Urocortin 3: haemodynamic, hormonal, and renal effects in experimental heart failure

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Received 23 March 2006; revised 8 June 2006; accepted 15 June 2006; online publish-ahead-of-print 6 July 2006

Aims To investigate the haemodynamic, hormonal, and renal effects of peripheral urocortin 3 (Ucn3) administration for the first time in either normal health or heart failure (HF).

Methods and results Eight sheep received incremental intravenous bolii of Ucn3 (10, 50, and 100 μg at 2-h intervals) before (normal) and during pacing-induced HF. Compared with controls, Ucn3 induced immediate, dose-dependent increases in cardiac output (normal 4.21 ± 0.23 vs. 5.65 ± 0.32 L/min, P < 0.001; HF 2.20 ± 0.14 vs. 4.43 ± 0.31 L/min, P < 0.001) and decreases in peripheral resistance (normal 20.8 ± 1.0 vs. 16.2 ± 0.8 mmHg/L/min, P < 0.01; HF 34.4 ± 1.7 vs. 16.2 ± 0.5 mmHg/L/min, P < 0.001) and left atrial pressure (normal 4.5 ± 0.3 vs. 0.6 ± 0.2 mmHg, P < 0.001; HF 23.0 ± 0.6 vs. 5.8 ± 1.9 mmHg/L/min, P < 0.001). Arterial pressure was minimally elevated in normals (P < 0.001) and reduced in HF (P < 0.05). In HF only, Ucn3 decreased plasma vasopressin (3.33 ± 0.36 vs. 1.73 ± 0.21 pmol/L, P < 0.05), endothelin-1 (3.56 ± 0.28 vs. 2.64 ± 0.24 pmol/L, P < 0.001), renin (2.74 ± 1.17 vs. 1.04 ± 0.22 mmol/L/h, P < 0.001), aldosterone (1494 ± 400 vs. 726 ± 168 pmol/L, P < 0.05), and epinephrine (1608 ± 278 vs. 1039 ± 75 pmol/L, P < 0.05), and increased urine output (P < 0.05), sodium excretion (P < 0.01), and creatinine clearance (P < 0.05).

Conclusion Ucn3 has significant cardiovascular effects in normal and HF sheep, supporting a role for this peptide in circulatory regulation. In HF, more prominent haemodynamic changes were associated with beneficial endocrine and renal effects, suggesting Ucn3 has therapeutic potential in this disease.

Introduction

Urocortin 1 (Ucn1)1 and the more recently identified urocortins 2 and 3 (Ucn2 and Ucn3, respectively)2-3 are structurally related members of the corticotropin releasing factor (CRF) family of peptides. Like its homologues,4,5 Ucn3 is widely distributed in multiple tissues throughout the body5-7 and has recently been located in high concentrations in the myocardium of the human heart—possibly at levels greater than those of Ucn1.7 Basal expression of Ucn3 in rat cardiomyocytes is also reported in larger quantities than Ucn2, with levels significantly increased in response to hypoxic stress.8 The actions of the Ucn peptides are mediated through the two G protein-coupled receptors, CRF1 and CRF2, which exhibit differences in distribution, ligand affinities, and pharmacology. The CRF1 receptor is expressed primarily in the brain,9 whereas CRF2 receptors are found in both central and peripheral tissues, with high density demonstrated in cardiac myocardium and blood vessels.4,10 Although Ucn1 binds strongly to both receptor subtypes, Ucn2 and Ucn3 are reported to be highly selective for the CRF2 receptor.3

