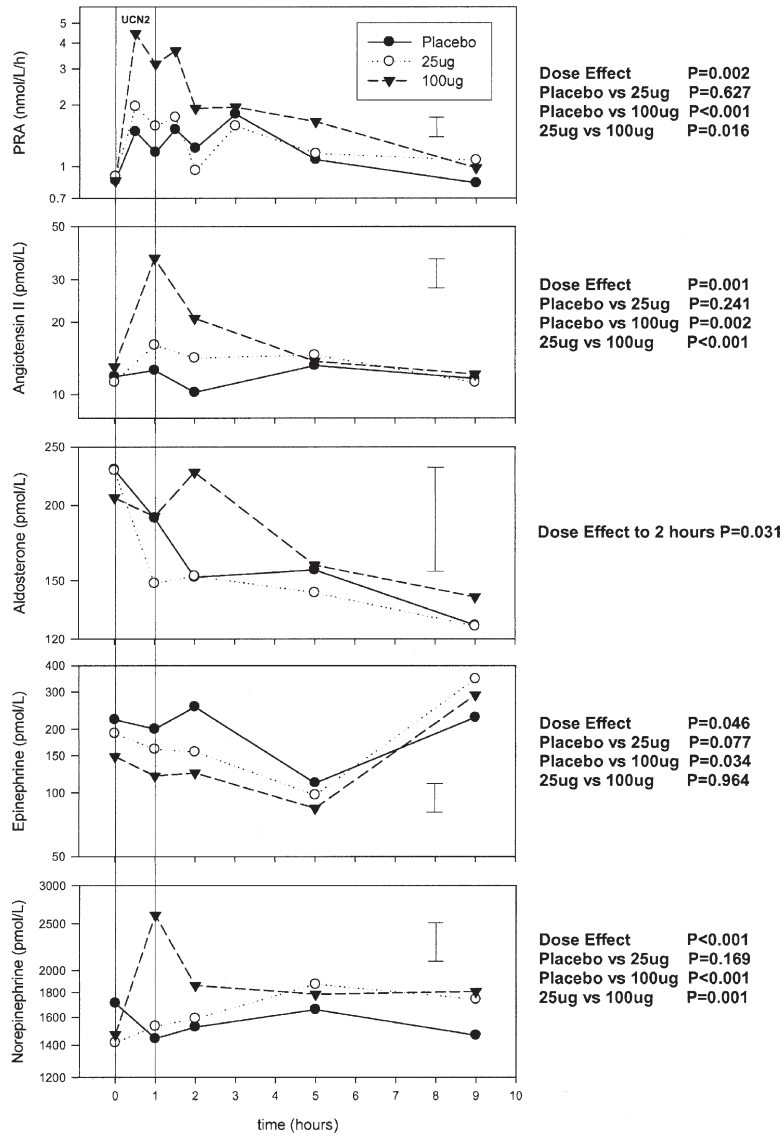


Figure 6 PRA, Angiotensin II, Aldosterone, Epinephrine, and Norepinephrine Responses to UCN 2 Infusion

Plasma renin activity (PRA), angiotensin II, aldosterone, epinephrine, and norepinephrine (geometric mean and pooled 95% confidence intervals) responses to urocortin (UCN) 2 infusion in 8 healthy men.

consistent with enhanced relaxation, an effect seen also in isolated mouse heart (6), the mechanism of which is unclear. A limitation of our echocardiographic data is its nonblinded interpretation.

It is likely UCN 2 vasodilates by both endothelial nitric oxide/cyclic guanosine monophosphate-dependent and -independent mechanisms as seen with UCN 1 (5). Endothelium-independent vasodilation also has been demonstrated with the administration of UCN 2 (11). It is possible that the significant increase in cyclic guanosine monophosphate noted is related to nitric oxide and not likely to be from natriuretic peptides because we saw no

increase in N-terminal pro-brain natriuretic peptide, although atrial natriuretic peptide was not measured. The sustained decreases in diastolic blood pressure and reduced mean atrial pressure on active days may be mediated by a cyclic guanosine monophosphate-dependent mechanism given its concurrent elevation, but other intermediary mechanisms we have not identified also may play a part. The absence of a cardiac natriuretic peptide response in the face of pronounced effects on cardiac output, heart rate, and arterial pressure suggests either the effects of UCN 2 produce no net increment in cardiomyocyte strain or that increased natriuretic peptide

