Interactions of Enhanced Urocortin 2 and Mineralocorticoid Receptor Antagonism in Experimental Heart Failure

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Background—Mineralocorticoid receptor antagonists (MRAs) have become established therapy in heart failure (HF). Urocortin 2 (Ucn2) is a novel peptide with potential in the treatment of this disease. The present study investigated the interactions of acute administration of Ucn2 and an MRA in experimental HF.

Methods and Results—Ucn2 and an MRA (canrenoic acid [CA]) were infused for 4 hours, both singly and together, in 8 sheep with pacing-induced HF. Ucn2, when administered as an adjunct to CA, further improved hemodynamic indices relative to that achieved by CA alone, producing additional increases in cardiac output and decreases in left atrial pressure and peripheral resistance but without eliciting a supplementary reduction in arterial pressure. Ucn2 cotreatment reversed CA-induced rises in circulating aldosterone levels, and also significantly reduced plasma renin activity, angiotensin II, and vasopressin concentrations. Although both CA and Ucn2 infusion produced a diuresis and natriuresis, responses with Ucn2 and Ucn+CA were 2- to 3-fold greater than that elicited by separate CA. Ucn2 cotherapy additionally increased urine potassium and creatinine excretion. In contrast to the rise in plasma potassium induced by CA, Ucn2 cotreatment reduced potassium concentrations.

Conclusions—Ucn2 cotherapy with an MRA in HF further improved hemodynamics relative to that achieved by CA alone, while also reducing plasma renin activity, angiotensin II, aldosterone and vasopressin levels, and enhancing renal function. Importantly, Ucn2 prevented CA-induced rises in plasma potassium. These data demonstrate a favorable profile of effects with short-term adjunct Ucn2 therapy and an MRA in HF. (Circ Heart Fail. 2013;6:825-832.)

Key Words: heart failure ■ mineralocorticoid receptor antagonism ■ urocortins

The steroid hormone aldosterone, an important downstream component of the renin–angiotensin–aldosterone system (RAAS), is now accepted as contributing significantly to the development and progression of heart failure (HF) through adverse mineralocorticoid receptor (MR)–mediated actions on volume/pressure homeostasis, and promotion of oxidative stress, inflammation, and fibrosis—all of which provoke tissue remodeling and end organ damage.1,2 Although angiotensin-converting enzyme inhibitors and angiotensin type 1 receptor antagonists reduce aldosterone secretion in the short term, the frequent occurrence of breakthrough or escape results ultimately in elevated circulating aldosterone levels. Direct blockade of aldosterone’s actions with MR antagonists (MRAs) such as spironolactone and eplerenone, now recognized as a third class of RAAS inhibitor, has been shown in a number of large interventional clinical trials to reduce morbidity and mortality in patients with HF.3-5 However, although MRAs are now an important accessory in our pharmaceutical management of HF, they remain the most underused of all medications, largely because of the fear of hyperkalemia,6 hypotension, and renal impairment.7 Urocortin 2 (Ucn2) belongs to a group of peptides (Ucn1–3) sharing structural similarities with the hypothalamic hormone corticotropin-releasing factor (CRF). Ucn2 is reported to bind with high affinity to the G-protein–coupled CRF receptor subtype CRF2,8 which is localized in diverse tissues throughout the brain and periphery (with strong expression demonstrated in the heart and vasculature).9 This receptor is thought to mediate the majority of actions seen with systemically administered peptide, including direct vasodilation and positive cardiac chronotropic and lusitropic activity9-10—effects which have identified Ucn2 as a potential therapeutic agent in heart disease. Several studies have shown that Ucn2 improves cardiovascular and renal function in experimental HF, in association with suppression of a number of vasoconstrictor/volume-retaining hormone systems (including the RAAS).11-15 In humans with stable systolic HF, Ucn2 increases left-ventricular ejection fraction and reduces systemic vascular resistance and cardiac work, with relative suppression of the RAAS and augmentation of renal parameters in the face of substantial falls in blood pressure.16 These promising results prompted the recent trialing of Ucn2 as a short-term parenteral therapy in patients with acute decompensated HF.17 Current evidence-based guidelines suggest that MR blockade should be an integral component of HF therapy. If, as
seems possible, Ucn2 is introduced as a treatment for human HF, the 2 agents will be used in conjunction. The effects of combined Ucn2 and MRAs in HF have not, however, been studied. This information is especially pertinent in light of the interactions reported between the Ucn2 and the RAAS. Accordingly, we investigated for the first time the hemodynamic, hormonal, electrolyte, and renal effects of Ucn2 and an MRA administered separately and together in an ovine model of experimental HF.