Increasing evidence supports roles for Ucn1 and Ucn2 in the regulation of cardiovascular function, with each peptide shown to reduce blood pressure11,12 and increase heart rate and cardiac contractility in vivo.11,12—effects mediated via the CRF2 receptor.11,13 In addition, we have recently demonstrated that administration of both Ucn114 and Ucn215 in experimental heart failure (HF) improves haemodynamic status in association with significant suppression of multiple vasoconstrictor hormone systems and augmentation of renal function. Of the three peptides, Ucn3 is the least well studied with respect to its potential cardiovascular bioactivity. Although recent investigations have shown that the peptide produces potent vasodilatation of rat16 and human17 arteries in vitro, and intracerebroventricular (ICV) Ucn3 elevates blood pressure and heart rate in the rat,17 there is currently no information concerning the haemodynamic effects of peripheral administration of the peptide. While Ucn3 might be expected to produce equivalent effects to those of Ucn2 given that both peptides preferentially bind the CRF2 receptor, a recent report indicates that they exhibit different affinities for microdomains of the receptor.18 In addition, an increasing number of studies indicate dissimilarities in the actions of the two peptides, including reports that ICV Ucn2 (but not Ucn3) increases anxiety in mice,19 whereas Ucn3 produces more pronounced

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antiapoptotic, cardioprotective, and natriuretic peptide-secretory activity in rat cardiomyocytes compared with either Ucn1 or Ucn2.

In view of Ucn3’s likely contribution to circulatory regulation and the reported differences in bioactivity from that of Ucn2, we investigated for the first time, the haemodynamic, hormonal, and renal effects of intravenously administered Ucn3 in sheep both before and during pacing-induced HF.

Methods

Identification of Ovine Ucn3 sequence

The sequence of ovine Ucn3 was determined by cloning and sequencing cDNA fragments generated by reverse-transcription polymerase chain reaction (RT-PCR). Sheep colon and small intestine (tissues shown to express Ucn3 in man) were rapidly dissected, snap frozen in liquid nitrogen, and stored at −80°C. Total RNA was extracted by TRIzol® (InVitrogen, Carlsbad, CA, USA) and cDNA reverse transcribed with Superscript II (Invitrogen) using an oligo-dt primer. The cDNA (5 μL) was amplified by an initial PCR reaction performed with partially nested primers, Ucn3-A and Ucn3-C (see Table 1), using QIagen Taq polymerase (Valencia, CA, USA) and buffer (2.5 mM magnesium chloride).

The initial PCR product was run on an electrophoresis gel (0.75% agarose) and a band of the expected size (491 bp) excised under UV light and dissolved in 0.5 mL sterile water in a boiling water bath. A 5 μL aliquot of this product was re-amplified by PCR using the Ucn3-B and Ucn3-C primers, yielding a single band 472 bp on the electrophoresis gel. This Ucn3 cDNA product was cloned into a PCR-Script plasmid using the PCR-Script cloning kit (Stratagene, La Jolla, CA, USA).

The ovine sequence of Ucn3 was confirmed by automated sequencing on an ABI Prism 3100 sequence detection system (Foster City, CA, USA) as being identical to murine Ucn3 (Figure 1). Consequently, commercially available murine Ucn3 was used in the following sheep physiological studies.

| Table 1 Primer sequences, Ucn3-A and Ucn3-C, used to amplify the ovine cDNA |
|-----------------|-----------------|-----------------|
| Primer name     | Primer sequence | Ucn3 sequence   |
| Ucn3-A upstream (outer) | 5’ ctgatgccacacctcttct 3’ | Product A-C equivalent to nucleotides 4-494a |
| Ucn3-B upstream (inner) | 5’ gctgcacttctgtgc 3’ | |
| Ucn3-C downstream | 5’ tactttctctctcaaccttg 3’ | Product B-C equivalent to nucleotides 24-494a |

*Designed from mouse Ucn3 sequence; Genebank accession number AF361944.

Surgical preparation

Eight Coopworth ewes (52-70 kg) were instrumented via a left lateral thoracotomy under general anaesthesia (induced by 17 mg/kg thiopentone; maintained with halothane/nitrous oxide). Two catheters were inserted in the left atrium for blood sampling, left atrial pressure (LAP) determination, and drug administration; a Konigsberg pressure-tip transducer was inserted into the aorta to record mean arterial pressure (MAP); an electromagnetic flow probe was placed around the ascending aorta to measure cardiac output (CO); and a 7 French His-bundle electrode was stitched subepicardially to the wall of the left ventricle for left-ventricular pacing. A bladder catheter was inserted per urethra for urine collections. Animals recovered 14 days before commencing the study protocol. During the experiments, the animals were held in metabolic cages and had free access to water and food (containing 80 mmol/day sodium; 200 mmol/day potassium).

