Integrated Hemodynamic, Hormonal, and Renal Actions of Urocortin 2 in Normal and Paced Sheep

Beneficial Effects in Heart Failure

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Background—Urocortin 2 (Ucn2) has potent cardiovascular actions and may participate in the pathophysiology of heart failure (HF). The integrated hemodynamic, endocrine, and renal effects of Ucn2 are unknown.

Methods and Results—Eight sheep received incremental intravenous boluses of murine Ucn2 (10, 50, and 100 μg at 2-hour intervals) before (normal) and during pacing-induced HF. Compared with control data, Ucn2 induced rapid and dose-dependent increases in cardiac output (peak effects: normal 4.3±0.2 versus 6.1±0.2 L/min, P<0.001; HF 2.3±0.1 versus 4.5±0.2 L/min, P<0.001) and reductions in peripheral resistance (normal 20.2±1.0 versus 15.2±0.8 mm Hg/L per minute, P<0.01; HF 32.2±1.7 versus 13.6±0.5 mm Hg/L per minute, P<0.001) and left atrial pressure (normal 4.3±0.3 versus 0.5±0.2 mm Hg, P<0.01; HF 22.9±0.6 versus 5.1±1.8 mm Hg, P<0.001). Mean arterial pressure was minimally elevated in normals and decreased in HF (both P<0.01). In both states, Ucn2 reduced plasma atrial natriuretic peptide levels (normal 13±2 versus 10±2 pmol/L; HF 200±20 versus 72±10 pmol/L) and similarly increased corticotropin, cortisol, and Ucn1 (all P<0.001). In HF only, Ucn2 dose dependently decreased plasma vasopressin (3.09±0.36 versus 1.62±0.12 pmol/L, P<0.01), renin (2.98±1.7 versus 0.69±0.10 pmol/L per hour, P<0.001), aldosterone (1186±303 versus 364±122 pmol/L, P<0.001), endothelin-1 (3.39±0.23 versus 2.56±0.18 pmol/L, P<0.01), epinephrine (1633±260 versus 657±142 pmol/L, P<0.01), and brain natriuretic peptide (36±3 versus 18±4 pmol/L, P<0.001) concentrations. Renal effects, including increased urine volume (1.7-fold, P<0.05), sodium excretion (>12-fold, P<0.01), and creatinine excretion (1.3-fold, P<0.001), also occurred only in HF.

Conclusions—Ucn2 has marked and beneficial hemodynamic, hormonal, and renal effects in experimental HF. These results support a role for Ucn2 in pressure/volume homeostasis in HF and suggest that the peptide may have therapeutic potential in this disease. (Circulation. 2005;112:3624-3632.)

Key Words: heart failure • hemodynamics • hormones • renal function • UCN2 protein, human
TABLE 1. Ucn2 Primers

<table>
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<th>Primer Name</th>
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<th>Ucn2 Sequence</th>
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<tr>
<td>Ucn2-A upstream (outer)</td>
<td>5’ atgaccaggtggcactgtt 3’</td>
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<tr>
<td>Ucn2-C downstream</td>
<td>5’ tcagcgccggccacatgg 3’</td>
<td>Product (B–C) equivalent to nucleotides 21–340*</td>
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</table>

*Designed from the mouse Ucn2 sequence; Genbank accession No. AF331517.

Methods

Identification of Ovine Ucn2 Sequence

The sequence of ovine Ucn2 was determined by cloning and sequencing cDNA fragments generated by reverse transcription–polymerase chain reaction (RT-PCR). Sheep kidney and left atrium (tissues shown to express Ucn2 in humans) were rapidly dissected, snap-frozen in liquid nitrogen, and stored at −80°C. Total RNA was extracted by TRIzol (Invitrogen), and cDNA was reverse transcribed with Superscript II (Invitrogen) with the use of an oligo(dT) primer.

Identification of Ovine Ucn2 Sequence

This initial PCR product was run on an electrophoresis gel (0.75% agarose), and a band of the expected size (340 bp) was excised under magnification. A 5′-nested PCR was performed with partially nested primers, Ucn2-A and Ucn2-C (Table 1), with the use of Qiagen Taq polymerase and buffer (2.5 mmol/L magnesium chloride).