**Methods**

**Surgical Preparation of Sheep**

Eight Coopworth ewes (40–62 kg; Lincoln University Farm, Christchurch, New Zealand) were instrumented as previously described via a left lateral thoracotomy under general anesthesia (induced by IV thiopentone 15 mg/kg; maintained with 2.5% isoflurane/2 L/min nitrous oxide/2 L/min oxygen) and using approved peri- and postoperative antibiotics (IV cephalzin 20 mg/kg and IV enrofloxacin 2.5 mg/kg) and analgesia (intercostal bupivacaine 0.5%/lignocaine 2%; IV carprofen 4 mg/kg; IV buprenorphine 0.005–0.01 mg/kg). The level of perioperative anesthesia was monitored by pedal withdrawal and careful observation of respiration and heart rate. Briefly, 2 polyvinyl chloride catheters were inserted into the left atrium for blood sampling and measurement of left atrial pressure (LAP); a Konigsberg pressure-tip transducer was inserted into the aorta to record mean arterial pressure (MAP) and into the apex of the left ventricle to obtain maximum derivatives of pressure over time (dP/dt max) as an index of contractility; an electromagnetic flow probe was placed around the ascending aorta to measure cardiac output (CO); a Swan–Ganz catheter was inserted into the pulmonary artery for infusions; and a 7 French His-bundle electrode was stitched subepicardially to the wall of the left ventricle for subsequent pacing. A bladder catheter was inserted into the urethra for urine collections. Animals recovered for 24 hours before commencing the study protocol. During the experiments, the animals were held in metabolic cages housed in a light-controlled room, received a diet of lucerne chaff and food pellets providing 75 mmol sodium and 150 mmol potassium/d, and had free access to water.

**Study Protocol**

The study protocol was approved by the University of Otago, Christchurch Animal Ethics Committee. After induction of HF by 7 days of rapid left-ventricular pacing (225 beats per minute), each sheep received in a balanced, random order crossover design, a 4-hour infusion of a vehicle control (0.9% saline), Ucn2 (50-μg bolus+75-μg/h infusion; American Peptide Company, Inc, Sunnyvale, CA), an MRA (canrenonic acid [CA], 200-ng bolus+75-μg/h infusion; Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand), and Ucn2+CA (administered at the same doses as above) on days 7, 9, 11, and 13 of pacing. All treatments were administered in a total volume of 50 mL of 0.9% saline via the pulmonary artery catheter.

MAP, LAP, CO, dP/dt max, and calculated total peripheral resistance (MAP/CO) were recorded at 15-minute intervals in the hour before infusion (baseline), at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 minutes during the 4-hour infusion period, and at 30-minute intervals during the 2-hour postinfusion period. Hemodynamic measurements were determined by online computer-assisted analysis (PowerLab Systems; ADInstruments, Dunedin, NZ) using established methods. Blood samples were drawn from the left atrium 30 minutes and immediately preinfusion (baseline) at 30, 60, 120, 180, and 240 minutes during the 4-hour infusion period and at 60-minute intervals during the 2-hour postinfusion period. Samples were taken into chilled EDTA tubes, centrifuged at 4°C and stored at either −20°C or −80°C before assay for plasma renin activity (PRA), angiotensin II (Ang II), aldosterone, arginine vasopressin, cortisol, atrial natriuretic peptide, brain natriuretic peptide, and catecholamines. For each hormone, all samples from individual animals were measured in the same assay to avoid interassay variability. Hematocrit was determined with every blood sample taken, and plasma electrolytes (sodium, potassium, and creatinine) measured 2 hourly.

Urine was collected hourly for the measurement of volume and sodium, potassium, and creatinine excretion. Creatinine clearance (CrCl) was calculated as urine creatinine×volume/plasma creatinine.