Study protocol

Each sheep received incremental intravenous bolus of mouse (ovine) Ucn3 (10, 50, and 100 μg at 2-h intervals) and a vehicle control (10 mL 0.9% saline) on two separate days, a day apart in a balanced random order, both before (normal) and after induction of HF by rapid left ventricular (LV) pacing (7 days at 225 bpm).

Heart rate, MAP, LAP, CO, and calculated total peripheral resistance (CTPR = MAP/CO) were recorded at 15 min intervals in the hour preceding the first bolus at 1000 h (baseline), at 15, 30, 45, 60, 90, and 120 min succeeding each bolus, and at 1000 h the following day. Haemodynamic measurements were determined by online computer assisted analysis. Blood samples were drawn from the left atrium following haemodynamic measurements into tubes on ice, centrifuged at 4°C, and stored at −80°C before assay for Ucn1, cyclic adenosine monophosphate (cAMP), arginine vasopressin (AVP), adrenocorticotrophic hormone (ACTH), cortisol, endothelin-1, plasma renin activity (PRA), aldosterone, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and catecholamines. Plasma electrolytes and haematocrit were measured with every blood sample taken. Cross-reactivity in the Ucn1 radioimmunoassay to Ucn3 (Peninsula Laboratories, USA) was <0.07% and to Ucn2 (Peninsula Laboratories, USA) was <0.026%.

Urine volume and samples for the measurement of cAMP, sodium, potassium, and creatinine excretion were collected at 2-h intervals before (baseline) and after each bolus, and overnight (1600, 1000 h). The study protocol was approved by the Christchurch School of Medicine Animal Ethics Committee.

Statistics

Results are expressed as mean ± SEM. To test for baseline differences between normal and HF sheep, baseline data from each state (mean of measurements made within the hour pre-treatment) were compared using paired t-tests. To test for the effects of Ucn3, control and Ucn3 study limbs (in both normal and HF sheep separately) were compared using repeated measures analysis of variance (ANOVA). Where significant differences were identified by ANOVA, the level of significance at individual time points in Figures 2-6 and Table 2 was determined by Fisher’s protected least-significant difference tests. To test for differences in the response to Ucn3 between normal and HF states, Ucn3 study limbs in each state

Figure 1  Sequences of ovine urocortins and human Ucn3. Predicted amino acid sequences of mature ovine Ucns and human Ucn3. Residues identical to Ucn1 sequences are in bold. Human Ucn3 residues different from ovine Ucn3 are underlined.
were compared by covariate ANOVA using baseline data as covariates. Significance was assumed at $P < 0.05$.

**Results**

**Ovine Ucn3 sequence**

The amino acid sequence encoding ovine Ucn3 was found to be identical to the mouse sequence published by Lewis et al. for the entire length of the precursor peptide (1-164aa). The predicted 38-amino acid sequence of mature ovine Ucn3 (as proposed for the murine peptide) is shown in Figure 1. This peptide demonstrates 90% homology with human Ucn3, and 42 and 18% sequence homology with the ovine forms of Ucn2 and Ucn1, respectively (Figure 1). It should be noted, however, that the mature, circulating form of ovine Ucn3 has yet to be established. The N-terminal cleavage site is uncertain, with a potential cleavage site with paired basic residues (Lysine at −1 and Arginine at −4) that would generate the 38-amino acid peptide shown in Figure 1, as proposed for both human and mouse Ucn3. An alternative potential cleavage site is found three residues upstream (Arginine at −1 and Lysine at −4), that would generate a 41-amino acid peptide analogous to human stresscopin peptide (SCP). Interestingly, a recent study by Takahashi et al. performing reverse-phase HPLC demonstrates both Ucn3 (38aa) and SCP (40aa) forms to be present in human plasma (the SCP peak ~40% that of the Ucn3 peak). It is possible therefore, that both forms of the peptide also circulate in sheep. Given that the sequences of ovine and mouse Ucn3 are identical, and the

![Figure 2](image-url)  
*Figure 2* Mean ± SEM haemodynamic responses to incremental bolus of Ucn3 and a vehicle control in eight sheep before (normal; left panel) and after induction of HF (right panel). Significant differences shown by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.  