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Surgical Preparation of Sheep

Eight Coopworth ewes (weight, 45 to 67 kg) were instrumented via a left lateral thoracotomy under general anesthesia (induced by 17 mg/kg thiopental; maintained with halothane/nitrous oxide). Two polyvinyl chloride 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 CO; and a 7F 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 for 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/d sodium; 200 mmol/d potassium).

Study Protocol

Each sheep received incremental intravenous boluses of mouse Ucn2 (10, 50, and 100 μg at 2-hour intervals) (Phoenix Pharmaceuticals Inc) and a vehicle control (10 mL 0.9% saline) on 2 separate days a day apart in a balanced random order, both before (normal) and after induction of HF by rapid left ventricular pacing (7 days at 225 bpm).

Heart rate (HR), MAP, LAP, CO, and calculated total peripheral resistance (CTPR = MAP/CO) were recorded at 15-minute intervals in the hour preceding the first bolus at 1000 hours (baseline); at 15, 30, 45, 60, 90, and 120 minutes succeeding each bolus; and at 1000 hours the following day. Hemodynamic measurements were determined by online computer-assisted analysis by established methods. Blood samples were drawn from the left atrium at 30 minutes and immediately preceding the first bolus at 1000 hours (baseline); at 30, 60, and 120 minutes succeeding each bolus; and at 1000 hours the following day. Samples were taken into tubes on ice, centrifuged at 4°C, and stored at either −20°C or −80°C before assay for Ucn1, cAMP, arginine vasopressin (AVP), corticotropic, cortisol, endothelin-1, plasma renin activity (PRA), aldosterone, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and catecholamines. For each hormone, all samples from individual animals were measured in the same assay to avoid interassay variability. Plasma electrolytes and hematocrit were measured with every blood sample taken.

Cross-reactivity in the Ucn1 radioimmunoassay to murine Ucn2 (Peninsula Laboratories) was <0.026%; to human Ucn3 (Peninsula Laboratories) was <0.026%; and to ovine CRF (Peninsula Laboratories) was <0.001%.

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

Statistical Analysis

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 before treatment) were compared with paired t tests. To test for the effects of Ucn2, control and Ucn2 study limbs (in both normal and HF sheep separately) were compared with repeated-measures ANOVA. Where significant differences were identified by ANOVA, the level of significance at individual time points in Figures 1 to 5 and Table 3 was determined by Fisher protected least significant difference tests. To test for differences in the response to Ucn2 between normal and HF states, Ucn2 study limbs in each state were compared by covariate ANOVA with baseline data used as covariates. Significance was assumed at P<0.05.

Results

Ovine Ucn2 Sequence

The predicted 38–amino acid sequence of mature ovine Ucn2 is shown in Table 2. Comparison with the human and murine forms of the peptide demonstrates 76% and 97% homology, respectively. In view of the close sequence identity of sheep and mouse Ucn2 (only 1 amino acid difference), commer-
cially available murine Ucn2 was used in the present sheep physiological studies.

**Ucn2 Effects in Normal and HF Sheep**

Rapid left ventricular pacing for 7 days induced the hemodynamic, endocrine, and sodium-retaining hallmarks of congestive HF, with significant declines in MAP and CO, increases in LAP and CTPR (all $P < 0.001$), activation of multiple hormone systems (plasma ANP, BNP, endothelin-1 [all $P < 0.001$], aldosterone, AVP, epinephrine, cAMP, urine cAMP [all $P < 0.05$]), and reduced renal function (urine volume, urine sodium [both $P < 0.001$], urine potassium, creatinine clearance, hematocrit [all $P < 0.01$], urine creatinine [all $P < 0.05$]) (Figures 1 to 5, Table 3).

