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

Rademaker at al: Combined MR Antagonism and Ucn2 in Heart Failure

Miriam T. Rademaker, PhD; Christopher J. Charles, PhD;
M. Gary Nicholls, MD, FRACP; A. Mark Richards, MD, PhD, FRACP

Christchurch Heart Institute, University of Otago, Christchurch
Christchurch, New Zealand

Correspondence to
Assoc. Prof. Miriam Rademaker
Department of Medicine
University of Otago, Christchurch
P.O. Box 4345, Christchurch, New Zealand
Phone: 64-3-3641112
Fax: 64-3-3640525
Email: miriam.rademaker@otago.ac.nz

DOI: 10.1161/CIRCHEARTFAILURE.112.000205

Journal Subject Codes: [110]Congestive; [118]Cardiovascular Pharmacology; [130]Animal models of human disease
Abstract

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 eight 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 co-treatment reversed CA-induced rises in circulating aldosterone levels, and also significantly reduced plasma renin activity, angiotensin II and vasopressin concentrations. While both CA and Ucn2 infusion produced a diuresis and natriuresis, responses with Ucn2 and Ucn+CA were 2-3-fold greater than that elicited by separate CA. Ucn2 co-therapy additionally increased urine potassium and creatinine excretion. In contrast to the rise in plasma potassium induced by CA, Ucn2 co-treatment reduced potassium concentrations.

Conclusions—Ucn2 co-treatment with an MRA in HF further improved hemodynamics relative to that achieved by CA alone, whilst 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.

Key Words: urocortin 2, mineralocorticoid receptor antagonism, heart failure
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 (ACEI) and angiotensin type 1 (AT1) 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 underutilized 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 CRF\(_2\)\(^8\) 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 activity\(^9,\(^10\) – 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).\textsuperscript{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.\textsuperscript{16} These promising results prompted the recent trialing of Ucn2 as a short-term parenteral therapy in patients with acute decompensated HF (ADHF).\textsuperscript{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 two 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 Ucn2 and the RAAS.\textsuperscript{13,15,18,19} 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\textsuperscript{20} via a left lateral thoracotomy under general anesthesia (induced by IV thiopentone 15mg/kg; maintained with 2.5\% isoflurane / 2L/min nitrous oxide / 2L/min oxygen) and using approved peri-/post-operative antibiotics (IV cephazolin 20mg/kg; IV enrofloxacin 2.5mg/kg) and analgesia (intercostal bupivacaine 0.5\% / lignocaine 2\%; IV carprofen 4mg/kg; IV buprenorphine 0.005-0.01mg/kg). The level of peri-operative anesthesia was monitored by pedal withdrawal and careful observation of respiration and heart rate. Briefly, two 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 per urethra for urine collections. Animals recovered for at least 14 days before commencing the study protocol. During the experiments the animals were held in metabolic cages housed in an light-controlled room, received a diet of lucerne chaff and food pellets providing approximately 75mmol sodium and 150mmol potassium/day, and had free access to water.

**Study Protocol**

The study protocol was approved by the University of Otago, Christchurch Animal Ethics Committee.

Following induction of HF by 7 days of rapid left ventricular pacing (225 beats/minute), each sheep received in a balanced, random order crossover design, a 4-hr infusion of a vehicle control (0.9% saline), Ucn2 (50ug bolus + 75ug/hr infusion; American Peptide Company, Inc., Sunnyvale, CA, USA), an MRA (canrenoic acid [CA] - 200mg bolus + 75mg/hr 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 50mls of 0.9% saline via the pulmonary artery catheter.

MAP, LAP, CO, dP/dt(max) and calculated total peripheral resistance (CTPR=MAP/CO) were
recorded at 15-minute intervals in the hour prior to 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 post-infusion period. Hemodynamic measurements were determined by on-line computer assisted analysis (PowerLab Systems, ADInstruments, Dunedin, NZ) using established methods.22

Blood samples were drawn from the left atrium 30 minutes and immediately pre-infusion (baseline), at 30, 60, 120, 180 and 240 minutes during the 4-hour infusion period, and at 60-minute intervals during the 2-hour post-infusion 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 (AngII), aldosterone, arginine vasopressin (AVP), cortisol, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and catecholamines.20,22-24 For each hormone, all samples from individual animals were measured in the same assay to avoid inter-assay variability. Hematocrit was determined with every blood sample taken, and plasma electrolytes (sodium, potassium, creatinine) measured 2-hourly.