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38aa Ucn3 form is present in greater quantities than the 40aa SCP peptide in human plasma, we chose to administer commercially available murine Ucn3 in the present study.

**Ucn3 effects in normal and HF sheep pacing effects**

Rapid left-ventricular pacing for 7 days induced the haemodynamic, endocrine, and sodium retaining hallmarks of congestive HF—with significant declines in MAP and CO, rises in LAP and CTPR (all $P < 0.001$), activation of multiple hormone systems [plasma ANP, BNP, endothelin-1 (all $P < 0.001$), cAMP, Ucn1 (both $P < 0.01$), PRA, aldosterone, AVP, norepinephrine, epinephrine (all $P < 0.05$)], and reduced renal function [haematocrit, creatinine clearance (both $P < 0.001$), urine volume, urine sodium, urine potassium, urine creatinine (all $P < 0.01$), plasma creatinine ($P < 0.05$)] (see Figures 2–6, Table 2).

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**Figure 3**  Mean ± SEM hormone responses to incremental bolus of Ucn3 and a vehicle control in eight sheep before (normal; left panel) and after induction of HF (right panel). Significant differences shown by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.**
Haemodynamics

Compared with time-matched control data, Ucn3 administration in normal sheep produced prominent and dose-dependent increases in CO ($P < 0.001$) and heart rate ($P < 0.001$), with minor concomitant falls in LAP ($P < 0.001$; Figure 2), and CTPR ($P < 0.01$; Table 2). Responses were rapid but transitory, with peak effects observed at the first recording post-bolus (15 min). MAP was elevated relative to control ($P < 0.001$), although in a less immediate fashion (Figure 2). All haemodynamic indices had returned to vehicle control levels by 24 h. Haematocrit was not significantly altered by Ucn3 (Table 2).

In HF sheep, Ucn3 markedly increased CO ($P < 0.001$), and decreased CTPR ($P < 0.001$) and LAP ($P < 0.001$), returning these parameters temporarily to non-paced levels immediately following the highest dose (Figure 2). Peripheral resistance was still significantly reduced relative to control the following day. In contrast to the response in normal sheep, MAP was reduced by Ucn3 administration (although not in a dose-dependent manner) ($P < 0.05$; Figure 2), as was haematocrit ($P < 0.001$; Table 2).

Comparison of Ucn3 effects between the two states indicated significantly greater changes in CO ($P < 0.01$), CTPR, and LAP (both $P < 0.001$) in HF relative to normals, and directionally opposite responses by MAP ($P < 0.001$).

Hormones

In normal sheep, Ucn3 boli acutely increased plasma ACTH ($P < 0.001$), cortisol ($P < 0.001$), and Ucn1 ($P < 0.05$) levels compared with controls, with a similar trend evident for AVP ($P = 0.0785$; Figure 3). Ucn3 also elevated circulating BNP levels in normals ($P < 0.05$), and tended to reduce ANP ($P = 0.065$) and epinephrine ($P = 0.0521$). Plasma cAMP, PRA, aldosterone, endothelin-1, and norepinephrine concentrations were unchanged compared with control data (Figures 3–5).

In HF, Ucn3 induced rises in plasma ACTH ($P < 0.05$), cortisol ($P < 0.05$), and Ucn1 ($P < 0.05$) similar to those observed in the normal state (Figure 3). In contrast to responses in non-paced animals, however, Ucn3 treatment in HF caused marked and largely dose-dependent falls in vasopressin ($P < 0.05$), cAMP ($P < 0.05$; Figure 3), renin ($P < 0.001$), aldosterone ($P < 0.05$), endothelin-1 ($P < 0.001$; Figure 4), ANP ($P < 0.001$), BNP ($P < 0.001$), and epinephrine ($P < 0.05$) concentrations (Figure 5). Plasma norepinephrine also tended to be reduced.