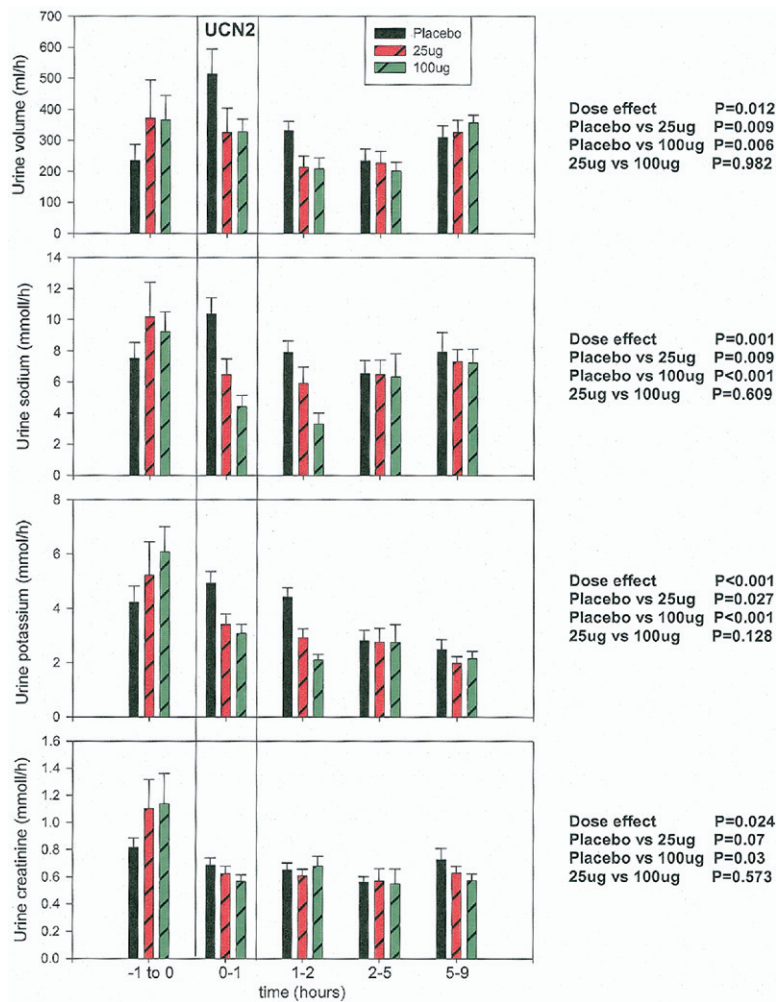


Figure 7 Urine Volumes and Excretion of Sodium, Potassium, and Creatinine Responses to UCN 2 Infusion

Urine volumes and excretion of sodium, potassium, and creatinine (mean ± SEM) responses to urocortin (UCN) 2 infusion in 8 healthy men.

secretion is balanced by a matched increase in natriuretic peptide clearance.

In previous studies, we observed no hemodynamic response to administration of 50 µg of UCN 1 in normal subjects or patients with heart failure, despite UCN 1 having a similar affinity as UCN 2 for the CRF₂ receptor (8). This result may be because of the CRF binding protein, which binds UCN 1 but not UCN 2 (18), potentially reducing in vivo UCN 1 availability to the receptor. This hypothesis is supported by peak plasma levels induced by 50 µg of UCN 1 in a similar group of healthy humans being less than that of 25 µg of UCN 2, given the molecular weights of the 2 peptides are similar as were the weights of the subjects studied (13).

Neurohormones. The lack of an ACTH-cortisol or “stress” response to UCN 2 is in striking contrast to our previous experience with UCN 1 administered to humans

(13,14). It supports the hypothesis that the pituitary-adrenal axis effects of the urocortins are mediated by CRF₁ receptors and, therefore, will not be induced by UCN 2 because of its lack of affinity for this receptor.