**Hemodynamics**

In normal sheep, Ucn2 induced marked and dose-dependent increases in CO and HR (both $P < 0.001$), in conjunction with minor reductions in CTPR ($P < 0.05$) and LAP ($P < 0.01$) (Figure 1, Table 3). Responses were both rapid and transient, with peak effects evident at 15 minutes after bolus. MAP tended to drop initially after Ucn2 administration but was elevated overall compared with control ($P < 0.01$) (Figure 1), with the reverse pattern apparent for hematocrit ($P < 0.05$) (Table 3). Hemodynamic measurements had returned to control levels by 24 hours.

In HF, Ucn2 doubled CO and more than halved CTPR and LAP (all $P < 0.001$), restoring these indices temporarily to normal (nonpaced) levels after administration of the highest dose (Figure 1). In contrast to the response in normal animals, MAP was reduced relative to control ($P < 0.05$), although not in a dose-dependent manner. Hematocrit was also decreased by Ucn2 in HF animals ($P < 0.001$) (Table 3). As in the normal state, no effects were noted the following day.

Comparison of Ucn2 effects in HF and normal states established that CTPR and LAP decreases were significantly greater in HF sheep (both $P < 0.001$) and CO rises tended to be greater in HF ($P = 0.067$), whereas MAP responses were directionally opposite ($P < 0.01$).

**Hormones**

In the normal state, Ucn2 produced acute rises in plasma AVP ($P < 0.05$), corticotropin ($P < 0.001$), and cortisol ($P < 0.001$) (Figure 2). Ucn1 levels were also elevated ($P < 0.001$), although in a dose-related and more sustained fashion, with raised concentrations persisting at 24 hours (Figure 2). Ucn2 administration reduced plasma ANP ($P < 0.001$) but increased BNP levels ($P < 0.05$), whereas there were trends for plasma aldosterone ($P = 0.081$) and epinephrine ($P = 0.077$) to fall (Figures 3 and 4). Plasma cAMP, PRA, endothelin, and norepinephrine concentrations were unchanged compared with those of controls (Figures 2 to 4).

In HF sheep, plasma corticotropin ($P < 0.001$), cortisol ($P < 0.001$), and Ucn1 ($P < 0.001$) (Figure 2) demonstrated rises similar to those observed in normals (Figure 2). However, whereas cAMP and AVP rose initially after each Ucn2 bolus, concentrations were decreased overall compared with control ($P < 0.05$ and $P < 0.01$, respectively) (Figure 2). Ucn2 also substantially and dose dependently reduced PRA ($P < 0.001$), aldosterone ($P < 0.001$), endothelin-1 ($P < 0.01$), ANP ($P < 0.001$), BNP ($P < 0.001$), and epinephrine ($P < 0.01$) levels in these animals (Figures 3 and 4). Plasma AVP, PRA, and epinephrine were still decreased relative to control at...
1000 hours the following day, whereas BNP was elevated. Plasma norepinephrine concentrations tended to be reduced relative to control ($P < 0.079$).

Comparison of Ucn2 effects in normal and HF states showed significantly different responses in PRA ($P < 0.001$), aldosterone ($P < 0.001$), endothelin-1 ($P < 0.001$), epinephrine ($P < 0.001$), and cAMP ($P < 0.001$), all of which fell prominently in HF sheep but did not change significantly in normal animals. Plasma ANP reductions were markedly greater in HF ($P < 0.001$), whereas directionally opposite responses were demonstrated by AVP ($P < 0.05$), BNP ($P < 0.001$), and norepinephrine ($P < 0.01$) (which all fell in HF).

**Urine and Plasma Electrolytes**

In normal sheep, Ucn2 tended to elevate urine cAMP excretion ($P = 0.09$) but had no effect on any other urinary parameter measured (Figure 5, Table 3). Ucn2 reduced plasma potassium ($P < 0.001$), whereas directionally opposite responses were demonstrated by AVP ($P < 0.05$), BNP ($P < 0.001$), and norepinephrine ($P < 0.01$) (which all fell in HF).

In HF sheep, Ucn2 dose dependently increased urine volume ($P < 0.05$); urine sodium ($P < 0.01$), potassium ($P < 0.05$), creatinine ($P < 0.001$), and cAMP excretion ($P < 0.01$) (Figure 5); and creatinine clearance ($P < 0.001$) (Table 3). There were no differences evident at 24 hours. Ucn2 decreased plasma potassium ($P < 0.05$) concentrations and tended to reduce water intake ($P = 0.086$) but did not alter other plasma electrolytes relative to control (Table 3).