Figure 4 Mean ± SEM hormone responses to incremental boli of Ucn3 and a vehicle control in eight sheep before (normal; left panel) and after induction of HF (right panel). Significant differences shown by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

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(P = 0.0801; Figure 5). Attenuation of circulating AVP, ANP, and aldosterone levels was still evident the following day.

Comparison of Ucn3 effects in normals and HF demonstrated significantly different responses by plasma cAMP (P < 0.05), PRA (P < 0.001), aldosterone (P < 0.05), endothelin-1 (P < 0.001), ANP (P < 0.001), and norepinephrine (P < 0.01)—all of which fell appreciably in HF but did not change significantly in the normal state. Directionally opposite responses were demonstrated by AVP (P < 0.001) and BNP (P < 0.001), which both rose in normal sheep and fell in HF.

Urine and plasma electrolytes

In normal animals, Ucn3 did not significantly alter plasma electrolytes or any renal parameter measured (Figure 6; Table 2). In the HF state, urine output (P < 0.05), urinary sodium (P < 0.01), potassium (P < 0.001), cAMP (P < 0.05), and creatinine (P < 0.001) excretion (Figure 6) and creatinine clearance (P < 0.05) (Table 2) were all enhanced by Ucn3 administration. Plasma creatinine tended to be reduced (P = 0.056) (Table 2), whereas sodium and potassium levels (data not shown) were unchanged. Comparison between normal and HF states showed significantly greater effects of Ucn3 on sodium (P < 0.05) and creatinine excretion (P < 0.05), creatinine clearance (P < 0.001), and plasma creatinine (P < 0.05) in HF sheep.

Discussion

This study is the first to report the effect of systemic Ucn3 administration on haemodynamic, hormonal, and renal indices in either health or HF. Whereas Ucn3 exhibited limited bioactivity in normal sheep (modest haemodynamic responses and negligible effects on vasoconstrictor...
hormones and renal parameters), incremental Ucn3 bolus in HF produced marked, dose-dependent improvements in CO and reductions in CTPR and LAP, slight decreases in MAP, significant suppression of multiple hormone systems (including AVP, endothelin-1, renin-angiotensin II-aldosterone and epinephrine), and enhanced renal performance.

Haemodynamics

The haemodynamic effects induced by Ucn3 in the present study, particularly striking in HF, are similar to those observed previously for both Ucn1\textsuperscript{14} and Ucn2\textsuperscript{15} in normal and HF sheep, and are likely due to similar underlying mechanisms. Ucn3-induced rises in CO presumably reflect a combination of positive inotropic activity, as reported formerly for both Ucn1 and Ucn2,\textsuperscript{11,22} and reductions in cardiac afterload.\textsuperscript{11} This latter mechanism may be especially important in HF where significantly greater increases in CO (compared with the normal state) were associated with considerably larger concurrent reductions in peripheral resistance (from elevated levels), and a fall rather than a rise in MAP.

The observed decreases in CTPR are consistent with the potent direct vasodilator actions of the peptide as demonstrated in both rat\textsuperscript{16} and human\textsuperscript{10} arteries in vitro. The hypotensive effect of Ucn3, evident in HF but not in normal animals, may be accredited in part to the significant reductions in this state of a number of elevated vasoconstrictor hormones (endothelin-1, angiotensin-II, AVP, epinephrine), as well as to the pre-constricted status of the arterial vasculature (evidenced by raised baseline peripheral resistance). Indeed, Ucn3 has been shown to potently reverse endothelin-1-induced arterial constriction,\textsuperscript{10} indicating that the peptide may play a particularly important role in regulating vascular tone in states such as HF, in which the endothelin-1 system is activated and vasoconstriction prevails.