**Statistics**

Data are expressed as mean±SEM. Baseline hemodynamic and hormone values represent the mean of the 4 and 2 measurements, respectively, made within the hour immediately before preinfusion. Differences between nonpaced laboratory normal sheep (n=20) and HF study animals (vehicle control baseline data; n=8) were compared using independent Student t tests (Table 1). Comparison of the baseline data for the 4-treatment arms of the study (Control, Ucn2, CA, and Ucn2+CA) by repeated measures 1-way ANOVA (using SPSS statistical package version 11.022) showed no significant differences for any variable before commencement of treatment. Differences between the 4-study arms were determined using 2-way repeated measures ANOVA. Significance was assumed when P<0.05. Where significant differences were identified by ANOVA, the level of significance at individual time points in Table 2 and bar graphs was determined by Fisher protected least-significant difference tests.

**Table 1. Effects of Rapid Left-Ventricular Pacing (7 Days at 225 Beats per Minute)**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Nonpaced</th>
<th>Paced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output, L/min</td>
<td>4.9±0.4</td>
<td>2.9±0.3†</td>
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<tr>
<td>dP/dt max, mm Hg/s</td>
<td>2089±153</td>
<td>1206±167‡</td>
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<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>84±2</td>
<td>74±2‡</td>
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<td>Left atrial pressure, mm Hg</td>
<td>4.1±0.3</td>
<td>23.5±0.7‡</td>
</tr>
<tr>
<td>Total peripheral resistance, mm Hg/L per minute</td>
<td>16±1</td>
<td>27±2‡</td>
</tr>
<tr>
<td>Atrial natriuretic peptide, pmol/L</td>
<td>17±2</td>
<td>219±34‡</td>
</tr>
<tr>
<td>Brain natriuretic peptide, pmol/L</td>
<td>3±1</td>
<td>38±4‡</td>
</tr>
<tr>
<td>Plasma renin activity, nmol/L per hour</td>
<td>0.39±0.06</td>
<td>2.76±0.96‡</td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>12±2</td>
<td>71±23‡</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>225±24</td>
<td>2997±933‡</td>
</tr>
<tr>
<td>Vasopressin, pmol/L</td>
<td>1.7±0.1</td>
<td>3.8±1.1†</td>
</tr>
<tr>
<td>Norepinephrine, pmol/L</td>
<td>2683±507</td>
<td>13252±4504‡</td>
</tr>
<tr>
<td>Epinephrine, pmol/L</td>
<td>490±88</td>
<td>1817±1254*</td>
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<td>Plasma sodium, mmol/L</td>
<td>142±1</td>
<td>141±1</td>
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<tr>
<td>Plasma potassium, mmol/L</td>
<td>3.95±0.06</td>
<td>3.83±0.20</td>
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<td>Plasma creatinine, μmol/L</td>
<td>68.5±1.8</td>
<td>83.9±5.0‡</td>
</tr>
<tr>
<td>Urine volume, mL/h</td>
<td>81±11</td>
<td>96±30</td>
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<tr>
<td>Urinary sodium excretion, mmol/h</td>
<td>2.62±0.30</td>
<td>0.33±0.12‡</td>
</tr>
<tr>
<td>Urinary potassium excretion, mmol/h</td>
<td>9.0±0.6</td>
<td>6.9±1.9*</td>
</tr>
<tr>
<td>Urinary creatinine excretion, mmol/h</td>
<td>0.50±0.02</td>
<td>0.41±0.04*</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>121±9</td>
<td>89±8‡</td>
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<tr>
<td>Hematocrit, %</td>
<td>31.2±1.2</td>
<td>25.3±0.9*</td>
</tr>
</tbody>
</table>

Mean±SEM measurements in normal sheep (nonpaced; laboratory normal data; n=20) and sheep with heart failure induced by 7 days of rapid left-ventricular pacing at 225 beats per minute (paced; vehicle control baseline data; n=8). Significant differences are shown by *P<0.05, †P<0.01, ‡P<0.001.
Results
Rapid left-ventricular pacing at 225 beats per minute for 7 days produced the hemodynamic, endocrine, and sodium-retaining hallmarks of congestive HF,\textsuperscript{20,21} with reduced CO, MAP and renal function, elevated LAP and peripheral resistance, and widespread hormonal activation (Table 1).

Compared with time-matched vehicle control data, separate Ucn2 and CA administration both significantly increased CO (Ucn2>CA; $P<0.001$ and $P<0.05$, respectively) and decreased LAP (Ucn2>CA; both $P<0.001$) and calculated total peripheral resistance (Ucn2>CA; $P<0.001$ and $P<0.05$, respectively; Figure 1). These changes persisted $\leq 2$ hours after infusion.