Comparison between normal and HF states showed significantly greater effects of Ucn2 on urine volume ($P < 0.05$), sodium excretion ($P < 0.05$), creatinine excretion ($P < 0.001$), and creatinine clearance ($P < 0.05$) in HF sheep.

**Discussion**

The present study is the first to report the integrated hemodynamic, hormonal, and renal effects of Ucn2 in normal and HF conditions. In HF, Ucn2 induced pronounced and dose-dependent decreases in CTPR and cardiac preload and augmentation of CO, small reductions in blood pressure, marked attenuation of vasoconstrictor/volume-retaining factors (including endothelin-1, renin-angiotensin II–aldosterone, AVP, and epinephrine), and improvements in renal function. In contrast, the majority of these responses in normal sheep were either greatly blunted or absent.

**Hemodynamics**

Ucn2 treatment induced prominent dose-dependent increases in CO in both normal and HF states. Similar observations have been reported recently in healthy and HF mice and appear to be due at least in part to the inotropic actions of Ucn2 as indicated by the rise in dP/dt in these animals. Further evidence of the inotropy of this peptide is provided by in vitro work demonstrating that Ucn2 improves intracellular calcium handling and contractility in isolated mouse cardio-
myocytes. A reduction in arterial load is also likely to have contributed to CO rises in the present study, as evidenced by the concomitant reductions in CTPR. Indeed, Bale et al. calculated that afterload reduction accounted for ~40% of the increase in ejection fraction after Ucn2 administration in normal mice and hypothesized that this effect may prove even more notable in the failing heart, in which significant afterload mismatch occurs. This appears to be the case in the present study because there was a trend for the increase in CO to be greater in the HF state, in which concurrent falls in peripheral resistance were substantially larger than those seen in normals.

The marked reductions in CTPR and decrease in MAP observed in HF suggest an effect of Ucn2 on arterial tone. This concurs with in vitro data demonstrating a direct vasodilator action of the peptide in human intramyocardial blood vessels and rat thoracic aorta. In contrast, Ucn2 raised MAP in normal sheep, although this was preceded by...
an initial acute fall. The dissimilarity in blood pressure responses between HF and normal sheep may be attributed to the preconstricted state of the arterial vasculature in HF. Ucn2 has been shown to be remarkably effective in reversing endothelin-1–induced arterial constriction, suggesting that the peptide is especially important in modulating vascular tone in pathophysiological conditions such as HF, in which endothelin-1 levels are increased. Furthermore, the actions of Ucn2 to considerably reduce elevated circulating levels of the potent vasoconstrictors endothelin-1, angiotensin II, AVP, and epinephrine in HF are likely to have contributed to its hypotensive effects in this setting. In the normal state, in which Ucn2 had minimal impact on either CTPR or plasma levels of vasoconstrictor peptides, it is conceivable that the vigorous increase in CO overwhelmed the vasodilator actions of the peptide.

Ucn2 administration in HF also resulted in an impressive reduction in LAP to normal state levels, presumably a reflection of the large increase in CO and reported lusitropic actions of the peptide. A possible contribution from reduced circulating filling pressures through venodilator actions (as demonstrated previously for Ucn1) cannot be excluded from our data. It is unlikely that a reduction in plasma volume contributed to the hemodynamic changes because, despite an increase in urine excretion, there was no corresponding rise in hematocrit.

The type and magnitude of hemodynamic responses induced by Ucn2 in the present study are comparable to those elicited by similar doses of Ucn1 in earlier work. However, the onset and duration of effect of the 2 peptides differ, with peak Ucn2 effects evident at 15 minutes (the first measurement made) after administration, whereas the maximum hemodynamic responses to Ucn1 occurred at 30 to 120 minutes and were still evident the following day. The more rapid onset and shorter duration of effect demonstrated for Ucn2 is likely to reflect a smaller volume of distribution and more rapid clearance (and thus shorter plasma half-life) of this peptide compared with Ucn1. Pharmacodynamic study of Ucn2 awaits development of a suitable assay.