The substantial reductions in LAP in HF to near normal levels is likely due to the sizable increase in CO, and perhaps also to possible lusitropic and/or venodilator actions—effects previously reported for the peptide’s homologues.\textsuperscript{11,23}

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**Figure 6** Mean ± SEM renal responses to incremental bolus of Ucn3 (black columns) and a vehicle control (grey columns) in eight sheep before (normal; left panel) and after induction of HF (right panel). Overnight (O/N) sample collected from 1600–1000 h. Significant differences shown by: *P < 0.05, **P < 0.01, ***P < 0.001.
Whereas the haemodynamic responses produced by Ucn3 are similar in type and amplitude to those generated by equivalent doses of Ucn1 and Ucn2 in previous studies, \(^14,15\) we found that the onset of action (Ucn3 > Ucn2 > Ucn1) and duration of effect (Ucn1 > Ucn2 > Ucn3) of these peptides differ appreciably with those of Ucn3, the most rapid and short lived of this peptide family. These data are in keeping with findings by Kageyama \(et\ al\). \(^16\) showing that although Ucn2 and Ucn3 produce equipotent vasodilation in rat thoracic aorta, the initiation of Ucn3 activity was significantly faster than that of Ucn2. The observed kinetic profiles of Ucn bioactivity suggest a smaller volume of distribution and more rapid clearance (and thus shorter plasma half-life) of Ucn3 compared with both Ucn1 and Ucn2 in sheep. Confirmation awaits development of valid assays for Ucn3 and Ucn2.

### Hormones

A single study has reported endogenous Ucn3 to circulate in normal human plasma at a level of \(\approx 50\) pmol/L. \(^7\) Although the origin of circulating Ucn3 has yet to be investigated, possible sources are numerous and include a range of tissues such as the heart, brain, kidney, adrenal gland, stomach, intestines, pancreas, muscle, and vasculature, where the peptide is found in substantial concentrations. \(^5,6,7,24\) Although the distribution overlap of ligand and receptor in many of these tissues may indicate local regulation by Ucn3, Takahashi \(et\ al\). \(^7\) have suggested that Ucn3 may also act as a circulating hormone, at least in some conditions. Of interest, the source of circulating Ucn1 has recently been investigated in normal and HF sheep via trans-organ arteriovenous sampling, \(^25\) revealing small but significant positive arteriovenous gradients across the renal and hepatic tissue beds in both states, with rises across the hind limb also significant in normal animals and across the head in HF. These findings are consistent with the expression of Ucn1 in the kidney, adrenal gland, liver, gastrointestinal tract, skeletal muscle, and brain. \(^9,26,27\) Secretion from the vascular endothelium may also contribute to plasma concentrations of the peptide. \(^28\) Surprisingly, no evidence for net production of Ucn1 from the heart was observed in either normal or HF sheep, \(^23\) despite numerous reports of strong cardiac expression of the peptide \(^4,9\) and increased immunoreactivity in diseased hearts. \(^29\) It is possible that Ucn1 in this tissue plays a more paracrine/autocrine role.

### Table 2  Effects of Ucn3 boli in eight sheep before (normal) and after induction of HF

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<td>104 ± 7</td>
<td>104 ± 7</td>
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<tr>
<td>Ucn3</td>
<td>108 ± 6</td>
<td>108 ± 5</td>
<td>111 ± 7</td>
<td>109 ± 8</td>
<td>109 ± 8</td>
<td>109 ± 8</td>
<td>109 ± 8</td>
</tr>
<tr>
<td>HF Cont</td>
<td>89 ± 7</td>
<td>80 ± 6</td>
<td>88 ± 5</td>
<td>82 ± 6</td>
<td>82 ± 6</td>
<td>82 ± 6</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>Ucn3</td>
<td>88 ± 5</td>
<td>91 ± 5*</td>
<td>95 ± 8*</td>
<td>103 ± 7**</td>
<td>103 ± 7**</td>
<td>103 ± 7**</td>
<td>103 ± 7**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Ucn3 boli administered at 2-h intervals. N, Normal; Cont, Control. Significant differences (compared with time-matched control) shown by: *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\).
Ucn3 for the CRF2 receptors to be in the 1–5 nM range. In addition, Ucn3 was found to be functionally more potent for cAMP production by cells expressing these receptors, with an EC50 ≈ 0.1 nM. Despite the evidence for potent Ucn3-induced production of cAMP, a proposed intracellular second messenger, no increases in plasma concentrations were detected following administration of Ucn3 in the present study. It is probable, however, that cAMP levels were raised amply at the target tissue level in order to generate the bioactivity observed, although it is also possible that the peptide may operate through alternative signal transduction pathways, as reported for Ucn1.23,30 The decrease in plasma cAMP seen with Ucn3 treatment in HF is likely a consequence of haemodynamic improvement.