Table 2. Effects of Ucn2 and CA in Sheep With Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>6 h</th>
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<td>Hematocrit, %</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Vehicle</td>
<td>25.3±0.9</td>
<td>24.6±1.0</td>
<td>24.4±1.0</td>
<td>24.4±1.0</td>
<td>24.2±1.0</td>
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<tr>
<td>Ucn2</td>
<td>25.6±1.0</td>
<td>23.4±1.0</td>
<td>22.9±0.9</td>
<td>22.3±0.9</td>
<td>22.1±0.9</td>
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<td>25.5±0.8</td>
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<td>24.8±0.7</td>
<td>24.8±0.7</td>
<td>24.9±0.8</td>
<td>24.6±0.7</td>
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<tr>
<td>Ucn2+CA</td>
<td>25.8±1.0</td>
<td>23.5±0.8</td>
<td>22.9±0.7</td>
<td>22.6±0.8</td>
<td>22.4±0.7</td>
<td>22.8±0.5</td>
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<td>Plasma epinephrine, pmol/L</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vehicle</td>
<td>1817±1254</td>
<td>2424±1387</td>
<td>2170±1391</td>
<td>1882±1002</td>
<td>1798±1085</td>
<td>1695±850</td>
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<tr>
<td>Ucn2</td>
<td>780±194</td>
<td>1280±436</td>
<td>675±171</td>
<td>998±385</td>
<td>951±396</td>
<td>1120±436</td>
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<tr>
<td>Canrenoic</td>
<td>1138±284</td>
<td>1762±353</td>
<td>1371±315</td>
<td>1314±298</td>
<td>1615±399</td>
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<tr>
<td>Ucn2+CA</td>
<td>1423±534</td>
<td>2144±616</td>
<td>1584±485</td>
<td>1234±359</td>
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<td>Plasma norepinephrine, nmol/L</td>
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<tr>
<td>Vehicle</td>
<td>13.3±4.5</td>
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<tr>
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<td>20.1±5.4</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Plasma potassium, mmol/L</td>
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<td>3.03±0.17</td>
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<td>116.2±15.2‡</td>
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<td>105.5±12.4†</td>
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<td>108.0±10.0†</td>
<td>104.6±15.7</td>
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<td>Water intake, mL/2 h</td>
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<td>271±120</td>
<td>...</td>
<td>128±42</td>
<td>309±102</td>
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</table>

Values are mean±SEM. Significant differences from control arm in respective state shown by: *$P<0.05$, †$P<0.01$, ‡$P<0.001$. CA indicates canrenoic acid; and Ucn2, Urocortin 2.
MAP was reduced in a sustained fashion by CA \((P<0.001)\) but fell only transiently with Ucn2 treatment before rising \((P<0.001)\). Ucn2 alone also induced a pronounced rise in \(dP/dt_{\text{max}}\) \((P<0.001)\), whereas CA alone had no significant impact on cardiac contractility (Figure 1).

Combined Ucn2+CA therapy had effects on \(dP/dt_{\text{max}}\) \((P<0.001)\), CO \((P<0.001)\), LAP \((P<0.001)\), and peripheral resistance \((P<0.001)\), which were comparable with those induced by Ucn2 alone and were significantly greater than the responses elicited by CA alone (all \(P<0.001)\). The blood pressure response to combined therapy was intermediate between the 2 treatments \((P<0.001)\); Figure 1). Hematocrit was similarly decreased by Ucn2 and Ucn2+CA (both \(P<0.001)\); Table 2).

Separate Ucn2 and Ucn2+CA induced persistent reductions in circulating levels of PRA \((P<0.001)\) and \((P<0.01)\), respectively), Ang II \((P<0.001)\); Figure 2), arginine vasopressin \((P<0.05)\) and \(P<0.01)\), respectively), atrial natriuretic peptide \((both P<0.001)\), and brain natriuretic peptide \((both P<0.001)\); compared with control, whereas CA alone had no significant impact on any of these indices. However, plasma aldosterone levels were significantly increased by CA \((P<0.001)\) and decreased by Ucn2 \((P<0.01)\), whereas dual therapy was characterized by an initial transient rise \((30–60 \text{ minutes})\) equal to that seen with CA alone followed by a decline relative to control data \((P<0.001)\); Figure 2). The blunting of the CA-induced aldosterone rise by the addition of Ucn2 demonstrated a significant difference between the CA and Ucn2+CA study arms \((P<0.01)\). Plasma cortisol was increased by all active treatments \((all P<0.001)\), with levels peaking at 1 hour before gradually declining (Figure 2). Circulating epinephrine and norepinephrine levels were unaltered by any agent (Table 2).