Urine was collected 2-hourly for the measurement of volume and sodium, potassium and creatinine excretion. Creatinine clearance (CrCl) was calculated as urine creatinine x volume/plasma creatinine.

**Statistics**

Data are expressed as mean±SEM. Baseline haemodynamic and hormone values represent the mean of the four and two measurements, respectively, made within the hour immediately pre-infusion. Differences between non-paced laboratory normal sheep (n =20) and HF study animals
(vehicle control baseline data, n=8) were compared using independent Student’s \( t \)-tests (Table 1). Comparison of the baseline data for the study’s four treatment arms (Control, Ucn2, CA, Ucn2+CA) by repeated measures 1-way analysis of variance (ANOVA) (using SPSS statistical package version 11.022) showed no significant differences for any variable prior to commencement of treatment. Differences between the four 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's protected least-significant difference tests.

### Results

Rapid left ventricular pacing at 225bpm for 7 days produced the hemodynamic, endocrine and sodium-retaining hallmarks of congestive HF\(^2\),\(^2\) with reduced CO, MAP and renal function, elevated LAP and peripheral resistance, and widespread hormonal activation (Table 1).

Compared to 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 left atrial pressure (Ucn2>CA; both \( p<0.001 \)) and CTPR (Ucn2>CA; \( p<0.001 \) and \( p<0.05 \), respectively) (Figure 1). These changes persisted up to 2 hours post-infusion. 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(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(max) (\( p<0.001 \)), CO (\( p<0.001 \)), LAP (\( p<0.001 \)) and peripheral resistance (\( p<0.001 \)) which were comparable to 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 two 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), AngII (both p<0.001) (Figure 2), AVP (p<0.05 and p<0.01, respectively), ANP (both p<0.001) and BNP (both p<0.001) (Figure 3) compared to control, whereas CA alone had no significant impact on any of these indices. Plasma aldosterone levels, on the other hand, were significantly increased by CA (p<0.001) and decreased by Ucn2 (p<0.01), while dual therapy was characterized by an initial transient rise (from 30-60 mins) 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 1hr 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 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 three 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 2-3-fold greater than that achieved by CA alone (all p<0.05).
(Figure 4). In addition, urine potassium and creatinine excretion (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-utilized because of fears of hyperkalemia, hypotension and renal
impairment.6,7 If Ucn2 is likewise to find a place in the treatment of HF, it should ideally
enhance any beneficial effects of MRAs whilst 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) re-absorption, 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 appreciable rise in plasma potassium concentrations (by 0.47mmol/L)
over the 6-hour study period. These results are in accord with those demonstrated in previous
studies of acute MR blockade.25-27 Nephrotoxicity is also well-recognized concern with clinical
use of MRAs,7 and there was a clear trend for CrCl to fall serially from baseline to 6 hrs
(82.6±6.8 vs 75.0±6.4 ml/min) during CA treatment. When Ucn2 was co-administered 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 ANP/BNP, are similar to those seen in our earlier work with the peptide in ovine HF,\textsuperscript{12-15} and are likely a consequence of improvements in glomerular filtration (as judged by the increase in CrCl), renal vasodilatation\textsuperscript{28} and attenuation of circulating anti-natriuretic/anti-diuretic factors (AngII, aldosterone, AVP). Direct tubular actions of Ucn2 are also plausible given that not only is the CRF\textsubscript{2} receptor present in the proximal tubules of the kidney,\textsuperscript{29} but administration of the peptide is accompanied by increases in urine cyclic adenosine monophosphate (Ucn2’s intracellular second messenger).\textsuperscript{12,15} Of interest, Ucn2 treatment in diabetic rodents improves renal structure and function in association with reduced kidney levels of transforming growth factor-\beta1, vascular endothelial growth factor and malonaldehyde (a marker of oxidative stress), and increased superoxide dismutase activity (an antioxidant with powerful anti-inflammatory actions).\textsuperscript{30,31} These Ucn2 effects, directly counter the oxidative, inflammatory and pro-fibrotic actions of aldosterone.\textsuperscript{1,2} 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/AngII and those with already impaired potassium excretion due to progressive age or disease-related decline in glomerular filtration rate, who are particularly vulnerable to the development of hyperkalemia.\textsuperscript{32} In addition, in ADHF with total body under-perfusion, acidosis is present or threatened, and falls in pH are a powerful stimulus for elevated potassium, as are falls in renal function (itself a cause of acidosis) - things which render significant hyperkalaemia (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 CA had little impact on either PRA or plasma AngII - results comparable to those reported by other investigators following acute MR antagonism,\(^2\),\(^3\),\(^4\) 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 AngII. While the Ucn2-induced natriuresis (3-fold greater than that seen 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\(^8\) and receptor\(^2\) in the kidney), or to inhibit sympathetic drive to the juxtaglomerular cells (with Ucn2 previously shown to suppress cardiac sympathetic drive).\(^3\)\(^5\) Moreover, there is some evidence suggesting that the Ucns may antagonize AngII production via suppression of ACE levels.\(^1\)\(^8\)