**Hormones**

Although plasma levels of cAMP, a proposed intracellular second messenger of Ucn2, were principally unchanged by Ucn2 in normal sheep, levels were presumably elevated sufficiently at the tissue level to induce the biological responses observed, whereas the falls in circulating concentrations in HF likely reflect hemodynamic improvement. Immediately succeeding each Ucn2 bolus, however, cAMP levels rose acutely in association with transient and remarkably well-matched increments in plasma AVP, corticotropin, and cortisol. Although these responses are similar to those elicited by Ucn1 in sheep, activation of the corticotropin/cortisol axis by Ucn2 is surprising given that the peptide is reported to have little affinity for the CRF1 receptor (which mediates the corticotropin response) and fails to stimulate corticotropin secretion in either cultured rat anterior pituitary cells or the intact animal. It is conceivable that Ucn2/receptor binding affinities may be species specific, with the ligand exhibiting greater affinity for ovine CRF1 receptors than that demonstrated for the mouse and rat receptor. Alternatively, the concurrent increases in plasma Ucn1 (shown to stimulate corticotropin/cortisol at a low threshold), presumably a consequence of competitive inhibition for the CRF2 receptor by Ucn2, may account for the observed increases in corticotropin. Whatever the mechanism, the effect of Ucn2 administration on the corticotropin/cortisol axis in humans is awaited with great interest.
The present study is the first to investigate the effects of Ucn2 on other vasoactive peptide systems. Acute rises in plasma AVP levels after Ucn2 treatment in normal sheep may indicate a direct effect on AVP secretion, a concept in keeping with the colocalization of Ucn2 mRNA and both CRF receptors with AVP mRNA-expressing cells in the hypothalamic paraventricular and supraoptic nuclei.25,26 In contrast, plasma AVP was significantly and persistently reduced at the higher doses of Ucn2 in the HF state. This result is analogous to that observed with Ucn1 administration in ovine HF10 and likely relates to the attenuation of AVP-stimulatory mechanisms operating in this setting, including any direct positive effect of Ucn2 on AVP secretion.

The action of Ucn2 to markedly and dose dependently suppress the renin-angiotensin-aldosterone system in HF is similar to that produced by Ucn1 in this state,10 although less sustained. The fall in PRA occurred despite the stimulatory effect of reductions in blood pressure, but whether as a result of direct inhibition of renin release at the juxtaglomerulus, increased sodium (and chloride) delivery to the macula densa (indicated by the significant natriuresis), reduction in sympa-

### TABLE 2. Ucn2 Amino Acid Sequences

<table>
<thead>
<tr>
<th>Human Ucn2</th>
<th>Murine Ucn2</th>
<th>Ovine Ucn2</th>
</tr>
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Predicted amino acid sequences of mature human, murine, and ovine Ucn2. Residues identical to all 3 sequences are in bold. Ovine residue different from murine sequence is underlined.

### TABLE 3. Effects of Ucn2 in 8 Sheep Before (Normal) and After Induction of HF

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Values are mean±SEM. Incremental Ucn2 boluses were administered at 2-hour intervals.

*P<0.05, †P<0.01, ‡P<0.001, significant differences compared with time-matched control.
thetic drive to the juxtaglomerular cells, or some other PRA-inhibitory mechanism remains to be seen. The substantial decreases in plasma aldosterone levels to normal state concentrations after Ucn2 administration presumably reflect reduced circulating angiotensin II, as assessed by the falls in PRA (and perhaps plasma potassium levels). It is possible that Ucn2 has a direct effect on aldosterone secretion at the adrenal glomerulosa, consistent with message expression of the peptide in the adrenals,13 although this cannot be concluded from our data. However, aldosterone declines in the face of significant increases in corticotropin might suggest inhibition of corticotropin-induced aldosterone secretion.