Plasma potassium concentrations were elevated relative to control data by CA \((P<0.05)\), and decreased by Ucn2 \((P<0.05)\) and Ucn2+CA \((P<0.05)\); Table 2). Similar trends

**Figure 1.** Mean±SEM hemodynamic responses to 4-hour infusions of vehicle (-), canrenoic acid (200-mg bolus+75-mg/h infusion; A), Ucn2 (50-μg bolus+75-μg/h infusion; ●), and canrenoic acid+Ucn2 combined (■) in 8 sheep with heart failure.

**Figure 2.** Mean±SEM hormonal responses to 4-hour infusions of vehicle (-), canrenoic acid (200-mg bolus+75-mg/h infusion; A), Ucn2 (50-μg bolus+75-μg/h infusion; ●), and canrenoic acid+Ucn2 combined (■) in 8 sheep with heart failure.
were observed for plasma creatinine responses to the various
treatments (rises with CA and falls with Ucn2/Ucn2+CA; all
0.1>P>0.05). Plasma sodium was not affected by any agent
(Table 2).

All 3 active treatments induced a diuresis (Ucn2, P<0.01; CA,
P<0.05; Ucn2+CA, P<0.01) and natriuresis (Ucn2,
P<0.01; CA, P<0.05; Ucn2+CA, P<0.01); however, the
responses with Ucn2 (alone and combined with CA) were 2-
to 3-fold greater than that achieved by CA alone (all P<0.05;
Figure 4). In addition, urine potassium and creatinine excre-
tion (Figure 4) and CrCl (Table 2), which tended to be reduced
by CA treatment alone, were significantly enhanced by Ucn2
and Ucn2+CA (all P<0.05). Water intake did not vary notably
with any treatment versus control (Table 2).

Discussion
MRAs are already a mainstay of pharmacotherapy for HF,
although, as previously mentioned, they are presently under-
used because of fears of hyperkalemia, hypotension, and renal
impairment.6,7 If Ucn2 is likewise to find a place in the treat-
ment of HF, it should ideally enhance any beneficial effects
of MRAs while reducing—or at least not exacerbating—the
adverse consequences of such therapy. In the present study in
experimental HF, we have demonstrated for the first time that
Ucn2, when added to the MRA, CA, improved hemodynamic,
hormone, electrolyte, and renal indices.

Aldosterone is recognized as playing a critical role in
volume homeostasis, and its main action in the kidney is to
increase sodium (and water) reabsorption, while at the same
time increasing the excretion of potassium ions.1 In keeping
with the blockade of these actions, CA in the present study
induced a natriuresis and diuresis, and although the decline in
potassium excretion was not significant, there was an apprecia-
tible rise in plasma potassium concentrations (by 0.47 mmol/L)
over the 6-hour study period. These results are in accord with
those demonstrated in previous studies of acute MR block-
ade.25–27 Nephrotoxicity is also a well-recognized concern
with clinical use of MRAs,7 and there was a clear trend for
CrCl to fall serially from baseline to 6 hours (82.6±6.8 ver-
sus 75.0±6.4 mL/min) during CA treatment. When Ucn2 was
coadministered with CA, it greatly enhanced the natriuretic
and diuretic responses (in addition to inducing a kaliuresis),
essentially rescued the decline in CrCl, and prevented the
CA-induced rise in plasma potassium.

The observed Ucn2-induced renal responses, which
occurred in the face of prominent reductions in plasma atrial

Figure 3. Mean±SEM hormonal responses to 4-hour infusions
of vehicle (○), canrenoic acid (200-mg bolus+75-mg/h infusion;
▲), Ucn2 (50-μg bolus+75-μg/h infusion; ●), and canrenoic
acid+Ucn2 combined (■) in 8 sheep with heart failure.