Notably, increments in renin, angiotensin II, and norepinephrine were observed only at the higher (100 µg) dose of UCN 2, which is suggestive of a secondary effect mediated by hemodynamic changes (i.e., decrease in SVR) exceeding a certain threshold. The increase in plasma norepinephrine may reflect baroreceptor-mediated increments in sympathetic traffic. Renin increases in response to decreased afferent arterial pressure and flow, changes in delivery of sodium to the macula densa, and to increased sympathetic traffic to adrenoceptors on the juxtaglomerular cells. It is likely a decrease in renal perfusion pressure and/or increased sympathetic drive, at least partially, underlies the pronounced rise in renin with high-dose UCN 2. Clarification of

whether or not UCN 2 exerts a more direct effect on renin secretion may require *in vitro* experiments.

The increase in an angiotensin II presumably reflects the increase in renin, and the trend toward increased aldosterone (at high doses of UCN 2) is a plausible response to the spike in plasma angiotensin II. Our work in normal sheep elicited no change in plasma renin activity or aldosterone to UCN 2 infusions, but the decreases in mean arterial pressure and SVR were comparatively smaller (3). Regional hemodynamic studies in rats that were given intravenous bolus doses of UCN 2 showed little or no change in renal vascular conductance (19). There is a paucity of work looking at the effect of UCN 2 on renal cellular activity.

We observed divergent plasma catecholamine responses with stimulation of norepinephrine and subtle, relative suppression of epinephrine. The CRF₂ receptors in the adrenal medulla mediate norepinephrine secretion in rat pheochromocytomas cell lines (10). However, our data permit no conclusions on possible direct effects of UCN 2 on catecholamine release from the adrenal or elsewhere.

Renal function. The effects of UCN 2 on renal function, although statistically significant, were generally modest. Our results do not allow conclusions on the underlying mechanisms. One of the clearest effects was on sodium excretion, which fell in a dose-related fashion, which may be the net effect of reductions in renal perfusion pressure, together with increments in renin, angiotensin II, and aldosterone (at least at the higher dose of UCN 2) and sympathetically mediated changes in intrarenal vasomotor tone. These all occurred in the absence of any increment in circulating cardiac natriuretic peptide levels. Potassium excretion also was reduced, presumably secondary to the aforementioned factors. Further clarification might be provided by experiments in the isolated perfused kidney and by closer study of possible direct effects on glomerular mesangium, renal tubular function, and juxtaglomerular cells.

Pharmacokinetics. The 10-min half-life of UCN 2 in healthy humans is shorter than that of UCN 1, which we have previously reported (*i.e.*, 52 min) (13). The volume of distribution was approximately that of plasma volume indirectly supporting lack of binding to the CRF-binding protein, cell membrane binding, or lipid solubility. The mechanism of clearance has yet to be established.

Conclusions. In this first report of UCN 2 administered to healthy humans, this peptide induced marked dose-related increases in cardiac output, heart rate, and left ventricular ejection fraction with decreased SVR. Lusitropism is suggested by the dose-related increase in Em. The activation of renin, angiotensin II, and norepinephrine occurred only with the greater (*i.e.*, 100 µg) dose, with its more powerful hemodynamic effects. It remains unclear whether these neurohormonal responses purely reflect a secondary response to hemodynamic perturbation or are partly the result of direct effects of UCN 2 on the kidney and/or adrenal. The decrease in urine sodium excretion and more modest changes in urine volume,

potassium, and creatinine may be partially secondary to reduced renal perfusion pressure and increased renal sympathetic traffic but, again, possible direct renal effects of UCN 2 warrant investigation.

Urocortin 2 has powerful cardiac actions in healthy humans and in experimental (ovine) heart failure. The neurohormonal activation and modest decrease in sodium excretion observed in our current report are largely consistent with findings in normal sheep and contrast with the pronounced suppression of volume-retaining vasoconstrictor neurohormonal systems, and augmentation of renal function observed in experimental ovine heart failure (3), which suggests the response to exogenous UCN 2 is dependent on the neurohumoral and hemodynamic milieu into which it is introduced. Natriuretic and neurohormone-suppressing effects may only be unmasked in settings with activation of powerful volume- and sodium-retaining mechanisms as in heart failure. Taken together, experimental and the human preclinical studies to date warrant further investigation of the role of UCN 2 in circulatory regulation and potential therapeutic effects in human heart failure or a malignant hypertension/hypertensive crisis.

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