As with Ucn1,10 Ucn2 treatment produced sizable falls in circulating endothelin-1 concentrations in HF. Although Ucn2 is reported to reverse endothelin-1–induced constriction,9 a direct effect on secretion of the peptide has not been investigated. Although Ucn2 did not alter norepinephrine levels, plasma epinephrine tended to be attenuated in normal sheep and was clearly reduced in HF. Whether this is due to a direct action of the peptide at the adrenal medulla (as it occurred despite concomitant falls in blood pressure) or possibly to an improvement in HF status27 or some other mechanism necessitates further study.

Ucn2-induced falls in plasma ANP are likely a result of the respective decreases in cardiac transmural pressures (reflected by the declines in LAP), leading to reduced stimulus for secretion. Surprisingly, BNP concentrations increased in nonpaced sheep and appeared to be reduced to a lesser extent (compared with ANP) in HF, actually rising to above control levels the following day (when LAP was equal to control measurements). Although this latter finding concurs with a report demonstrating augmentation of natriuretic peptide secretion (~2-fold) by Ucn2 in rat cardiomyocytes,28 the difference in response between ANP and BNP is unexpected but may reflect a greater impact of falls in LAP on ANP secretion from atrial stores.

Renal Effects

This is the first report of the renal actions of Ucn2. In contrast to the lack of renal effects demonstrated in normal animals, Ucn2 significantly and largely dose dependently increased urine output and urine sodium and creatinine excretion in HF. Again, these effects are similar to those observed with Ucn1 administration in ovine HF (with the exception of sustained overnight activity)10 and occurred despite falls in MAP and marked reductions in circulating natriuretic peptide levels. It is likely that an effect of Ucn2 on renal hemodynamics played a role in the natriuresis/diuresis observed in the present study given the increase in glomerular filtration (indicated by the substantial rise in creatinine clearance) and evidence of Ucn2-induced renal vasodilatation in the rat.29 Direct tubular actions may also have occurred in light of the relative increase in urine cAMP excretion and reports of Ucn2 expression within the kidney.13 In addition, the prominent declines in plasma concentrations of antinatriuretic/antidiuretic factors including AVP, angiotensin II, and aldosterone are very likely to have contributed to these renal responses. The diuretic effect of Ucn2 occurred notwithstanding a trend for water intake to be reduced, an effect previously observed after intracerebroventricular administration in the rat.30

In conclusion, in contrast to limited Ucn2 bioactivity in normal sheep, administration of the peptide in HF induced significant decreases in peripheral resistance and left ventricular preload and afterload and improvements in CO, in association with marked attenuation of a range of vasoconstrictor/volume-retaining systems and augmentation of urine and sodium excretion. Although the exact mechanisms underlying many of these varied responses (especially endocrine and renal) clearly require investigation, they are undeniably salutary in a disease characterized by poor pump performance, sodium/volume retention, and abnormal and (often) adverse hormonal activation. These data support a role for Ucn2 in both cardiovascular and volume regulation in HF and point toward a therapeutic application of this peptide in clinical cardiac failure.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Some of the most successful pharmacological treatments for heart failure (HF) to date are based on the inhibition of hormones abnormally activated in response to cardiac injury and/or dysfunction (for example, renin-angiotensin-aldosterone and sympathetic nervous systems). Despite improvements in therapy, however, the prognosis remains poor, and further progress will require manipulation of alternative neurohumoral responses in addition to established antifailure strategies. In the present study we report on the effects of the novel peptide urocortin 2 (Ucn2) in an experimental ovine model of HF. We found that acute intravenous Ucn2 significantly improves cardiac output and reduces peripheral resistance and left ventricular preload and afterload, in association with marked attenuation of a range of vasoconstrictor/volume-retaining systems (renin-angiotensin-aldosterone, endothelin-1, and arginine vasopressin) and augmentation of renal function (natriuresis, diuresis, increased creatinine clearance). This combination of responses is undeniably salutary in a disease characterized by poor pump performance, sodium/volume retention, and aberrant and (often) adverse hormonal activation. Thus, these data support a role for Ucn2 in both cardiovascular and volume regulation in HF and point toward the therapeutic application of this peptide in clinical cardiac failure. Future investigations into the effects of Ucn2 administration both chronically and in humans are required to fully elucidate the potential of this peptide in this setting.
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