The significant acute activation of the pituitary-adrenal axis by Ucn3 observed in the current study might be somewhat unexpected given reports that (human) Ucn3 has minimal effect on ACTH release in the rat5 and mouse,19 although the response is similar to that produced by Ucn2 in sheep,15 a peptide also reported to display low affinity for the CRF1 receptor5 (the receptor responsible for ACTH activation).31 It is possible that ovine Ucn3 exhibits greater affinity for the ovine CRF1 receptor than that demonstrated in the aforementioned studies by human Ucn3 for rodent receptors.5,19 Alternatively, the concomitant acute rises in plasma Ucn1 (likely as a result of competitive interaction for receptors and/or degradative pathways by Ucn3) may be responsible for the observed ACTH/cortisol activation. Regardless of the mechanism, the impact of human Ucn3 on the ACTH/cortisol axis in man is anticipated with interest.

The marked attenuation of plasma AVP by Ucn3 is similar to that noted previously with administration of both Ucn114 and Ucn215 in our ovine model of HF, and is likely attributable to improvements in CO and thus pressure to sino-aortic volume receptors, as well as to declining circulating levels of angiotensin II (evidenced by reduced PRA) and the natriuretic peptides—factors than respectively enhance and inhibit AVP secretion.32

Although Ucn3 is reported to stimulate natriuretic peptide secretion,20 both ANP and BNP plasma concentrations were substantially decreased in HF sheep in the present study. These reductions are in keeping with the prominent falls in LAP (an indicator of cardiac transmural pressure) resulting in attenuated secretion of the peptides. In normal animals, however, while plasma ANP also tended to fall, BNP levels were actually increased. This difference may reflect a greater responsiveness of ANP relative to BNP to the minor acute falls in LAP (due to higher ANP content within atrial stores), and/or a greater impact of Ucn3 on BNP secretion—with Chanalaris et al.20 recently demonstrating that Ucn3 is more than twice as potent in inducing BNP mRNA expression in rat cardiomyocytes than ANP.

We observed significant attenuation by Ucn3 of multiple (activated) vasoconstrictor peptide systems in HF in the present study. Plasma renin levels were more than halved in a dose-dependent manner in the face of falls in arterial pressure, but whether due to increased sodium (and chloride) concentrations at the macula densa (evidenced by significant increases in urine sodium excretion), a direct inhibitory effect (in keeping with Ucn3’s presence within the kidney),5 or some other mechanism is unknown. Plasma aldosterone levels were likewise reduced, presumably as a consequence of declines in circulating angiotensin II. A more direct effect of Ucn3 on aldosterone secretion is also possible given that the peptide is expressed in human adrenal tissue,5 although it is reported to be predominantly located in the cortex (where it is colocalized in >85% of adrenocortical cells with the CRF2 receptor).6

Significant alleviation of elevated plasma endothelin-1 concentrations by Ucn3 was also observed in the current study. Although a direct inhibitory effect of Ucn3 (and the other Ucn peptides) on endothelin-1 secretion may be suggested by our recent work showing marked augmentation of plasma endothelin-1 levels following CRF receptor-blockade in ovine HF,13 definitive evidence requires in vitro investigation. In contrast to increases in plasma epinephrine levels noted following central administration of Ucn3 in rats,17 we observed appreciable falls in circulating concentrations of the peptide succeeding peripheral Ucn3 treatment in HF sheep, with a similar trend demonstrated in the normal state. Whether these findings reflect an improvement in haemodynamic status or another inhibitory mechanism remains to be seen.