As noted by others investigating the short-term effects of MR antagonism,\(^2\),\(^3\),\(^4\),\(^6\) CA in the present study was characterized by marked (more than 2-fold) increases in plasma aldosterone levels, indicating successful blockade of the MR throughout the experiment. Ucn2, on the other hand, induced a significant decline in circulating aldosterone that presumably reflects the coincident decreases in plasma AngII and perhaps potassium levels, although the latter was not reduced in as sustained a fashion (up to 2 hours post-infusion) as were both AngII and aldosterone. However, it is conceivable that the peptide also has a direct inhibitory effect on aldosterone secretion in light of reports that Ucn2\(^8\) and the CRF\(_2\) receptor\(^3\)\(^7\) 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. While the RAAS-inhibitory actions of Ucn2, noted previously by our group,\textsuperscript{12-15} are obviously beneficial in the setting of HF, as an adjunct therapy to MR antagonism it may have additional clinical benefits given that aldosterone appears to also have non-MR-mediated effects.\textsuperscript{38,39}

Both Ucn2 and CA treatments enhanced plasma cortisol levels in the present study. A stimulatory effect of MR blockade by CA on the hypothalamus-pituitary-adrenocortical (HPA) axis is consistent with previous reports,\textsuperscript{34,40} while the Ucn2-induced rise in cortisol may be due to an attendant increase in plasma Ucn1 (which we have formerly shown occurs following Ucn2 administration\textsuperscript{12} presumably as a consequence of competition for the CRF\textsubscript{2} receptor) with subsequent activation of CRF\textsubscript{1} and the HPA axis.\textsuperscript{41} While HPA 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\textsuperscript{15} and in the present study. Moreover, actions of Ucn2 to increase cortisol appear to be less pronounced in man.\textsuperscript{16}

In keeping with the diuretic and vasorelaxant effects of CA,\textsuperscript{42,43} its administration in the present study significantly reduced MAP and CTPR. 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 to 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. While 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 post-infusion follow-up. In severe ADHF (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 ADHF who are already hypotensive, with kidneys struggling at, or falling below, autoregulatory limits and with universal multi-tissue underperfusion, any further falls in blood pressure are potentially disastrous and to be avoided if at all possible.

In conclusion, acute Ucn2 co-treatment with an MRA in an experimental model of HF further improved hemodynamics relative to that achieved by CA alone, reduced PRA, AngII, aldosterone and AVP 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 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 National Heart Foundation of New Zealand and the Health Research Council of New Zealand.

Disclosures

None.