Figure 4. Mean±SEM renal responses to 4-hour infusions of
vehicle (open bars), Ucn2 (50-μg bolus+75-μg/h infusion; striped
bars), canrenoic acid (200-mg bolus+75-mg/h infusion; dot-
ted bars) and canrenoic acid+Ucn2 combined (closed bars) in
8 sheep with heart failure. Significant differences from time-
matched control data are shown by *P<0.05, †P<0.01, ‡P<0.001.
natriuretic peptide/brain natriuretic peptide, are similar to those seen in our earlier work with the peptide in ovine HF, and are likely a consequence of improvements in glomerular filtration (as judged by the increase in CrCl), renal vasodilation, and attenuation of circulating antinatriuretic/antidiuretic factors (Ang II, aldosterone, and arginine vasopressin). Direct tubular actions of Ucn2 are also plausible given that not only is the CRF2 receptor present in the proximal tubules of the kidney, but also administration of the peptide is accompanied by increases in urine cAMP (Ucn2’s intracellular second messenger). Of interest, Ucn2 treatment in diabetic rodents improves renal structure and function in association with reduced kidney levels of transforming growth factor-β1, vascular endothelial growth factor and malonaldehyde (a marker of oxidative stress), and increased superoxide dismutase activity (an antioxidant with powerful anti-inflammatory actions). These Ucn2 effects directly counter the oxidative, inflammatory, and profibrotic actions of aldosterone. Our data demonstrating favorable actions of Ucn2 to improve renal function and prevent rises in plasma potassium during MR antagonism in HF may be especially beneficial in subjects also receiving drugs that block renin/Ang II and those with already impaired potassium excretion because of progressive age or disease-related decline in glomerular filtration rate, who are particularly vulnerable to the development of hyperkalemia.

In addition, such actions to prevent rises in plasma potassium may also be beneficial in the setting of acute decompenated HF where, with total body underperfusion, acidosis is present or threatened. Falls in pH are a powerful stimulus for increasing plasma potassium, as are falls in renal function (which in turn can also cause acidosis)—things which render significant hyperkalemia (with its attendant arrhythmic threat) from MR antagonism more likely.

A marked difference was evident in the RAAS response to Ucn2 and CA administration in the present study. We found that CA had little impact on either PRA or plasma Ang II—results comparable to those reported by other investigators after acute MR antagonism, and although a stimulatory effect of CA on renin secretion might have been anticipated secondary to the fall in arterial pressure, this may have been countered by the concomitant renin-inhibitory increase in sodium excretion. In contrast, Ucn2, both alone and in combination with CA, significantly reduced circulating levels of both PRA and Ang II. Although the Ucn2-induced natriuresis (3-fold greater than that observed with CA alone) almost certainly contributed to the observed fall in PRA, it is also possible that Ucn2 may have actions to directly inhibit renin release (given expression of both peptide and receptor in the kidney) or to inhibit sympathetic drive to the juxtaglomerular cells (with Ucn2 previously shown to suppress cardiac sympathetic drive). Moreover, there is some evidence suggesting that the Ucn2s may antagonize Ang II production via suppression of ACE levels.

As noted by others investigating the short-term effects of MR antagonism, CA in the present study was characterized by marked (>2-fold) increases in plasma aldosterone levels, indicating successful blockade of the MR throughout the experiment. Contrarily, Ucn2 induced a significant decline in circulating aldosterone that presumably reflects the coincident decreases in plasma Ang II and perhaps potassium levels, although the latter was not reduced in a sustained fashion (≤2 hours postinfusion) as were with both Ang II and aldosterone. However, it is conceivable that the peptide also has a direct inhibitory effect on aldosterone secretion in light of reports that Ucn2 and the CRF receptor are present in the adrenal gland, with the receptor showing higher expression in the cortex (the region of aldosterone production) than in the medulla. Administration of the combined agents was distinguished by a prompt transient rise in aldosterone followed by a gradual decline to below control, likely due to levels initially rising with MR blockade followed by subsequent suppression of aldosterone secretion. Although the RAAS-inhibitory actions of Ucn2, noted previously by our group, are obviously not salutary, this consequence of Ucn2 administration presumably as a consequence of competition for the CRF receptor with subsequent activation of CRF, and the hypothalamic–pituitary–adrenocortical axis. Although hypothalamic–pituitary–adrenocortical activation is obviously not salutary, this consequence of Ucn2 administration has been shown to be a transient event (unlike the more sustained hemodynamic, vasoactive hormone, and renal responses) both previously and in the present study. Moreover, actions of Ucn2 to increase cortisol seem to be less pronounced in man.