The endocrine responses elicited by Ucn3 are comparable in nature and magnitude to those produced by both Ucn1 and Ucn2 in normal and HF sheep,14,15 but, as observed with the haemodynamics, tended to occur more rapidly (Ucn3 > Ucn2 > Ucn1), and be less sustained (Ucn1 > Ucn2 > Ucn3). This was particularly striking with the natriuretic peptides, where the maximum reductions following Ucn3 administration in the present study occurred at 30 min post bolus, whereas the peak effects subsequent to Ucn2 and Ucn1 bolus were at 60 and 120 min, respectively.14,15 This result is consistent with the more immediate and transient falls in LAP seen with Ucn3. Surprisingly, however, the significant attenuation of plasma aldosterone following Ucn3 persisted out to 24 h post bolus, a finding more closely resembling that induced by Ucn1 rather than Ucn2 (where aldosterone had returned to control levels by the following day). Whether Ucn3 does indeed demonstrate a more persistent suppression of aldosterone relative to Ucn2 requires further investigation.

**Renal**

Augmentation of renal function following systemic Ucn3 treatment was observed in the HF arm of the present study (absent in normal animals), and included significant and dose-dependent increases in urine volume, sodium and creatinine excretion. These responses are similar in type to those produced by both Ucn1 and Ucn2,14,15 though more closely resembling the latter in lack of overnight activity, and occurred despite a reduction in arterial pressure and considerable decreases in plasma ANP/BNP levels. While major concomitant falls in circulating levels of volume/sodium-retaining factors (AVP, aldosterone, angiotensin II) would most certainly have facilitated Ucn3’s renal effects, the reported presence of high concentrations of the peptide in the renal cortex, particularly distal tubules of the renal cortex,5 may point towards a direct role in tubular reabsorption. Significant elevations in urine cAMP excretion noted in the present study are consistent with this finding. Additional factors possibly contributing to the observed natriuresis/diuresis may include Ucn3 actions to induce renal vasodilation and increase
glomerular filtration (as judged by elevated creatinine clearance). Although exhibiting a similar range of renal actions to those generated by the other UcnS, the increase in sodium excretion elicited by Ucn3 is approximately a third that produced by identical doses of both Ucn1 and Ucn2 in earlier studies (high dose: Ucn1 13.49 ± 2.06 mmol/ml/h; Ucn2 13.55 ± 5.89 mmol/ml/h; Ucn3 4.47 ± 1.85 mmol/ml/h).14,15 The reasons for this comparatively attenuated natriuretic response cannot be determined from the present study, but may in part reflect the less persistent decrease in arterial tone (and thus reduced impact on renal haemodynamics) seen with Ucn3.

It should be kept in mind that Ucn3, and indeed all the UcnS, through their interaction with the CRF2 receptor, participate in the regulation of a range of other functions in addition to those related to the cardiovascular system and blood pressure/volume homeostasis (as seen in the present study). Activation of the CRF2 receptor is also reported to alleviate anxiety, depression and arousal, suppress appetite and gastric emptying, improve skeletomuscular mass and performance, and to influence learning, energy balance, and immune responses.34 Interestingly, some of the effects induced by CRF2 receptor activation counter-regulate those mediated by the CRF1 receptor, with the later receptor associated with increased anxiety, arousal, and blood pressure.

In conclusion, we have shown in the current study that whereas Ucn3 exhibits limited bioactivity in normal sheep, effects of the peptide in HF were multifarious and substantial—including increases in CO in conjunction with reductions in peripheral resistance, cardiac preload and plasma levels of a variety of undesirable vasoconstrictor peptides, and improvements in renal performance. This combination of effects is unequivocally favourable in HF and suggest a potential therapeutic role for Ucn3 in this disease. While we have shown that Ucn3 administration in sheep exhibits a comparable range of bioactivity to that of Ucn1 and Ucn2 for the indices measured, it should be noted that Ucn3 is reported to exhibit more potent anxiolytic, cardioprotective, and natriuretic peptide-secreting activity in the rat.6,20 The possible advantages of using one peptide over another as a potential treatment for HF requires further study, especially those of longer duration and in man.

Acknowledgements

We are grateful to the National Heart Foundation and Health Research Council of New Zealand for financial support and the staff of the Endocrine Laboratory for hormone assays, and the staff of the Christchurch School of Medicine Animal Laboratory for animal care.

Conflict of interest: none declared.

References