References


25. Gudmundsson FF, Viste A, Myking OL, Grong K, Svanes K. Effects of the aldosterone receptor antagonist potassium canrenoate on renal blood flow and urinary output during


Table 1. Effects of rapid left-ventricular pacing (7 days at 225 bpm)

<table>
<thead>
<tr>
<th></th>
<th>Non-paced</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>
</tr>
<tr>
<td>dP/dt max (mmHg/s)</td>
<td>2089±153</td>
<td>1206±167 ‡</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>84±2</td>
<td>74±2 ‡</td>
</tr>
<tr>
<td>Left atrial pressure (mmHg)</td>
<td>4.1±0.3</td>
<td>23.5±0.7 ‡</td>
</tr>
<tr>
<td>Total peripheral resistance (mmHg/L/min)</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/hr)</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>268±157</td>
<td>13252±4504 ‡</td>
</tr>
<tr>
<td>Epinephrine (pmol/L)</td>
<td>490±88</td>
<td>1817±1254 *</td>
</tr>
<tr>
<td>Plasma sodium (mmol/L)</td>
<td>142±1</td>
<td>141±1</td>
</tr>
<tr>
<td>Plasma potassium (mmol/L)</td>
<td>3.95±0.06</td>
<td>3.83±0.20</td>
</tr>
<tr>
<td>Plasma creatinine (umol/L)</td>
<td>68.5±1.8</td>
<td>83.9±5.0 †</td>
</tr>
<tr>
<td>Urine volume (ml/hr)</td>
<td>81±11</td>
<td>96±30</td>
</tr>
<tr>
<td>Urinary sodium excretion (mmol/hr)</td>
<td>2.62±0.30</td>
<td>0.33±0.12 ‡</td>
</tr>
<tr>
<td>Urinary potassium excretion (mmol/hr)</td>
<td>9.0±0.6</td>
<td>6.9±1.9 *</td>
</tr>
<tr>
<td>Urinary creatinine excretion (mmol/hr)</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>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>31.2±1.2</td>
<td>25.3±0.9 *</td>
</tr>
</tbody>
</table>

Mean±SEM measurements in normal sheep (Non-paced: Laboratory normal data, n=20) and sheep with heart failure induced by 7 days of rapid left-ventricular pacing at 225 bpm (Paced: Vehicle control baseline data, n=8). Significant differences are shown by: * p<0.05, † p<0.01, ‡ p<0.001.
Table 2. Effects of urocortin 2 (Ucn2) and Canrenoic acid (CA) in sheep with heart failure