In keeping with the diuretic and vasorelaxant effects of CA, its administration in the present study significantly reduced MAP and calculated total peripheral resistance. This decrease in cardiac afterload likely contributed to the accompanying rise in CO, with a subsequent decline in LAP. When CA was given in combination with Ucn2, the resulting hemodynamic responses were largely comparable with those produced by Ucn2 alone with marked improvements in CO in association with substantial reductions (a halving) in peripheral resistance and LAP—effects observed previously in this model of HF. Although the Ucn2-induced increase in CO is presumably in part a consequence of the large falls in peripheral resistance, with the peptide shown to directly reduce vascular tone, Ucn2 also exhibits potent inotropic activity, as evidenced here by the concurrent rise in dP/dt max. The reductions in LAP are probably secondary to the rise in CO, although the peptide is reported to have lusitropic and venodilator activity, which may also have contributed. Importantly, not only were the beneficial hemodynamic actions of Ucn2 not inhibited by concurrent MR antagonism, but the combination therapy negated the fall in blood pressure seen with CA alone for most of the infusion period and well on into the postinfusion follow-up. In severe acute decompensated HF (which is what this ovine model parallels) this is highly desirable. Although the falls in MAP with CA alone were not large, in patients with severe acute decompensated HF who are already hypotensive, with
kidneys struggling at, or falling below, autoregulatory limits and in patients with universal multitissue underperfusion, any further falls in blood pressure are potentially disastrous and to be avoided if at all possible.

In conclusion, acute Ucn2 cotreatment with an MRA in an experimental model of HF further improved hemodynamics relative to that achieved by CA alone, reduced PRA, ANG II, aldosterone, and arginine vasopressin levels; enhanced the natriuretic and diuretic responses; and essentially rescued the decline in CrCl. Importantly, Ucn2 also prevented the CA-induced rises in plasma potassium. Of note, the combination of treatments did not cause any major adverse effects, such as a profound fall in arterial pressure, which is a frequent, dangerous, and unwanted threat when combining multiple agents in HF; and the beneficial renal effects of Ucn2 were sustained when CA was on board.

Acknowledgments

We are grateful to staff of the University of Otago-Christchurch Animal Research Area for animal care, and Endocrine Laboratory staff for performance of hormone assays.

Sources of Funding

Funding was provided by the Heart Foundation of New Zealand and staff for performance of hormone assays. Animal Research Area for animal care, and Endocrine Laboratory

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**CLINICAL PERSPECTIVE**

Urocortin 2 (Ucn2) is a novel peptide with potential as a short-term parenteral therapy in acute decompensated heart failure (HF). Because any prospective new treatment is likely to be used conjointly with established HF therapies, such as mineralocorticoid receptor antagonists (MRAs), it is essential that the integrated effects of these agents be assessed. Ideally, Ucn2 would enhance any beneficial effects of MRA, while reducing—or at least not exacerbating—any adverse consequences. In the present study, we have demonstrated for the first time that acute Ucn2 cotreatment with an MRA in experimental acute decompensated HF further improved hemodynamics relative to that achieved by MRA alone, while also reducing plasma renin activity, angiotensin II, aldosterone and vasopressin levels, and enhancing renal function. Importantly, Ucn2 prevented MRA-induced rises in plasma potassium. Our data demonstrate a favorable profile of effects with adjunct Ucn2 and MRA therapy in acute decompensated HF. These findings may be of additional clinical relevance given that MRAs are viewed cautiously in clinical HF as being potentially prohyperkalemic. In addition, at present it is very unusual for MRAs to be introduced in the acute phase of treating acute decompensated HF because of fears regarding hyperkalemia and renal function. This new combination treatment holds out the possibility of earlier and safer introduction of MRA therapy, and if the benefit demonstrated from earlier and earlier introduction of β-blockers is any guide, then the earlier introduction of MRA therapy is also a strategy that should be tested.
Interactions of Enhanced Urocortin 2 and Mineralocorticoid Receptor Antagonism in Experimental Heart Failure
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Circ Heart Fail. 2013;6:825-832; originally published online June 10, 2013;
doi: 10.1161/CIRCHEARTFAILURE.112.000205

Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

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