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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-μg low-dose (LD), and 100-μg 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 ± 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 ± 1.4%; LD 6.6 ± 1.5%; HD 14.1 ± 0.8%; $p < 0.001$) while decreasing systemic vascular resistance (placebo −128 ± 50 dynes·s/cm^5; LD −407 ± 49 dynes·s/cm^5; HD −774 ± 133 dynes·s/cm^5; $p < 0.001$). Activation of plasma renin activity ($p = 0.002$), angiotensin II ($p = 0.001$), and norepinephrine ($p < 0.001$) occurred only with the higher 100-μg 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, CRF_1 and CRF_2 (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 CRF_1 receptors, and urocortin 1 binds to both CRF_1 and CRF_2 receptors, whereas UCNs 2 and 3 are selective agonists for the CRF_2 receptors. CRF_1 receptors are found in the brain, pituitary gland, and gut. There are at least 2 splice variants of the CRF_2 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 CRF_2 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 CRF_1 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 ± SD, 41.1 ± 11.7 years), weighing 60 to 104 kg (mean 80 ± 17 kg), with a body mass index 19 to 32 kg/m^2 (mean 25.6 ± 4.5 kg/m^2), plasma creatinine 0.08 to 0.012 mmol/l (mean 0.098 ± 0.014 mmol/l), and echocardiographic left ventricular ejection fractions of 58% to 77% (mean 66.5 ± 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 μg, and 100 μg 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 μg) 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 μg 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 μg 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 μg/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-μg and 100-μg 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-