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>6hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>
<td>24.1±1.0</td>
</tr>
<tr>
<td>Ucn2</td>
<td>25.0±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>
<td>23.5±0.7</td>
</tr>
<tr>
<td>Canrenoic</td>
<td>25.5±0.8</td>
<td>24.9±0.7</td>
<td>24.8±0.7</td>
<td>24.8±0.7</td>
<td>24.9±0.8</td>
<td>24.6±0.7</td>
</tr>
<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>
</tr>
<tr>
<td><strong>Plasma Epinephrine (pmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>Canrenoic</td>
<td>1138±284</td>
<td>1762±353</td>
<td>1371±315</td>
<td>1314±298</td>
<td>1615±399</td>
<td>1468±212</td>
</tr>
<tr>
<td>Ucn2+CA</td>
<td>1423±534</td>
<td>2144±616</td>
<td>1584±485</td>
<td>1234±359</td>
<td>1405±409</td>
<td>1395±358</td>
</tr>
<tr>
<td><strong>Plasma Norepinephrine (nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>141±1</td>
<td></td>
<td></td>
<td></td>
<td>142±1</td>
<td></td>
</tr>
<tr>
<td>Ucn2</td>
<td>140±2</td>
<td></td>
<td>139±2</td>
<td></td>
<td>137±3</td>
<td></td>
</tr>
<tr>
<td>Canrenoic</td>
<td>142±1</td>
<td></td>
<td>142±1</td>
<td></td>
<td>141±1</td>
<td></td>
</tr>
<tr>
<td>Ucn2+CA</td>
<td>139±2</td>
<td></td>
<td>139±1</td>
<td></td>
<td>139±1</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma sodium (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.83±0.20</td>
<td></td>
<td>3.92±0.18</td>
<td></td>
<td>3.99±0.20</td>
<td>3.95±0.19</td>
</tr>
<tr>
<td>Ucn2</td>
<td>3.75±0.15</td>
<td></td>
<td>3.17±0.12‡</td>
<td></td>
<td>3.03±0.17‡</td>
<td>3.35±0.12‡</td>
</tr>
<tr>
<td>Canrenoic</td>
<td>3.88±0.15</td>
<td></td>
<td>4.07±0.21*</td>
<td></td>
<td>4.25±0.15‡</td>
<td>4.35±0.11‡</td>
</tr>
<tr>
<td>Ucn2+CA</td>
<td>3.95±0.12</td>
<td></td>
<td>3.40±0.15‡</td>
<td></td>
<td>3.30±0.16‡</td>
<td>3.67±0.19*</td>
</tr>
<tr>
<td><strong>Plasma potassium (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>83.9±5.0</td>
<td></td>
<td>81.9±4.6</td>
<td></td>
<td>83.1±4.9</td>
<td>81.0±5.6</td>
</tr>
<tr>
<td>Ucn2</td>
<td>84.9±6.8</td>
<td></td>
<td>80.8±5.9</td>
<td></td>
<td>78.0±6.6</td>
<td>77.8±6.7</td>
</tr>
<tr>
<td>Canrenoic</td>
<td>82.4±3.9</td>
<td></td>
<td>85.5±4.2</td>
<td></td>
<td>86.0±4.2</td>
<td>84.3±3.7</td>
</tr>
<tr>
<td>Ucn2+CA</td>
<td>83.3±6.4</td>
<td></td>
<td>80.9±6.5</td>
<td></td>
<td>77.9±6.1</td>
<td>75.8±6.1</td>
</tr>
<tr>
<td><strong>Creatinine clearance (ml/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>89.8±7.9</td>
<td></td>
<td>82.7±9.4</td>
<td></td>
<td>87.4±8.4</td>
<td>95.1±12.2</td>
</tr>
<tr>
<td>Ucn2</td>
<td>90.4±12.2</td>
<td></td>
<td>99.1±13.7†</td>
<td></td>
<td>116.2±15.2‡</td>
<td>108.6±11.3*</td>
</tr>
<tr>
<td>Canrenoic</td>
<td>82.0±6.8</td>
<td></td>
<td>82.1±5.5</td>
<td></td>
<td>79.6±6.2</td>
<td>75.0±6.4</td>
</tr>
<tr>
<td>Ucn2+CA</td>
<td>84.3±8.8</td>
<td></td>
<td>105.5±12.4†</td>
<td></td>
<td>108.0±10.0†</td>
<td>104.6±15.7</td>
</tr>
<tr>
<td><strong>Water intake (ml/2hrs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>475±125</td>
<td></td>
<td>315±71</td>
<td></td>
<td>179±58</td>
<td>265±78</td>
</tr>
<tr>
<td>Ucn2</td>
<td>744±209</td>
<td></td>
<td>304±145</td>
<td></td>
<td>284±92</td>
<td>158±63</td>
</tr>
<tr>
<td>Canrenoic</td>
<td>573±164</td>
<td></td>
<td>325±135</td>
<td></td>
<td>286±108</td>
<td>174±78</td>
</tr>
<tr>
<td>Ucn2+CA</td>
<td>439±164</td>
<td></td>
<td>271±120</td>
<td></td>
<td>128±42</td>
<td>309±102</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Significant differences from Control arm in respective state shown by: * p<0.05, † p<0.01, ‡ p<0.001.
Figure Legends

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

Figure 3. Mean±SEM hormonal responses to 4-hour infusions of vehicle (○), canrenoic acid (200mg bolus+75mg/hr infusion) (▲), Ucn2 (50ug bolus+75ug/hr 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 (50ug bolus+75ug/hr infusion) (striped bars), canrenoic acid (200mg bolus+75mg/hr infusion) (dotted 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.
Vehicle (○), Canrenoic acid (▲), Ucn2 (♦), Canrenoic + Ucn2 (▪)

Left ventricular dP/dt (max) (mmHg/s)

Cardiac output (L/min)

Mean arterial pressure (mmHg)

Left atrial pressure (mmHg)

Calculated total peripheral resistance (mmHg/L/min)

Time (hours)
Interactions of enhanced urocortin 2 and mineralocorticoid receptor antagonism in experimental heart failure.
Miriam T. Rademaker, Christopher J. Charles, M. Gary Nicholls and A. Mark Richards

Circ Heart Fail. published online June 10, 2013;
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/early/2013/06/10/CIRCHEARTFAILURE.112.000205